Original article

The Potential Effect of *Mucuna pruriens* Seed Extract on Sperm Quality experimental study on mice exposed to cigarette smoke

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Abstract

Background: Infertility has been more common problems among couple of reproductive age. One of the factors causing this disorder is unhealthy environmental factors including exposure to cigarette smoke. Polynuclear aromatic hydrocarbons (PAHs) in cigarette smoke can cause testicular atrophy, while the free radicals can inhibit the stages of spermatogenesis, and nicotine in cigarettes affects the brain dopamine levels affecting the levels of GnRH, and subsequently affect the levels of FSH and LH needed in spermatogenesis. The use of *Mucuna pruriens* seed extract containing antioxidants and L-dopa is expected to improve the quality of sperm after exposure to cigarette smoke. ***Objective***: This study aimed to determine the effect of *Mucuna pruriens* seed extract on the sperm quality in mice exposed to cigarette smoke. ***Methods:*** This study was an experimental study with a post test only control group design. A total of 20 mice were divided into 4 groups of five mice each. All groups were exposed to cigarette smoke. Group 1 was the negative control exposed to cigarette smoke. Groups 2, 3, 4 were exposed to cigarettes smoke and given *Mucuna pruriens* seed extracts at the dose of 250; 300; and 350 mg/Kg BW/day. Parameters of sperm quality included concentration, morphology, motility and viability. ***Results:*** Post hoc tests showed there were significant differences among treatment groups. ***Conclusion:*** the administration of *Mucuna pruriens* seed extract affects the sperm quality of BALB/c mice exposed to cigarettes smoke.

Keywords: cigarette smoke; *Mucuna pruriens* seed extract; sperm quality

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Introduction

About 12-15% of couples of reproductive age have problems to conceive and produce offspring [1]in which 20-40% are due to male factors. One of the causes of fertility disorders in men is unhealthy environmental conditions that directly or indirectly affect the sperm quality.[2] The unhealthy environment can be caused by exposure to cigarette smoke[3,4]. Earlier study showed that after 30 days of exposure to cigarette smoke sperm quality significantly decreased.[5] This is due to the components of cigarette smoke affecting the male reproductive system. Free radicals in cigarette smoke may cause oxidative stress in the male reproductive system, disrupting spermatogenesis, the nicotine reduce brain dopamine levels leading to reduced brain Gonadotropin-releasing hormone (GnRH) levels required in the secretion of LH and FSH hormones needed in the process of sperm production, and Polynuclear Aromatic Hydrogen (PAH) can

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cause testicular atrophy.[5,6] Shukla stated the *Mucuna pruriens* seeds contained L-dopa compounds and antioxidants. The content is expected to neutralize the effects of oxidative stress and decrease dopamine due to exposure to cigarette smoke.[7,8] Another study by Pradipta and Winarni et al showed that administration of *Mucuna pruriens* seed extract improves the sperm quality in normal mice.[9,10] However, there have been no studies on the effect of seed extract of *Mucuna pruriens* on the condition of spermatozoa after cigarette smoke exposure. This study specifically was aimed to determine the effects of *Mucuna pruriens* seed extract on the concentration, morphology, quality, and viability of sperm in mice exposed to cigarette smoke.

Material and Methods

This laboratory experimental research method was done by post test randomized control group design. A total of 20 male BALB/c mice aged 2 months weighing 20-30 grams were adapted for one week before being randomized into 4 groups, of 5 mice each. All groups were exposed to smoke from 1 cigarette per day, for 30 days. Group 1 was negative control group which was given cigarette smoke exposure only, while group 2,3 and 4 were given Mucuna pruriens seed extract at different doses for 30 days along with cigarette smoke. Group 2 was given Mucuna pruriens seed extract at a dose of 250 mg / kg, group 3 was given *Mucuna pruriens* seeds at the dose of 300 mg/kg, group 4 was given *Mucuna pruriens* seed extract at a dose of 350 mg / kg. All groups were given standard diet and drink every day.

