Effect of Curcumin *(Curcuma xanthorriza)* and Red Ginger *(Zingiber officinale* var. *rubrum)* Ethanol Extract on Improvement of Mice Sperm Quality Exposed by Monosodium Glutamate

Niken Herny Lidya Aprilia[1], Sri Rahayu[2], Agung Pramana Warih Marhendra[2]

[1]Master Program of Biology, Departemen of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia

Abstract

Temulawak (*Curcuma xanthorriza*) and red ginger (*Zingiber officinale* var. rubrum Theilade) contain antioxidant compounds that play an important role in inhibiting the negative effects of the excessive administration of free radicals. Excessive free radicals affect the spermatogenesis process. Which results in decreased sperm quality. This research determined the effect of administering ethanol extract of ginger and red ginger on the sperm quality of mice exposed to MSG. This study used 25 male mice (*Mus musculus*), aged 2.5 – 3 months and weighing 25 – 30 g, which were randomly divided into five groups: P0 (control), P1 MSG 4 mg.g^{-1} bw, P2 MSG 4 mg.g^{-1} bw and *C. xanthorriza* extract 0.2 mg.g^{-1} bw, P3 MSG 4 mg.g^{-1} bw and *Z. officinale* extract 0.4 mg.g^{-1} bw, P4 4 mg.g^{-1} bw, and a combination of *C. xanthorriza* extract 0.1 mg.g^{-1} bw and *Z. officinale* extract 0.2 mg.g^{-1} bw, MSG, and all extracts were administered orally for 30 days. On the 31st day, the mice were dissected, and epididymis was collected for sperm quality analysis, such as motility, viability, abnormality, and spermatozoa concentration. Subsequently, the sperm quality data were analyzed using One-way ANOVA through the SPSS 16.0 program for Windows ($P<0.05$), followed by Tukey's Honestly Significant Difference (HSD) test. The addition of both single and combination from *C. xanthorriza* and *Z. officinale* can ameliorate motility, viability, and spermatozoa compared with the group that was only given by MSG. Thus, adding temulawak (*C. xanthorriza*) and red ginger (*Z. officinale*) ethanolic extract can also ameliorate the mice's sperm quality.

Keywords: *C. xanthorriza*, MSG, sperm quality, *Z. officinale*

INTRODUCTION

Monosodium Glutamate (MSG) is a chemical substance classified as a derivate of glutamic acid. This chemical is often used as an additive in food to enhance the umami taste. Recommended MSG consumption for Asian countries ranges from 1.2 to 1.7 g daily. However, MSG consumption in Indonesia has increased to 4.32 g per day [1]. This increase in MSG consumption can trigger physiological disturbances in the body, particularly within the reproductive system. Excessive MSG consumption can lead to the formation of Reactive Oxygen Species (ROS) in the testes and brain [2].

The mechanism by which MSG can cause damage to the testes and brain is mediated by glutamate receptors, including N-Methyl-D-Aspartate (NMDA), Metabotropic Glutamate (mGLU), and kainate (ka) receptors. Glutamate diffuses through the calcium ions (Ca^{2+}). This rise in Ca^{2+} within mitochondria leads to ROS formation, particularly in the Tricarboxylic Acid (TCA) [3]. The produced ROS can cause cell damage due to oxidative stress. The damage to cells within the testicular tissue can lead to morphological alterations in spermatozoa, an imbalanced testosterone secretion by Leydig cells, and disrupted spermatogenesis [4].

Glutamate Toxicity also directly affects the Hypothalamic-pituitary-Gonadal (HPG) Axis, leading to changes in reproductive homeostasis [5]. The HPG axis plays a crucial role in hormone secretion, particularly Gonadotrophin-releasing Hormone (GnRH) secretion. The ROS produced due to glutamate metabolism can trigger a decrease in the secretion of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH), both of which are essential in the process of spermatogenesis [6].

