









Effect of the Extract of Dayak Onions (*Eleutherine palmifolia*) on the Sperm Quality of Mice (*Mus musculus*) Induced with Monosodium Glutamate

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ABSTRACT

This study aims to determine the effect of the extract of Dayak onions (*Eleutherine palmifolia*) on the motility, viability, and concentration of spermatozoa in mice (*Mus musculus*) induced with monosodium glutamate (MSG). This study involved 25 male mice aged 11 weeks and weighing approximately 20 g. The mice were divided into five groups, namely C- (CMC-Na 0.5%), C+ (4 mg/g BW of MSG), T1 (4 mg/g BW of MSG and 30 mg/kg BW of Dayak onion extract), T2 (4 mg/g BW of MSG and 60 mg/kg BW of Dayak onion extract), and T3 (4 mg/g BW of MSG and 120 mg/kg BW of Dayak onion extract). All treatments were administered for 52 days. The results showed no significant differences in the motility and concentration of spermatozoa between the C- (81.50 ± 4.18 ; 7.21 ± 0.37) and T3 (77 ± 2.74 ; 7.04 ± 0.13) groups ($p < 0.05$). In addition, the results showed significant differences in the viability of spermatozoa between the C- (83.30 ± 3.40) and T3 (77.20 ± 4.06) groups. Therefore, it can be concluded that the administration of Dayak onion extracts orally to mice induced with MSG can maintain the motility, viability, and concentration of spermatozoa.

Keyword: Dayak onion, mice, MSG, sperm quality

INTRODUCTION

The globalization of the food market has led to changes in consumption patterns and lifestyles, resulting in an increase in the use

of flavor enhancers from time to time. One of the flavor enhancers that is commonly used by the general public is monosodium glutamate (MSG) (Niaz et al., 2018). In Indonesia, MSG consumption has increased

from 1.53 g/capita/day in 1998 to 9.62 g/capita/day in 2011 (Nuraida *et al.*, 2014). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) classify MSG as a food additive with an acceptable daily intake of 120 mg/kg body weight (BW). Overconsumption of MSG can cause adverse effects on the body, such as headache, nausea, flushed face, Chinese restaurant syndrome (Bera *et al.*, 2017), palpitations, weakness, numbness in the neck, and difficulty breathing (Datta *et al.*, 2019). Overconsumption of MSG can also cause damage to the tissues and organs, including the reproductive system (Kayode *et al.*, 2020).

Furthermore, excessive consumption of MSG can cause damage to the arcuate nucleus and ventromedial nucleus of the hypothalamus (Nurhayati, 2014). This can disrupt the secretion of gonadotropin-releasing hormone (GnRH), which in turn affects the adenohypophysis in the secretion of gonadotropic hormones. As a result, the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) may decrease (Giovambattista *et al.*, 2003). Decreased levels of FSH and LH lead to decreased levels of testosterone. This disrupts spermatogenesis (Oduwole *et al.*, 2021) and leads to decreased sperm quality (Dutta *et al.*, 2019).

Excessive consumption of monosodium glutamate (MSG) can cause the excessive formation of free radicals and oxidative stress, leading to an increase in the production of reactive oxygen species (ROS). This can damage DNA transport and spermatogenic cells (Sukmaningsih *et al.*, 2011), resulting in a decrease in the number of spermatozoa (Bilondatu *et al.*, 2016).

Therefore, an increase in ROS levels can cause an imbalance between ROS and antioxidants, leading to oxidative stress that can reduce the motility and viability of spermatozoa (Susilowati, 2018).

