THE EFFECT OF CHRONIC EXPOSURE OF NICOTINE INHALATION TO THE COUNT OF SPERMATOGONIA, SERTOLI CELLS AND LEYDIG CELLS OF YOUNG WHITE RAT WISTAR STRAIN

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ABSTRACT

Objective: *To analyze the difference in the number of spermatogonia, leydig cells and sertoli cells in young age of white mice Wistar strain after inhalation of chronic nicotine exposure.* ***Material & Method:*** *Laboratory experimental study with post test only control group design, measurement of spermatogonium, leydig cell, sertoli cell in 5 groups of young male Wistar strain, negative control group and treatment group given nicotine exposure 0.5 mg, 1 mg, 2 mg, and 4 mg/kg body weight/day for 30 days.* ***Results:*** *A significant reduction in spermatogonium was found in the group given nicotine 0.5 mg/kgBW/day (p=0.048), 1 mg/kgBW/day (p=0.002), 2 mg/kgBW/day (p=0.002) and 4 mg/kgBW/day (p=0.000) when compared to the control group. Significant decreases were also seen in the group receiving 4 mg of nicotine exposure compared with 0.5 mg (p=0.018). Significant decrease in sertoli cell count was seen only in the nicotine group of 4 mg/kgBW/day compared with the control group (p=0.047). A significant decrease in leydig cell count was found in the nicotine 2 mg/kgBW/day (p=0.037) and nicotine group 4 mg/kgBW/day (p=0.023) when compared with the control group. Significant decreases were also found in the 4 mg/kgBW/day group compared to the 0.5 mg/kgBW/day group (p=0.004). In this study there were also a decrease in the number of spermatogonia, sertoli cells, and leydig cells in the increased dose of nicotine given although not statistically significant.* ***Conclusion:*** *Chronic exposure of nicotine per inhalation may decrease the number of spermatogonia, sertoli cells, and leydig cells. The higher the dose of nicotine given the greater the decrease in the number of spermatogonium cells, sertoli cells, and leydig cells that occur. This proves that nicotine is one of the causes of infertility in men.*

Keywords: *Nicotine, spermatogonia, sertoli cells, leydig cells, infertility.*

ABSTRAK

Tujuan: *Untuk menganalisis perbedaan jumlah spermatogonium, sel leydig dan sel sertoli pada tikus putih strain Wistar usia muda paska paparan kronis nikotin secara inhalasi.* ***Bahan & Cara:*** *Penelitian eksperimental laboratorium dengan post test only control group design, dengan pengukuran variabel jumlah spermatogonium, sel leydig, sel sertoli pada 5 kelompok tikus witar jantan muda yaitu kelompok kontrol negatif dan kelompok perlakuan yang diberikan paparan nikotin 0.5 mg, 1 mg, 2 mg, dan 4 mg/kgBB/hari selama 30 hari.* ***Hasil:*** *Didapatkan penurunan jumlah spermatogonium yang signifikan pada kelompok yang diberikan nikotin 0.5 mg/kgBB/hari (p=0.048), 1 mg/kgBB/hari (p=0.002), 2 mg/kgBB/hari (p=0.002) dan 4 mg/kgBB/hari (p=0.000) jika dibandingkan dengan kelompok kontrol. Penurunan yang signifikan juga terlihat pada kelompok yang mendapatkan paparan nikotin 4 mg dibandingkan dengan 0.5 mg (p=0.018). Penurunan jumlah sel sertoli yang signifikan hanya terlihat pada kelompok nikotin 4 mg/kgBB/hari dibandingkan dengan kelompok kontrol (p=0.047). Penurunan jumlah sel leydig yang signifikan didapatkan pada kelompok nikotin 2 mg/kgBB/hari (p=0.037) dan kelompok nikotin 4 mg/kgBB/hari (p=0.023) jika dibandingkan dengan kelompok kontrol. Penurunan yang signifikan juga didapatkan pada kelompok 4 mg/kg/BB dibandingkan dengan kelompok 0.5 mg/kgBB/hari (p=0.004). Pada penelitian ini juga tampak penurunan jumlah spermatogonium, sel sertoli, dan sel leydig pada peningkatan dosis nikotin yang diberikan walaupun tidak signifikan secara statistik.* ***Simpulan:*** *Pemberian paparan kronis nikotin per inhalasi dapat menurunkan jumlah spermatogonium, sel sertoli, dan sel leydig. Semakin tinggi dosis nikotin yang diberikan maka semakin besar penurunan jumlah sel spermatogonium, sel sertoli, dan sel leydig yang terjadi. Hal ini membuktikan bahwa nikotin merupakan salah satu penyebab infertilitas pada pria.*

Kata Kunci: *Nikotin, spermatogonium, sel sertoli, sel leydig, infertilitas.*

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INTRODUCTION

Infertility according to the World Health Organization (WHO) is the failure of a couple to get pregnant for at least 12 months of regular sex without contraception. Infertility includes both men and women. Disturbance on one side or on both sides can disrupt the fertility of couples. So fertility disorders in the husband will be able to cause infertility in couples, and vice versa.[1]

