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Original article

Ethanolic extract of Dayak onion (*Eleutherine palmifolia*) prevented sperm membran damage in mice exposed to monosodium glutamate

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ABSTRACT

Monosodium glutamate (MSG) could cause increased production of reactive oxygen species (ROS), causing oxidative stress in the testicles, which adversely affected sperm quality. Dayak onion extract which is known for its high antioxidant content, could help alleviate oxidative stress caused by MSG. This research examined the effect of Dayak onion (*Eleutherine palmifolia*) on sperm abnormalities and plasma membrane integrity in mice (*Mus musculus*) exposed to MSG. Twenty-five male mice, 8 weeks old with a body weight approximately 20g, were divided into five groups, C– (received 0.5% Na-CMC), C+ (received 4 mg/g bw MSG), and three treatment groups (T1, T2, T3) which were given 4 mg/g bw MSG accompanied by Dayak onion extract at doses of 30, 60, and 120 mg/kg bw respectively. All treatments lasted for 52 days. Significant differences ($p < 0.05$) in sperm abnormalities and plasma membrane integrity were observed among the groups. Sperm abnormalities found were (12.3 ± 1.92), (61.1 ± 3.10), (41.6 ± 2.87), (30.4± 1.91), and (18.2 ± 2.10) % respectively for C–, C+, T1, T2 and T3. Meanwhile, sperm plasma membrane integrity found were (33.0 ± 3.24), (69.3 ± 2.32), (41.8 ± 2.42), (55.4 ± 3.11), and (64.2 ± 1.27) % respectively for C–, C+, T1, T2 and T3. These results indicate that Dayak onion extract could help reduce sperm abnormalities and maintain plasma membrane integrity in mice exposed to MSG.

Keywords: Dayak onion, mice, MSG, reproductive health, sperm quality

INTRODUCTION

The free market had played a crucial role in promoting globalization in the food sector, and had significantly influenced people's lifestyles and food consumption habits over time. These shifts included changes in the types, sizes, shapes, and nutritional content of food products (Kearney, 2010). As a result, there had been a surge in processing technology and increased competition in food production, pushing the market to develop more attractive and flavorful food products. This had led to a significant increase in the use of flavoring ingredients (Niaz *et al*., 2018). One prominent additive was monosodium glutamate (MSG), discovered by

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Japanese chemist Ju Miao Ikeda (Wang *et al*., 2021). MSG mainly consisted of the L-glutamate sodium salt, which enhanced the savory taste by stimulating human taste receptors (Thuy *et al*., 2020). Prolonged MSG consumption might result in sperm degeneration, alterations in sperm count, and various abnormalities (Anzila *et al*., 2019). MSG had been found to negatively affect gonadotropin-releasing hormone (GnRH), disrupt the neuroendocrine system, and lower levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, thereby impairing testicular spermatogenesis and increasing cell apoptosis (Tolulope *et al*., 2024). The harmful effects of MSG on sperm were related to excessive production of reactive oxygen species (ROS), which could degrade sperm quality and elevate levels of testicular malondialdehyde (MDA) (Abdou *et al*., 2020). The metabolism of MSG led to oxidative stress due to the overproduction of ROS (Okwudiri *et al*., 2012). When oxidative stress occurred, antioxidants struggled to effectively managed free radicals, resulting in increased ROS production and diminished levels of natural antioxidants (Martemucci *et al*., 2022). This imbalance disrupted the spermatogenesis cycle, leading to higher sperm abnormalities and reduced integrity of sperm plasma membranes (Agarwal *et al*., 2020).

Indonesia has rich biodiversity, home to many medicinal plants, including Dayak onions (*Eleutherine palmifolia*). Dayak onions contained high levels of antioxidants which could help protect against organ damage caused by free radicals by inhibiting or slowing down oxidation (Ilmiah *et al*., 2023). Onions contained various chemical compounds including tannins, polyphenols, flavonoids, quinones, glycosides, stearic acid, gallic acid, eleutherinone, eleutherol, eleutherine, and isoeleutherine (Supomo *et al*., 2019). A study demonstrated that administering Dayak onion extract to rats exposed to cigarette smoke caused an improved rate of sperm abnormalities and a higher sperm counts (Martaningtyas *et al*., 2015). These positive results were due to the strong antioxidant properties of the flavonoid and phenolic compounds found in Dayak onions.