Dayak onions (*Eleutherine palmifolia*) are rich in antioxidants. Antioxidants can help reduce the risk of organ damage due to free radicals by inhibiting or slowing down the oxidation process (Prastyaningtyas *et al.*, 2021). Antioxidants work by reducing the excessive production of free radicals and preventing the resulting damage (Ravi *et al.*, 2022). Dayak onions contain various chemical compounds, including tannins, polyphenols, flavonoids, quinones, glycosides, stearic acid, gallic acid, eleutherin-one, eleutherol, eleutherine, and iso-eleutherine (Raga *et al.*, 2012). Phenolic and flavonoid compounds found in Dayak onion extract can act as hydrogen donors and neutralize ROS. Therefore, consumption of Dayak onion extract can neutralize ROS and prevent damage to sperm DNA (Angela & Sumbayak, 2017). Based on the aforementioned explanation, this study aims to determine the protective effect of Dayak onion extract on the sperm quality of mice induced with MSG.

MATERIALS AND METHODS

Ethical Approval

This study received approval from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number 1.KEH.081.07.2022.

Research Design

This study was conducted from July to September 2022. The extraction of Dayak onions took place at the Pharmacology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga. The experimental animals were kept at the Experimental Animal Facility, while the examination of spermatozoa was conducted at the Embryology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga. This study used an experimental design and involved 25 male mice. The mice were divided into five treatment groups, each with five repetitions. The negative control group (C-) was administered with only 0.5% CMC-N orally, while the positive control group (C+) was administered with only MSG at a dose of 4mg/g BW orally. On the other hand, the treatment groups, namely treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3), were orally administered with Dayak onion extract at doses of 30, 60, and 120 mg/kg BW respectively. One hour after administration, the treatment groups were induced with MSG at a dose of 4 mg/g BW orally. This dose is based on research conducted on Dayak onion extract (Jayanti & Raudah, 2021) and MSG (Pebrianti, 2013). The experiment was conducted for 52 days. At the end of the experiment, the mice were euthanized by atlanto-occipital dislocation. Sperm was obtained by squeezing the cauda epididymis and examined under a

microscope at 400x magnification for quality assessment.

Statistical Analysis

Statistical data analysis was performed using SPSS Statistics 20. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to analyze the differences between groups. Superscripts (a, b, c, d, e) indicate different values and significant differences between the groups.

RESULTS AND DISCUSSION

This study found a significant difference in the motility, viability, and concentration of sperm in the C+ group compared to the C- group (see Figures 1, 2, and 3, and Table 1). The results of the sperm quality test showed similar patterns for motility and viability, while the sperm concentration had a significant difference ($p < 0.05$) in each treatment group. In addition, this study found no significant difference in the motility, viability, and concentration of sperm in C+ group compared to the T3. These results are consistent with the mechanism of the damage caused by excessive exposure to MSG. This increases the levels of free radicals in the body and results in oxidative stress, which is known to significantly reduce sperm quality (Kayode *et al.*, 2020).

Table 1. Mean and Standard Deviation of the Extract of Dayak Onions (*Eleutherine palmifolia*) on the Sperm Quality of Mice (*Mus musculus*) Induced with Monosodium Glutamate (MSG)

Treatments	Mean ± SD		
	Motility ± SD(%)	Viability ± SD (%)	Concentration ± SD (million/mm ³)
C-	81.50 ^c ± 4.18	83.30 ^e ± 3.40	7.21 ^d ± 0.37
C+	28 ^a ± 2.74	33.20 ^a ± 1.79	3.61 ^a ± 0.65
T1	32.50 ^a ± 5.86	38.3 ^b ± 3.56	4.83 ^b ± 0.27
T2	56.50 ^b ± 5.18	58.8 ^c ± 1.39	5.85 ^c ± 0.21
T3	77 ^c ± 2.74	77.20 ^d ± 4.06	7.04 ^d ± 0.13

Note: Different superscripts (a, b, c, d, e) indicate significant differences ($p < 0.05$). The C- group received 0.5% CMC-Na orally for 52 days. The C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. The T1 group received 30 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T2 group received 60 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T3 group received 120 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days.