In 50 percent of infertility cases, male infertility factors are found along with cement fluid abnormalities. Fertile couples can compensate for male infertility problems so that infertility problems usually arise as both partners have a disruption to infertility.[2]

Some of the contributing factors of male infertility are congenital or acquired urogenital abnormalities, malignancy, urogenital tract infection, elevated scrotum temperature (eg, varicocele), endocrine disorders, genetic disorders and immunologic factors.[2]

In 30-40 percent of male infertility cases are an ideopathic or infertility that not found cause or abnormalities in physical, laboratory, endocrine, genetic or biochemical examination. Idiopathic male infertility is suspected to result from endocrine disorders due to external factors such as environmental pollution, reactive oxygen species, or genetic and epigenetic disorders.[1]

Reactive Oxygent Species (ROS) is one of free radical and can destruct body tissues including testicular tissue. Testicular tissue is sensitive to ROS. They are bound testicular cell membran. ROS induced lipid peroxidation in germinal cells, which resulting in both cells apoptotic and necrotic.[1,3-5]

Nicotine is one of the existing ROS in the commonly used tobacco in cigarettes. Indonesia is the country with the third largest number of smokers after China and India which is about 28% of the population or about 65 million people. This number increased 0.9% in the period 2000-2008.[6,7]

Accumulation of chronic exposure of nicotine can cause damage from the testicular tissue, both morphologically damaged (spermatogonium, sertoli and leydig cells) and functionally (the regulation of the hormone testosterone) that will disrupt the spermatogenesis process. If it happens for a long time, testicular tissue will become irreversible damage and will cause male infertility.[8]

OBJECTIVE

This study aimed to analyze whether there was an effect of giving chronic inhalation of nicotine to the number of spermatogonia, sertoli cells, and leydig cells that have a role in producing sperm that has an significant role in male fertility.

MATERIAL & METHODS

The experimental animals used in this study were white mice (Rattus Norvegicus) Wistar strain of young males aged 2 months, looking healthy without defects, weighing 150-250 grams, which has certification of animal requirements obtained from Faculty of Veterinary Medicine of Airlangga University Surabaya.

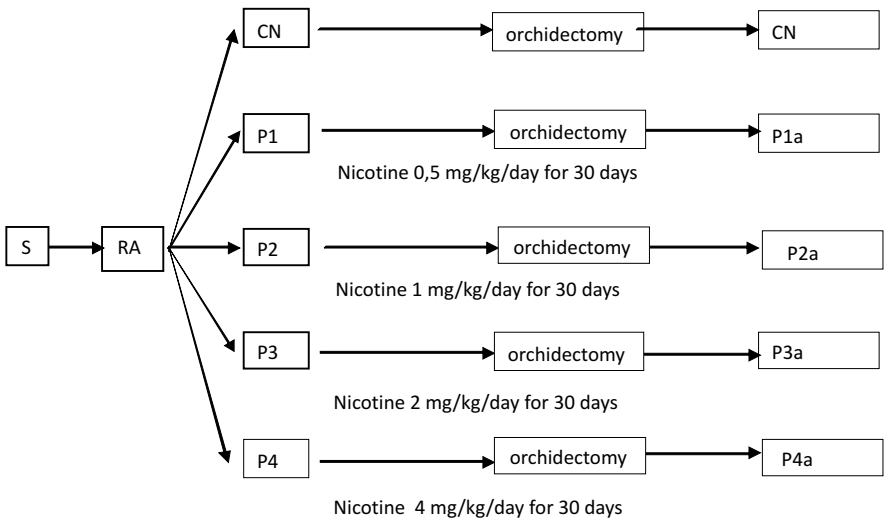

Figure 1. Research design.

The research was conducted in animal laboratory of Faculty of Veterinary Medicine of Airlangga University. Rats were first done in the adaptation process in the enclosure/research environment for 2 weeks.

The study sample was divided into 5 groups with 1 negative control group, and 4 other treatment groups. This grouping was done randomly. The number of samples of this study in accordance with the Federer formula to obtain each of 5 mouse groups. Due to the possibility of dropout samples, each group is added to 6 rats. The total number of mice was 30 rats. The dropout criterion in this study was when the mice died during the study.

The experimental design used was a laboratory experimental study with post test only control group design, with measurements of spermatogonium, leydig, sertoli and spermatogenesis variables performed after experimental animals, randomized groupings of animals by repetition of five experimental animals each group and there was a control group as a comparison (negative control). The research design can be seen in figure 1.

Nicotine (C10H14N2) is an alkaloid organic compound, which generally consists of Carbon, Hydrogen, Nitrogen and sometimes Oxygen. In this study the pure nicotine fluid (Nicotine RTS Vapes, USA) was diluted with 0.9% NaCl, administered by inhalation to experimental animals using a nebulizer (OneMed) inside a specially designed chamber designed for this study made of glass of 38 x 28.5 x 22.5 cm equipped with ventilation. Pure nicotine dose used 0.5 mg/KgBB/times of administration, 1mg/KgBB/administration time, 2 mg/KgBB/administration time, and 4 mg/KgBB/Inhalation is done for 90 minutes every day, for 4 weeks.

