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Protective Effect of The Extract of Dayak Onions (*Eleutherine palmifolia*) on Sertoli and Leydig Cell Necrosis in 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 number of necrotic Sertoli and Leydig cells 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- (0.5% CMC-Na), C+ (4 mg/g BW of MSG and 0.5% CMC-Na), 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 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 mice were euthanized on day 53 of the experiment. Their testicles were removed and used to prepare histological specimens with the H&E staining method. The results showed significant differences ($p < 0.05$) in the number of necrotic Sertoli and Leydig cells between the C+ group and the T1, T2, and T3 groups with gradually decreasing values. The results suggested that the administration of the extract of Dayak onions can prevent Sertoli and Leydig cell necrosis in mice induced with MSG at an optimal dose of 120 mg/kg BW.

Keyword: Dayak onion, Leydig cell necrosis, mice, MSG, Sertoli cell necrosis

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INTRODUCTION

Monosodium glutamate (MSG) is a common and increasingly used food additive (Niaz et al., 2018). Its average consumption in several countries is as follows: Taiwan (3g/day), Korea (2.3g/day), Japan (1.6g/day), India (0.4g/day), and the United States of America (0.35g/day). In Indonesia, the average consumption of MSG is 0.6g/day (Yonata & Iswara, 2016). Overconsumption of MSG can have adverse effects on the reproductive system by causing free radicals to overproduce, leading to infertility (Kayode et al., 2020).

MSG can potentially cause infertility by activating several glutamatergic receptors, including metabotropic glutamate receptors (mGluR), ionotropic GluR, and N-methyl-D-aspartate receptors (NMDAR). The activation of these receptors triggers the phospholipase C (PLC) signaling pathway by stimulating G proteins, resulting in an increase in intracellular calcium levels within cells (Jakaria et al., 2018). Elevated calcium levels can lead to reactive oxygen species (ROS) production and damage in hypothalamic neural synapses. This damage disrupts the hypothalamic signaling axis, including the anterior pituitary gland and the testes. This disruption can interfere with the production of reproductive hormones, such as follicle-stimulating hormone (FSH) and interstitial cell-stimulating hormone (ICSH). These hormones play crucial roles in regulating the reproductive system. Any interference with their production can lead to reproductive issues, including infertility (Kayode et al., 2020).

In addition, excessive production of ROS within the testicular tubules can cause damage to Sertoli and Leydig cells, resulting in oxidative stress. Oxidative stress is characterized by an increase in the levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, and a decrease in the levels of glutathione (GSH), an important endogenous antioxidant (Agarwal et al., 2018).

The damage caused by ROS can be mitigated or prevented by exogenous antioxidants. Dayak onions (*Eleutherine palmifolia*) are rich in antioxidants, such as phenolic and flavonoid compounds, that are able to donate hydrogen ions and neutralize ROS, thereby reducing their harmful effects on testicular cells (Angela & Sumbayak, 2017). By neutralizing ROS, exogenous antioxidants protect Sertoli and Leydig cells from oxidative damage and maintain normal testicular function, which is essential for reproductive health. Based on the aforementioned explanation, this study aims to determine the protective effect of Dayak onion extract on the number of necrotic Sertoli and Leydig cells in mice induced with MSG.

MATERIALS AND METHODS

This study, which was conducted from July to September 2022, received

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ethical approval from the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number 1.KEH.081.07.2022. 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 histopathology was performed 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 two large groups: the control group, consisting of negative control (C-) and positive control (C+), and the treatment group, consisting of treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3). The C- group was administered with only 0.5% CMC-Na orally, while the C+ group was administered with only MSG at a dose of 4mg/g BW orally. On the other hand, the T1, T2, and T3 groups were administered with Dayak onion extract orally at doses of 30, 60 and 120 mg/kgBW, respectively. One hour after administration, the treatment groups were induced with MSG at a dose of 4mg/g BW orally. This dose is based on research conducted on Dayak onion extract (Jayanti & Raudah, 2021) and MSG (Pebrianti, 2013). The experiment was carried out for 52 days.

