

DIFFERENCES OF TESTOSTERONE LEVELS TO THE DURATION OF INHALED NICOTINE EXPOSURE IN SPRAGUE-DAWLEY MALE WHITE RATS

¹Ido Putra Ramadhan, ¹Tarmono Djojodimedjo, ¹Doddy M. Soebadi.

¹Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.

ABSTRACT

Objective: To analyze the differences in testosterone level in young Sprague-Dawley white rats after exposure of inhaled nicotine and the recovery effect that occurs when nicotine exposure is stopped. **Material & Methods:** This is an experimental study, which variable measurements were taken after the experimental animals were treated. The experimental grouping process was carried out randomly, with repetitions of 12 experimental animals in each group and the control group as a comparison (negative control). This study using male Sprague-Dawley white rats as samples. Nicotine administration was carried out by inhalation using pure nicotine (C₁₀H₁₄N₂), which was diluted using distilled water. Treatment group 1 (P1) was given nicotine exposure for 30 days, treatment group 2 (P2) for 15 days, and treatment group 3 (P3) for 15 days and then stopped for 15 days compared to the control group. Testosterone levels are measured using the ELISA (Enzyme-Linked Immunosorbent Assay) method in the morning. **Results:** The lowest testosterone levels were found in the P1 and significantly different from the P2 ($p=0.029$) and P3 ($p=0.001$). The results of the analysis show that a decrease in testosterone levels in mice is related to the duration of nicotine exposure. **Conclusion:** Testosterone levels in the control group were significantly higher compared to the treatment group that exposed by nicotine inhalation with a dose of 4 mg/kgBW/day for 15 days, and also there is a significant recovery effect on testosterone levels after the treatment was halted for 15 days.

Keywords: Nicotine, testosterone, recovery.

ABSTRAK

Tujuan: Menganalisis perbedaan kadar testosteron pada tikus putih strain Sprague-Dawley usia muda setelah diberikan paparan nikotin secara inhalasi, serta efek pemulihan yang terjadi saat paparan nikotin dihentikan. **Bahan & Cara:** Penelitian ini dirancang sebagai studi eksperimental laboratorium, dimana pengukuran variabel dilakukan setelah hewan coba diberikan perlakuan. Pengelompokan hewan coba dilakukan secara acak, dengan pengulangan sebanyak 12 ekor hewan coba pada masing-masing kelompok, dan kelompok kontrol sebagai pembanding (kontrol negatif). Penelitian ini menggunakan tikus putih Sprague-Dawley jantan sebagai sampel. Pemberian nikotin dilakukan secara inhalasi dengan menggunakan nikotin murni (C₁₀H₁₄N₂) yang telah diencerkan menggunakan aquadest. Kelompok perlakuan 1 (P1) diberikan paparan nikotin selama 30 hari, kelompok perlakuan 2 (P2) selama 15 hari, dan kelompok perlakuan 3 (P3) selama 15 hari lalu dihentikan selama 15 hari dibandingkan dengan kelompok kontrol. Kadar testosteron diukur menggunakan metode ELISA (Enzyme-Linked Immunosorbent Assay) pada pagi hari. **Hasil:** Kadar testosteron paling rendah didapatkan pada kelompok P1 dan berbeda secara signifikan terhadap kelompok P2 ($p=0,029$) dan terhadap kelompok P3 ($p=0,001$). Hasil analisis pada penelitian ini menunjukkan bahwa penurunan kadar testosteron pada tikus berkaitan dengan durasi paparan nikotin secara inhalasi. **Simpulan:** Kadar testosteron kelompok kontrol lebih tinggi secara signifikan dibandingkan dengan kelompok perlakuan paparan nikotin inhalasi dengan dosis 4 mg/kgBW/hari selama 15 hari, dan didapatkan efek pemulihan yang signifikan pada kadar testosteron setelah perlakuan paparan nikotin inhalasi dihentikan selama 15 hari.

Kata Kunci: Nikotin, testosteron, pemulihan.