The left orchidectomy was performed by the mouse positioned supine in the intraperitoneal 75 mg/KgBB, scrotum skin incision to the left testis and the funiculus was apparent, funiculus in the kIem at 2 adjacent sites and cut off, the proximal pieces tied with 3-0 silk threads (Ethicon Inc., Johnson & Johnson Co., Somerville), removable clamps and testicular samples were then removed and immediately fixed using a 7.4-phin formalin buffer, scrotum skin incision was sewn back one layer using a 3-0 plain catgut yarn (Ethicon Inc., Johnson & Johnson Co., Somerville). Within 1 hour after the testicular sample is taken, it will be processed for histological examination by making paraffin blocks, where the sample will be cut with a thickness of 4 microns. To see spermatogonia, leydig cells, sertoli cells and spermatogenesis used Hematoxylin-Eosine (HE) painting on testicular preparations. After all the research process ends, the experimental animals will be left alive. All actions were performed using aseptic methods.

Spermatogonia in human divided into 3 type, according to cell nucleus. 3 type spermatogonia were dark type A, pale type A, and type B. Spermatogonia were calculated from 5 seminiferous tubules cross sections with 400 times enlargement and HE staining.

Sertoli cells have pyramid shape, polimorf and pale nucleus, contain few of fine chromatin, and attached to the lamina basement. Sertoli cells were calculated from 5 seminiferous tubules cross sections with 400 times enlargement and HE staining.

Leydig cells, also known as Leydig intersisial cells, are complementary to the seminiferous tubules of the testes. Leydig cells produce testosterone and are always associated with the nervous system. Leydig cells were marked with round shaped nucleus and the cytoplasm was granular and eosinophilic. Leydig cells were calculated from 5 seminiferous tubules cross sections with 400 times enlargement and HE staining.

From the calculation of sertoli cell count, leydig cell, and spermatogonium number (numerical variable), normality test (to know normal data distribution or not) and variance test (to know the data variant is the same or not). Normality test results and data variance were used as the basis for determining hypothesis testing to determine the difference of sertoli cell cell count, leydig cell, and spermatogonia between control group and treatment group. If the distribution of normal and homogeneous data, then used the test parametric hypothesis (one way Anova). If the distribution of data is not normally distributed and homogeneous, then the alternative is selected non parametric test (Kruskall-Wallis).[9,10]

Hypotheses tested with value of significance gained. Significant value is $p<0.05$. If one way Anova with $p<0.05$, then continue with multiple comparison test or Post Hoc Test or if Kruskall-Wallis test with $p<0.05$ followed by Mean Whitney test, that is to know more detailed couples of different treatment groups were significantly different and did not differ significantly. All data is analyzed with computerized statistical software

product and service solution 20 for windows (SPSS 20).[9,10]

RESULTS

Of the 30 rats were grouped into 5 groups by randomization. The rats were weighed at before and after 30 days of treatment. After that done orchidectomy. The testes were weighed and the staining process was done with haematoxylin eosin. The characteristics of the study subjects were as in table 1. In the table it can be seen that the initial body weight, final weight, weight of the testes of each group was homogeneous.

In this research, the influence of nicotine inhalation on the number of spermatogonium can be seen in table 2. Based on the results of statistical tests using shapiro-wilk test, the data distribution of each group was normal ($p>0.05$). On homogeneity test showed homogeneous data $p>0.005$, so this research used one way Anova test to analyze the differences between groups (Table 2).

In this research one way Anova test results showed that there were significant differences between groups ($p<0.005$), therefore the data was continued with Post Hoc test to know the differences between groups. Based on the statistical test the homogeneity of the variants between groups was

Table 1. Basic characteristics.

Characteristics	Group					p value
	KN	KP-1	KP-2	KP-3	KP-4	
Initial BW (gr)*	150 ± 17.89	163.33 ± 13.66	163.33± 15.05	151.67 ± 21.37	171.67 ± 28.58	0.32
Final Bw (gr)*	170 ± 18.97	185 ± 24.29	176.67 ± 13.66	176.67 ± 31.41	193.33 ± 37.24	0.60
Testes weight (gr)	1.23 ± 0.31	1.30 ± 0.17	1.26 ± 0.07	1.41 ± 0.10	1.39 ± 0.23	0.44

Table 2. Comparison of the count of spermatogonia in each group.