Sperm quality is vital to reproductive health, particularly in assessing individual fertility levels [7]. Parameters used in sperm quality analysis include motility, viability, abnormalities, and spermatozoa concentration. Treatment of Monosodium Glutamate (MSG) at a dose of 4 mg.g^{-1} body weight MSG to Rattus norvegicus for 30 days also leads to reduced sperm quality, decreased testosterone hormone levels, and progression of spermatogenic cell damage. One critical factor that can influence sperm quality is nutrition. Nutrition rich in antioxidants can reduce oxidative stress levels in sperm and increase sperm quality [7].

*Correspondence address:
Muhammad Sasmito Djati
Email : msdjati@ub.ac.id
Address : Dept. Biology, University of Brawijaya, Veteran Malang, 65145.

ISSN. 2087-2852
E-ISSN. 2338-1655

Curcumin, a major component of *C. xanthorriza* that acts as an antioxidant. A previous study has demonstrated that adding 0.2 $mg.g^{-1}$ bw Curcumin to *Mus musculus* that exposed to MSG can improve the mice's sperm quality. Another plant known for its antioxidant properties is red ginger *Zingiber officinale*, which contains gingerol, gingerdiol, and gingerdione as antioxidants [8]. The *Z. officinale* can increase sperm production by acting as a suppressor of oxidative damage, thereby promoting cell development [9]. The oral addition of ethanol extract of *Z. officinale* to *Rattus norvegicus* for 26 days has been shown to improve sperm motility and viability [10]. Previous studies also indicate that adding *Z. officinale* extract at a dose of 0.05 $mg.g^{-1}$ body weight can increase sperm quality parameters and the number of spermatogenic cells in *Mus musculus* [11].

Despite the *fact that C. xanthorriza* and *Z. officinale* are widely known as sources of antioxidants, research on the effect of adding ethanol extract of *C. xanthorriza* and *Z. officinale*, both individually and in combination, in the field of reproduction, particularly on sperm quality in MSG-exposed mice, remains limited. The aim of this research was to determine the effect of administering ethanol extract of ginger and red ginger on the sperm quality of mice exposed to MSG.

MATERIAL AND METHOD

Ethical approval and Research design

This research procedure has earned an agreement from the Animal Care and Use Committee of Brawijaya University, number: 004-KEP-UB-2023. Twenty-five male mice (*Mus musculus*) aged 2.5 – 3 months and weighing 25 – 30 g were randomly assigned to five treatment groups for a duration of 30 days (Table 1).

Table 1. Group of Mice (n= 5 per group)

Group Code	MSG ($mg.g^{-1}$ bw)	CX extract ($mg.g^{-1}$ bw)	ZO extract ($mg.g^{-1}$ bw)
P0*	-	-	-
P1	4	-	-
P2	4	0.2	-
P3	4	-	0.4
P4	4	0.1	0.2

Notes: P0 (control) received a standard diet of pellets and *ad libitum* water, MSG = Monosodium Glutamate, CX = *Curcuma xanthorriza*, ZO = *Zingiber officinale*, bw = body weight.

Sperm Quality Analysis

On the 31st day, *Mus musculus* were euthanized by cervical dislocation, and epididymal organs were isolated. The caudal epididymal was placed in an object glass containing 1.5 mL PBS solution at 37°C. Sperm quality was assessed based on various parameters, including motility, viability, abnormalities, and spermatozoa concentration [12], using a microscope at magnifications of 100x and 400x.

Sperm Motility

The 10 µL of semen was collected and placed onto an object glass. Sperm motility analysis was conducted by observing progressively moving spermatozoa under a microscope at 100x magnification in five fields of view. The progression of spermatozoa was categorized into several classifications [13].

