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ORIGINAL ARTICLE

Effects of Javanese Ginseng Root Extracts (*Talinum triangulare W.*) on Thickness of Seminiferous Tubules of Male Rats (*Rattus norvegicus*) Exposed by Cigarette Smoke

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A B S T R A C T

Introduction: Cigarette smoke contains various radical chemical compounds that can increase oxidative stress, which may cause lipid peroxidation in the body and affect various different organs. This study aimed to examine the effects of Javanese ginseng root extracts (*Talinum triangulare W.*) on the thickness of seminiferous tubules of white male rats (*Rattus norvegicus*) exposed to cigarette smoke.

Methods: This was an experimental study with a randomized controlled method and a post-test control group design. Post-test control group design with 15 white male rats was divided into five groups: a normal group, a control group (exposed to cigarette smoke), and three treatment groups (exposed to cigarette smoke and Javanese ginseng root extract with doses of 12.5mg, 25mg, and 50mg/200grBW, respectively) for 30 days. The seminiferous tubule thickness was observed using H&E staining under a light microscope with 200x magnification in five fields view.

Results: One-way ANOVA showed that Javanese ginseng root extracts significantly affected seminiferous tubule thickness (p = 0.000). With a dose of 12.5mg/200mgBW, Javanese ginseng root extract started to show a significant effect (p = 0.000) on the thickness of the epithelium of the seminiferous tubules. The group exposed to cigarette smoke had an average thickness of seminiferous tubules of 7.92 μm, the normal group had an average thickness of 9.13 μm, and treatment groups with doses 12.5mg, 25mg, and 50mg/200gBW had an average thickness of 11.85 μm, 12.7μm, and 14.69 μm, respectively.

Conclusion: Javanese ginseng root extract increased seminiferous tubule thickness in white male rats exposed to cigarette smoke.

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Introduction

Smoking is a very common behavior among people all around the world. The Tobacco Atlas 6th edition reported that in 2018 as many as 942 million men and 172 million women were smokers aged >15 years old. The number of smokers in Indonesia is 46.16%, and Indonesia has the largest percentage of smokers in the ASEAN region.[1] The World Health Organization (WHO) stated that there are around 6 million deaths per year caused by tobacco. The Global Youth Tobacco Survey in 2014 estimated that the number of deaths would grow to over 8 million in 2030.[2]

Cigarette smoke contains various components of gas and particles which can induce the formation of free radicals in the body. Continuous free radical production can cause oxidative stress when there is an imbalance between the production of free radicals and antioxidants, which increases oxidants.[3] One of the causes of oxidative stress is the reactive oxygen species (ROS), a metabolite from cellular metabolism in the form of oxygen molecules which is normal if there is no overproduction. When there is an excessive level of ROS, it will cause a negative effect towards the cells in the body.[4] The occurrence of oxidative stress can trigger damage to body cells. In addition to ROS, reactive nitrogen species (RNS) can damage body cells.[5]

Cigarette smoke also contains polynuclear aromatic hydrogen (PAH), which results in atrophy of the testes, inhibits spermatogenesis, and damages the morphology of spermatozoa. The nicotine in cigarette smoke can also cause testosterone levels to decrease.[6]

One of the health problems that arise affecting the reproductive organ is infertility. Infertility is the failure of a couple to get pregnant in a period of 12 months of regular sexual intercourse with no contraception.[7,8] Smoking habits in men can increase the risk of infertility up to 50%.[9] One of the causes of infertility is impairment in spermatogenesis which occur in the seminiferous tubules.[10] A good spermatogenesis can be observed from the thickness of the seminiferous tubular wall. The thicker wall of the seminiferous tubules indicates that the Sertoli and spermatogenic cells can conduct good spermatogenesis.[11]

A high level of free radicals in the body can be suppressed by administering antioxidants. When free radicals in the body are higher than the level of antioxidants, the body will undergo oxidative stress. To prevent oxidative stress from occurring, the body needs an intake of external antioxidants. One plant with antioxidant properties is *Talinum triangulare W.* which is known as Javanese ginseng, *som Jawa*, or *Krokot Belanda*. A previous study found that the natural antioxidants in Javanese ginseng roots include alkaloids, terpenoids, tannins, saponins, and flavonoids.[12] Ginsenoside (triterpene glycoside), or saponin, is the most abundant chemical compound in Javanese ginseng roots. The ginsenoside of *Talinum triangulare W.* ranges from 20-30%.[13]