Group	N	Mean ± SD	p value
Control	6	28.967 ± 4.236	0.001
Nicotine 0.5 mg/kg/day	6	24.433 ± 5.234	
Nicotine 1 mg/kg/day	6	21.433 ± 1.777	
Nicotine 2 mg/kg/day	6	21.267 ± 3.326	
Nicotine 4 mg/kg/day	6	18.900 + 3.443	

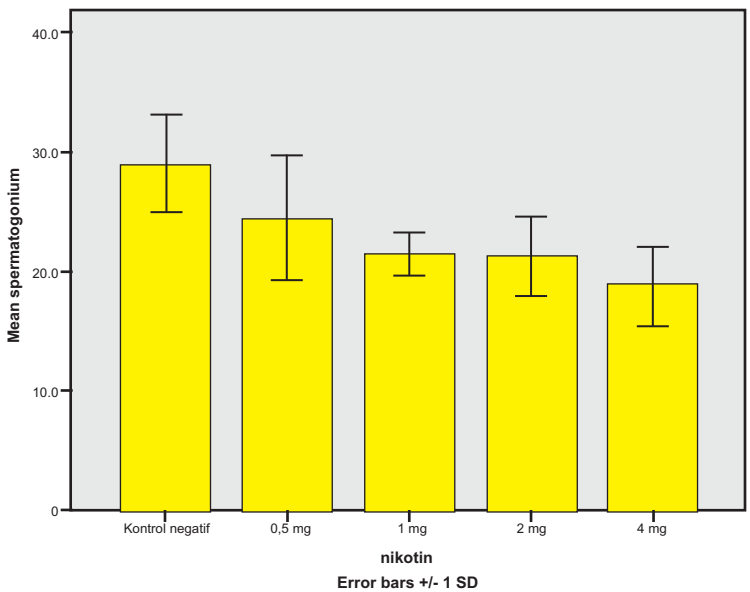

Figure 2. Graph of the ratio of spermatogonia in each group.

homogeneous (p=0.001), therefore the Post Hoc test was tested by the LSD test (Table 3).

Based on post hoc test result, there were significant difference mean spermatogonia between control group and nicotine 0.5 mg/kg/day, 1 mg/kg/day, 2 mg/kg/day and 4 mg/kg/day 5 mg/day.

In this study, when compared between groups receiving nicotine, there were significant difference between the groups receiving nicotine 0.5 mg/kg/day and the group receiving nicotine 4 mg/kg/day (p=0.018) in which the spermatogonium in the group who received nicotine 4 mg/kg/day had a lower amount compared to those receiving nicotine 0.5 mg/kg/day. However, for comparison of spermatogonium counts between the other groups there was no significant difference even though descriptively found the higher the dose given the less the number of spermatogonium cells.

In this study the influence of nicotine inhalation on the count of sertoli cells can be seen in table 4. Based on the results of statistical tests using shapiro-wilk test, the data distribution of each group was normal (p>0.05). In homogeneity test showed non homogeneous data p<0.005, then in this study Anova Brown Forsythe test to see the differences between groups (Table 4).

Table 3. Post Hoc LSD analysis compares the number of spermatogonia in each group.

Group	0.5 mg/kgBW	1 mg/kgBW	2 mg/kgBW	4 mg/kgBW
Control (-)	0.048*	0.002*	0.002*	0.000*
0.5 mg/kgBW		0.181	0.159	0.018*
1 mg/kgBW			0.940	0.257
2 mg/kgBW				0.288
4 mg/kgBW				

Table 4. Anova Brown Forsythe test compares nicotine inhalation between groups.

Group	N	Mean	p value
Control	6	13.733 ± 2.276	0.00
Nicotine 0.5 mg/kg/day	6	12.300 ± 2.035	
Nicotine 1 mg/kg/day	6	12.233 ± 2.107	
Nicotine 2 mg/kg/day	6	10.500 ± 0.953	
Nicotine 4 mg/kg/day	6	9.467 ± 0.665	

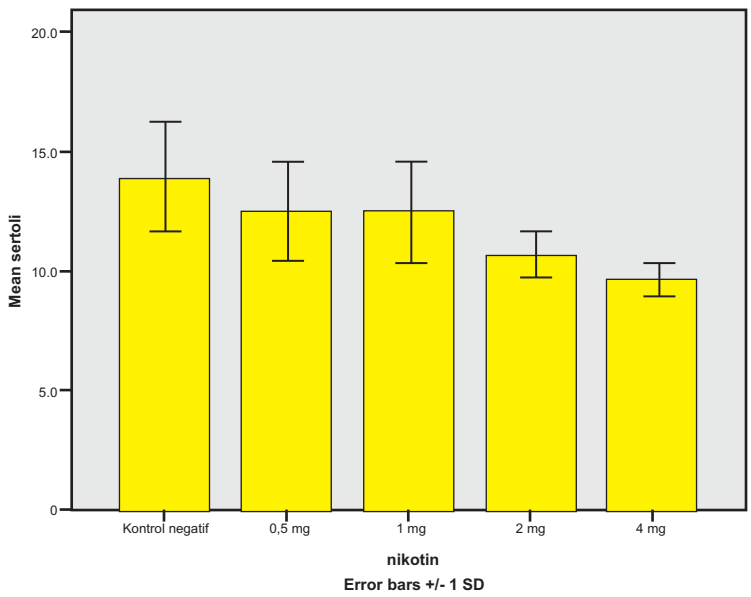

Figure 3. Graph of the number of sertoli cells in each group.

