

The Effect of Red Ginger (*Zingiber officinale* var. *rubrum*) Extract Administration on the Number of Spermatogenic Cells and Leydig Cells in White Rats (*Rattus norvegicus*) Exposed to Monosodium Glutamate

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ABSTRACT

The purpose of this study was to examine the effect of red ginger extract (*Zingiber officinale* var. *rubrum*) on the number of spermatogenic cells and Leydig cells in white rats (*Rattus norvegicus*) exposed to monosodium glutamate (MSG). This study used 25 male white rats divided into five treatment groups. The K- group received 1% CMC-Na, the K+ group received MSG at a dose of 5 g/kg body weight (BW), and the P1 to P3 groups received MSG at 5 g/kg BW along with red ginger extract at doses of 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW, respectively, for 42 days. The rats' testes were collected, processed into histopathological preparations, and observed under a microscope at 400× magnification. Data were analyzed using one-way ANOVA followed by Duncan's test. The results showed significant differences ($p < 0.05$) in the number of spermatogenic cells and Leydig cells among the K-, K+, P1, and P2 groups, but no significant differences ($p > 0.05$) were observed between the K- group ($57.60^d \pm 1.490$ and $14.92^d \pm 1.487$) and the P3 group ($57.20^d \pm 1.208$ and $14.84^d \pm 1.459$). The optimal dose of red ginger extract was 200 mg/kg BW (P3). In conclusion, administration of red ginger extract can maintain the number of spermatogenic cells and Leydig cells in white rats (*Rattus norvegicus*) exposed to MSG.

Keywords: red ginger, monosodium glutamate, spermatogenic cells, Leydig cells, white rats

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INTRODUCTION

Monosodium glutamate (MSG) is a widely consumed food additive in Indonesia (Sulastri, 2015). MSG is the sodium salt of glutamic acid, a non-essential amino acid found in food. Its composition consists of

78% glutamate, 12% sodium, and 10% water (Muntaza et al., 2020).

MSG poses health risks when consumed in excess. The FDA and WHO have established a safe daily intake limit of 120 mg/kg body weight (Yonata et al., 2016).

Overconsumption can lead to MSG symptom complex syndrome. Intraperitoneal administration of MSG at 4 g/kg body weight in white rats for 15–30 days has been shown to disrupt spermatogenesis (Nurhayati, 2015). Spermatogenesis is the process of sperm cell formation. Disorders of spermatogenesis are categorized as pretesticular, testicular, or posttesticular. Pretesticular disorders impair the hypothalamic–pituitary hormonal regulation system (Hasbi & Gustina, 2018). Reduced levels of Interstitial Cell Stimulating Hormone (ICSH) and Follicle-Stimulating Hormone (FSH) disrupt spermatogenesis and decrease Leydig cell activity (Salsabilla, 2024).

MSG can induce testicular dysfunction by activating glutamate receptors and transporters, thereby increasing free radical production (Budiman *et al.*, 2015). Free radicals stabilize themselves by extracting electrons from surrounding molecules, damaging biomolecules and increasing Reactive Oxygen Species (ROS) levels (Arnanda *et al.*, 2019). ROS triggers lipid peroxidation, damaging cell membranes rich in polyunsaturated fatty acids (PUFAs), reducing adenosine triphosphate (ATP) production, and promoting apoptosis. This cascade decreases spermatogenic cell numbers (Fambudi *et al.*, 2020).

The damage caused by MSG can be mitigated by exogenous antioxidants such as red ginger (*Zingiber officinale* var. *rubrum*). Red ginger contains higher levels of starch (52.9%), essential oil (3.9%), and alcohol-soluble extract (9.93%) compared to emprit ginger (41.48% starch, 3.5% essential oil, and 7.29% alcohol-soluble extract) and elephant ginger (44.25% starch, 2.5% essential oil, and 5.81% alcohol-soluble extract) (Hernani &

Hayani, 2001). Its bioactive compounds, such as gingerol, shogaol, and zingerone, exhibit strong antioxidant properties.

Red ginger influences the reproductive hormone cycle via the hypothalamic–pituitary axis. The hypothalamus secretes Gonadotropin-Releasing Hormone (GnRH), which stimulates the anterior pituitary gland to release FSH and ICSH. These hormones promote testosterone production in Leydig cells, which is essential for spermatogenesis (Rahma *et al.*, 2023). Additionally, red ginger demonstrates androgenic effects. Kamtchouing *et al.* (2002) reported that red ginger extract significantly increases testis weight, serum testosterone levels, testicular cholesterol levels, and epididymal alpha-glucosidase activity in rats.