Indonesians still prefer to undergo herbal treatments with medicinal plants available in nature, including helping to deal with infertility problems. Plants are commonly used for their antioxidant components, which benefit the body. One of the plants with the most potent antioxidant properties is the Javanese ginseng roots.[14,15] The flavonoid compounds and ginsenoside contained in the Javanese ginseng roots have a stimulant effect and also act as aphrodisiacs which increase the stamina and fitness of men.[16]

Javanese ginseng roots contain many chemical compounds, such as flavonoids at 140 mg/g, tannins at 36 mg/g, and saponins at 30 mg/g. Substances derived from saponins that are considered efficacious and are widely contained in ginseng roots are ginsenosides. Ginsenoside is a saponin from the triterpene saponin group. Ginsenoside in ginseng roots increases resistance to stress, disease, and fatigue.[13,17]

The antioxidant activity in Javanese ginseng root extract can increase the production of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone hormones, which play a role in helping accelerate the process of spermatogenesis. This situation will cause an increase in the number of spermatogenic cells in the seminiferous tubules, causing the walls of the seminiferous tubules to thicken.

Javanese ginseng roots have high antioxidant activity. However, the antioxidant effect of Javanese ginseng extract on the thickness of the seminiferous tubules is still unclear. Therefore, this study aimed to examine the effects of Javanese ginseng root extracts on the thickness of seminiferous tubules of white male rats (*Rattus norvegicus*) exposed to cigarette smoke.

Methods

This was an experimental study with a randomized controlled method and post-test control group design. Data collection was conducted only at the end of the study period after administering treatments. This study had received ethical clearance from the Ethics Committee for Health Research, University of Muhammadiyah Malang, Malang.

Extraction of Javanese Ginseng Roots

Javanese ginseng roots were obtained from Materia Medika, Batu, and then extracted at the Biomedical Laboratory, Faculty of Medicine, University of Muhammadiyah Malang, Malang, using the maceration method with 96% ethanol for three days at room temperature. The extraction product was then filtrated to separate the sediment from the filtrate and was condensed using a rotary evaporator at 40°C, followed by drying. The dried extract was then dissolved in NaCl solution for administration into the rats via gastric tube.

Treatment to Experimental Animal

The experimental animal used in this study were white male rats (*Rattus norvegicus*). There were 20 rats divided into five groups, each consisting of three samples and one backup. Before the administration of treatments, the rats were given an adaptation time of one week to adapt to the experimental environment. The groups were divided into two control groups, positive and negative control, and three treatment groups. In the negative control group, the rats

were not given any treatment. In the positive control group, the rats were exposed to cigarettes. The cigarettes used were non-filter kretek cigarettes, the *Dji Sam Soe* brand (with 39 mg tar and 2.3 mg nicotine), two sticks/day. For the treatment group 1, 2, and 3, each was exposed to cigarette smoke two sticks/day and was given Javanese ginseng root extract with doses of 12.5mg/200gBW; 25mg/200gBW; 50mg/200gBW, respectively, for 30 days.

Measurement of Seminiferous Tubules Epithelial

After 30 days, 24 hours after the last treatment, the right testes of the rats were excised and made into preparations with H&E staining, then proceeded with an evaluation of the thickness of seminiferous tubules under a light microscope using 100x magnification. The measurement of the thickness of seminiferous tubules was conducted at 4 points, at 12, 3, 6, and 9 o'clock directions. For each sample, five tubules were chosen and then the average number was calculated.[18]

Data Analysis

The data collected from this study were analyzed using a normality test, homogeneity test, post-hoc test, and regression analysis using IBM SPSS Statistic 25 for Windows with a 95% confidence level.

Results

This study was conducted for 37 days, including the adaptation period of experimental animals. It used several experimental animals, as many as 15 white male rats, with three experimental rats in each group. This study was conducted at the Biomedical Laboratory, Faculty of Medicine, University of Muhammadiyah Malang, Malang.