Mucuna pruriens seed extract were obtained from 500 grams fresh *Mucuna pruriens* seeds that were boiled for 30 minutes, continued with soaked for 24 hours in water, and dried using 60^0 Celsius oven for 5 hours. Dried seeds were mashed up into powder shape and weighed. Seed powder was continued to be extracted using ethanol solvent and water in 1:1 comparison resulting in 5,8 grams seed extract. This extract was then aliquot for the treatment doses.

At the end of the treatment the experimental animals were terminated using cervical dislocation and sperm samples were taken from epididymis and subjected to the evaluation of concentration, morphology, motility and viability.

The sperm concentration was examined using haemocytometer with 10 times dilution and evaluated under microscope using 400 time magnification. While sperm morphology was assessed under microscope using giemsa smear. Sperm motility was directly observed under 400 times magnification under microscope and recorded using optilab camera. Viability of the sperm was evaluated under microscope on sperm smeared with eosin negrosin smear to distinguish the dead and alive sperm. [11]

Data on sperm quality were presented in images. The data scoring tested by Shapiro Wilk, the normal distribution of data followed by a different test of ANOVA, and not normally distributed data were analyzed using different test of nonparametric Kruskal Wallis test.

Ethical clearance: The study was approved by the Ethical Review Committee Board of Faculty of Medicine, UNISSULA, Semarang, Indonesia.

Results

Sperm quality was measured by measuring 4 parameters, concentration, morphology, motility and viability. At the end of the study the following results are obtained.

Sperm Concentration

The results of observing the concentration of spermatozoa between treatment groups are shown in the following figure:

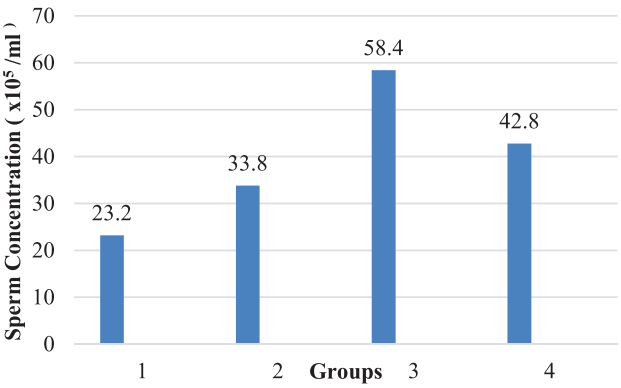


Figure 4. 2. Mean Sperm Concentration

The highest mean sperm concentration ($58.4x10^5$ / ml) was found in group 3 who received *Mucuna pruriens* seed extract at a dose of 300 mg / kgBW / day. The One way ANOVA test results show a p value of 0.0000 indicating that there were significant differences between treatment groups. Furthermore, the LSD post hoc test showed the following results:

Table 1. Mean number of sperm concentration

Group (I)	group (II)	P	
	Group 2	0.105	not significant
group 1	Group 3	0.000	significant
	Group 4	0.006	significant
Group 2	Group 3	0.001	significant
	Group 4	0.164	Not significant
Group 3	Group 4	0.022	significant