Viability and Abnormality Spermatozoa

The 10 µL of semen was aspirated using a micropipette and placed onto an object glass. Subsequently, a stain containing 1% Eosin and 5% Negrosin was added. The percentage of spermatozoa viability and abnormalities were observed under a light microscope at a magnification of 400x, following the formula [1] :

$$Viability\ (\%) = \frac{number\ of\ live\ spermatozoa}{total\ number\ of\ spermatozoa}\ x\ 100\%$$

$$Abnormality\ (\%) = \frac{number\ of\ live\ spermatozoa}{total\ number\ of\ spermatozoa}\ x\ 100\%$$

Spermatozoa Concentrations

The 10 µL of semen was collected and mixed with fixative (physiological NaCl) in a 1:10 ratio, resulting in a total of 100 µL. The two solutions were homogenized, and then 10 µL of the mixture was loaded into a Neubauer Haemocytometer. Spermatozoa concentration was determined in triplicated under a microscope at a magnification of 400X, employing the formula as provided [3]:

$$SC = n \times k \times FP \times 10^4$$

Description:
SC : Spermatozoa concentration
n : the number of spermatozoa
10^4 : volume of Haemocytometer counting chamber
FP : dilution factor (10)
K : number of small squares

Statistical analysis

The data about sperm quality, encompassing motility, viability, abnormalities, and spermatozoa concentration, were statistically analyzed by One-way ANOVA with the SPSS 16.0 software program for Windows ($p<0.05$). It was then followed by the Tukey Honestly Significant Difference (HSD) post-hoc test.

ISSN. 2087-2852
E-ISSN. 2338-1655

RESULT AND DISCUSSION

Data on sperm quality results are contained in Table 2. Parameters of sperm quality were used, including motility (Fig. 1), viability, abnormality, and spermatozoa concentrations.

Table 2. The effect of MSG and extract *C. xanthorriza* as well as *Z. officinale* on quality sperm of *Mus musculus*

Sperm quality ±SD				
Group	**SM (%)**	**SV (%)**	**SA (%)**	**SC (10^6.mL^{-1})**
P0	58 ± 3.49^a	84 ± 1.10^a	26 ± 1.51^a	9 ± 2.7^a
P1	48 ± 2.31^a	62 ± 4.96^a	45 ± 12.3^c	12 ± 3.0^a
P2	51 ± 8.58^a	70 ± 11.5^a	35 ± 4.56^b	21 ± 6.5^c
P3	73 ± 4.99^c	81 ± 12.2^a	25 ± 4.81^a	18 ± 4.0^b
P4	71 ± 10.3^b	78 ± 9.41^a	27 ± 4.62^a	15 ± 3.4^a

Notes: Different letters within the same column show a statistically significant ($p \leq 0.05$). Group P1 that was exposed to MSG had effects on sperm quality. In contrast, groups P3 and P4 with extract of *C. xanthorriza* and red ginger have improved quality of sperm although exposed to MSG.

SM (%) = Percentage of sperm motility
SV (%) = Percentage of sperm viability
SA (%) = Percentage of sperm abnormality
SC (10^6.mL^{-1}) = spermatozoa concentrations

P0 = control
P1 = 4 mg.g^{-1} bw MSG
P2 = 4 mg.g^{-1} bw MSG + 0.2 mg.g^{-1} bw *C. xanthorriza*
P3 = 4 mg.g^{-1} bw MSG + 0.4 mg.g^{-1} bw *Z. officinale*
P4 = 4 mg.g^{-1} bw MSG + 0.1 mg.g^{-1} bw *C. xanthorriza* + 0.2 mg.g^{-1} bw *Z. officinale*

Sperm Motility

The results of this study reveal that the group exposed to a dose of 4mg.g^{-1} body weight of MSG (P1) exhibited a motility percentage of 48.66±2.31% (Table 2). This result aligns with prior research, which demonstrated a significant reduction in sperm motility in *Mus musculus* following the addition of 4 mg.g^{-1} body weight MSG for 30 days [19]. There is a decrease of 10% compared to the group control (P0), which was not exposed to MSG (Table 2). Furthermore, the addition of MSG at a dose of 4 mg.g^{-1} bw for 21 days was found to elevate testicular Malondialdehyde (MDA) production, serving as a biomarker for lipid peroxidation. This increase in MDA production points to oxidative stress due to the generation of augmented Reactive Oxygen Species (ROS) and decreased antioxidant levels in the body after MSG exposure [14].

