RESEARCH NOTE

PREVENTIVE EFFECTS OF *Apis dorsata* HONEY ON THE SPERMATOGENIC CELLS AND SERTOLI CELLS COUNT OF MICE *(Mus Musculus)* EXPOSED TO MONOSODIUM GLUTAMATE

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ABSTRACT

The purpose of this study was to determine the preventive effect of *Apis dorsata* honey (AH) on the spermatogenic and Sertoli cells count of mice (*Mus musculus*) exposed to monosodium glutamate (MSG). This study used 25 male BALB/c mice which were divided into five treatments. The negative control (C-) received aquadest, the positive control (C+) received MSG 4 mg/gBW, T1 received AH 2.7 mg/gBW + MSG 4 mg/gBW, T2 received AH 5.4 mg/gBW + MSG 4 mg/gBW, and T3 received AH 8.1 mg/gBW and MSG 4 mg/gBW. All groups were treated for 52 days. The testes were then prepared as histopathology slides and examined under a microscope. The results of this study showed that there was a significant difference in spermatogenic cells and Sertoli cells between C-, C+, T1, and T2 ($p<0.05$) and showed no significant difference ($p>0.05$) in spermatid and Sertoli cells count between C- (90.28 ± 1.361 and 13.60 ± 0.374) and T3 treatment group (88.04 ± 1.212 and 13.04 ± 0.434). The best preventive dosage of AH was the T3 with 8.1 mg/gBW. It can be concluded that administration of AH can maintain spermatogenic and Sertoli cells count in mice exposed to MSG.

Keywords: *reproductive health, Apis dorsata honey, MSG, spermatogenic cells, sertoli cells, Mus musculus.*

Philipp. J. Vet Med., 59(2): 193-198

INTRODUCTION

Monosodium glutamate is one of the foods additive that serves an umami taste (Al-Harbi *et al.,* 2014). Monosodium glutamate is a sodium salt with the main constituent of glutamic acid and it is a pure crystalline powder (Al-Harbi *et al.,* 2014). The American Food and Drug Administration (FDA) 1995 stated that MSG is a flavor enhancer in food that is safe for consumption with a safety level of 6 mg/kgBW (Sukmaningsih *et al.,* 2011). Excessive consumption of MSG in the long term in humans and experimental animals can cause
serious problems, such as a male reproduction disorder, that ends in infertility (Das and Ghosh, 2010).

One of the toxic effects of MSG is related to by causing infertility and spermatogenesis disorders (Elfiana, 2012). Spermatogenesis disorders can occur through pre-testicular, testicular, and post-testicular mechanisms (Sukmaningsih *et al.,* 2011). Pre-testicular mechanism inhibits spermatogenesis through regulation of the hypothalamus-anterior pituitary-gonads axis, decreasing the secretion of Interstitial Cell Stimulating Hormone (ICSH) and Follicle Stimulating Hormone (FSH) thus, reducing spermatogenesis efficiency (Sukmaningsih, 2011; Singh, 2016). On the other hand, MSG increases the production of Reactive Oxygen Species (ROS) by the oxidation of

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spermatogenic membrane cells (Okwudiri *et al.,* 2012; Hamza and Al-Harbi, 2014). The sudden increase of ROS and lipid peroxidation causes cell membrane permeability disruption and oxidative stress thus, bringing about cellular function loss and spermatogenic cell damage (Alalwani, 2014; Mukti *et al.,* 2020).

Antioxidants play an important role in inhibiting oxidative stress by acting as a first defense line to neutralize ROS thus, reducing the spermatogenic cell's damage (Hartati *et al.,* 2018). *Apis dorsata* honey (AH) is a honey produced by *Apis dorsata* bees. Mohamed *et al.,* (2010) stated that *Apis dorsata* honey has higher antioxidant levels and activities than *Apis cerana* honey and *Apis mellifera* honey. It is because AH is multi-floral honey that comes from many flowers and nectars different from *Apis cerana* and *Apis mellifera* that onlyorginates from one kind of flower (Pribadi and Wiratmoko, 2019).