At the end of the experiment, the mice were euthanized by atlanto-occipital cervical dislocation. The specimens were dissected and placed in 10% formalin solution for histopathological examination with hematoxylin. The histopathological examination was performed using a Nikon Eclipse microscope at 400x magnification to observe the number of necrotic Sertoli and Leydig cells by calculating the average number of necrotic cells in five visual fields.

Statistical Analysis

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

RESULTS AND DISCUSSION

This study found a significant increase in necrotic cells in the C+ group compared to the C- group for all observed parameters (see Table 1 and Figures 1, 2, and 3). The numbers of necrotic Sertoli and Leydig cells differed significantly among the treatment groups ($p < 0.05$). In addition, the number of necrotic cells decreased gradually from the C+ group to the T1, T2, and, T3 groups. The results of the T3 group closely resembled those of the C- group, although no significant differences were observed between them. This suggested that the administration of Dayak onion extract has been shown to be effective in preventing the necrosis of

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Sertoli and Leydig cells in mice induced with MSG.

Table 1. The average number of necrotic Sertoli and Leydig cells in each group

Treatments	Mean ± SD	
	Sertoli cell necrosis	Leydig cell necrosis
C-	$2^{e} \pm 0.14$	$3.12^{e} \pm 0.23$
C+	$7.16^{a} \pm 0.48$	$12.28^{a} \pm 0.41$
T1	$6^{b} \pm 0.49$	$10.04^{b} \pm 0.57$
T2	$4.48^{c} \pm 0.36$	$8.2^{c} \pm 0.45$
T3	$2.8^{d} \pm 0.45$	$4.16^{d} \pm 0.55$

Note: Different superscripts indicate significant differences ($p < 0.05$). The C- group received 0.5% CMC-Na orally for 52 days, while the C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. Moreover, 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. Finally, 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.

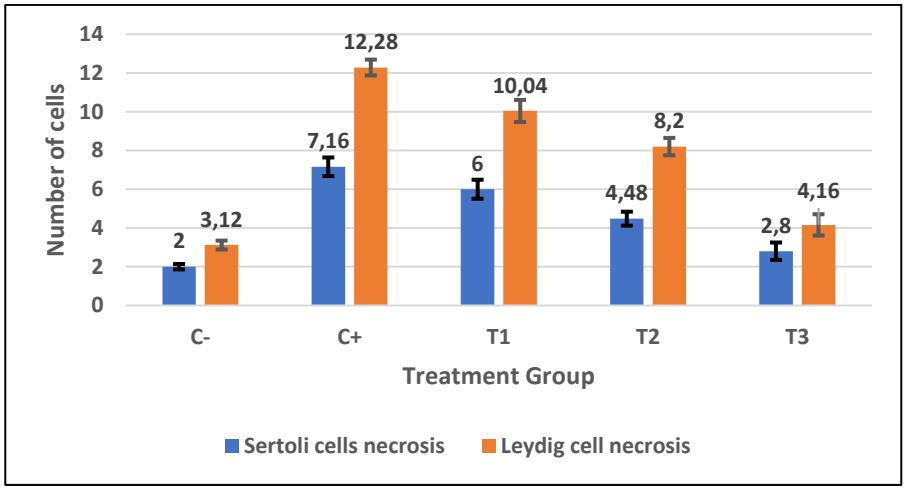

Figure 1. The number of necrotic Sertoli and Leydig cells in mice administered with Dayak onion extract and induced with MSG. The C- group received 0.5% CMC-Na orally for 52 days. The C+ group received distilled MSG at a dose of 4 mg/kg BW orally for 52 days. The T1 group received 30 mg/kg BW of Dayak onion extract and MSG at a dose of 4 mg/kg BW orally for 52 days. The T2

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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.