The decline in sperm motility caused by oxidative stress is closely associated with the bioavailability of energy within spermatozoa. Consequently, ATP production, essential for bioenergy in spermatozoa, decreases due to cellular dysfunction, which can result in apoptosis or necrosis of cells [15]. The redominate reduction in sperm motility is linked to mitochondrial dysfunction in spermatozoa, leading to increased ROS and decreased ATP production [16].

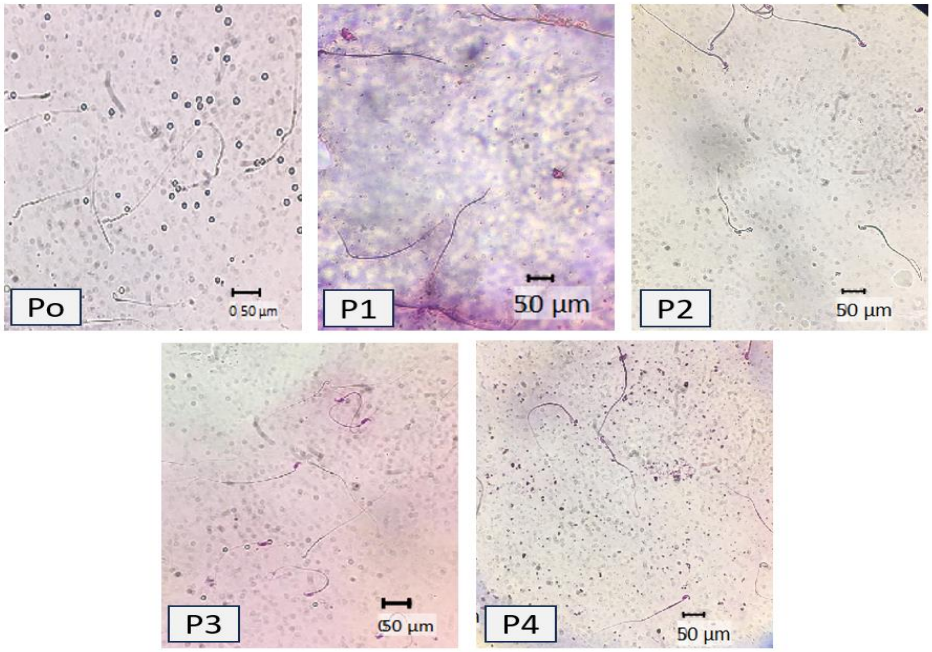

Figure 1. Sperm motility in each treatment; P0 was only given feed, P1 was given MSG treatment, P2 was given *C. Xanthorria* extract treatment with MSG exposure, P3 was given red ginger extract treatment with MSG exposure, and P4 group were given a combination of *C. xanthorriza* and red ginger extract treatment with MSG exposure.

ISSN. 2087-2852
E-ISSN. 2338-1655

The addition of *Z. officinale* extracts at a dose of 0.4 $mg.g^{-1}$ bw results in increased sperm motility, with a percentage group P3 of 73±4.99%. Prior research mentioned that providing ethanol extract of red ginger for 26 days to *Rattus norvegicus* re-increase sperm motility [18]. The *Z. officinale* extract also contains phytochemicals that can modulate androgenic activity, particularly by elevating testosterone levels [18]. The antioxidative and chemoprotective compounds within *Z. officinale* increase the activity of superoxide Dismutase (SOD) and catalase (CAT) in the testes, thereby safeguarding against the free radical that triggers DNA damage and ultimately improve sperm quality in mice [12].

The combination of *C. xanthorriza* and *Z. officinale* extracts yields an increase in sperm motility, with a percentage of 71±10.3%. The curcumin content within *C. xanthorriza* acts as a scavenger of free radicals, preventing ROS formation. The phytochemical content in *Z. officinale* modulates androgenic activity, optimizing spermatogenesis through increased testosterone levels. Protective agents found in *Z. officinale* include gingerol and shogaol. The addition of *Z. officinale* extract can maintain the Hypothalamic-pituitary-Gonadol (HPG) axis and modulate steroidgenesis [19].