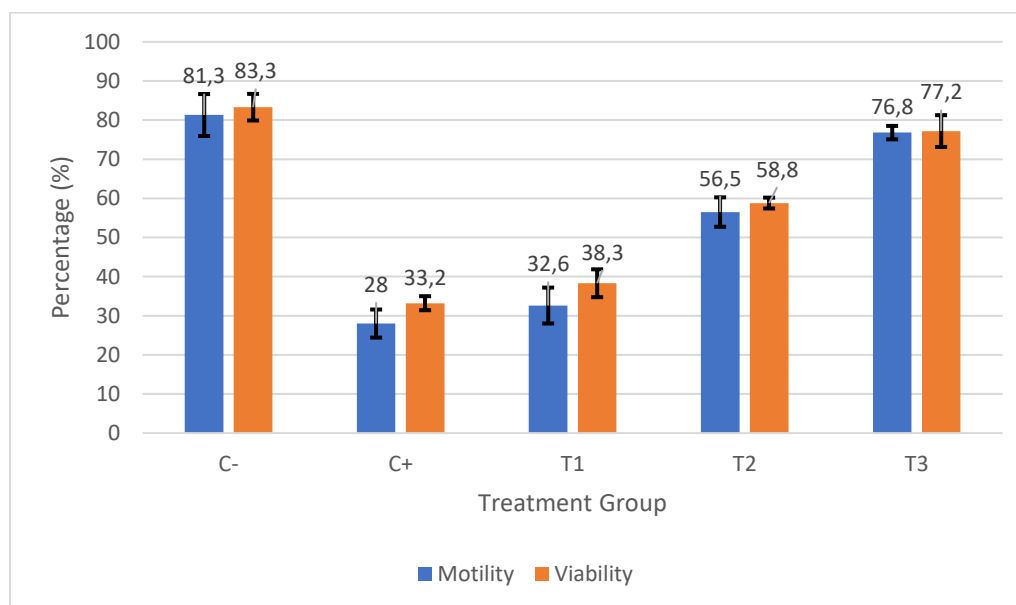


Figure 1. The effect of Dayak onion extract on the motility and viability of sperm in mice induced with MSG. The C- group received 0.5% CMC-Na orally for 52 days. The C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. The T1 group received 30 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T2 group received 60 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T3 group received 120 mg/kg BW of Dayak onion extract and MSG at a dose 4 mg/kg BW orally for 52 days.

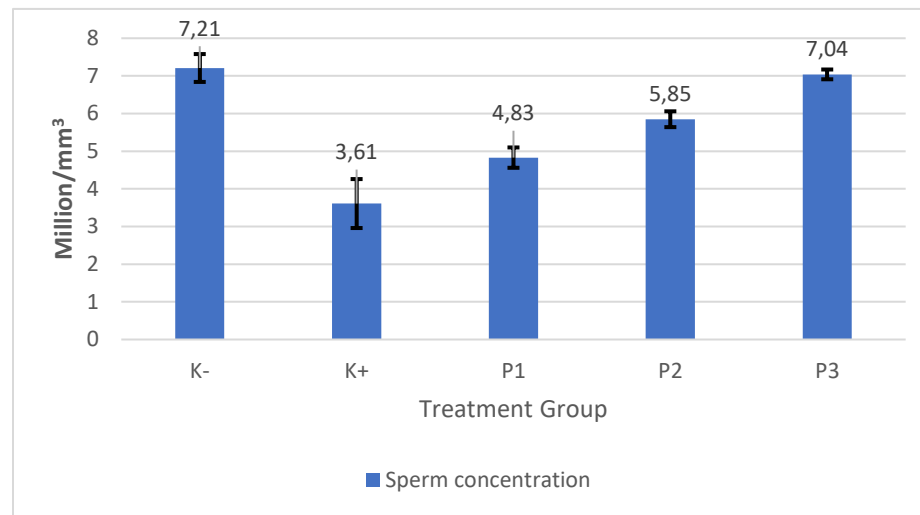


Figure 2. The effect of Dayak onion extract on the concentration of sperm in mice induced with MSG. The C- group received 0.5% CMC-Na orally for 52 days. The C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. The T1 group received 30 mg/kg BW of Dayak onion extract and MSG at a dose 4 mg/kg BW orally for 52 days. The T2 group received 60 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T3 group received 120 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days.