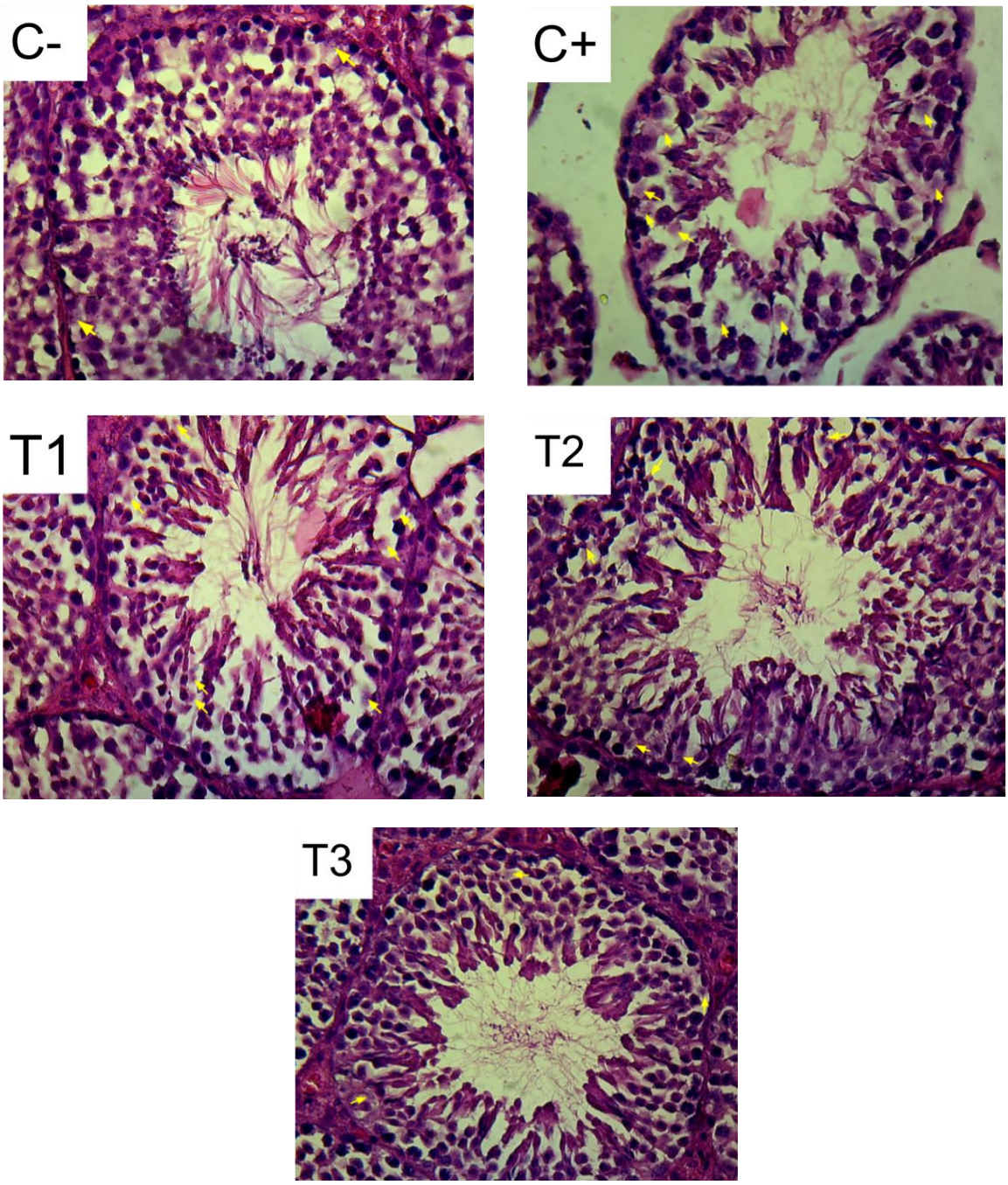

Figure 2. The testicular histopathology (HE) of mice induced with MSG and administered with Dayak Onion extract as a preventive antioxidant at a magnification of 400x. The yellow arrows indicate necrotic Sertoli cells. The C- group received 0.5%

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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.