Sperm Viability

The addition of MSG at a dose of 4 $mg.g^{-1}$ body weight for 30 days led to a reduction in sperm viability in the P1 group by 22% compared to the control (P0) group (Table 2). The increased production of Reactive Oxygen Species (ROS) from MSG consumption by the body elevates Ca^{2+} production within the mitochondria. Ca^{2+} serves as a primary regulator of various cellular [20]. Accumulation of Ca^{2+} can increase ROS levels, impacting spermatozoa's fluidity and membrane integrity [21]. Previous research by Firstiantono *et al.* [3] demonstrated that adding MSG at a dose of 0.4 $mg.g^{-1}$ body weight for 30 days significantly reduced sperm viability compared to the control group.

The Spermatozoa membrane contains numerous polyunsaturated fatty acids (PUFA) susceptible to ROS. An imbalance in ATP production can lead to axonemal damage, resulting in decreased sperm viability [22]. Adding *C. xanthorriza* and *Z. officinale* extracts can increase sperm viability with percentages of 70±11.5% and 81±12.2%, respectively, compared to the MSG treatment. The combined addition of *C. xanthorriza* and *Z. officinale* extracts also increases sperm viability percentage of 81±12.2%. The curcumin content within *C. xanthorriza* extract protects the spermatozoa membrane against the reactivity of free radicals. Curcumin is a scavenger of free radicals, thus preserving the fluidity and integrity of the spermatozoa membrane.

Previous research has indicated that curcumin can elevate testosterone and Lutenizing Hormone (LH) levels in MSG-exposed rats [23]. The extract of *Z. officinale* contains active phenolic compounds such as gingerol, shogaol, zingerol, and gingerdiol, all exhibiting antioxidant activity. Prior studies have shown that adding ethanol extract of *Z. officinale* for 26 days can increase sperm viability in rats [24]. Supplementation with turmeric and rats with a high blood pressure diet can increase testosterone levels and sperm quality [24].

Sperm Abnormality

Based on the result of this study, it is evident that abnormalities in spermatozoa were observed across all treatment groups. The addition of MSG at a dose of 4 $mg.g^{-1}$ body weight resulted in the highest percentage of spermatozoa abnormalities, reaching 45±12.3% compared to the control group (P0) (Table 2).

Exposure to MSG can increase antioxidant activity, such as glutathione (GSH) [25]. The High production of Reactive Oxygen Species (ROS) due to MSG exposure can impact the plasma membrane of spermatozoa, which contains a significant amount of phospholipids and unsaturated fatty acids. These components are particularly vulnerable to ROS, especially the highly reactive hydroxyl radical. The hydroxyl radical can induce lipid peroxidation, damaging the fatty acid chains and forming toxic products to spermatozoa and ethane (C_2H_6) [26]. Such products can trigger morphological damage to spermatozoa.

This study's results reveal the presence of spermatozoa abnormalities, such as those lacking heads and coiled tails (Fig. 2). Oxidative stress conditions can also impact changes in the morphology of spermatozoa heads [27]. The addition of ethanol extracts of *C. xanthorriza* and *Z. Officinale* can decrease abnormalities in spermatozoa exposed to MSG, with respective percentages of 4.56% and 4.81% (Table 2).

The combined addition of *C. xanthorriza* and *Z. officinale* extracts resulted in a percentage of spermatozoa abnormalities at 27±4.62%,

ISSN. 2087-2852
E-ISSN. 2338-1655

compared to the MSG treatment group (P1). The Curcumin content within *C. xanthorriza* plays a role in inhibiting lipid peroxidation by absorbing free radicals and stimulating endogenous antioxidant activities, such as superoxide Dismutase (SOD) and catalase (CAT) [28]. This protective mechanism is attributed to curcumin and gingerol in *Z. officinale*, which exhibits antioxidant activity. Previous studies have demonstrated that adding 6-gingerol isolated from *Z. officinale* resulted in the lowest percentage of spermatozoa abnormalities among treatment groups and improved testicular function [29]. Other bioactive compounds found in *Z. officinale* possess antioxidant activity and contribute to protecting DNA in spermatozoa [30].