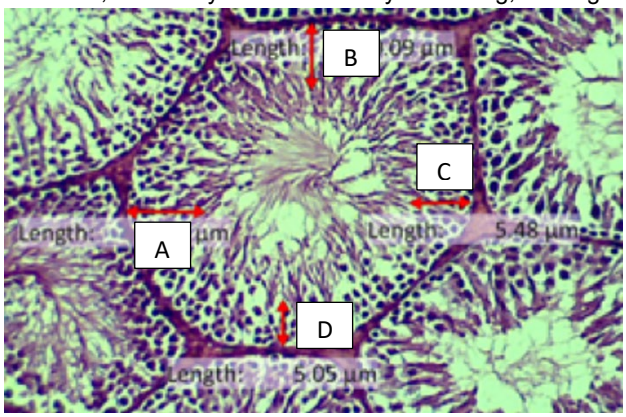

Figure 1. The result of seminiferous tubule preparation (H&E) on light microscopic with objective lens 100x. (A) length 6.67µm, (B) length 10.09µm, (C) length 5.48µm, (D) length 5.05µm.

Table 1. The average thickness of seminiferous tubules after treatment in every group

Group	Thickness of seminiferous tubules (µm)
K+	7.92
K-	9.13
P1	11.85
P2	12.7
P3	14.69

Source: Research data, processed

Table 2. Result analysis of one-way ANOVA

Indicator	Signification	Conclusion
Thickness of seminiferous tubule	0.000	Significant

Source: Research data, processed

Data analysis using one-way ANOVA got the results of $p = 0.000$ ($p < 0.05$), meaning that Javanese ginseng root extract affected the thickness of the seminiferous tubules of white male rats exposed to cigarette smoke.

Table 3. Result analysis of post hoc Bonferroni

Group	Treatment	Thickness of seminiferous tubule	Conclusion
K+	12.5mg/200grBW	0.000	Significant
	25mg/200grBW	0.000	Significant
	50mg/200grBW	0.000	Significant

Source: Research data, processed

Based on Table 3, it was found that Javanese ginseng root extract had a significantly different effect on the thickness of the seminiferous tubule epithelium in white male rats between the positive control group and all treatment groups. It can be concluded that Javanese ginseng root extract can increase the thickness of the seminiferous tubule epithelium in white male rats exposed to cigarette smoke starting at a dose of 12.5mg/200grBW.

Table 4. Results of linear regression analysis

Indicator	Coefficient R	Adjusted R^2	Sig.	Conclusion
Thickness of seminiferous tubule	0.123	0.817	0.000	Significant

Source: Research data, processed

From the analysis results in Table 4, it is known that the administration of Javanese ginseng root extract had a significant effect on increasing the thickness of the seminiferous tubule epithelium in white male rats with a significance of 0.000. The results of the linear regression test showed that the Adjusted R^2 for the thickness of the seminiferous tubules was 81.7%, while internal and external factors from experimental animals caused 18.3%.

Discussion

This study showed that exposure to cigarette smoke two sticks per day could decrease the thickness of seminiferous tubules in the positive control group compared to the normal group, which is in accordance with a study by Putra (2014), in which the group exposed to cigarette smoke would have a decrease in the thickness of epithelial cells of seminiferous tubules due to dysfunction of the Sertoli and spermatogenic cells which inhibit spermatogenesis process.[18,19]

Cigarette smoke contains various chemical substances which can induce the formation of free radicals (ROS) in the body. The testis is rich in polyunsaturated fatty acid (PUFA) and susceptible to free radicals. Free radicals from cigarette smoke will increase lipid peroxidation, which

will convert unsaturated acids into unstable lipid peroxidase. The conversion of these fatty acids will inhibit spermatogenesis in the testis.[20] Oxidative stress will cause damage to deoxyribonucleic acid (DNA), protein, and glucose. As a result, intracellular adenosine triphosphate (ATP) will diminish rapidly. This will affect the production of testosterone and abnormal hormonal production, which will cause apoptosis of the cells in the testes. Apoptotic testicular cells will result in decreased spermatogenesis.[21,22]

High levels of ROS in the body can trigger damage to tight junctions between the plasma membranes of Sertoli cells. Therefore, it can cause blood testes barrier (BTB) damage. This damage will result in decreased spermatogenic cell protection. Hence, it will disrupt the spermatogenic process in the testes.[23] Sertoli cells are important in the process of spermatogenesis. In general, Sertoli cells have the function of providing nutrition for the developing testicular germ cells. The growth of testicular germ cells is highly dependent on the metabolism of the Sertoli cell. These cells are columnar in shape, attached at the base to the basal lamina, and the end extends into the lumen of the seminiferous tubules. Sertoli cell bonds are very strong and will form two compartments. Basal and adluminal luminal compartments lead to the lumen of the seminiferous tubules. These two compartments are called BTB, which are composed of tight junctions of Sertoli cells. The main function of BTB is to protect spermatogenic cells in the seminiferous tubules from the immune system of the body flowing through blood vessels.[24]

