The Effect of Red Ginger Extract (*Zingiber officinale var rubrum*) on The Thickness of The Epitelial and Diameter of Seminiferous Tubules in Albino Rat (*Rattus norvegicus*) Exposed Monosodium Glutamat

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ABSTRACT

Background: This study examined administration of MSG intraperitoneally to white rats resulted in a decrease in testicular weight, an increase in lipid peroxidase levels The testes, as the site of spermatogenesis, are highly vulnerable to oxidation processes caused by free radicals, which can disrupt spermatogenesis. Free radicals can be countered by antioxidants. One plant believed to have antioxidant activity is red ginger. **Purpose:** To determine the effect of red ginger against histopathology seminiferous tubules of white rat (*Rattus norvegicus*) exposed by monosodium glutamate. **Methods:** This study used 30 rats which were divided into 5 groups. The positve control group (K+) were given only monosodium glutamate 5g/kgBW and CMC Na orally, the treatments groups P1 were given monosodium glutamte 5g/KgBW + red ginger 50mg/rat/day, P2 were given 5g/KgBW + red ginger 100mg/rat/day, and P3 were given 5g/KgBW + red ginger 200mg/rat/day. All treatments were administered for 42 days. After 42 days of treatments, all rats were sacrificed and seminiferous tubules were observed with microscope from pathology laboratory. **Results:** Administration of red ginger extract can defend seminiferous tubules epithelial thicknes and diameter from exposure of monosodium glutamate with 100mg/KgBW dosage as the most effective dosage. Seminiferous tubules epitelial thickness and diameter were tested using one-way ANOVA and Duncan's multiple range test. **Conclusion:** Red Ginger Extract (Zingiber officinale var rubrum) has shown potential effects to counteract Monosodium Glutamat consequences on The Thickness of the epitelial and diameter of seminiferous tubules of white rat (*Rattus norvegicus*).

Keywords: *Zingiber officinale var rubrum*, histopathologic, seminiferous tuble, monosodium glutamate

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INTRODUCTION

The advancement of food processing technology and production competition have led to an increasing use of flavor enhancers over time (Shabrina, 2020). One commonly used flavor enhancer is Monosodium Glutamate (MSG), which was first isolated in crystal form from seaweed (Laminaria japonica) and identified as glutamic acid that enhances the delicious taste of food (Nurhayati, 2015). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) classify MSG as a food additive with an acceptable daily intake (ADI) of 120 mg/kg body weight/day (Nurhayati, 2015).

According to Yonata and Iswara (2016), consuming MSG in amounts exceeding 0.5 grams can result in manifestations affecting various organs, including the heart, nervous system,

respiratory system, gastrointestinal tract, muscles, genital and urinary tract, skin, and vision. These symptoms are referred to as MSG complex syndrome. These damages not only occur in humans but also in experimental animals. Administration of MSG to Wistar rats for 14 days (short-term exposure) and 30 days (long-term exposure) led to a decrease in testicular weight and disrupted spermatogenesis, resulting in abnormalities in the number and morphology of normal spermatozoa (Nurhayati, 2015).

Administration of MSG intraperitoneally to white rats resulted in a decrease in testicular weight, an increase in lipid peroxidase levels characterized by the formation of aldehyde compounds, particularly MDA, oxidative damage, and a decrease in testicular ascorbic acid (Nayanatara *et al.*, 2008). The testes, as the site of spermatogenesis, are highly vulnerable to oxidation processes caused by free radicals, which can disrupt spermatogenesis (Sukmaningsih *et al.*, 2011).

Free radicals can be countered by antioxidants. One plant believed to have antioxidant activity is red ginger. Red ginger contains volatile and non-volatile compounds. The non-volatile compounds in red ginger consist of flavonoids and polyphenols, which have been proven to possess antioxidant activity in suppressing free radical activity. Some key bioactive components in red ginger are 4- diarylheptanoids, shogaols, 6- and 8- gingerols, and gingerones, which exhibit antioxidant activity surpassing that of vitamin E. Gingerols and shogaols exhibit antioxidant activity due to the presence of benzene rings and hydroxyl groups (Zakaria, 2000; Revindran, 2005; Ghasemzadeh *et al.*, 2016). Red ginger also contains arginine, which is one of the essential amino acids that functions as an antioxidant and produces nitric oxide (Srivastava, 2006; Susilowati *et al.*, 2019).