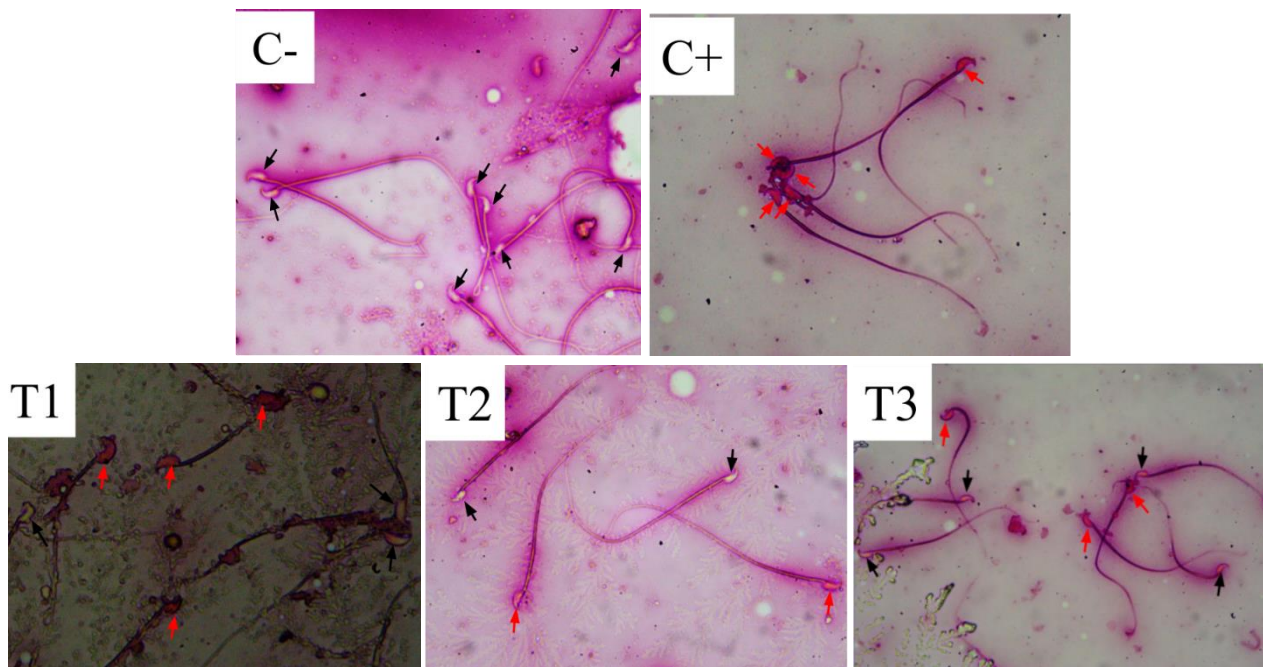


Figure 3 The viability of mice sperm under a microscope at 400x magnification. Black arrows indicate alive spermatozoa. Red arrows indicate dead spermatozoa. The C- group received 0.5% CMC-Na orally for 52 days. The C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. The T1 group received 30 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T2 group received 60 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T3 group received 120 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days.

Sperm motility can be determined by measuring the number of alive spermatozoa in a sample (Susilowati *et al.*, 2022). Sperm movement is facilitated by the presence of energy (ATP) produced by the mitochondria and the dynein motor (cytoskeleton) in the flagellum or tail of the spermatozoa (Suprayogi *et al.*, 2018). The C+ group showed a decrease in sperm motility. This is consistent with previous research which suggested that the administration of MSG results in an increase in the number of quiescent spermatozoa and a decrease in the number of moving spermatozoa (Pebrianti, 2013). In addition, high doses of MSG can cause oxidative stress and increase ROS levels (Ochiogu *et al.*, 2015). The plasma membrane of spermatozoa is rich in polyunsaturated fatty acids, which can undergo lipid peroxidation reactions when exposed to free radicals. Lipid peroxidation leads to an increase in membrane fluidity, disruption of membrane integrity, and inactivation of membrane binding to enzymes and receptors (Asadi *et al.*, 2017).