METHODS

Experimental Design

This study employed an experimental research design. Treatments were conducted at the Animal Testing Facility, and observations were carried out at the Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

The experimental subjects consisted of 25 male white rats (*Rattus norvegicus*), aged 3 months, with body weights ranging from 100–150 g. The rats were randomly divided into five treatment groups, each consisting of five animals. The K– group received 1 mL of aquades orally, while the K+ group received 1 mL of aquades orally followed by monosodium glutamate (MSG) at a dose of 5 g/kg body weight (BW) administered orally (1 mL). The P1, P2, and P3 groups received MSG at a dose of 5 g/kg BW followed by oral administration of red ginger extract at doses of 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW (1 mL), respectively.

The organs were collected 24 hours after the final treatment. The rats were euthanized, and necropsy was performed to collect the testes. The testes were processed into histopathological preparations using hematoxylin–eosin (HE) staining. Observations were conducted under a light microscope at 400× magnification across five visual fields.

Data Analysis

Quantitative data on spermatogenic cells and Leydig cells were tabulated and expressed as mean $\pm$ standard deviation (SD). Prior to hypothesis testing, the data were assessed for normality and homogeneity of variance to ensure compliance with the assumptions required for parametric analysis. Differences among treatment groups were then analyzed using a one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Duncan's multiple range test was performed as a post-hoc analysis to identify specific differences between groups. All statistical analyses were conducted at a 95% confidence level.

RESULT AND DISCUSSION

Data analysis using the one-way ANOVA test indicated significant differences across all research parameters. The asymptotic significance (Asymp. Sig.) values for spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and Leydig cells were 0.0001 ($p < 0.05$).

Duncan's test results in Table 1 showed significant differences ($p < 0.05$) in spermatogonia between K– and K+, K– and P1, K– and P2, K+ and P1, K+ and P2, K+ and P3, P1 and P2, P1 and P3, and P2 and P3.

However, K– and P3 showed no significant difference ($p > 0.05$).

For primary and secondary spermatocytes, significant differences ($p < 0.05$) were observed between K– and K+, K– and P1, K– and P2, K– and P3, K+ and P1, K+ and P2, K+ and P3, P1 and P2, P1 and P3, and P2 and P3.

Similarly, spermatids exhibited significant differences ($p < 0.05$) between K– and K+, K– and P1, K– and P2, K– and P3, K+ and P1, K+ and P2, K+ and P3, P1 and P2, P1 and P3, and P2 and P3.

For Leydig cells, significant differences ($p < 0.05$) were found between K– and K+, K– and P1, K– and P2, K+ and P1, K+ and P2, K+ and P3, P1 and P2, P1 and P3, and P2 and P3. Again, K– and P3 showed no significant difference ($p > 0.05$).

A consistent pattern was observed in spermatogenic and Leydig cell counts across groups, ordered as $K+ < P1 < P2 < P3 < K-$. The mean counts of spermatogonia and Leydig cells in the K– control group ($57.60^d \pm 1.490$ and $14.92^d \pm 1.487$) were not significantly different ($p > 0.05$) from those in the P3 treatment group ($57.20^d \pm 1.208$ and $14.84^d \pm 1.459$). This indicates that the P3 group (200 mg/kg BW red ginger extract, *Zingiber officinale* var. *rubrum*) represents the optimal dose for maintaining spermatogenic and Leydig cell numbers in MSG-exposed white rats.

Histopathological observations of rat testes administered red ginger extract and exposed to MSG showed that the order of spermatogenic and Leydig cell counts from highest to lowest was $K- > P3 > P2 > P1 > K+$. This finding indicates that red ginger extract can maintain spermatogenic and Leydig cell counts in MSG-exposed rats.