METHODS

Experimental Animals

This study is an experimental research conducted using 30 male white rats, divided into five groups including the negative control group (K-), positive control group (K+), P1, P2, and P3. Red ginger extract was administered orally to the P1 group (50 mg/kgBW), P2 group (100 mg/kgBW), and P3 group (200 mg/kgBW). The K+ and K- groups were only given 1% CMC-Na. One hour after the administration of the extract, the rats were orally given 5g/kgBW of MSG. The treatment was carried out for 42 days.

Extraction of Red Ginger

Red ginger extract was made using the maceration method. Fresh red ginger was cleaned, dried, and ground into powder. Two kilograms of red ginger powder were soaked in four liters of 96% ethanol for one day. Next, a filtration process was conducted to separate the red ginger residue from the soaking solution. Then, evaporation was carried out for 4-5 hours using a rotary evaporator at a temperature of 60°C to obtain the red ginger extract.

Sample Collection

On the 43rd day, the white rats were euthanized using the cervical dislocation method, and the testes were extracted and placed in a 10% formalin solution to create histopathological preparations with Hematoxylin Eosin staining.

Data Observation

Observations of epithelial thickness and seminiferous tubule diameter were conducted using a light microscope at a magnification of 100x to

measure five seminiferous tubules in one preparation. The measurement of epithelial thickness involved identifying seminiferous tubules with a shape close to a circle and drawing a line from the nearest distance between the basement membrane and the spermatogonia cell layer to the surface of the lumen of each seminiferous tubule. Four measurements were taken at different locations in each seminiferous tubule (Altoe *et al.*, 2014).

The tubule diameter was measured by identifying tubules with a shape close to a circle and measuring the farthest distance between two opposing points that pass through the midpoint. Then, a perpendicular measurement was taken with the first diameter and had to intersect the midpoint of the tubule. The horizontal and vertical diameters were summed, and their average was calculated (Altoe *et al.*, 2014).

Data Analysis

The data were analyzed using SPSS (Statistical Product and Service Solutions) with a one-way Analysis of Variance (ANOVA) test, followed by the Duncan's Multiple Range Test to determine the differences between each treatment (Kusriningrum, 2011).

RESULT AND DISCUSSION

Microscopic observations of the histopathology of the testes of white rats (*Rattus norvegicus*) induced with monosodium glutamate at a dose of 5g/kg body weight orally for 42 days resulted in testicular damage, leading to a decrease in the diameter and epithelial thickness of the seminiferous tubules. The reduction in size observed in the seminiferous tubules is believed to be caused by a decrease in LH secretion activity in the anterior pituitary. This is due to the main MSG receptors located in the brain, making the brain one of the organs at high risk of damage due to MSG. If damage occurs in the hypothalamus, it will result in a decrease in GnRH, which in turn leads to a decrease in the production of FSH and LH in the anterior pituitary. The disruption of LH secretion, in turn, leads to a reduction in testosterone production in Leydig cells, which can interfere with the process of spermatogenesis. It is suspected that the decrease in testosterone and LH levels may cause atrophy of the seminiferous tubules (Cholifah *et al.*, 2014).

The epithelial thickness of seminiferous tubules in the testes of male rats in the K+ group experienced a decrease in comparison to the K- group. This could be due to a reduction in the number of Sertoli cells and spermatogenic cells or inhibition of spermatogenesis. Additionally, there is also a possibility of germ cells undergoing apoptosis (Kovacevik *et al.*, 2006). The reduction in epithelial thickness of seminiferous tubules may indicate infertility in male white rats (Clermont and Perey, 2008).

Table 1. Mean Rank of Epithelial Thickness of Seminiferous Tubules in White Rats

Group	Mean Rank (µm) ± SD
Negative Control (K-)	65.678^{b}± 1.800
Positive Control (K+)	52.332^{a} ± 4.557
P1	62.527^{b} ± 3.488
P2	64.300^{b} ± 1.495
P3	55.585^{a} ± 2.566

Different superscripts in the same column indicate significant differences ($p<0.05$).