Nicotine in cigarette smoke can affect how the central nervous system works by inhibiting the action of gonadotropin-releasing hormone (GnRH), which results in the inhibition of the formation of FSH and LH. The inhibited formation of FSH and LH results in the process of spermatogenesis being abnormal.[25]

Data obtained from the one-way ANOVA test showed a significant difference in the thickness of seminiferous tubules epithelial between the positive control group and the treatment group with a significance level of 0.000. This significant difference in the thickness of seminiferous tubules was due to the administration of Javanese ginseng root extract in the treatment group. This was due to the antioxidant activity of Javanese ginseng root extract with an IC_{50} value of 42.7 ± 2.7 ppm, which is included in the very strong antioxidant category.[26]

Javanese ginseng roots contain various chemical compounds, including flavonoids which is a compounds with antioxidant activity that can cause the reduction of free radicals in the body.[27] The mechanism by which flavonoids reduce the level of free radicals in the body is by donating hydrogen atoms to radical compounds. Therefore, they will become relatively more stable by using their ability to bind to metal compounds.[28] Ginsenoside is a derivative compound of triterpene saponin, which can increase stress resistance in the body. A body with higher stress resistance will increase the secretion of reproductive hormones, including FSH, LH, and testosterone. A good production of reproductive hormones will result in a good spermatogenesis process.[29] LH will induce increased Leydig cells and testosterone production, resulting in increased sperm production. Meanwhile, FSH will increase the production of Sertoli cells, which provide nutrition during spermatogenesis. If the production and nutrition of sperm increase, the process of spermatogenesis will also increase.[30]

To evaluate the difference between each group, the Post Hoc Bonferroni test was conducted. This test showed that the thickness of seminiferous tubules epithelial in the treatment group with doses of 12.5mg, 25mg, and 50mg/200gBW had identical significance levels, which was 0.000 ($p < 0.05$). Therefore, it can be concluded that all three doses could significantly increase the thickness of seminiferous tubules epithelial of rats since the significance level of all three doses was $p < 0.05$. A dose of 12.5mg/200gBW produced the effect of increasing the thickness of seminiferous tubules of rats and was the most optimum dose compared to another dosing because it was the minimum dose to produce the effect of increasing the thickness of seminiferous tubules of rats.

The administration of Javanese Ginseng root extract significantly affected the increased thickness of seminiferous tubules of white male rats with a significance of 0.000. The results of linear regression analysis showed that the Adjusted R^2 for the thickness of seminiferous tubules was 81.7%, while 18.3% was due to factors other than Javanese Ginseng root extract. Increasing 1mg dose of Javanese ginseng root extract can increase the thickness of the seminiferous tubule epithelium by 0.12 μm.

From the linear regression test results, it can be concluded that Javanese ginseng root extract had an effect of 81.7% in increasing the thickness of the seminiferous tubule epithelium. This study has a greater effect than a previous study by Rahman N. (2017), which found that Javanese ginseng leaf extract had a 72% effect on reducing malondialdehyde (MDA) levels in hypercholesterolemic rats.[31] This is because the content of phenolic levels in the roots of Javanese ginseng is more than in the leaves. According to Aghata, the total content of phenolic compounds in the roots of Javanese ginseng is 78.5 mg, and in the leaves is only 23.5 mg. This makes Javanese ginseng root has a greater effect on fighting free radicals in the body.[26]

Several factors can influence the results of this study. In general, these factors are divided into endogenous and exogenous factors. Endogenous factors affect research results originating from within the body of the experimental animal. Endogenous factors that influence include the genetic factors of each experimental animal and also the stress experienced by each experimental animal. Genetic factors of experimental animals can affect the thickness of the seminiferous tubules. Stress experienced by experimental animals can inhibit processes in the hypothalamus and cause hormonal disturbances. Therefore, the Leydig cell process to produce the hormone testosterone will decrease. Decreased testosterone will interfere with the process of spermatogenesis. Hence, there will be a decrease in male reproductive function.

Strength and Limitations

The strength of this study was the variables studied could be measured more precisely, because it was processed using a software with a high level of trust. The limitations were this study was not able to control the stress factors that were obtained by each experimental animal with each other, genetic factors from experimental animal could not be controlled, and this study took a long time.

Conclusion

Based on the results, it can be concluded that seminiferous tubule thickness in white male rats exposed to cigarette smoke was increased by Javanese ginseng root extract.