MATERIALS AND METHODS

This study employed an experimental design using 25 male mice divided into five groups based on treatment. The negative control group (C–) was only given 0.5% Na-CMC orally, while the positive control group (C+) received MSG at a dose of 4 mg/g body weight (bw) orally. Treatment groups T1, T2, and T3 were given Dayak onion extract at doses of 30, 60, and 120 mg/kg bw, respectively, followed by the same dose of MSG one hour later. This dosing scheme referred to earlier studies on Dayak onion extract (Jayanti and Raudah, 2021) and MSG (Pebrianti, 2013).

The entire treatment period lasted 52 days, then the mice were euthanized using atlanto-occipital dislocation. Sperm samples were collected by slicing the cauda epididymis and mixing them with 0.5 mL of physiological saline. Sperm were then examined microscopically at a magnification of 400x to assess sperm abnormalities and sperm plasma membrane integrity. The Faculty of Veterinary Medicine Animal Ethics Committee at Universitas Airlangga approved the experiment. All protocols adhered to the guidelines of the Ethics Committee regarding animal care, ensuring that the procedures did not cause discomfort or pain to the animals (certificate registration number: 1.KEH.081.07.2022).

Extraction of Dayak onion

Thin slices of Dayak onion bulbs were first dried and then ground into a fine powder. This powder was macerated in a 96% ethanol solution while stirring, ensuring that the ethanol level was maintained 1 cm above the surface of the powder. The mixture was then filtered through flannel cloth to remove the residue, then macerated again until the filtrate became clear. Extraction was carried out over three consecutive 24-hour periods, with the addition of new solvent each day. The resulting filtrate was evaporated using a rotary evaporator at a

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temperature of 40°C and a speed of 40 rpm until all the liquid was evaporated. The filtrate was further evaporated over a water bath, and the final extract was weighed (Martaningtyas *et al*., 2015).

Sperm abnormality examination

To assess sperm abnormalities, a drop of sperm suspension was placed on a glass slide, followed by the addition of one drop of eosin nigrosin. The combination was mixed thoroughly and dried briefly over a Bunsen burner for 5-10 seconds. The prepared slides were then examined under a microscope at a magnification of 400x (Abd-Elrazek *et al*., 2020).

Sperm plasma membrane integrity examination

To evaluate the integrity of the spermatozoa plasma membrane, a mixture was prepared in a 1:9 ratio of 0.1 mL of sperm suspension to 0.9 mL of hypoosmotic solution (composed of 0.735 g of sodium citrate and 1.351 g of fructose). This mixture was then incubated at 37°C for 30 minutes. Following the incubation, it was transferred to a glass slide, covered with a cover slip, and examined under a microscope at a magnification of 400x (Fadhilah *et al*., 2023).

Data analysis

Data were analyzed using analysis of variance, followed by Duncan's test to show significant differences between groups.

RESULTS

This study showed significant differences ($p < 0.05$) in sperm abnormalities and plasma membrane integrity between the C+ group and the C– group. Apart from that, it was also seen that there were significant differences ($p < 0.05$) in sperm abnormalities and plasma membrane integrity between the control group and each treatment group (Table 1). With increasing doses of Dayak onions, spermatozoa abnormalities and plasma membrane integrity became close to those of the normal group (C–). In contrast, the C+ group showed the highest level of abnormalities and the lowest spermatozoa plasma membrane integrity. These results supported the theory of the mechanism of damage caused by high levels of MSG, which increased the production of free radicals in the body, and caused oxidative stress which significantly reduced spermatozoa quality (Kayode *et al*., 2020).