The main antioxidants of AH is phenolics and flavonoids (Mokosuli *et al.,* 2019). Phenolics and flavonoids work by breaking free radical chain reactions on spermatogenic cell and sertoli cell membranes thus, preventing cell damage and maintaining spermatogenic and sertoli cell count of mice (*Mus musculus)* exposed to MSG (Hamza and Al-Harbi, 2014; Dong *et al.,* 2019). Due to its rich content of antioxidants including phenolic and flavonoids, AH has ameliorative properties and can repair the damage caused by oxidative stress (Sahlan *et al.,* 2018). On the other hand, the anthraquinone bioactive compound in AH is a powerful ROS scavenger and prevents it from binding into polyunsaturated fatty acid (PUFA) and thus, inhibiting auto-oxidation (Luqman *et al.*, 2021). From these backgrounds, this research aims to prove the preventive effects of *Apis dorsata* honey on the spermatogenic cells and Sertoli cells count of mice (*Mus Musculus*) exposed to monosodium glutamate.

MATERIALS AND METHODS

This research received ethical clearance number 1. KE.075.08.2020 released by the Animal Care and Use Committee, Faculty of Veterinary Medicine Universitas Airlangga. It is an experimental laboratory study and was carried out for 52 days at the Experimental Animal Laboratory and Embryology Laboratory, Faculty Veterinary Medicine, Universitas Airlangga. This study used 25 male BALB/c mice (*Mus musculus)* with body weights of around 30-35 grams and are three months old (Pusat Veterinaria Farma, Surabaya Indonesia), *Apis dorsata* honey from Tesso Nilo® for guaranteed purity, Purified MSG (Merck®), Buffered Neutral Formalin (BNF) 10%, alcohol (80%, 95%, and 96%), xylol, paraffin, and *Haematoxylin Eosin* (HE), animal cage, minor surgical instruments, and Nikon® Eclipse E100 microscope complete with Optilab professional series.

Experimental animals were randomly divided into five treatments: control negative (C-) was given only aquadest, control positive (C+) was given MSG 4 mg/gBW, the T1 treatment group was given AH 2.7 mg/30gBW and MSG 4 mg/gBW an hour later, T2 treatment group was given AH 5.4 mg/30gBW and MSG 4 mg/gBW an hour later, and the T3 treatment group was given honey 8.1 mg/gBW and MSG 4 mg/gBW an hour later. All treatments were orally given for 52 days (Widayati *et al*, 2018; Rista and Yuziani, 2014).

After 52 days of treatment, all mice were anesthetized using 0.1 cc of ketamine by intramuscular injection. Specimens were collected by cutting the aorta to isolate the testes by incising the abdomen, pushing the scrotal sac to find the testes and cutting it then placing it into a BNF 10% solution for fixation then processed further using histopathological preparations of *Haematoxylin Eosin* (HE) staining. Histopathological slides were examined using the Nikon® Eclipse E100 with 400x magnification to calculate the average number of spermatogenic cells and Sertoli cells in five fields of view (FoV) of seminiferous tubules in each study group. Data analysis using ANOVA and Duncan as post-hoc tests.

RESULT AND DISCUSSION

The results of statistical analysis using ANOVA and Duncan as a post-hoc test showed a significant difference ($p<0.05$) in spermatogenic cells and Sertoli cells with C+, C-, T1, and T2. However, there was no significant difference ($p>0.05$) in spermatid cells and Sertoli cell count between C- and T3. Figure 1 (C-) shows spermatid and Sertoli cells that are surrounded by peritubular myoid cells. The administration of AH can maintain/increase spermatogenic cells and Sertoli cells count along with the increased dosage of AH due to the ameliorative nature of AH (Sahlan *et al.,*, 2018) (Table 1, Figure 1).