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Conflict of Interest

The authors declared there is no conflict of interest.

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Ethical Clearance

This study had received ethical clearance from the Ethics Committee for Health Research, University of Muhammadiyah Malang, Malang (no. E.5.a/035/KEPK-UMM/II/2022) on 21 February 2022.

References

1. Ministry of Health Republic of Indonesia. Perilaku Merokok Masyarakat Indonesia. *Ministry of Health Republic of Indonesia*, https://www.kemkes.go.id/article/view/16011100002/perilaku-merokok-masyarakat-indonesia.html (2015).
2. World Health Organization. Global Youth Tobacco Survey. *WHO*, https://www.who.int/docs/default-source/searo/indonesia/indonesia-gyts-2019-factsheet-(ages-13-15)-(final)-indonesian-final.pdf?sfvrsn=b99e597b_2 (2019).
3. Bundhun PK, Janoo G, Bhurtu A, *et al*. Tobacco Smoking and Semen Quality in Infertile Males: A Systematic Review and Meta-Analysis. *BMC Public Health* 2019; 19: 36. [PubMed]
4. Elsayed AA, Adwas AA, Ibrahim EAS, *et al*. Oxidative Stress and Antioxidant Mechanisms in Human Body. *J Appl Biotechnol Bioeng* 2019; 6: 43–47. [Journal]
5. Pizzino G, Irrera N, Cucinotta M, *et al*. Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev* 2017; 2017: 1–13. [PubMed] [Semantic Scholar]
6. Milla MN, Istadi Y, Shaula V, *et al*. The Potential Effect of Mucuna pruriens Seed Extract on Sperm Quality experimental study on mice exposed to cigarette smoke. *Bangladesh J Med Sci* 2021; 20: 768–773. [Journal]
7. Alahmar AT. Role of Oxidative Stress in Male Infertility: An Updated Review. *J Hum Reprod Sci* 2019; 12: 4–18. [PubMed]
8. Sun H, Gong TT, Jiang YT, *et al*. Global, Regional, and National Prevalence and Disability-Adjusted Life-Years for Infertility in 195 Countries and Territories, 1990-2017: Results from a Global Burden of Disease Study, 2017. *Aging (Albany NY)* 2019; 11: 10952–10991. [PubMed]
9. Ministry of Health Republic of Indonesia. Situasi Umum Konsumsi Tembakau di Indonesia. *Ministry of Health Republic of Indonesia*, https://www.kemkes.go.id/folder/view/01/structure-publikasi-pusdatin-info-datin.html (2018).
10. Loegito M, Sargowo D, Hinting A, *et al*. Effect of Cigarette Smokes Density on Histophatologis Testis of Rats. *Int J Dev Res*; 7, https://www.journalijdr.com/effect-cigarette-smokes-density-histophatologis-testis-rats (2017).
11. Barati E, Nikzad H, Karimian M. Oxidative Stress and Male Infertility: Current Knowledge of Pathophysiology and Role of Antioxidant Therapy in Disease Management. *Cell Mol Life Sci* 2020; 77: 93–113. [PubMed]
12. Ilodibia C, Igboabuchi N. Evaluation of Phytochemical and Nutritional Potential of Talinum Triangulare (Jacq) Leaf, Stem and Root on Human Health. *Int J Biol Res* 2016; 5: 6. [Journal]
13. Ahmad F, Anggita VS. Enhancement of Saponin Accumulation in Adventitious Root Culture of Javanese Ginseng (Talinum paniculatum Gaertn.) through Methyl Jasmonate and Salicylic Acid Elicitation. *African J Biotechnol* 2019; 18: 130–135. [Semantic Scholar]
14. Agarwal A, Leisegang K, Majzoub A, *et al*. Utility of Antioxidants in the Treatment of Male Infertility: Clinical Guidelines Based on a Systematic Review and Analysis of Evidence. *World J Mens Health* 2021; 39: 233. [PubMed]
15. Fang C, Wang F, Shen Y, *et al*. The Roles and Potential Mechanisms of Traditional Chinese Medicine on Male Infertility. *Pharmacol Res - Mod Chinese Med* 2022; 4: 100129. [ScienceDirect]
16. Santoso A, Amin M, Sumitro S, *et al*. *LC MS Determination of Java Ginseng (Talinum paiculatum) Ginsenoside*. Universitas Nusantara PGRI Kediri, http://repository.unpkediri.ac.id/4275/ (2016).
17. Bamidele OP, Fasogbon MB, Adebowale OJ, *et al*. Effect of Blanching Time on Total Phenolic,Antioxidant Activities and Mineral Content of Selected Green Leafy Vegetables. *Curr J Appl Sci Technol* 2017; 24: 1–8. [Journal]
18. Putra Y. Pengaruh Rokok terhadap Jumlah Sel Spermatozoa Mencit Jantan (Mus Musculus, Strain Jepang). *Sainstek J Sains dan Teknol*; 6. Epub ahead of print 2014. DOI: http://dx.doi.org/10.31958/js.v6i1.101.
19. Utomo B, Daningtia NR, Yuliani GA, *et al*. *Effect of a Standardized 40% Ellagic Acid Pomegranate (Punica Granatum L.) Extract on Seminiferous Tubule Histopathology Diameter, and Epithelium Thickness in Albino Wistar Rats After Heat Exposure*. Universitas Airlangga, https://repository.unair.ac.id/85540/ (2019).
20. Martantiningtyas DC, Nurliani A, Rusmiati R. Antioxidant Effect of Ethanol Extract of Bulbus Dayak Onion (Eleutherine americana) to the Quality of Spermatozoa Exposed by Cigarette Smoke in Rats (Rattus norvegicus). *J Sain Vet*; 33. Epub ahead of print 2015. DOI: https://doi.org/10.22146/jsv.8111.
21. Asadi A, Ghahremani R, Abdolmaleki A, *et al*. Role of