In this study Anova Brown Forsythe test results can be seen that there are significant differences between groups p=0.005, therefore the data continued with the Post Hoc test to analize the differences between groups. Based on the statistical test the homogeneity of the variants between the groups was not homogeneous (p=0.004), therefore the Post Hoc test was tested by Tamhanne test (Table 5).

From the results of Tamogen post hoc test, there was no significant difference in number of sertoli cells between the control group and the group receiving nicotine 0.5 mg/kg/day, nicotine 1 mg/kg/day and nicotine 2 mg/kg/day, but between the control group and the nicotine-treated group of 4 mg/kg/day there was a significant difference in the number of sertoli cells (p=0.047) in which the group in the nicotine group received 4 mg/kg/day had a lower number than the control group.

In this study, if we compare the number of sertoli cells between the groups, there was no statistically significant difference, but although not statistically significant, it was found that the higher the dose of nicotine given the lowering in the number of sertoli cells.

In this study the influence of nicotine inhalation on the count of Leydig cells can be seen in table 6. Based on the results of statistical tests using

Table 5. Post Hoc Tamhone analysis compares the count of sertoli cells in each group.

Group	0.5 mg/kgBW	1 mg/kgBW	2 mg/kgBW	4 mg/kgBW
Control (-)	0.961	0.953	0.147	0.047*
0.5 mg/kgBW		1.000	0.610	0.161
1 mg/kgBW			0.685	0.200
2 mg/kgBW				0.447
4 mg/kgBW				

Table 6. Comparison of the count of leydig cells in each group.

Group	N	Mean ± SD	p value
Control	6	29.167 ± 7.797	0.00
Nicotine 0.5 mg/kg/day	6	20.133 ± 2.930	
Nicotine 1 mg/kg/day	6	15.500 ± 4.414	
Nicotine 2 mg/kg/day	6	14.233 ± 3.347	
Nicotine 4 mg/kg/day	6	12.067 ± 2.265	

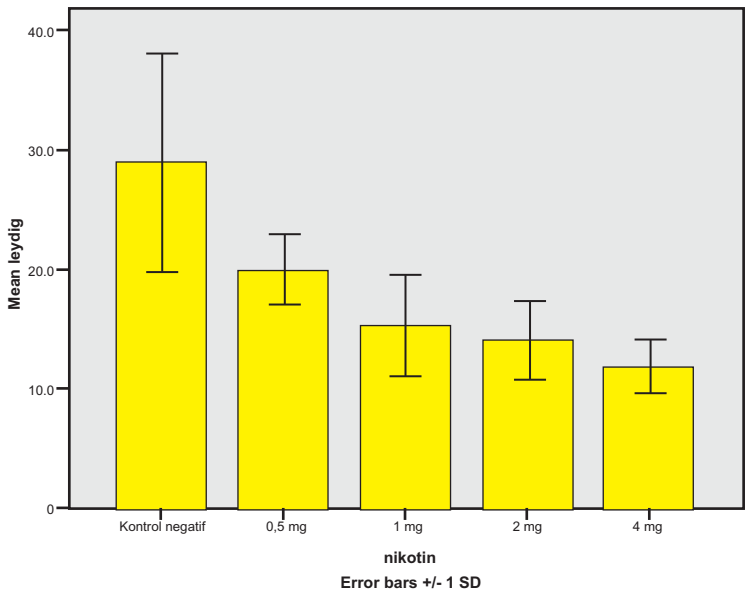

Figure 4. Graph of the ratio of Leydig cell counts to each group.