In Table 1 and Figure 1, it can be seen that spermatogonia cells and primary spermatocytes, in the control negative (C-), which was given only aquadest, showed the highest cell count (43.72 ± 1.154 cells and 55.00 ± 1.296 cells) statistically different ($p<0.05$) compared to C+, T1, T2, and T3 groups. The treatment groups T1, T2, and T3 showed a significant difference ($p<0.05$) in spermatogonia cells count and primary

Table 1. The average and deviation standard on spermatogenic cells and Sertoli cells count of mice (*Mus musculus*) exposed to monosodium glutamate (MSG).

Group	Spermatogonia	Primary Spermatocyte	Spermatid	Sertoli
C-	$43.72^{e} \pm 1.154$	$55.00^{e} \pm 1.296$	$90.28^{d} \pm 1.361$	$13.60^{d} \pm 0.374$
C+	$30.92^{a} \pm 0.460$	$40.52^{a} \pm 1.712$	$78,08^{a} \pm 3.964$	$8.84^{a} \pm 0.297$
T1	$33.08^{b} \pm 0.540$	$44.72^{b} \pm 0.901$	$81.76^{b} \pm 1.480$	$9.96^{b} \pm 0.713$
T2	$35.52^{c} \pm 0.593$	$48.20^{c} \pm 0.663$	$84.92^{c} \pm 0.782$	$11.64^{c} \pm 0.297$
T3	$39.44^{d} \pm 0.932$	$51.48^{d} \pm 1.425$	$88.04^{d} \pm 1.212$	$13.04^{d} \pm 0.434$

Different superscript (^{a,b,c,d,e}) in one column showed significant differences ($p<0.05$).