Table 1. Mean rank values of spermatogenic cells and leydig cell counts in rats treated with red ginger extract and exposed to MSG

Group	Spermatogonium	Primary and Secondary Spermatocyte	Spermatid	Leydig cell
K-	57.60 ^d ± 1.490	75.52 ^e ± 1.238	118.48 ^e ± 2.189	14.92 ^d ± 1.487
K+	41.84 ^a ± 1.228	53.48 ^a ± 1.301	80.72 ^a ± 1.559	8.04 ^a ± 1.203
P1	47.96 ^b ± 1.090	62.60 ^b ± 1.140	94.32 ^b ± 1.339	10.32 ^b ± 1.521
P2	52.68 ^c ± 0.976	66.88 ^c ± 1.205	105.92 ^c ± 1.331	12.68 ^c ± 2.166
P3	57.20 ^d ± 1.208	73.76 ^d ± 1.472	113.96 ^d ± 1.519	14.84 ^d ± 1.459

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

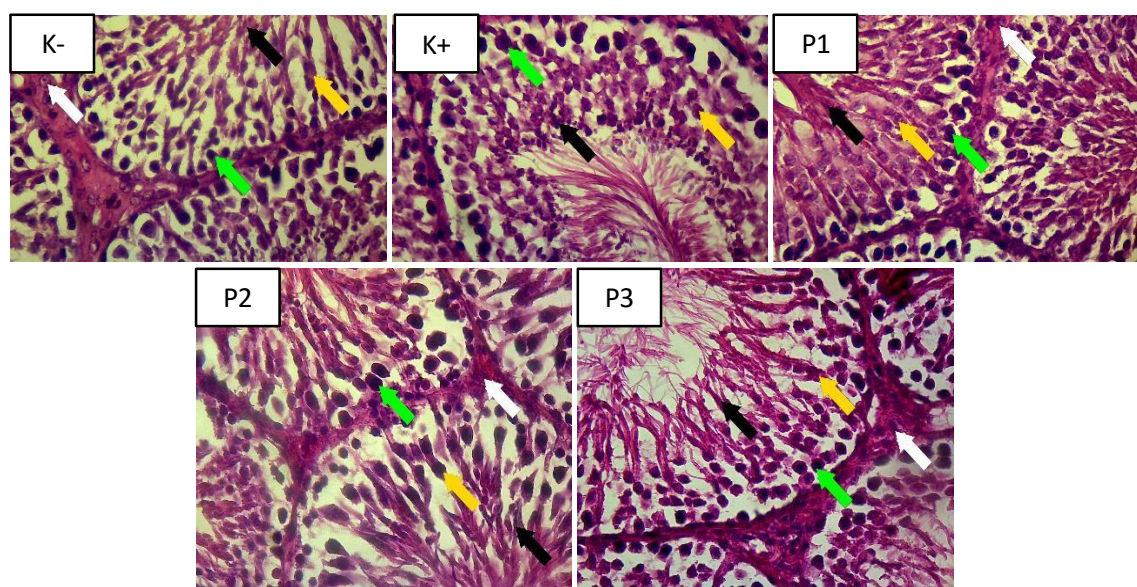


Figure 1. Histopathological description of testis preparations in white rats administered red ginger extract and exposed to MSG (HE staining, 400× magnification). Light green arrows indicate spermatogonia, yellow arrows indicate primary and secondary spermatocytes, black arrows indicate spermatids, and white arrows indicate Leydig cells. The K- group shows normal spermatogenic and Leydig cell counts in adult rats (control group). The K+ group shows spermatogenic and Leydig cell counts in adult rats exposed to 5 g/kg BW MSG (untreated). The P1, P2, and P3 groups show spermatogenic and Leydig cell counts in rats administered red ginger extract at 50, 100, and 200 mg/kg BW, respectively, followed by 5 g/kg BW MSG. The cell counts from highest to lowest are K-, P3, P2, P1, and K+.

Administration of MSG at 5 g/kg BW for 42 days significantly reduced spermatogenic and Leydig cell counts due to

increased free radical production (Lestari *et al.*, 2021). Free radicals damage cells through lipid peroxidation of cell membranes, DNA

mutation, and impaired cellular function via protein crosslinking (Sayuti & Yenrina, 2015). Lipid peroxidation disrupts spermatogenic cell membranes rich in polyunsaturated fatty acids (PUFAs) and overwhelms endogenous antioxidants such as superoxide dismutase, glutathione peroxidase, and catalase (Hartati *et al.*, 2018).