Table 2. Mean Rank of Diameter of Seminiferous Tubules in White Rats

Group	Mean Rank (µm) ± SD
Negative Control (K-)	293.724^{c}± 8.846
Positive Control (K+)	256.730^{a} ± 7.259
P1	276.669^{b} ± 4.311
P2	288.866^{c} ± 2.679
P3	260.335^{a} ± 10.873

Different superscripts in the same column indicate significant differences ($p<0.05$).

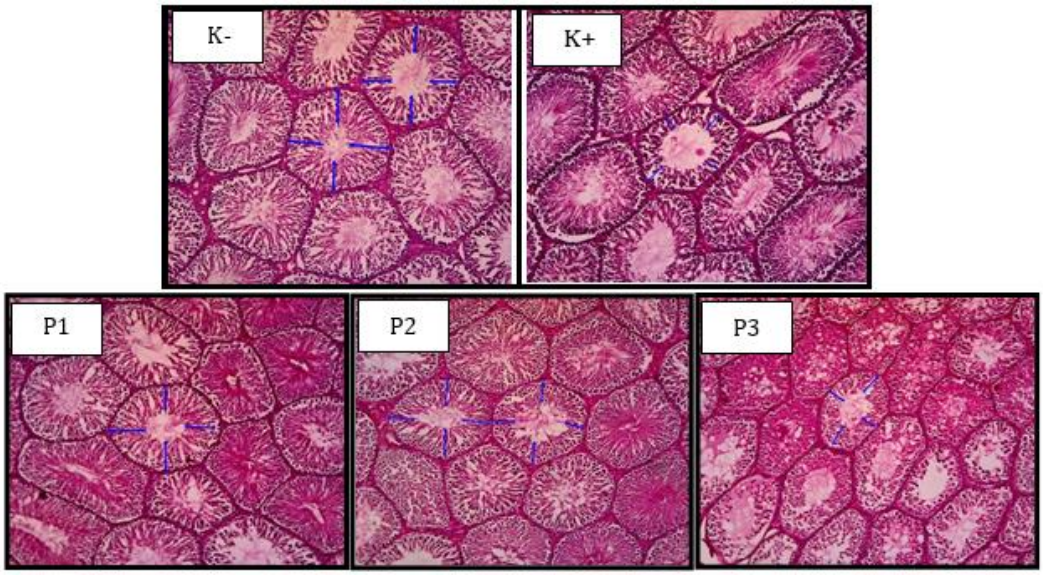

Figure 1. Histopathological image of epithelial thickness of seminiferous tubules at 100x magnification with HE staining.

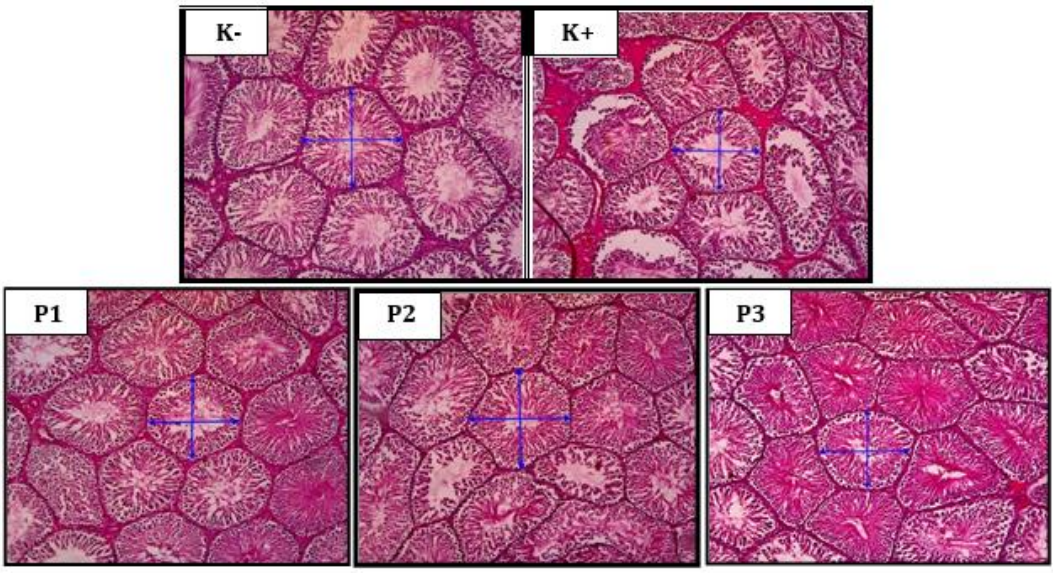

Figure 2. Histopathological image of diameter of seminiferous tubules at 100x magnification with HE staining.