Correspondence: Doddy M. Soebadi; c/o: Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya. Jl. Mayjen. Prof. Dr. Moestopo 6-8 Surabaya 60286. Phone: +62315501318; Fax: +62315024971. Mobile phone: +62816500755. Email: dmsuebadi@gmail.com.

INTRODUCTION

Infertility is defined as the failure of a partner to get a pregnancy after an active sexual relationship without using a contraceptive device within 12 months. In the case of male infertility, 50% of cases show abnormalities in semen testing based on the World Health Organization reference value. Testosterone is the main hormone in male and an anabolic steroid that plays an important function on male reproductive tissue, such as testicles and prostate also develop secondary sexual characteristics of male. Testosterone in men is produced primarily in the testis, especially by the Leydig cells.

Nicotine is one of the free radicals contained in tobacco that commonly found in cigarettes.¹ Nicotine abuse by smoking is a big problem for public health. Smoking causes oxidative stress (OS), genetic and epigenetic changes that cause disruption in sperm function and infertility.² The effect of smoking on infertility is thought to originate from toxins formed when cigarettes are burned, including benzopyrene, nicotine, cadmium, and lead.

OBJECTIVE

To analyze the differences in testosterone levels in young Sprague-Dawley white rats after

exposure of inhaled nicotine and the recovery effect that occurs when nicotine exposure is stopped.

MATERIAL & METHODS

This study is as an experimental laboratory study, which variable measurements were taken after the experimental animals were treated, the experimental grouping process was carried out randomly, with repetitions of 12 experimental animals in each group, and the control group as a comparison (negative control). This study using male Sprague-Dawley white rats as samples with the following criteria: 2-3 months old, body weight about 250-300 gram, healthy without any defects or congenital disorders, and were obtained from Animal Laboratory of Faculty of Veterinary Medicine, Universitas Airlangga.³

This research has already obtained an ethics feasibility certificate at the Faculty of Veterinary Medicine, Universitas Airlangga. Nicotine administration was carried out by inhalation using pure nicotine (C₁₀H₁₄N₂) which was diluted using distilled water. Nicotine is given at a dose of 4 mg/kgBW/day in each treatment group. Treatment group 1 (P1) was given nicotine exposure for 30 days, treatment group 2 (P2) for 15 days, and treatment group 3 (P3) for 15 days and then stopped

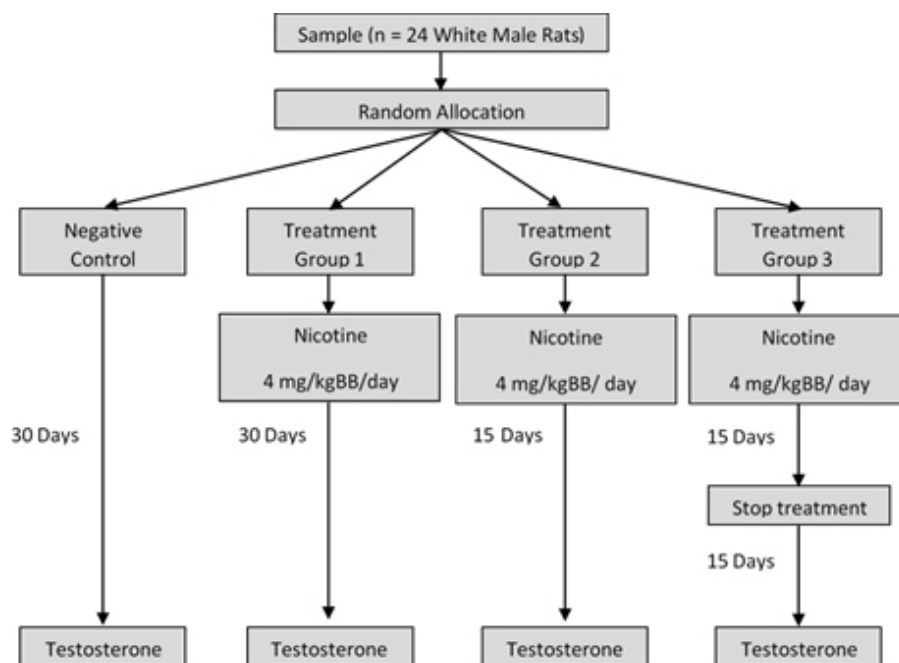


Figure 1. Research conceptual framework.