Sperm Apoptosis and Oxidative Stress in Male Infertility: A Narrative Review. *Int J Reprod Biomed*. Epub ahead of print 27 July 2021. DOI: 10.18502/ijrm.v19i6.9371. [PubMed]
22. Jiang X, Zhang N, Yin L, *et al*. A Commercial Roundup® Formulation Induced Male Germ Cell Apoptosis by Promoting the Expression of XAF1 in Adult Mice. *Toxicol Lett* 2018; 296: 163–172. [PubMed]
23. Mao BP, Li L, Yan M, *et al*. Regulation of BTB Dynamics in Spermatogenesis—Insights From the Adjudin Model. *Toxicol Sci* 2019; 172: 75–88. [PubMed]
24. Shi F, Zhang Z, Cui H, *et al*. Analysis by Transcriptomics and Metabolomics for the Proliferation Inhibition and Dysfunction through Redox Imbalance-Mediated DNA Damage Response and Ferroptosis in Male Reproduction of Mice and TM4 Sertoli Cells Exposed to PM2.5. *Ecotoxicol Environ Saf* 2022; 238: 113569. [ScienceDirect] [PubMed]
25. Omolaoye TS, El Shahawy O, Skosana BT, *et al*. The Mutagenic Effect of Tobacco Smoke on Male Fertility. *Environ Sci Pollut Res* 2022; 29: 62055–62066. [PubMed]
26. Aghata A. *Kandungan Fenolik Total dan Aktivitas Antioksidan Ekstrak Tanaman Ex vitro dan In vitro Ginseng Jawa (Talinum Triangulare wild)*. Institut Teknologi Bandung, https://digilib.itb.ac.id/index.php/gdl/view/42949 (2019).
27. Salehi B, Martorell M, Arbiser J, *et al*. Antioxidants: Positive or Negative Actors? *Biomolecules* 2018; 8: 124. [PubMed]
28. Yang QY, Lai XD, Ouyang J, *et al*. Effects of Ginsenoside Rg3 on Fatigue Resistance and SIRT1 in Aged Rats. *Toxicology* 2018; 409: 144–151. [ScienceDirect] [PubMed]
29. Xu X, Qu Z, Qian H, *et al*. Ginsenoside Rg1 Ameliorates Reproductive Function Injury in C57BL/6J Mice Induced by di-N-Butyl-Phthalate. *Environ Toxicol* 2021; 36: 789–799. [PubMed]
30. Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of Follicle-Stimulating Hormone in Spermatogenesis. *Front Endocrinol (Lausanne)* 2018; 9: 763. [PubMed]
31. Rahman F. *Pengaruh Ekstrak Daun Ginseng Jawa (Talinum triangulare) Terhadap Kadar Malondialdehyde (MDA) Pada Tikus (Rattus novergicus) Hiperkolesterolemia*. University of Muhammadiyah Malang, http://eprints.umm.ac.id/41245/ (2017).