Disruption of the sperm membrane integrity inhibits the need for nutrients and adenosine triphosphate (ATP) within the cell. As a result, spermatozoa are unable to contract their flagella and become immotile (Hafaz, 2017). Sperm motility increased in the T1, T2, and T3 groups due to the administration of Dayak onion extract, which has antioxidant properties that neutralize free radicals (Jayanti & Raudah, 2021). The components of Dayak onions that act as potent antioxidants are phenolic compounds and flavonoids. These compounds can inhibit antioxidants through

a radical scavenging mechanism by donating an electron to an unpaired electron in free radicals, thereby reducing their levels (Santos-Sanchez *et al.*, 2019). They can also protect spermatozoa from damage caused by free radicals and maintain their motility.

Sperm viability is determined by measuring the number of alive spermatozoa in a sample (Susilowati *et al.*, 2019). The C+ group showed a decrease in sperm viability. This is consistent with previous research that showed a decrease in the number of alive spermatozoa and an increase in the number of dead spermatozoa in mice due to an increase in ROS levels on the administration of MSG (Pebrianti, 2013). Plasma membrane damage is responsible for the decreased sperm viability. The plasma membrane of spermatozoa serves to protect cell organelles and regulate electrolyte balance in the metabolism. This damaged disrupts the metabolism of spermatozoa, causing them to lose their fertility by releasing cellular components and losing of the inactivating power of important protein-enzyme components in the acrosome. This results in the death of spermatozoa and affect their viability (Setyawan *et al.*, 2019).

The T1, T2, and T3 groups showed an increased in sperm viability, which was attributed to the presence of flavonoids in Dayak onions. Flavonoids protect the plasma membrane from ROS, maintaining the integrity of the membrane and allowing for proper ATP formation. ATP is a source of energy for spermatozoa, and with the maintenance of ATP formation, sperm viability remains high (Utomo *et al.*, 2021).

The C+ group showed a decrease in sperm concentration. High doses of MSG disturb spermatogenesis through cytotoxic testicular mechanism. MSG leads to the formation of excess free radicals and causes oxidative stress. As a result, the physiological function of spermatozoa is disrupted. In addition, oxidative stress can lead to an increase in lipid peroxidation, which can cause damage to cells and result in sperm apoptosis, ultimately reducing the number of spermatozoa (Rahmah, 2020). In contrast, The T1, T2, and T3 groups showed a significantly increase in sperm concentration. The increase in sperm concentration was attributed to the antioxidant properties of Dayak onions, which neutralize free radicals that cause DNA damage and due to excessive ROS production. Therefore, the administration of Dayak onion extract can improve sperm quality, especially sperm concentration (Pratiwi *et al.*, 2017).

The antioxidant activity of the ethanol extract of Dayak onions is higher than that of red onions and garlic, as evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The IC₅₀ values of the extracts of Dayak onions, garlic, and red onions are 25.33 ppm (Kuntorini & Astuti, 2010), 149.49 ppm (Putri, 2020). and 166.97 ppm (Martha, 2019), respectively. The IC₅₀ value is the concentration of the compound that can scavenge 50% of the free radicals. The higher the free radical scavenging activity increases as the IC₅₀ value decreases. Antioxidants are classified as very potent if the IC₅₀ value is less than 50 ppm, potent if the IC₅₀ value is between 50 and 100 ppm, moderate if the IC₅₀ value is between 100 and 150 ppm, and

weak if the IC₅₀ value is between 150 and 200 ppm (Mokoginta *et al.*, 2020).

CONCLUSION

This study concluded that Dayak onion extract (*Eleutherine palmifolia*) can maintain the sperm quality of mice (*Mus musculus*) induced with monosodium glutamate (MSG) at an optimal dose of 120 mg/kg BW.

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