Lipid peroxidation occurs in three stages. The initiation stage begins with oxygen activation by Reactive Oxygen Species (ROS), such as OH^- and HO_2 , which combine with hydrogen atoms to form water and fatty acid radicals. The propagation stage occurs when fatty acid radicals react with free fatty acids, producing additional radicals, lipid peroxides, and cyclic peroxides. This cycle continues as lipid radicals interact with each other continuously. The termination stage occurs when radical reactions cease as two radicals combine to form non-radical compounds (Ghosh, 2015).

Malondialdehyde (MDA), a cytotoxic and mutagenic end-product of lipid peroxidation (Singh *et al.*, 2014), binds to DNA and proteins, causing protein crosslinking—covalent bonding between amino acid residues within or across polypeptides. Crosslinking alters biomolecules, leading to failure of cell division and cellular damage (McKerchar *et al.*, 2019). MDA also induces mitochondrial apoptosis by binding to mitochondrial membrane proteins, increasing ROS production (superoxide anions and hydroxyl radicals) and Ca^{2+} accumulation (Moazamian *et al.*, 2015). This disrupts mitochondrial membrane permeability, triggering the release of cytochrome C, apoptosis-inducing factor (AIF), and Smac/Diablo into the cytoplasm. Cytochrome C activates

Apoptosis Protease Activating Factor-1 (APAF-1), which stimulates procaspase-9 to become caspase-9 (Samik & Safitri, 2017; Luqman *et al.*, 2019). Caspase-9 initiates apoptosis by activating caspase-3, which stimulates DNA-damaging endonucleases and DNases. Elevated caspase-3 levels in spermatogenic and Leydig cells cause excessive apoptosis (Akhigbhe & Ajayi, 2020).

The adverse effects of MSG and lipid peroxidation can be mitigated by red ginger (*Zingiber officinale* var. *rubrum*). Its active compounds—gingerol, shogaol, zingiberene, gingerdiol, and zingerone—enhance the activity and concentration of endogenous antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase. These enzymes function optimally when antioxidant levels balance free radicals (Iqra *et al.*, 2023). Red ginger antioxidants halt free radical chain reactions through free radical scavenging during the initiation phase and peroxyradical scavenging during the propagation phase (Berlina *et al.*, 2019).

CONCLUSION

Administration of red ginger extract can maintain the number of spermatogenic cells and Leydig cells in white rats (*Rattus norvegicus*) exposed to MSG, with the optimal dose being 200 mg/kg body weight.

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Author Contribution

All authors participated in all aspects of this work, including study preparation, research implementation, data collection and analysis, manuscript drafting, and approval of the final version for publication.

Competing Interest

The authors declare that there are no competing interests.

Ethical Approval

All experimental procedures involving animals in this study were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. All efforts were made to minimize animal suffering during the experimental procedures.

REFERENCES

- Akhigbhe, R. and Ajayi, A. 2020. Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up-regulation of NO/TNF- α and caspase-3 activities. *PLoS ONE*, 15(3), p.e0224052. <https://doi.org/10.1371/journal.pone.0224052>
- Arnanda, Q.P. and Nuwarda, R.F. 2019. Penggunaan radiofarmaka teknesium-99m dari senyawa glutation dan senyawa flavonoid sebagai deteksi dini radikal bebas pemicu kanker. *Jurnal Farmaka*, 17(2), pp.236–243.
- Berlina, C.R. et al. 2019. Pengaruh pemberian ekstrak rumput kebar (*Biophytum petersianum* Klotzsch) terhadap jumlah sel Sertoli mencit (*Mus musculus*) yang dipapar 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Journal of Basic Medicine Veterinary*, 6(1), pp.45–52.
- Budiman, J., Istiadi, H. and Amravati, S. 2015. Pengaruh madu terhadap gambaran mikroskopis testis pada tikus Wistar yang diinduksi monosodium glutamat. Doctoral dissertation. Universitas Diponegoro.
- Ersanto, E. and Sukarjati, S. 2017. Potensi ekstrak jahe merah (*Zingiber officinale* Rosc.) sebagai antimikroba dan peningkat kualitas spermatozoa mencit yang diinfeksi *Staphylococcus aureus* secara intraurethra. *Jurnal Universitas PGRI Adi Buana*, 69(2).
- Fambudi, D.A.S. et al. 2020. The influence of kebar grass (*Biophytum petersianum* K.) ethanol extract towards the amount of spermatogenic cells of mice (*Mus musculus*) induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Advances in Animal and Veterinary Sciences*, 8(9), pp.976–980.
- Ghosh, M. 2015. Introduction to oxidative cytotoxicity and cellular defense mechanism. *ResearchGate*.
- Hartati, S.M., Pangkahila, W. and Aman, I.G.M. 2018. Pemberian dexpanthenol intraperitoneal menghambat penurunan jumlah sel Leydig dan sel Sertoli pada testis tikus putih (*Rattus norvegicus*) galur Wistar yang dipapar monosodium glutamate. *Indonesian Journal of Anti-Aging Medicine*, 2(1), pp.9–13.