Based on the observation of histological preparations that have been conducted, the results of measuring the epithelial thickness of seminiferous tubules in the P1 and P2 groups showed an increase compared to the K+ group, while the P3 group did not show a significant effect compared to the K+ group. This is due to the presence of flavonoid content in red ginger extract, which has antioxidant properties.

The main flavonoid compounds in red ginger that function as antioxidants are gingerol and shogaol, which contain benzene rings and hydroxyl groups. These compounds can donate electrons, thereby neutralizing free radicals (Tejasari and Zakaria, 2006). Red ginger also contains arginine, a non-essential amino acid that plays a role in the immune system, cellular immunity, and spermatozoa formation processes (Srivastava *et al.*, 2006). Research conducted by Sakr *et al.* (2011) also proves that the antioxidant effects of ginger can increase testosterone and LH hormones. Red ginger also contains other compounds called triterpenoids, which can improve the activity of the pituitary membrane, thereby increasing the pituitary's sensitivity to the GnRH hormone produced by the hypothalamus. This increased sensitivity is directly proportional to the increase in LH and FSH hormones (Fadhilah *et al.*, 2022).

The size of the seminiferous tubules can decrease due to free radicals, which are the effects of MSG, causing a decrease in the cells in the testes and hindering the process of spermatogenesis. Gurmeet *et al.* (2014) state that the decrease in the diameter of the seminiferous tubules caused by a decrease in the number of cells leads to impaired spermatogenesis. The shrinking of the seminiferous tubules is attributed to the inhibition of testosterone and FSH hormones due to free radicals. This is believed to be the cause of seminiferous tubule atrophy (Eboetse and Victor, 2011).

Groups P1 and P2 showed a significant increase in the diameter of the seminiferous tubules compared to the K+ group. Group P3 did not show a significant difference compared to K+. The changes observed in each treatment group are believed to be due to the antioxidant activity of the red ginger extract used.

According to Rukhayah (2022), the phenolic compounds present in red ginger have the potential to prevent hemolysis and inhibit free radical reactions. Additionally, due to its antioxidant activity, ginger stimulates androgenic activity in the testicular organ by increasing LH, FSH, and testosterone hormones (Ali and Hasan, 2008). Moreover, the presence of arginine compounds in red ginger rhizomes can produce Nitric Oxide, which functions to enhance spermatozoa motility by increasing the metabolic rate and generating more ATP, while also protecting the axonemal membrane from lipid peroxidation processes (Irawati, 2019).

The active compounds in red ginger such as alkaloids, triterpenoids, and tannins are also known to have a similar structure to estrogen. As a result, they can occupy estrogen receptors in the testes and elicit a negative response in the brain, leading to a decrease in the production of FSH and LH.

CONCLUSION

Administration of red ginger extract can maintain the epithelial thickness and diameter of seminiferous tubules in white rats induced with an effective dose of 100 mg/kg body weight of MSG. Further research is needed to investigate macroscopic parameters such as testis weight. Additionally, studies can be conducted to assess the

sperm quality of white rats.

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Author Contribution

N.R and R.K conceptualization, Y.D and T.V.W contributed to the animal study, K.R analyzed data and draft preparation, S.P.M and N.R were writing review and editing.

Competing Interest

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

All trials were examined and approved by the Faculty of Veterinary Medicine at Airlangga University's Ethical Approval Committee assessed this work, and it was given ethical approval under No. 1.KEH.081.06.2023.

Data Availability

The article includes data that was used to support the study's conclusions.

REFERENCES

Cholifah, S., Arsyad and Salni. 2014. Pengaruh Ekstrak Pare (Momordica charantina) Terhadap Struktur Histoogi Testis dan Epididimis Tikus Jantan (*Rattus norvegicus*) Sparaque Dawley. Majalah Kedokteran Sriwijaya. Vol 46: No 2 (2014).

Clermont, Y. and Perey. 2008. Quantitive Study of The Cell Population of The Seminiferous Tubules in Immature Rats. The American Journal of Anatomy 100(2): 242-267.