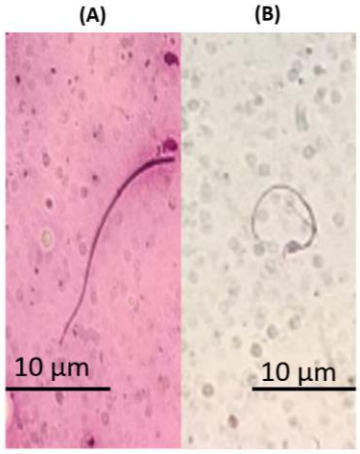

Figure 2. Morphology sperm abnormality correct the number of abnormality (400X). (A) Detache head, (B) Sperm coiled tail abnomality found in this study.

Spermatozoa Concentration

The results of this study demonstrate that the highest spermatozoa concentration was observed in the group treated with *C. xanthorriza* extract at a dose of 17.27 cell.mL^{-1}, and this deferred significantly from the group exposed solely to MSG at a dose of 4 mg.g^{-1} body weight over 30 days (Table 1). Previous research has indicated that *C. xanthorriza* extract is capable of increasing the spermatozoa concentration in *Mus musculus* exposed to MSG [31].

The addition of MSG can lead to decreased levels of Luteinizing Hormone (LH) and testosterone. These hormones play pivotal roles in maintaining the normal function of the testes and spermatogenesis [32]. Elevated production of Reactive Oxygen Species (ROS) can trigger lipid peroxidation, leading to membrane and DNA damage in spermatozoa, resulting in cellular apoptosis or necrosis. This progression of cell death consequently leads to a decline in spermatozoa concentration [33].

CONCLUSION

The addition of *C. xanthorriza* extract at a dose of 0.2 mg.g^{-1} body weight, and *Z. Officinale* extract at a dose of 0.4 mg.g^{-1} body weight, as well as their combination, proves to be effective in increasing the quality of spermatozoa exposed to MSG. There is a need for further research regarding the use of Curcumin (*Curcuma xanthorriza*) and red ginger on infertile organisms who suffer from reproductive disorders. It is necessary to carry out a histopathological analysis of the liver and kidneys to determine the toxicity caused by MSG.

REFERENCES

[1] Rahayu, S., R. Annisa, I. Anzila, Y.I. Christina, A. Soewondo, A.P.W. Marhendra, M.S. Djati. 2021. *Marsilea crenata* ethanol extract prevents monosodium glutamate adverse effects on the serum levels of reproductive hormones, sperm quality, and testis histology in male rats. *Vet. World.* 14(6). 1529–1536. DOI: 10.14202/vetworld. 2021.1529-1536.

[2] Anzila, I., A.P.W. Marhendra, S. Rahayu. 2019. The effect of monosodium L-glutamate (MSG) treatment for short and long terms to the semen quality of adult male rats. *J. Exp. Life. Sci.* 9(2). 116–121. DOI: 10.21776/ub.jels.2019.009.02.09.

[3] Firstiantono, A., S. Rahayu., A.P.W. Marhendra, A. Soewondo. 2022. Potential of combination *Marsilea crenata* And *Curcuma xanthorriza* to improve sperm quality of male mice exposed by monosodium glutamate. *Biotropika.* 10(1). 33–39. DOI: 10.21776/ub.biotropika.2022. 010.01.04.

[4] Jubaidi, F.F., R.D. Mathialagan, M.M. Noor, I.S. Taib, S.B. Budin. 2019. Monosodium glutamate daily oral supplementation: study of its effects on male reproductive system on rat model. *Syst. Biol. Reprod. Med.* 65(3). 194–204. DOI: 10.1080/19396368.2019. 1573274.

[5] Oduwole, O.O., H. Peltoketo, I.T. Huhtaniemi. 2018. Role of follicle-stimulating hormone in spermatogenesis. *Front. Endocrinol.* 9. 1–11. DOI: 10.3389/ fendo.2018.00763.