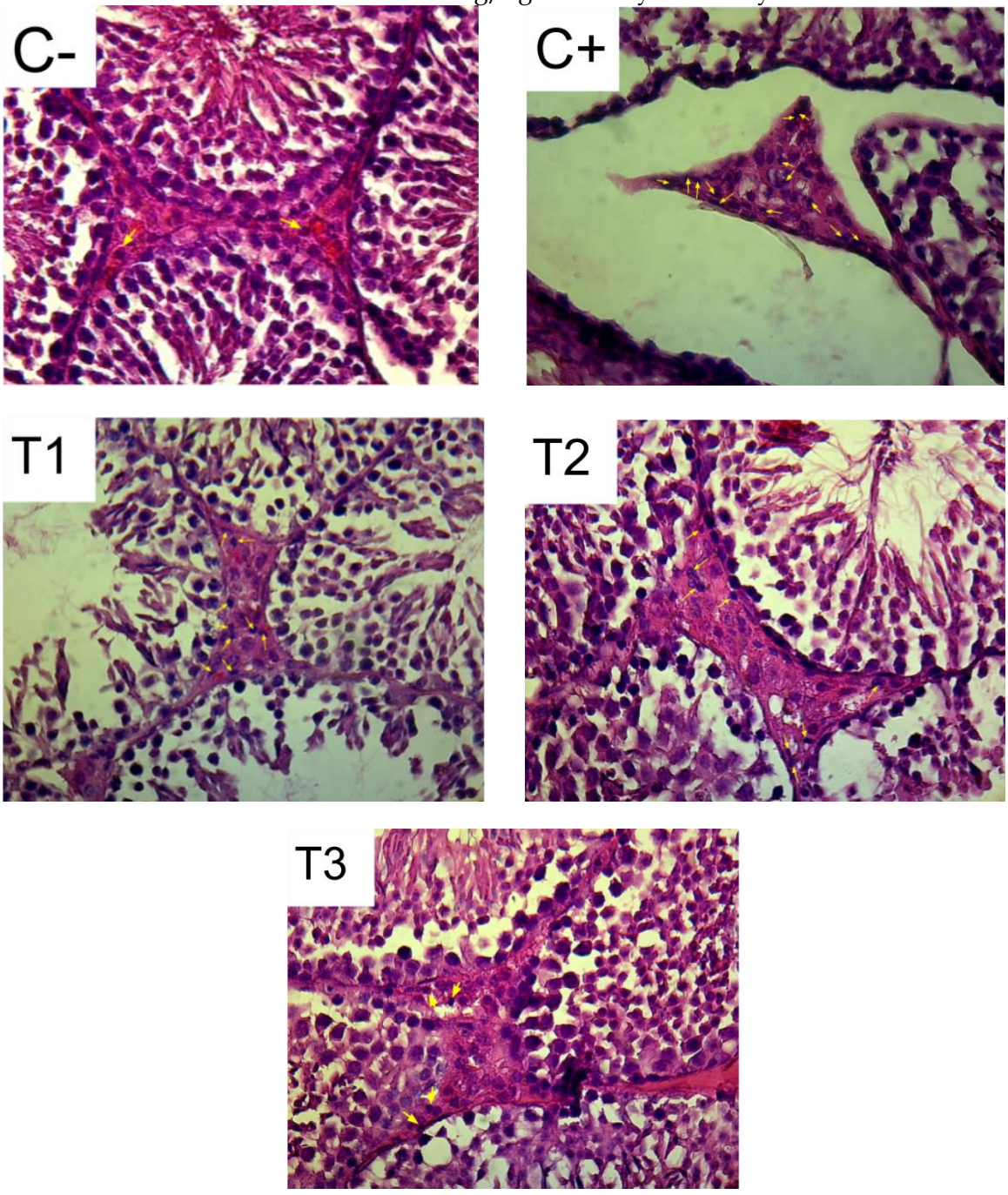

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Figure 3. The testicular histopathology (HE) of mice induced with MSG and administered with Dayak onion extract as a preventive antioxidant at a magnification of 400x. The yellow arrows indicate necrotic Leydig cells. The C- group received 0.5% CMC-Na 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.