Eboetse, Y.O. and A.O. Victor. 2011. Pertubation of The Hypothalamic-Ptuitary-Ovarian Axis in Experimentally Inflicted Third Degree Burns in Wistar Rats. European Journal of Experimental Biology. 1(4): 6-11

Fadhilah, A.F. Fitranto, A. Nur Signa, A.G. 2022. Perbedaan Jumlah Sel Sertoli Pasca Pemberian Ekstrak Etanol Akar Purwoceng (Pimpinella alpina). Gunung Djati Conference Series, Volume 15 (2022) Prosiding Seminar Nasional Kimia 2022 ISSN: 2774-6585

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, 2016. Variation of The Phytochemical Constituents And Antioxidant Activities of Zingiber officinale var. rubrum Theilade Associated with Different Drying Methods And Polyphenol Oxidase Activity. Molecules, 21(6).

Gurmeet., I. Rosnah, M.K. Normadiah and A.M. Srijit. 2014. Detrimemental Effects Bisphenol on Development and Functions of The Male Reprductive System in Experimental Rats. EXCLI Journal, 151-6.

Irawati, N.B.I., Sutriningsih., Anggraini, W.S. 2019. Efek Pemberian Ekstrak Jahe Merah (Zingiber officinale var. Rubrum) Terhadap Sel Spermatogenik Mencit Jantan yang diinduksi Cyproterone acetate. Wellness and Healthy. 1(2): 295-304.

Kovacevic, K., T. Budefeld and Majdic. 2006. Reduction Seminiferus Tubule Diameter in Mice Neonatally Exposed to Perfume. Slov Vet Res: 43 (4): 177-83.

Nayanatara A. 2008. Role of Ascorbic Acid in Monosodium Glutamate Mediated Effect on Testicular

Weight, Sperm Morphology, and Sperm Count, in Rat testis. Journal of Chinese Clinical Medicine. 3(1).

Nurhayati, N. 2014. Pengaruh Monosodium Glutamate (MSG) Terhadap Jumlah Dan Morfologi Spermatozoa Tikus Jantan Dewasa (*Rattus norvegicus*). JPP (Jurnal Kesehatan Poltekkes Palembang). 1(14).

Revindran, P. N., and Babu, K. N. 2005. Ginger The Genus Zingiber, (RC Press, New York, hal. 87-90).

Sakr SA, Badawy GM. 2011. Effect of Ginger (Zingiber officinale r.) On Metiraminhibited Spermatogenesis and Induced Apoptosis in Albino Mice. Journal of Applied Pharmaceutical Science. Vol. 1 (04) :131-136.

Srivastava, S., Desai, P., Coutinho, E., and Govil, G. 2006. Mechanism of Action of L-arginine on The Vitality of Spermatozoa is Primarily Through Increased Biosynthesis of Nitric Oxide. Biology of reproduction. 74(5): 954–958.

Sukmaningsih, A. S. A., Ermayanti, I. G. A. M., Wiratmini, N. I., dan Sudatri, N. W. 2011. Gangguan Spermatogenesis Setelah Pemberian Monosodium Glutamat pada Mencit (Mus musculus).Jurnal Biologi Udayana.

Susilowati S, Triana IN, Wurlina W, Arimbi A, Srianto P, and Mustofa I. Addition of L-arginine in skim milk extender maintains goat spermatozoa quality in chilled temperature for five days. Vet World. 12(11):1784-1789.

Tejasari dan Zakaria, F. R., 2006, Senyawa Bioaktif Rimpang Jahe (Zingiber officinale Roscue) Meningkatkan Respon Sitolitik Sel NK terhadap Sel Kanker Darah K-562 In Vitro, Jurnal Teknologi dan Industri Pangan. 2 (17).

Yonata, A., dan Iswara, I. 2016. Efek Toksik Konsumsi Monosodium Glutamate. Jurnal Majority. 5(3): 100-104.

Zakaria, 2000. Pengaruh Konsumsi Jahe (Zingiber officinale Roscoe) Terhadap Kadar Malonaldehida dan Vitamin E Plasma Pada Mahasiswa Pesantren Ulil Albaab Kedung Badak, Bogor. Buletin Teknologi dan Industri Pangan, Vol. XI, No. 1, Th. 2000. IPB. Bogor.

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