- Hasbi, H. and Gustina, S. 2018. Androgen regulation in spermatogenesis to increase male fertility. *Wartazoa*, 28(2), pp.13–22.
- Hernani and Hayani, E. 2001. Identification of chemical components in red ginger (*Zingiber officinale* var. *rubrum*) by GC–MS. In: *Proceedings of the International Seminar on Natural Products Chemistry and Utilization of Natural Resources*. Jakarta: UI–UNESCO, pp.501–505.
- Iqra, K.N. et al. 2023. Pengaruh pemberian ekstrak jahe merah (*Zingiber officinale* var. *rubrum*) dalam meningkatkan motilitas dan morfologi spermatozoa mencit (*Mus musculus* L.). *Jurnal Biocelebes*, 17(2).
- Kamtchouing, P. et al. 2002. Evaluation of androgenic activity of *Zingiber officinale* and *Pendadiplandra brazzeana* in male rats. *Asian Journal of Andrology*, 4(4), pp.299–301.
- Lestari, S.A. Wardani, D.P.K. and Sudarsono, T.A. 2021. Efek monosodium glutamat terhadap gambaran histologi hepar tikus putih (*Rattus norvegicus*) galur Wistar. *Jurnal Media Analisis Kesehatan*, 12(1), pp.56–63.
- McKerchar, H.J. et al. 2019. Protein–protein crosslinking in food: Proteomic characterisation methods, consequences and applications. *Trends in Food Science & Technology*, 86, pp.217–229. <https://doi.org/10.1016/j.tifs.2019.02.018>
- Moazamian, R. et al. 2015. Oxidative stress and human spermatozoa: Diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. *Molecular Human Reproduction*, 21(6), pp.502–515. <https://doi.org/10.1093/molehr/gav021>
- Muntaza, Y. and Adi, A.C. 2020. Hubungan sumber informasi dan pengalaman dengan tingkat pengetahuan tentang penggunaan monosodium glutamate (MSG) pada ibu rumah tangga. *Amerta Nutrition*, 4(1), pp.72–78. <https://doi.org/10.20473/amnt.v4i1.2020.72-78>
- Nurhayati, N. 2015. Pengaruh monosodium glutamate (MSG) terhadap jumlah dan morfologi spermatozoa tikus jantan dewasa (*Rattus norvegicus*). *Jurnal Kesehatan Poltekkes Palembang*, 2(14).
- Rahmi, H. 2017. Aktivitas antioksidan dari berbagai sumber buah-buahan di Indonesia. *Jurnal Agrotek Indonesia*, 2(1), pp.34–38.
- Salsabilla, S. 2024. Pengaruh ekstrak bunga telang terhadap jumlah sel Leydig dan diameter tubulus seminiferus pada testis tikus putih jantan galur Wistar yang diinduksi monosodium glutamate (MSG). Undergraduate thesis. Universitas Islam Sultan Agung.
- Samik, A. and Safitri, E. 2017. Potency of mycotoxin binders on MDA level, expressions of caspase-9 and caspase-3 in the uterus of mice exposed to zearalenone. *Iraqi Journal of Veterinary Sciences*, 31(1), pp.29–33. <https://doi.org/10.33899/ijvs.2017.145676>
- Sayuti, K. and Yenrina, R. 2015. *Antioksidan Alami dan Sintetik*. Padang: Andalas University Press.
- Singh, Z. et al. 2014. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies.

Iranian Journal of Public Health, 43(3),
pp.7–16.

Sulastri, S. 2015. Analisis kadar monosodium glutamat pada bumbu mi instan yang diperjualbelikan di koperasi wisata

Universitas Indonesia Timur. *Media Laboran*, 7, pp.5–9.

Yonata, A. and Iswara, I. 2016. Efek toksik konsumsi monosodium glutamate. *Majority*, 5(3), pp.100–104.