The number of necrotic Sertoli and Leydig cells necrosis was higher in the C- group compared to all other groups. This is due to the continuous administration of MSG, which increases L-glutamate levels in the bloodstream. This in turn activates mGluR receptors and ultimately increases the binding activity of D-aspartate with NMDAR (Widayati & Hayati, 2018). During the steroidogenesis process, NMDAR is activated by the mitogen-activated protein kinases (MAPK) and cyclic adenosine monophosphate signaling pathways, which leads to the activation of the steroidogenic acute regulatory (STAR) protein complex, which converts cholesterol to testosterone through testosterone biosynthesis (Zhang et al., 2007).

Chronically elevated levels of L-glutamate in the blood can lead to increased Ca^{2+} influx at hypothalamic neural synapses, causing nerve cell death due to excessive excitation, a condition known as excitotoxicity (Jakaria et al., 2018). This can result in the ablation of hypothalamic neuron cells, which affects the hypothalamus-pituitary-testis axis as well as FSH and ICSH production (Kayode et al., 2020). Decreased ICSH levels leads to decreased stimulation of Leydig cells, resulting in a decrease in testosterone secretion. Meanwhile, a decrease in FSH disrupts the development of Sertoli cells, which affects the formation of androgen binding protein (ABP). ABP binds free testosterone in the blood to assist in the spermatogenesis (Camihort et al., 2005).

Furthermore, excessive NMDAR stimulation can lead to excessive intracellular Ca^{2+} secretion and the activation of ROS-forming enzymes, including xanthine oxidase, lipoxygenase, and NADPH oxidase. Excessive ROS production lead to oxidative stress, in which endogenous antioxidants such as GSH and superoxide dismutase are unable to keep up with ROS production (Asadi et al., 2017). This excessive activation interferes with the MAPK signaling pathway and disrupts STAR-mediated steroidogenesis (Diemer et al., 2003). ROS binds to polyunsaturated fatty acids (PUFA) and initiates lipid peroxidation, initiating a chain reaction that produces radical lipids. Oxidized lipid cell membranes produce MDA and 4-hydroxynonenal (4-

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NHE), which are toxic to tissues, especially reproductive tissues, and increase the number of necrotic Leydig cells (Luqman et al., 2022). Lipid peroxidation can cause cell damage to cells, leading to apoptosis and necrosis in spermatogenic, Sertoli, and Leydig cells (Agarwal et al., 2008). In addition, MDA and 4-NHE are significant contributors to DNA damage and mutations. The reaction between MDA and DNA can result in cross-linking of DNA proteins, leading to changes in the biochemical properties of various biomolecules within the cell. This condition can make the cell unable to divide and more susceptible to damage (Ayala et al., 2014).

The gradual decrease in the number of necrotic Sertoli and Leydig cells from the T1, T2, and T3 groups was attributed to the antioxidants present in Dayak onions. Although the results in the T3 group were not as good as those in the C- group, they demonstrated that Dayak onion extract can prevent necrosis in Sertoli and Leydig cells in mice induced with MSG. The ethanol extract of *Eleutherine palmifolia* contains flavonoids, which act as antioxidants that are highly effective in scavenging free radicals. This is due to the presence of hydroxyl groups, which act as a reducing agent and a hydrogen donor for free radicals (Angela & Sumbayak, 2017). Flavonoids also play a role in increasing the quantity and activity of endogenous antioxidants, inhibit apoptosis in germ cells, and exhibit cytoprotective properties in testicular tissue (Khaki et al., 2009).

Moreover, the ethanol extract of *Eleutherine palmifolia* contains alkaloids, glycosides, flavonoids, phenolics, steroids, and tannins, which have the potential as antioxidants (Prayitno & Mukti, 2018). Flavonoids play a crucial role in suppressing ROS by inhibiting the activity of xanthine oxidase and nicotinamide adenine phosphate (NADPH) oxidase. They also act as metal chelators (Fe^{2+} and Cu^{2+}), which prevent chain reactions that can generate free radicals (Tang et al., 2018). This study showed that the administration of Dayak onion extract as a preventive antioxidant reduced the number of necrotic Sertoli and Leydig cells.

CONCLUSION

This study concluded that the extract of Dayak onion (*Eleutherine palmifolia*) can prevent Sertoli and Leydig cell necrosis in mice (*Mus musculus*) induced with monosodium glutamate (MSG) at an optimal dose of 120 mg/kg BW.

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