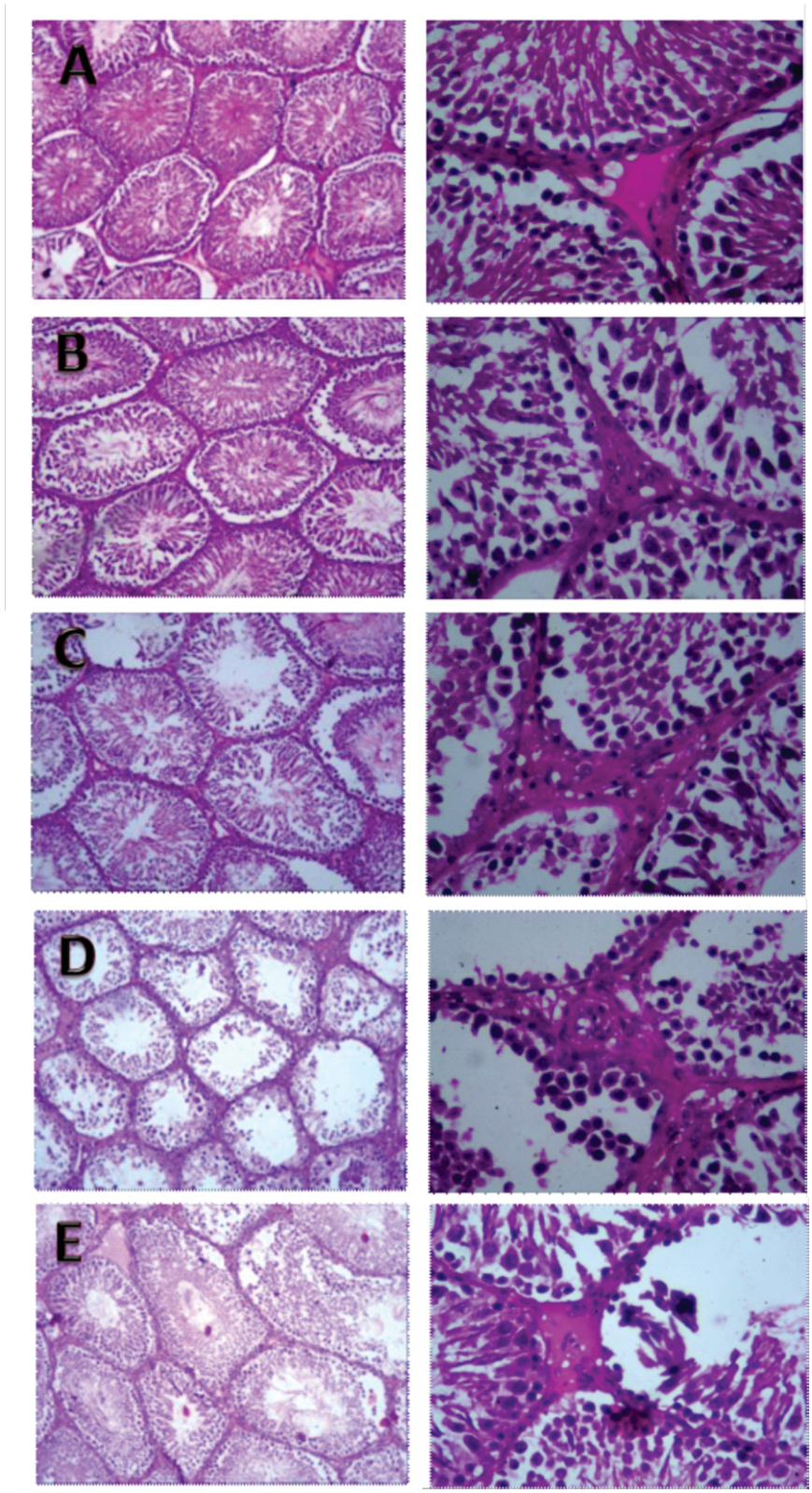

Figure 5. Microscopic cross-sectional view of seminiferous tubules in: A. rats of control group, B. Nicotine 0.5 mg/kg/day, C. Nicotine 1 mg/kg/day, D. Nicotine 2 mg/kg/day, E. Nicotine 4 mg/kg/day. 100x magnification and 400x magnification. Nikon eclipse e-100 microscope. Optilab Viewer 2.2. 400x.

Table 7. Post Hoc Tamhane's analysis comparisons the number of leydig cells in each group.

Group	0.5 mg/kg BW	1 mg/kg BW	2 mg/kg BW	4 mg/kg BW
Control (-)	0.304	0.057	0.037*	0.023*
0.5 mg/kg BW		0.472	0.086	0.004*
1 mg/kg BW			1.000	0.755
2 mg/kg BW				0.919
4 mg/kg BW				

shapiro-wilk test, the data distribution of each group was normal ($p>0.05$). On homogeneity test showed unhomogeneous data $p<0.005$, then in this research Anova Brown Forsythe test to see the differences between groups.

In this study Anova Brown Forsythe test results can be seen that there were significant differences between groups $p=0.000$, therefore the data continued with the Post Hoc test to analyze the differences between groups. Based on the statistical test the homogeneity of the variants between the groups was not homogeneous ($p=0.00$), therefore the Post Hoc test was tested by Tamhanne test (Table 7).

Based on Tamhane's post hoc test results, there was no significant difference in the count of Leydig cells between the control group and the group receiving nicotine 0.5 mg/kg/day and 1 mg/kg/day, but between the control group and the received group Nicotine 2 mg/kg/day and 4 mg/kg/day there was a significant difference in the count of Leydig cells in each group ($p=0.037$ and $p=0.023$), respectively in the group receiving nicotine 2 mg/kg/day and 4 mg/kg/day had a lower Leydig cell count than the control group.

In this study, if we compare between the nicotine-derived group, there was significant difference between the group receiving nicotine 0.5 mg/kg/day and the group receiving nicotine 4 mg/kg/day ($p=0.004$) in which the number of leydig cells in the group that received nicotine 4 mg/kg/day had a lower amount compared to those receiving nicotine 0.5 mg/kg/day. However, for comparison the number of leydig cells between the other groups was not found to be significantly different although descriptively found the higher the dose of nicotine administered the less the number of leydig cells.