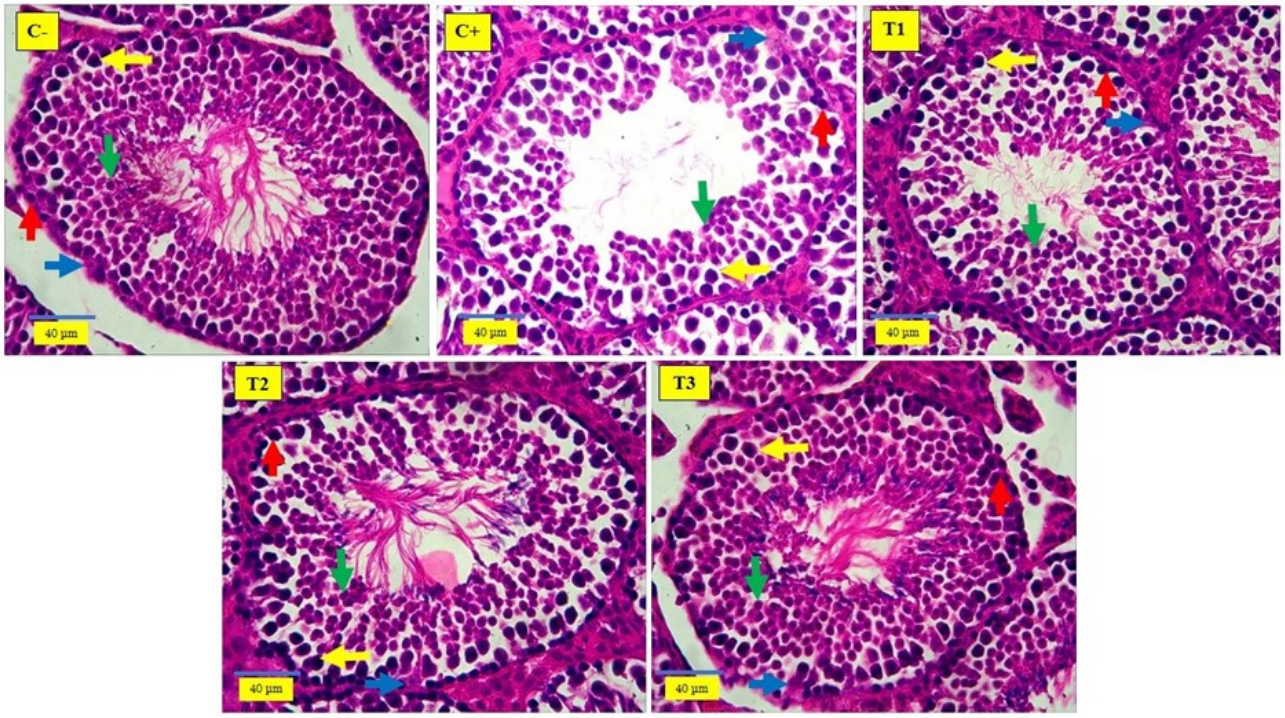

Figure 1. Histopathological slides of testes with HE staining (scale bar = 40 µ m). Normal histological structure of mice's testis (C-): Seminiferous tubules are made up of spermatogenic and Sertoli cells that are surrounded by peritubularmyoid cells. There was a drastic decrease in the number of spermatogenic and Sertoli cells when MSG (C+) was given. There was an increase in the number of spermatogenic and Sertoli cells in the group given MSG and AH along with the increase in dose (T1-T3). There was no significant difference in spermatid and Sertoli cell count between C- and T3 (Red arrows = spermatogonia, yellow arrows = spermatocyte, green arrows = spermatocyte, blue arrows= Sertoli).

spermatocyte count (Figure 1). The spermatid cells count in the control negative (C-), which was given aquadest, showed the highest number of 90.28±1.361 and revealed that a significant difference ($p<0.05$) compared to the C+, T1, and T2 groups, but showed no significant difference ($p>0.05$) compared to T3 group that was given AH 8,1 mg/gBW with 88.04 ± 1.212 cells (Figure 1). The Sertoli cells in the control negative (C-), which was given only aquadest, showed the highest cells count (13.60 ± 0.374 cells) and showed a significant difference ($p<0.05$) with C+, T1, and T2 groups, but showed no significant difference ($p>0.05$) with T3 treatment group that was given AH 8.1 mg/gBW of 13.04 ± 0.434 cells (Figure 1). On average, treatment groups that were given AH (T1 2.7mg/gBW, T2 5.4mg/gBW, and T3 8.1 mg/gBW) before the administration of MSG 4 mg/gBW showed a significant difference ($p<0.05$) in spermatogenic cells and Sertoli cells compared with control positive (C+) which was given MSG 4 mg/gBW.

In this study, the administration of MSG 4 mg/gBW for 52 days caused a decrease in spermatogenic cells and Sertoli cell count. Exposure to free radical sources such as a high dosage of MSG causes spermatogenic cell damage in three ways: cell membrane lipid peroxidation, cell mutation due to DNA damage, and impaired cellular function due to crosslinking protein (Sayuti and Yenrine, 2015).

Lipid peroxidation causes cell damage through cell membrane damage and reactive aldehydes. The reaction between free radicals and spermatogenic cell membrane components (unsaturated lipids) causes ion transport disruption and cell membrane permeability damage, as well as cell leakage due to loss of cell membrane integrity (Yin *et al.,* 2012). Lipid peroxidation causes an increase in lipid peroxide formation including malondialdehyde (MDA). Malondialdehyde (MDA) is a biomarker of cellular oxidative stress (Singh *et al.,* 2014).