Table 1 Sperm abnormalities and spermatozoa plasma membrane integrity of mice (*Mus musculus*) exposed to MSG

	spermatozoa abnormality (%)	spermatozoa plasma membrane integrity (%)
C–	12.3 ± 1.92 ^{e}	69.3 ± 2.32 ^{e}
C+	61.1 ± 3.10 ^{a}	33.0 ± 3.24 ^{a}
T1	41.6 ± 2.87 ^{b}	41.8 ± 2.42 ^{b}
T2	30.4 ± 1.91 ^{c}	55.4 ± 3.11 ^{c}
T3	18.2 ± 2.10 ^{d}	64.2 ± 1.27 ^{d}

Different superscripts (a,b,c,d,e) in the same column indicate significant differences ($p < 0.05$).

Table 1 illustrated the results of the Duncan test, which revealed that the C+ group exhibited the highest rate of spermatozoa abnormalities and the lowest integrity of the plasma membrane, showing significant differences compared to all treatment groups (C–, T1, T2, and T3). This indicates that the administration of MSG adversely impacted spermatozoa quality, leading to an increased number of abnormalities and diminished plasma membrane integrity. These findings were consistent with research showed that MSG adversely affected male reproductive structure and function in rats (Kayode *et al*., 2020). Such effects resulted in disrupted spermatogenesis from oxidative damage, histological changes, and an imbalance in gonadotropins, ultimately resulting in decreased spermatozoa quality. Furthermore, increasing the dose of Dayak onion extract (30, 60, and 120 mg/kg bw) resulted in a decrease in spermatozoa abnormalities and an increase in the number of

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spermatozoa with intact plasma membranes, resembling the normal levels (C–). This suggests that Dayak onion extract could mitigate the harmful effects of MSG.

Among the treatment groups, T3, which received a preventive dose of Dayak onion extract of 120 mg/kg bw, was most effective in reducing spermatozoa abnormalities and maintaining plasma membrane integrity. Evaluation of spermatozoa abnormalities in mice involved analysis of defects in important structural components of spermatozoa morphology, specifically the head, neck, tail, or overall structure. This assessment aims to identify abnormalities such as broken tails, heads lacking tails, and tails that showed deformities such as bends or coils (Figure 1).

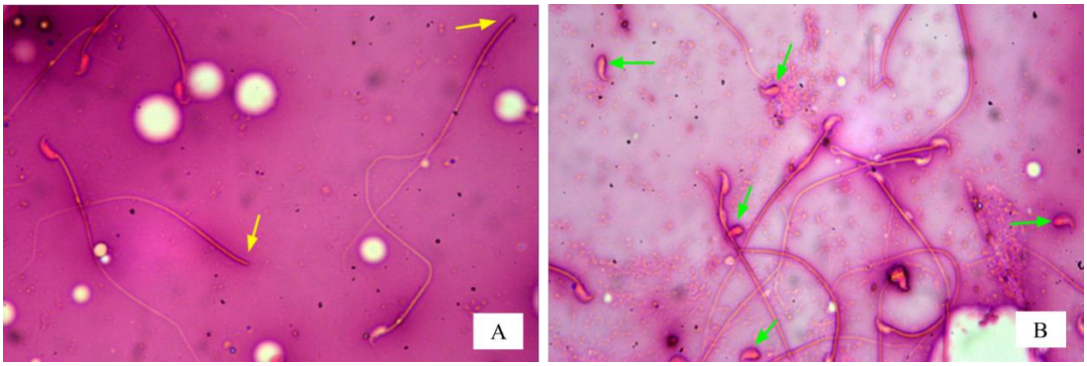

Figure 1 Mice spermatozoa abnormalities under a microscope with 400x magnification; A: headless spermatozoa tail (yellow arrows); B: spermatozoa head without tail (green arrows).

After incubation in a hypoosmotic solution, spermatozoa with intact plasma membranes would show specific morphological changes, including an increase in size at the tip of the tail, a curved tail, a shorter and thicker tail, and swelling of part or all of the curve that forms by the spermatozoa tail (Figure 2).