DISCUSSION

Nicotine is the main toxic substance of cigarette smoke as we know it have an significant role in the progress of cardiovascular disease and lung cancer in smokers. But often we ignore that there are mutagenic consequences in cigarette smoke and the potential effects on the production and maturation of germ cells as well as on the reproductive organ itself.[11,12]

In previous studies it has been shown that nicotine administration leads to elevated lipid levels and lipid peroxidation products in serum and mouse organ tissues. Other studies have also reported that nicotine administration affects the relationship between tissue and free radical defense system components, and nicotine also induces oxidative stress in germ cells.[13-16]

Free radicals such as superoxide and hydroxyl cause cell damage. Damage from the phospholipid membrane and lipid peroxidation releases unsaturated fatty acids from the phospholipid membrane. Membrane structure and fluidity will change.[17]

Effect of nicotine on decrease of sperm quality and testicular histology in mice probably due to accretion of oxidative degradation of phospholipids. Helen et al (2000) was report that in nicotine-treat mice, accretion of lipid peroxidation products concentration were also associated with decreased scavenger enzyme activity such as catalase and dismutase peroxide.[18,19]

Testes in mice given nicotine exposure show smaller and the seminiferous tubules are shrunk and separated by wider intertisial spaces. In some seminiferous tubules examined have collapse or degeneration of spermatogenic cells. Characteristic features of collapsed seminiferous tubules is the absence or low of spermatozoa at the middle of the tubule. Another effect is the presence of thickening of the basement membrane and degenerating cells. In other tubules, however, spermatogonia and sertoli cells appear normal shape and amount. The intersitial cells of leydig exhibit fewer amounts and atrophy than mice not given nicotine.[20]

In Reddy et al (1997) study, it was found that there was a decrease in the weight of the testes in mice given intraperitone injectable nicotine with a

dose of 0.2 mg, 0.4 mg and 0.6 mg/100 grBW for 15 days compared with negative controls. This reduction in the weight of the testes is thought to be due to a decrease in the availability of pituitary gonadrotropin (LH and FSH) resulting from the inhibitory effect of nicotine.[21,22]

In another study, Oyeyipo et al (2010), also found a significant decrease in testicular weight in mice who received nicotine peroral 0.5 mg/kgBW and 1 mg/kgBW administered for 30 days when compared with negative control group rats.[8]

In this study there was no significant difference in the weight of the testes between groups. This is in accordance with the study of Carvalho et al (2006), there was no significant decrease in testis weight in the male wistar rats group given nicotine 0.125 mg/kgBW for 90 days compared with the control group.[23]

The spermatogonium cell is the stem cell of all produced sperm and is a derivative of primordial germ cells that migrate to the gonaldal ridge of yolk sac early in intrauterine life. The spermatogenic cells functioning in the process of fertilization/ reproduction consist of various stages of cleavage ie the spermatogonium cell with distinctive features located near the tubular basal lamina, relatively large, and having irregular chromatin; primary spermatocyte cells with the greatest characteristics among the other spermatogenic cells and have clear clear chromatin; secondary spermatocytes with rare features, small in size with small nuclei and pale cytoplasm; spermatid cells typically of the smallest size compared to other spermatogenic cells, have little cytoplasm and solid chromatin; and spermatozoa cells with long-term characteristics such as exclamation marks and located in the central area of the lumen.[24,25]

In this study there were significant differences in spermatogonium between the groups of mice who did not receive nicotine exposure with mice receiving nicotine exposure at both doses of 0.5 mg/kg/day, 1 mg/kg/day, 2 mg/kg/day or 4 mg/kg/day is the number of spermatogonium decreased in mice that get nicotine exposure. When compared to the number of spermatogonium cells between groups who received nicotine exposure found only mice who received nicotine exposure of 0.5 mg/kg/day were significantly different with mice who received nicotine 4 mg/kg/day. However, it was found that there was a decrease in the count of spermatogonium cells in nicotine exposure which received higher doses, although the decrease was not significant statistically.

In the Nesseim et al (2011) study, it was found that the reduction in spermatogonium cell counts in nicotine-dependent mice depends on the dose and long of exposure of the nicotine itself. In a study using rats injected with nicotine 0.2 mg, 0.4 mg, and 0.6 mg/100gBB mice for 2 weeks, 4 weeks and 8 weeks, a significant decrease in spermatogonium counts when compared to the control group was found in group 0.4 mg/100gBB for 8 weeks and 0.6 mg/100gBW for 4 weeks and 8 weeks.[26]

In the nicotine-exposed testes there is an increase in cholesterol synthesis, triglycerides, phospholipids and free fatty acids that indicate the occurrence of specific gonadotoxic effects. Morphological abnormalities in sertoli cells that occur after nicotine exposure are similar to those in ischemic experiments, suggesting that vascular lesions may have an important role in nicotine toxicity.[27]

In the Nesseim et al (2011) study, it was found that the reduction in the count of sertoli cells in nicotine-dependent mice depends on the dose and long of exposure of the nicotine itself. In a study using rats injected 0.2 mg, 0.4 mg, and 0.6 mg/100gBB of mice for 2 weeks, 4 weeks and 8 weeks were found to decrease sertoli levels compared to the control group, but decreased sertoli cell which is significant only shown in the group of rats given nicotine 0.6 mg/100gBB either for 2 weeks, 4 weeks or 8 weeks.[26]

In this study, there is a decrease in the count of sertoli cells along with the increased dose of nicotine given. The decrease in sertoli cell count was seen in nicotine administration of 0.5 mg/kg/day, 1 mg/kg/day and 2 mg/kg/day, but significant reductions were seen only in the administration of nicotine dose 4 mg/kg/day when compared with negative control.