Malondialdehyde is an important contributor to DNA damage and DNA mutation. The reaction of MDA and DNA can cause DNA crosslink protein. This situation caused a change in the cell's biochemical properties thus, cells are unable to divide and are damaged (Yin *et al.,* 2012; Ayala *et al.,* 2014). Malondialdehyde can also induce an intrinsic pathway of apoptosis in mitochondria. The reaction of MDA and mitochondrial membrane protein can disrupt the electron transport chains, induce ROS mitochondria, and Ca^{2+} accumulation (Moazamian *et al.,* 2015; Luqman *et al.,* 2019). This situation caused a change in mitochondrial permeability and stimulated mitochondria to release cytochrome C, AIF (Apoptosis Induced Factor), and Smac/Diablo into the cytosol. Release of cytochrome C activated Apoptosis Protease Factor-I (APAF-I) followed by stimulation of procaspase 9 to activate caspase 9 (Samik and Safitri, 2017; Luqman *et al.,* 2019). Caspase 9 binds with procaspase 3 to activate caspase 3. Increased caspase 3 on spermatogenic cells and Sertoli cells indicates DNA damage and excessive apoptosis (Akhigbe and Ajayi, 2020). This cascade reaction is proven in this research by a significant decrease in spermatogenic cells and Sertoli cell count in the C+ group that were given with MSG 4mg/gBW to induce toxicity and increase the lipid peroxidation reaction by ROS and produces MDA that can cause DNA damage.

Excessive exposure to MSG also causes oxidative stress on Sertoli cells and decreases the number of Sertoli cells. Spermatogenesis depends on the normal function of Sertoli cells as it provides nutritional factors for the development of spermatogonia into spermatozoa, such as transferrin and androgen binding protein (ABP), which have a function in the remodeling and movement of germ cells towards the lumen of seminiferous tubules (Wiryawan and Wahyuniari, 2010; Singh, 2016, Suseno *et al.,* 2020). This study showed that administration of AH to the T1, T2, and T3 groups presented positive results by increasing the spermatogenic cells and Sertoli cells count compared with control positive (C+) that was given MSG 4 mg/gBW. AH is one of the natural products that contains a high level of antioxidants. The main antioxidants are phenolics and flavonoids (Mokosuli *et al.,* 2019). Antioxidants have a protective effect by preventing oxidative damage and cell damage from lipid peroxidation chain reactions in the cellular membrane by interfering with the initiation and propagation of free radicals (Ayala *et al.,* 2014).

Flavonoids work as free radical scavengers that donate hydrogen atoms to free radicals and stabilize ROS (Hamza and Al-Harbi, 2014; Dong *et al.,* 2019). Phenolics act as inhibitors of free radical oxidation through the mechanism of scavenging lipid peroxy-radicals (ROO^{-}) with their hydroxyl (H^{+}) groups (Budiman *et al.,* 2015). AH as a preventive exposure to MSG in this study contains antioxidants that are used against the toxic effects of MSG. The administration of AH aims to increase endogenous antioxidants and reduce ROS production (Dong *et al.,* 2019). The balance conditions of oxidant and antioxidant prevent oxidative stress and preserve spermatogenesisso the spermatogenic cells and Sertoli cells count close to the normal number (Dong *et al.,* 2019).

This is evidenced by control positive (C+) given MSG 4mg/gBW displayed the smallest cell count and treatment groups (T1, T2, and T3) given AH with varying doses showed greater cell counts of spermatogenic cells and Sertoli cells.

In conclusion, the administration of AH can maintain several spermatogenic cells and Sertoli cells of male mice (*Mus musculus)* exposed to monosodium glutamate, with the optimal dose of AH in the T3 group being 8.1 mg/gBW. The results of this study are expected to be used as a basis for informing people about the dangers of using MSG and the potential of Apis dorsata bee honey in preventing and improving male reproduction caused by MSG. Exploring the quality of spermatozoa and the ability to fertilize eggs can be carried out as further research as a step to obtain clinical data.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of the Faculy of Veterinary Medicine Universitas Airlangga, Surabaya, East Java, Indonesia.

AUTHOR'S CONTRIBUTION

Research concept and design JSA, c ollection and/or assembly of data EML, data analysis and interpretation: SSW, ESAM, APH, and VFH, writing the article JSA, and W, Critical revision of the article EML, final approval of the article JSA and EML.

STATEMENT ON COMPETING INTEREST

The authors have no competing interest to declare.

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