Sperm Morphology

Pictures below show the sperm morphology in each groups

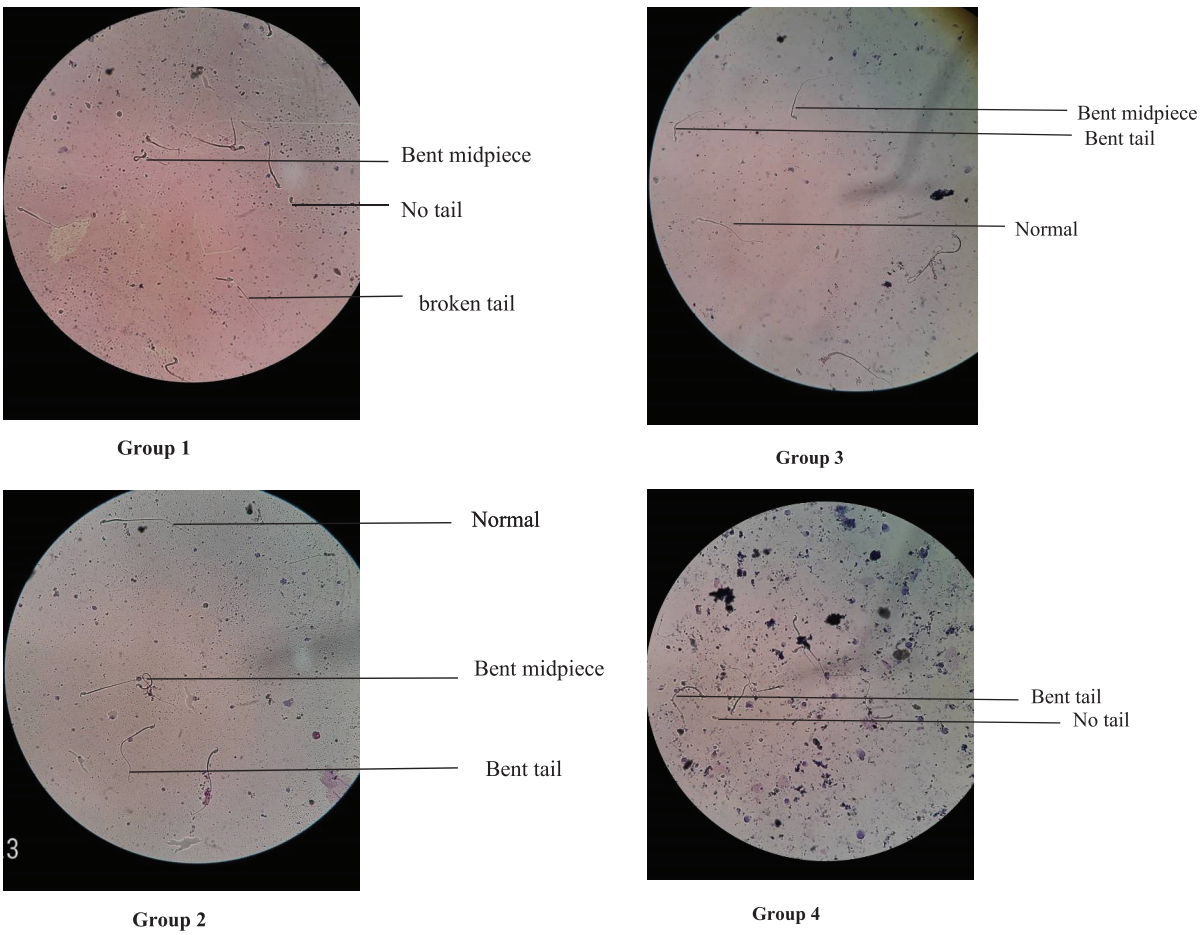


Figure 4.3. The mean sperm morphology in various treatment groups

The highest number of morphologically normal spermatozoa was found in group 3; with a dose of *Mucuna pruriens* seed extract 300 mg / kgBW/day (72%).

Statistical tests using the Kruskal Wallis showed morphological differences between various treatment groups (p 0.002), with significant differences found in the groups as follows:

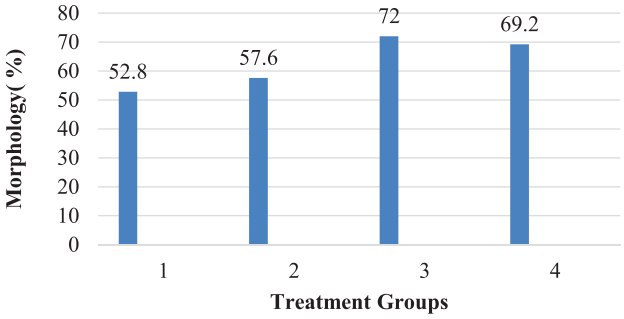


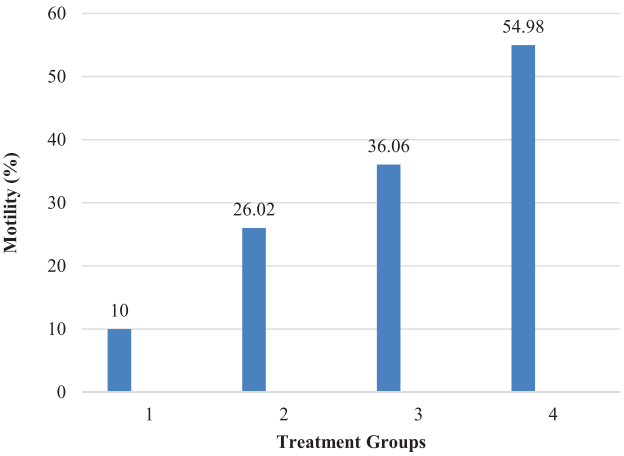
Table 4. 1 . Mann - Whitney Test Results

	p-value	Information
I><II	0.401	Not significant
I ><III	0.009 *	Significant
I><IV	0.009 *	Significant
II ><III	0.009 *	Significant
II ><IV	0.009 *	Significant
III ><IV	0.530	Not significant

Description: * mean differences between the two groups are significant

Sperm Motility

The results of sperm motility are illustrated in the figure as follows:



The highest mean number of motile spermatozoa was found in group 4, with the dose of *Mucuna pruriens* seed extract of dose 350 mg /kg BW/day (54.98 %).

The results of the one way ANOVA statistical test showed significant differences in the various treatment groups (P <0.000), and advanced post hoc tests showed different groups as follows:

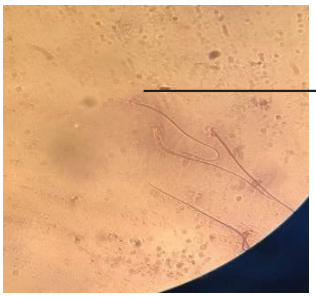
Tabel IV.4. Post hocLSD

Comparison of treated groups		P	
Control	*Mucuna pruriens* 250mg	0.011	Significant
	Mucuna pruriens 300mg	0.000	Significant
	Mucuna pruriens 350mg	0.000	Significant
Mucuna pruriens 250mg	*Mucuna pruriens*300mg	0.092	Not Significant
	*Mucuna pruriens*350mg	0.000	Significant
*Mucuna pruriens*300mg	*Mucuna pruriens*350mg	0.004	Significant

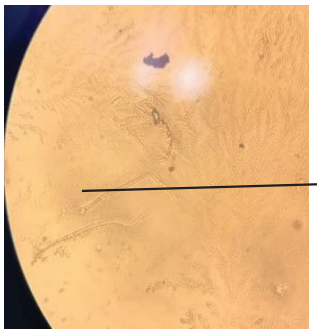
Description: * = difference in mean between two significant groups

Sperm Viability

Pictures below show the difference of dead and alive sperm after eosin negrosin smearing.



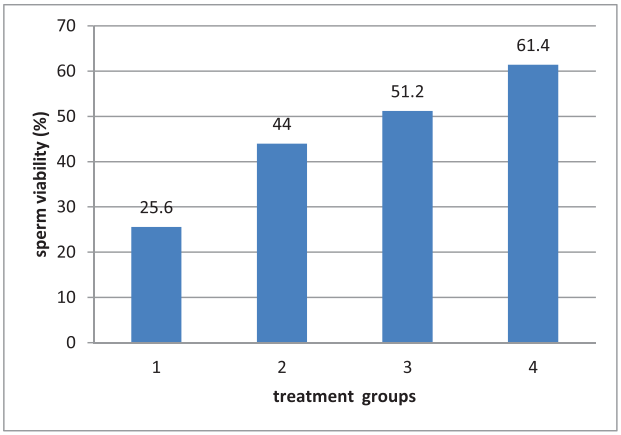
Dead sperm (red coloring head)