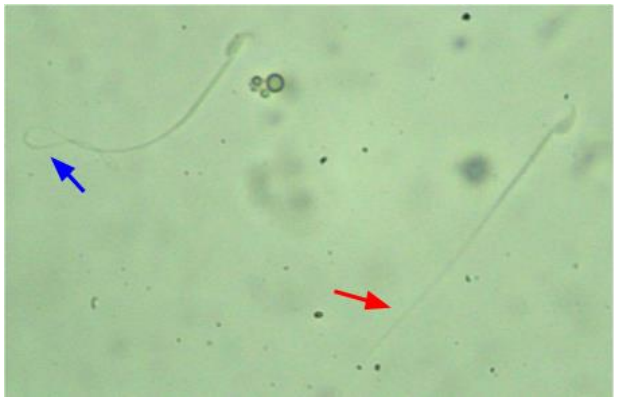

Figure 2 Examination of the integrity of the mice spermatozoa plasma membrane under a microscope with 400x magnification; a curved spermatozoa tail indicates an intact plasma membrane (blue arrow), while a straight spermatozoa tail indicates a damaged plasma membrane (red arrow).

DISCUSSION

Monosodium glutamate is known for its toxic properties, particularly its potential to increase the production of free radicals and ROS (Hamza and Al-Harbi, 2014). The first signs of spermatozoa damage appeared when the integrity of the plasma membrane was compromised (Ahmad *et al.*, 2015). Sperm membranes were highly susceptible to ROS due to their high levels of polyunsaturated fatty acids (PUFAs), which contained multiple double bonds in their hydrocarbon chains (Ogbuewu *et al.*, 2010). Lipid peroxidation began when free radicals attacked these fatty acids, resulting in the loss of hydrogen atoms from their side chains (Panasenko *et al.*, 2024). Fatty acids with more double bonds were more susceptible to hydrogen loss, thereby facilitating the peroxidation process (Ratcliffe *et al.*, 2020). When ROS interact with unsaturated fatty acids in spermatozoa membranes, peroxidation occurred, leading to

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reduced membrane enzyme activity, ion channels, and overall membrane fluidity.

This disruption negatively affected essential processes required for spermatozoa production and fertilization (Nowicka-Bauer and Nixon, 2020). The permeability of membranes was vital for nutrient transport, which was crucial for spermatozoa metabolism (Zhang *et al*., 2023). When spermatozoa membrane permeability was impaired, nutrient transport became hindered, resulting in disruptions in spermatogenesis and diminished spermatozoa quality, including an increase in abnormalities (Chao *et al*., 2023). MSG exposure significantly impaired spermatozoa quality in rats, evidenced by decreases in spermatozoa concentration, motility, viability, and membrane integrity, as well as an increase in morphological anomalies (Jubaidi *et al*., 2019). In addition to harming membranes and nutrient transport, free radicals could induce morphological changes in spermatozoa by damaging DNA, proteins, and mitochondrial functions, as well as altering apoptosis (Kaltsas *et al*., 2023). They could weaken the structural integrity of DNA within spermatozoa nuclei; for instance, ROS could induce adduct formation, leading to DNA fragmentation and mutations. This genetic damage disrupted cell division and differentiation, causing the production of spermatozoa with abnormal shapes and functions (Nguyen *et al*., 2022). Furthermore, the lipid membranes of spermatozoa, rich in unsaturated fatty acids, were particularly susceptible to oxidative injury. Free radicals such as superoxide and hydroxyl radicals could initiate lipid peroxidation, resulting in harmful byproducts like MDA. Such damage might lead to the leakage of crucial cellular components and alter spermatozoa morphology, resulting in abnormal structures (Colagar *et al*., 2013).