ISSN. 2087-2852
E-ISSN. 2338-1655

[6] Omu, A.E. 2013. Sperm parameters: Paradigmatic Index of Good Health and Longevity. *Med. Princ. Pract.* 22(1). 30–42. DOI: 10.1159/000354208.

[7] Ahangan, M.G., M.K. Dehkordi, A.A. Javar, M.H. Salehi, M. Ostadpoor. 2021. A systematic review on the effect of Ginger (*Zingiber officinale*) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. *Vet. Med. Sci.* 7(5). 1959–1969. DOI: 10.1002/vms3.538.

[8] Baliga, M.S., R. Haniadka, M.M. Pereira, J.J. D'Souza, P.L. Pallaty, H.P. Bhat, S. Popuri. 2011. Update on the chemopreventive effects of ginger and its phytochemicals. *Crit. Rev. Food Sci. Nutr*. 51(6). 499–523. DOI: 10.1080/10408391003698669.

[9] Herve, T., K.J. Raphaël, N. Ferdinand, F.T.L. Vitrice, A. Gaye, M.M. Outman, N.M.W. Marvel. 2018. Growth performance, serum biochemical profile, oxidative status, and fertility traits in male Japanese quail fed on ginger (*Zingiber officinale*, roscoe) essential oil. *Vet. Med. Int*. 1. 1–8. DOI: 10.1155/ 2018/7682060.

[10] Banihani, S.A. 2019. Effect of ginger (*Zingiber officinale*) on semen quality. *Andrologia*. 51(6). 1–10. DOI: 10.1111/and. 13296.

[11] Sutyarso., H. Busman, M. Kanedi, Muhartono. 2016. Rhizome extract of white ginger (*Zingiber officinale* Roxb) maintains testicular function of aging mice. *Int. J. Nutr. Food Sci.* 5(3). 175–178. DOI: 10.11648/ j.ijnfs.20160503.14.

[12] Aini, S., A.P.W. Marhendra, S. Rahayu. 2022. *Curcuma mangga* ethanol extract improves sperm quality of mice exposed to monosodium glutamate. *J. Exp. Life Sci.* 12(3). 98–104. DOI: 10.21776/ub.jels.2022. 012.03.04.

[13] EL- Sawy, H.B.I., M.M. Soliman, S.A. El Shazly, H.A.M. Ali. 2018. Protective effects of camel milk and Vitamin E against monosodium glutamate induced biochemical and testicular dysfunction. *Prog. Nutr.* 20(1). 76–85. DOI: 10.23751/pn. v20i1.5870.

[14] Celik-Ozenci, C., A. Tasatargil. 2013. Role of poly (ADP-ribose) polymerases in male reproduction. *Spermatogenesis*. 3(2). 1-9. DOI: 10.4161/spmg.24194.

[15] Bauer, K.N., B. Nixon. 2020. Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants.* 9(2). 1–22. DOI: 10.3390/antiox9020134.

[16] Jagetia, G.C., G.K. Rajanikant. 2015 Curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated γ-irradiation. *Antioxidants.* 4(1). 25–41. DOI: 10.3390/antiox4010025.

[17] Khaki, A., F. Fathiazad, M. Nouri, A.A. Khaki, C.C. Onzanci, M.G. Novin, M. Hamadeh. 2009. The effects of Ginger on spermatogenesis and sperm parameters of rat. *Iranian J. Reprod. Med.* 7(1). 7–12.

[18] Morakinyo, A.O., P.U. Achema, O.A. Adegoke. 2010. Effect of *Zingiber officinale* (ginger) on sodium arsenit induced reproductive toxicity in male rats. *Afr. J. Biomed. Res.* 13. 39–45.

[19] Nayantara, A., N. Vinodini, G. Damodar, B. Ahamed, C. Ramaswamy, dan B. R. Shabarinath. 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, 3,1-5.