Living sperm (white coloring head)

The following graph shows the calculation of the mean number of viable sperm of spermatozoa in BALB/mice in each treatment group

The sperm viability for each treatment group was as follow: Group 1 25.6%, group 2 44 %, group 3 was 51,2% while group 4 was 61,4%. Kruskal Wallis test showed p value of 0,001, and post hoc test declared that there were significant differences among group 1 with group 2,3, and 4. Furthermore significant



difference was also presented between group 2 and 4 (p=0,009) , and group 3 and 4 (p=0,009)

Discussion

Based on sperm quality parameters, including viability, concentration, morphology and motility, it can be concluded that *Mucuna pruriens* seed extract affects the quality of sperm in Balb/C mice exposed to cigarettes smoke. Based on the results of data of the statistical analysis, the lowest mean percentage of sperm motility of male BALB /c mice was found in the negative control group, namely the group exposed to cigarette smoke for 30 days without the provision of *Mucuna pruriens* seed extract. This study shows that exposure to cigarette smoke results in decreased sperma motility in male BALB/c mice. This occurs because of cigarette smoke containing free radicals [12] and nicotine. [13]The presence of free radicals contained in cigarette smoke affects the decrease in antioxidants in cement. if the amount of free radicals exceeds the antioxidants in cement, oxidative stress will occur.[14,15] The presence of nicotine is a component of cigarette smoke particles that is harmful to male reproduction. [3,11] Substance in nicotine has a direct effect on dopamine; by way of nicotine circulated to the brain it will trigger the release of dopamine.[13,16,17]. Decreasing dopamine levels will cause disruption of the process of spermatogenesis by inhibiting GnRH expenditure. GnRH affects the decrease in testosterone production, because it cannot stimulate the release of LH and FSH hormones. FSH, LH, and testosterone hormones work synergistically in the process of spermatogenesis. Decreasing the FSH, LH, and testosterone will interfere with the process of spermatogenesis.[18]

Seed extracts of *Mucuna pruriens* contains flavonoids as antioxidants and L-dopa content that helps in the process of increasing the sperm quality. The presence of flavanoids that have antioxidant effects serves to overcome the damage caused by oxidation. [19] and the content of L-dopa which will increase the dopamine level to support the process of spermatogenesis.[20] The parameter related to sperm viability are the sperm motility, in which low viability will produce immotile sperm. In the seed extract with the highest dose of 350 mg/kgBW/day showed the highest mean of viability and motility. As for concentration and morphology parameters, the highest mean was obtained in the group treated with 300 mg/kg BW/day (group 3).

The administration of *Mucuna pruriens* seed extract effected the quality of sperm in BALB/c mice exposed to cigarette smoke.

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Conflict of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper

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References:

1. Djuwantono T, Bayuaji H. Permadi W,Buku-Pengelolaan-Infertilitas. Jakarta. Sagung Seto, 2012
2. Ferreira, M.Silva, J.V, Silva, V, Barros, A, Silva, O.A.B.d.C.e, Fardhila, M. (2012). Lifestyle Influence Human Sperm Functional Quality, *Asia Pacific Journal of Reproduction*. 1(3), pp 224-230. https://doi.org/10.1016/S2305-0500(13)60081-0
3. Masoumi, S. Z., Parsa, P., Darvish, N., Mokhtari, S., Yavangi, M., dan Roshanaei, G., (2015). An Epidemiologic Survey On The Causes Of Infertility In Patients Referred To Infertility Center In Fatemieh Hospital In Hamadan. *Iranian Journal of Reproductive Medicine*, 2015, 13(8), 513. PMID: 26568755.
4. Sari PD. Effect of cigarette smoke in quality and quantity spermatozoa, *Journal Majority*, 2014, **3**(7), pp. 102-106.
5. Batubara, I. V. D., Wantouw, B., & Tendean, L. Pengaruh paparan asap rokok kretek terhadap kualitas spermatozoa mencit jantan (mus musculus). *Jurnal e-Biomedik,* 2013; **1**(1). https://doi.org/10.35790/ebm.1.1.2013.4367
6. Tremellen K, Oxidative stress and male infertility-a clinical perspective. *Human reproduction update*, 2008,; **14**(3), 243-258. https://doi.org/10.1093/humupd/dmn004
7. Shukla, K. K., Mahdi, A. A., Ahmad, M. K., Shankhwar, S. N., Rajender, S., & Jaiswar, S. P. . Mucuna pruriens improves male fertility by its action on the hypothalamus-pituitary-gondal axis. *Fertility and sterility*, 2009; **92**(6), 1934-1940. https://doi.org/10.1016/j.fertnstert.2008.09.045
8. Agarwal, A. dan Prabakaran, S.A. Oxidative stress and antioxidants in maleinfertility: a difficult balance. *International Journal of Reproductive BioMedicine*, 2012, **3**(1), pp.1-8.
9. Pradipta, V, Pengaruh Ekstrak Daging Biji KaraMucuna pruriens Asal Bantul (Mucuna Pruriens) Terhadap Fertilitas Mencit Balb C Jantan (Mus Musculus) (Doctoral dissertation, Universitas Pendidikan Indonesia), 2013
10. Winarni, S., Judiwati, R., Prajogo, B. dan Hayati, A, Fraksi Etanol 96% Biji Koro Benguk (Mucuna pruriens L.) Sebagai Peningkat Kualitas Spermatozoa Mencit (Mus Musculus). *Jurnal Kesehatan Reproduksi*, 2010, **1**(2 Apr), pp.60-66.
11. WHO, W. H. O., (2010).WHO Laboratory Manual For The Examination And Processing Of Human Semen (Vol. 5th Ed). Geneva: World Health Organization.
12. Halliwell, B. and Gutterigde, J., (1999).Free Radicals, other rective spesies and disease. In free radical in Biology Medicine. Nwe York: Oxford University. Dikutip http://www.ncbi.nlm.nihgov.*Dikutip tanggal Juni* 2006. Vol **141**(2).
13. Fowles, J. and Bates, M. The Chemical Constituents in Cigarettes and Cigarette Smoke: Piorities for Harm Reduction.Epidemiology and Toxiocology Group, New Zealand, 2000, pp 1-65
14. Sukmaningsih AA, Penurunan jumlah spermatosit pakiten dan spermatid tubulus seminiferus testis pada mencit (Mus musculus) yang dipaparkan asap rokok. *Jurnal Biologi,* 2009; **13**(2).
15. Agarwal, A. dan Prabakaran, S.A, Oxidative stress and antioxidants in maleinfertility: a difficult balance. *International Journal of Reproductive BioMedicine*,2012, **3**(1), pp.1-8.
16. Tirtosastro S & Murdiyati, AS, Kandungan kimia tembakau dan rokok. Buletin Tanaman Tembakau, *Serat & Minyak Industri,* 2017; **2**(1), 33-44. https://doi.org/10.21082/bultas.v1n1.2009.10-16
17. Fowles, J. and Bates, M., (2000). The Chemical Constituents in Cigarettes and Cigarette Smoke: Piorities for Harm Reduction.Epidemiology and Toxiocology Group, New Zealand, pp 1-65
18. Ferreira, M.Silva, J.V, Silva, V, Barros, A, Silva, O.A.B.d.C.e, Fardhila, M.,Lifestyle Influence Human Sperm Functional Quality, *Asia Pacific Journal of Reproduction*. 2012, **1**(3), pp 224-230. https://doi.org/10.1016/S2305-0500(13)60081-0
19. Mulyani, L. Benefit and Content of velvet beans (Mucuna pruriens L.) as herbal medicine, *Prosiding Farmasi*, 2016, **2**(2) pp. 351-357.
20. Katzung B, Basic and Clinical Pharmacology, New York, McGraw Hill, 2018