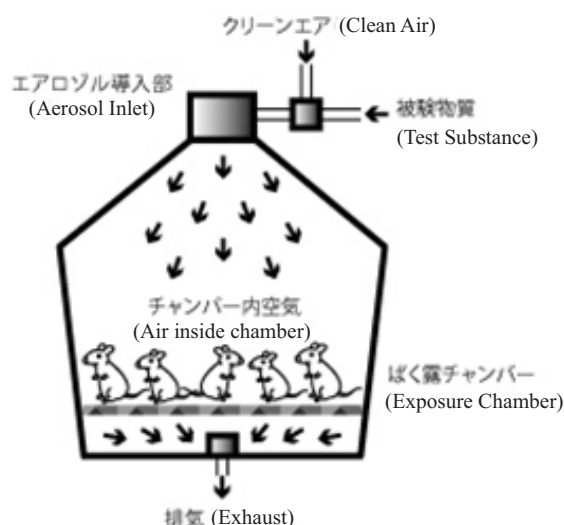


Figure 2. Smoking chamber sketch.

for 15 days compared to the control group. Testosterone levels are measured using the ELISA (Enzyme-Linked Immunosorbent Assay). Determining the duration of intervention, based on pre-existing theory, which states that nicotine exposure for 2-4 weeks has an impact on testicular damage in mice models.⁴

The data then processed and analyzed using statistical product and service solution 20 for windows software (SPSS 25).

RESULTS

The samples used in this study were male Sprague-Dawley white rats aged 10-12 weeks with total amounts of 36 subjects with each group having 12 subjects. The analysis showed a significant difference between the control group and the group exposed to inhaled nicotine for 30 days (P1)

($p=0.000$) or 15 days (P2) ($p=0.000$). There was no significant difference between the control group and the exposed group for 15 days and then stopped for 15 days before measuring the testosterone (P3) levels ($p=0.410$), but descriptively, the average testosterone level in the P3 group was lower than the group control (6.608 vs 6.525). Therefore, in this study, it was proven that exposure to nicotine inhalation can reduce testosterone levels in mice.

The lowest testosterone levels were found in the P1 group and significantly different from the P2 group ($p=0.029$) and in the P3 group ($p=0.001$). Testosterone levels in the P2 group were significantly lower compared to the P3 group ($p=0.017$). The results of the Games-Howell post-hoc test show that a decrease in testosterone levels in mice is related to the duration of exposure, duration without exposure, and dose of nicotine inhalation exposure.

Tabel 1. Post-hoc Games-Howell analysis of comparison of testosterone levels.

Comparison Between Groups	Mean Difference	CI95%		p value
		Lower Limit	Upper Limit	
K- vs P1	0.505	0.279	0.731	0.000*
K- vs P2	0.257	0.156	0.358	0.000*
K- vs P3	0.083	-0.065	0.230	0.410
P1 vs P2	-0.248	-0.474	-0.022	0.029*
P1 vs P3	-0.423	-0.665	-0.180	0.001*
P2 vs P3	-0.174	-0.321	-0.027	0.017*

* $p<0.05$, significantly different by statistic.

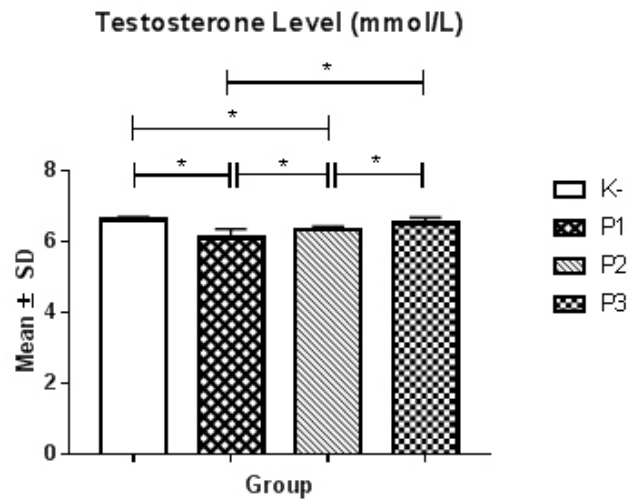


Figure 3. Comparison of testosterone level in each group.