[20] Giorgi, C., S. Marchi, P. Pinton. 2018. The machineries, regulation and cellular functions of mitochondrial calcium. *Nat. Rev. Mol. Cell Biol.* 19. 713–730.

[21] Agarwal, A., G. Virk, C. Ong, S.S. du Plessis. 2014. Effect of oxidative stress on male reproduction. *World J. Men's Health*. 32(1). 1–17. DOI: 10.5534/wjmh.2014.32.1.1

[22] Bansal, A.K., G.S. Bilaspuri. 2011. Impacts of oxidative stress and antioxidants on semen functions. *Vet. Med. Int.* 1–7. DOI: 10.4061/ 2011/686137.

[23] Belhan, S., S. Yildirim, Z. Huyut, U. Ozdek, G. Oto, S. Algul. 2020. Effects of curcumin on sperm quality, lipid profile, antioxidant activity, and histopathological changes in streptozotocin-induced diabetes in rats. *Andrologia*. 52(6). 1–8. DOI. 10.1111/and. 13584.

[24] Akinyemi, A.J., I.A. Adedara, G.R. Thome, V.M. Morsch, M.T. Rovani, L.K.S. Mujica, *et al.* 2015. Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats. *Toxicol. Rep*. 13(2). 1357–1366. DOI: 10.1016/ j.toxrep.2015.10.001.

[25] Patricio, A., D.F. Cruz, J.V. Silva, A. Padrao, B.R. Correia, L.K. Gregorio, *et al.* 2016. Relation between seminal quality and oxidative balance in sperm cells. *ACTA Urol.*

ISSN. 2087-2852
E-ISSN. 2338-1655

Port. 33(1). 6-15. DOI: 10.1016/j.acup.2015. 10.001.

[26] Tremallen, K. 2008. Oxidative stress and male infertility – a clinical perspective. *Hum. Reprod. Update*. 14(3). 1–16. DOI: 10.1093/ humupd/dmn004.

[27] Rahayu, S., T. Susilawati, A. Soewondo. 2020. Biologi reproduksi : Kajian seluler dan molekuler. UB Press. Malang.

[28] Lu, W.P., X.T. Mei, Y.Wang, Y.P. Zheng, Y.F. Xue, D.H. Xu. 2015. Zn(II)-curcumin protects against oxidative stress, deleterious changes in sperm parameters and histological changes in a male mouse model of sylophosphamide-induced reproductive damage. *Environ. Toxicol. Pharmacol*. 39(2). 515–524. DOI: 10.1016/j.etap.2014.12.014.

[29] Farombi, E.O., I.A. Adedara, B.O. Ajayi, T.E. Idowu, O.O. Eriomala, F.O. Akinbote. 2018. 6-Gingerol improves testicular function in mice model of chronic ulcerative colitis. *Hum. Exp. Toxicol*. 37(4). 358–372. DOI: 10.1177/0960327117703689.

[30] Roberta, V.L.M., A.M.S. Silva, A.P. Duarte, S. Socorro, S. Correia, C.J. Maia. 2021. Natural products as protective agents for male fertility. *BioChem.* 1(3). 122–147. DOI: 10.3390/biochem1030011.

[31] Soleimanzadeh, A., A. Saberivand. 2013. Effect of curcumin on rat sperm morphology after the freeze-thawing process. *Vet. Res. Forum.* 4(3). 185–189.

[32] Oliveira, N.N.P.M., M.A.R. Felix, T.C.S. Pereira, L.G.P. Rocha, J.R. Miranda, M.G. Zangeronimo, *et al.* 2015. Sperm quality and testicular histomorphometry of wistar rats supplemented with extract and fractions of fruit of *Tribulus terrestris* L. *Braz. Arch. Biol. Technol*. 58(6). 891–897. DOI: 10.1590/ S1516-89132015060278.

[33] Farombi, E.O., O.O. Onyema. 2006. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of Vitamin C, Vitamin E and quercetin. *Hum. Exp. Toxicol*. 25. 251–259. DOI: 10.1191/0960327106ht621oa.

ISSN. 2087-2852
E-ISSN. 2338-1655