The effect of nicotine on the number of sertoli cells has been proven but with a lighter rate than other spermatogenic cells. The phagocytic ability of sertoli cells is impaired, evident from the excess residual bodies in the luminae of the seminiferous tubules. The disrupted sertoli cell function affects spermatogenesis.[26]

The Leydig cell is present in the interstitial compartment of the testes that lies between the seminiferous tubules. In this interstitial compart-

ment there are also macrophages and lymphocytes. Leydig cells are the last cells in response to damage if there are destruction or degeneration of seminiferous tubules because leydig cell in interstitial compartment and they are the most resistant cell from oxidative stress occured in the testes.[28]

In this study there was a decrease of leydig cells significantly only in the group nicotine inhalation at doses of 2 mg/kg/day and 4 mg/kg/day when compared with negative control, for a dose of 0.5 mg/kg/day and 1 mg/kg/day whereas in the study of Kanwal et al, the count of leydig cells in the albino rats group given intraperitoneal nicotine with a dose of 0.5 mg/kg BW for 15 days had shown a significant decrease compared with the control group. This difference probably due to the different pathways of nicotine giving between inhalation and intraperitoneal.[29]

The decrease in the number of leydig cells is thought to be due to an increase of apoptosis, according to Kim et al (2005) study, found that nicotine activates a specific trigger path of intracellular death, possibly by the activation of bax-dependent caspase 3, which induces apoptosis in leydig cells. Thus, nicotine-induced nicotine-leydig cell apoptosis is thought to be one of the causes of male infertility.[30]

In various studies, the use of nicotine uses different ways. It is also possible for different results. The use of nicotine that is often used in research is the administration of intraperitoneal or subcutaneous injections, oral administration, and perinhalasi.

In a study of rotenberg et al (1980) showed that the absorption of nicotine in the first inhalation of cigarette smoke was rapid and was thought to have no early metabolism in the lungs. In addition, nicotine and metabolic pharmacokinetics are essentially similar to previous studies that used intravenous injection of a single dose of nicotine. In another study, rotenberg et al (1983), showed that inhalation of multiple cigarettes (one cigarette every 8 hours for 15 days) increased the nicotine absorption range, but not its absorption rate, reduced the rate and conversion rate to cotinine, and increased metabolite N- polar oxide. This change is seen in persistent plasma nicotine levels. Nicotine excretion and urinary metabolites further support the conclusion that nicotine pharmacokinetic changes occur after exposure to secondhand smoke (chronic).[31,32]

Nicotine absorption passes through biological membrane depending on the pH. The pH of smoke produced from cigarettes is alkaline, and nicotine especially in the form of unionized so it can quickly pass through the cell membrane. When cigarette smoke reaches the pulmonary alveoli, nicotine is absorbed rapidly regardless of the pH of the smoke. Concentration of nicotine in the blood increases rapidly during smoking and peak at the time of completion. Perhaps, rapid absorption of nicotine through the lung is the result of a large surface area of alveoli and nicotine smelting into the physiological pH fluid, which helps transfer across the cell membrane. Whereas in oral nicotine, the dose of nicotine found in the systemic is less than the oral nicotine dose itself.[12]

The process of smoking cigarettes is complex, and smokers can manipulate the dose of nicotine from the way the cigarette puffs it. Thus, the nicotine intake of cigarette smoking depends on the volume of suction, the depth of inhalation, the level of dilution with the air of the room, the amount of suction and the intensity of the suction. Due to the complexity of the process, the dose of nicotine can not be predicted from the amount of tobacco material or its absorber characteristics. To determine the dose, we need to measure the level of nicotine in the blood and find out how quickly the smoker eliminates nicotine.[12]

Based on that, the downside of this study is that authors only assign nicotine doses per exposure, but can not calculate how many nicotine doses are inhaled by each mouse. Subsequent studies may be able to measure the levels of nicotine in the blood or compare the results obtained from per inhalation exposure with per injection.[12]

CONCLUSION

Chronic inhalation of nicotine per inhalation with doses of 0.5 mg/kg/day, 1 mg/kg/day, 2 mg/kg/day and 4 mg/kg/day in young male wistar rats decreased cell count spermatogonia.

Chronic inhalation of nicotine with a dose of 4 mg/kg/day in young male wistar rats decreased the count of sertoli cells. Chronic inhalation of nicotine with a dose of 2 mg/kg/day, and 4 mg/kg/day in young male wistar rats decreased the count of leydig cells. The decrease in spermatogonia, leydig cells, and sertoli cells count are directly proportional to the dose of nicotine administered.

Later on, further research on the effects of nicotine on infertility in men is needed. The research that can be done is a study of the effect of nicotine influences given per inhalation by per injection, the effect of this nicotine on testosterone levels, antioxidant effects in preventing infertility caused by this nicotine, and research on whether the nicotine update is permanent or temporary.

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