DISCUSSION

One of the factors of infertility in men is the level of the testosterone hormone in the body. Testosterone is the main hormone in men and is an anabolic steroid that plays an important role in male reproductive tissue. Most testosterone (95%) in men is produced by the testes, and the rest by the adrenal glands.⁵ Testosterone is produced by Leydig cells found in interstitial tissue along with mast cells, and macrophages.⁶

In this study, it was found that exposure to inhaled nicotine in rats for 15 and 30 days had significantly lower testosterone levels compared to the control group. In the group given a 15-day recovery interval, there was no significant difference with the control group. The lowest testosterone levels were found in the group with inhaled nicotine exposure for 30 days and significantly lower testosterone levels compared with the group with inhalation nicotine exposure for 15 days. This is proof that exposure to nicotine inhalation can reduce testosterone levels in mice and the effect of decreased testosterone caused by nicotine is directly proportional to the duration of nicotine inhalation exposure means that the longer the duration of exposure to nicotine inhalation, the testosterone levels will also decline. Inhalation of nicotine for a long time can cause accumulation of nicotine and its metabolites, cotinine in the body.^{7,8} High levels of nicotine accumulation can suppress testosterone biosynthesis, reduce levels of mRNA and bcl-2 protein and increase p53 and caspase-3 which interfere with spermatogenesis resulting in cell

depletion or thinning.⁹ High nicotine accumulation will also trigger the formation of excessive reactive oxygen species (ROS). Nicotine also decreases the anti-oxidants in the testicles such as glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase and increases lipid peroxidase in the testicles.¹⁰ That will cause an imbalance between scavenger (superoxide dismutase (SOD) and catalase) and ROS which can lead to Leydig cell necrosis.¹¹

Similar results were found in the Oyeyipo study in 2010 and 2013 by Aprioku in 2015, and by Guo in 2017.¹²⁻¹⁵ Testosterone levels significantly decreased in the nicotine group compared to the control group. Decreasing testosterone levels in mice are caused by a disturbance or damage to testicular cytoarchitecture caused by nicotine.¹⁰ Testicular damage by nicotine is associated with increased ROS due to exposure of nicotine.¹⁴ Recent studies have shown that administration of nicotine caused a significant decrease in the number of Leydig cells compared to the control group.

Decreasing the number of Leydig cells causes disruption of Leydig cells function in producing testosterone so that finally testosterone levels in the body will decrease.¹⁶ In addition, nicotine can also inhibit testosterone biosynthesis.⁸ This result was consistent with a previous meta-analysis conducted by Zhao et al shows that higher testosterone among men is correlated with smoking, however the evidence was minimal in different gender. Smoking may provide additional causative insight into the harms of smoking.¹⁷ A study by Haifa et al also found that 34.8% of men who visited

infertility clinics and gave samples of semen and blood for testing were smokers and had significantly low semen parameters in comparison to the nonsmokers. The cause of effects of semen changes is causing the increased prevalence of primary infertility among the couples.¹⁸

Testosterone levels in the group given a 15-day recovery time lag were significantly higher compared to the group not given the recovery time lag. This illustrates that giving a recovery time lag in the group of mice that had previously been exposed to inhaled nicotine had a positive effect on rat testosterone levels. These results are also consistent with the results of the Oyeyipo study in 2010 and 2013.^{12,13} The effect of damage caused by nicotine in the testicles for 15 days which causes a decrease in testosterone levels, can be repaired or recovered by stopping the nicotine exposure. Nicotine exposure for 15 days illustrates that the damage effect caused by nicotine for 15 days is still temporary or reversible.

CONCLUSION

Testosterone levels in the control group were significantly higher compared to the treatment group that exposed by nicotine inhalation with a dose of 4 mg/kgBW/day for 15 days, and there is a significant recovery effect on testosterone levels after the treatment was halted for 15 days.

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