Alongside affecting lipids and DNA, free radicals could also impair proteins and enzymes essential for spermatozoa function. Oxidation of amino acid residues, especially those containing sulfur, such as cysteine could disrupt protein structure formation and stability. These oxidized proteins, which might become nonfunctional, could further contribute to morphological abnormalities in spermatozoa (Hussain *et al*., 2023). Moreover, the activation of apoptosis pathways in spermatozoa due to free radicals could lead to programmed cell death. This activation not only reduced the viable spermatozoa population but could also result in immature or defective spermatozoa due to abnormal cellular division (Ojo *et al*., 2023). In summary, the cumulative effects of free radicals on DNA, membranes, proteins, and cellular signaling processes significantly contributed to morphological abnormalities in spermatozoa, ultimately adversely affecting male fertility.

Antioxidants played a vital role in preserving the integrity of spermatozoa plasma membranes and ensuring normal morphology by neutralizing free radicals, protecting lipids and DNA, and enhancing antioxidant enzyme activity (Kowalczyk *et al*., 2022). They effectively captured and neutralized ROS, which threatened the structural integrity of lipid membranes. By inhibiting detrimental oxidative reactions, antioxidants contributed to overall cellular health (Jomova *et al*., 2024). Additionally, antioxidants specifically protected unsaturated fatty acids in lipid membranes from peroxidation, which was crucial for maintaining the structure and fluidity of these membranes. This function was essential for the optimal performance of spermatozoa, including motility (Qamar *et al*., 2024). Beyond membrane protection, antioxidants also shield spermatozoa DNA from oxidative damage. By reducing DNA damage, they supported normal cell division, ultimately leading to the production of healthy, high-quality spermatozoa (Pini *et al*., 2020). Antioxidants further mitigate inflammation caused by excess free radicals, which could disrupt testicular function and hinder spermatozoa production. By decreasing oxidative stress and inflammatory responses, antioxidants foster an environment conducive to spermatogenesis (Walke *et al*., 2023). They also enhanced the activity of endogenous enzymes like superoxide dismutase and glutathione peroxidase, which transformed free radicals into non-reactive substances, thus strengthening

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cellular defenses against oxidative damage (Ighodaro and Akinloye, 2018). By alleviating oxidative stress, antioxidants helped maintain redox balance within cells, which was essential for proper cell cycle regulation. This balance was critical for ensuring effective development of spermatozoa and preserving normal morphology (Walke *et al*., 2023).

In this context, Dayak onion extract (*Eleutherine palmifolia*) stood out for its high content of alkaloids, glycosides, flavonoids, phenolics, steroids, and tannins, all contributing to its antioxidant effects (Supomo *et al*., 2019). Flavonoids and phenolics in Dayak onion were particularly proficient at scavenging free radicals due to their hydroxyl groups, which acted as reducing agents by donating hydrogen to free radicals (Angela and Sumbayak, 2017).
Moreover, these antioxidant compounds inhibited ROS production by interfering with the enzymatic actions of xanthine oxidase and NADPH oxidase, as well as chelating metal ions (Fe2+ and Cu2+). This prevented chain reactions that generated free radicals (Tang *et al*., 2018). Research indicated that administering an ethanol extract of Dayak onion significantly improved spermatozoa abnormalities and spermatozoa plasma membrane integrity in mice (*Mus musculus*) exposed to MSG particularly at a dose of 120 mg/kg bw/day.

CONCLUSIONS

The results of the study indicate that administering Dayak onion extract (*Eleutherine palmifolia*) could reduce spermatozoa abnormalities in mice (*Mus musculus*) that were exposed to MSG. In addition, Dayak onion extract also contributed to preserving the integrity of spermatozoa plasma membranes in these mice, with an effective dose of 120 mg/kg bw.

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AUTHOR'S CONTRIBUTIONS

Syahraini Adhiya Lathifah (SAL), Aldin Akbar Rahmatullah (AAR), Boedi Setiawan (BS), Chairul Anwar Nidom (CAN), Sri Mulyati (SM), Nove Hidajati (NH), Tri Wahyu Suprayogi (TWS)

NP1: conceived the idea, designed the mainframe of this manuscript, acquisition, analysis and interpretation of data, and manuscript drafting under the supervision of NP2 and NP3. NP3, NP4, NP5 and NP6: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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