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Research Article

Protective Effects of *Apis dorsata* Honey on Leydig Cell Count and Seminiferous Tubule Diameter of Mice Exposed to Monosodium Glutamate

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ABSTRACT

The preventive effect of *Apis dorsata* honey (ADh) on the number of Leydig cells and seminiferous tubule diameter of mice (*Mus musculus*) exposed to monosodium glutamate (MSG) was investigated. 25 mice were divided into 5 groups. In C- group only distilled water was given. The C+ group was given 4mg/gBW of MSG while groups T1, T2 and T3 were given *Apis dorsata* forest honey with dosage of 53.82 mg/20g, 107.64 mg/20g and 161.46 g/20g respectively in addition to 4mg/gBW MSG. All treatments were given *per oral* for 52 days. Leydig cell population in the control group was 44±1.64. These values were significantly reduced in the animals exposed to MSG. Significant reversal of the effect of MSG was observed in the animals treated with *Apis dorsata* honey (28.56±1.47, 38.28±1.37 and 42.68±1.39 for T1, T2 and T3 respectively). Seminiferous tubular diameter was also significantly reduced by MSG (158.53±5.21 µm) when compared with the control (199.13±4.78 µm; $p<0.05$) while *Apis dorsata* honey administration attenuated the toxic effects of MSG. The results showed no significant difference ($p>0.05$) between the T3 and C- groups on the Leydig cell and seminiferous tubules diameter variable. It can be concluded that administration of *Apis dorsata* honey can maintain the number of Leydig cells and the diameter of the Seminiferous Tubules in mice exposed to MSG.

Keywords: *Apis dorsata honey, Leydig cells, monosodium glutamate, reproductive health, seminiferous tubules*.

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INTRODUCTION

Globalization and the high development of people mobility have made such drastic changes in human life, especially in the field of food consumption. To compensate faster lifestyle, fast food is a practical and easily available option to meet human primary needs (Sudargo *et al*, 2018).Fast food is required to have high palatability, additives are often added to enhance taste, one of them is monosodium glutamate (MSG) (Andarwulan *et al*, 2015).

MSG consumption in the community tends to increase every year.There has been a very rapid increase in consumption from 100,568 tons to 122,966 tons in the period 1998 - 2004 with an average daily consumption of around 1.53 g / capita/day to 9,62 g /capita/day in 2011 according to Nuri *et al*. (2011).Chronic consumption of MSG affects the reproductive system which ends in infertility (Kayode *et al*, 2020).

The degenerative effects of MSG on the male reproductive system begins with the increase in extracellular glutamic acid levels in the post synapses of brain nerve cells. The increase in these amino acids will increase the expression of Metabotropic Glutamic Receptor (mGluR), Ionotropic Glutamic Receptor (iGluR) and N-methyl-D-aspartate Receptor (NMDAR).In this receptor overexpression, there is an activation of the PLC pathway through G protein activity which will produce IP3 and increase intracellular Ca2+ concentrations due to the release of Ca2+ in the endoplasmic reticulum (Jakaria *et al*, 2018). Excess Ca2+ in cells leads to excessive production of Reactive Oxygen Species (ROS)known as excitotoxicity (Kritis *et al*, 2015).

Excitotoxicity causes neuron cell necrosis in the hypothalamic arcuate nucleus will interfere with the function of the hypothalamus-pituitary-gonad axis to release Follicle Stimulating Hormone (FSH) and Interstitial Cell Stimulating Hormone (ICSH) hormones (Kamalah, 2019). Disruption in

the FSH hormone will inhibit the spermatogenesis process and impaired ICSH release will affect the production of testosterone by Leydig cells.Damage to spermatogenic cells is also caused by excessive production of ROS in the tubules will cause oxidative stress and membrane lipid peroxidation which is marked by increasing levels of Malondialdehyde (MDA) and decreasing levels of Glutathione (GSH) (Liwikasari *et al*, 2018; Anbarkeh *et al*, 2019).

The activity of excessive ROS can be neutralized using antioxidants. Honey produced by *Apis dorsata* bees is multifloral honey that comes from many flowers and has a high antioxidant content compared to monofloral honey produced by *Apis melifera* bees (Saputri, 2017). The high antioxidant content has a high potential to overcome ROS, so this study aimed to determine the preventive effect of *Apis dorsata* honey with the parameters Leydig cells count and the seminiferous tubules diameter of mice (*Mus musculus*) given MSG.

MATERIALS AND METHODS

Ethical clearance: This research received ethical clearance number: 1.KE.075.08.2020 released by Animal Care and Use Committee, Faculty of Veterinary Medicine Universitas Airlangga.

Experimental Animals: This study was a laboratory experimental study using a completely randomized design (CRD) for 25 male mice divided into five treatments using preventive doses and for each treatment, there were five replications. The treatment was carried out for fifty-two days following one and a half cycles of spermatogenesis from mice. Male mice (Mus musculus) strain BALB /c obtained from the Pusat Veteriner Farma, Jalan Ahmad Yani No.68, Surabaya. The feed used during the maintenance of mice is the standard Hi-Pro-Vite Medicated 593 feed. The material used in this study was *Apis dorsata* honey with Tesso Nilo trademark and L-Glutamic acid from Mercks trademark.

Study design: The treatment group in this study consist of 5 groups, including: control negative (C) with placebo, control positive (C+) which had MSG 4mg/gBW, treatment 1 (T1) which was given with *Apis dorsata* honey 53.82 mg/20gBW and 1 hour later given MSG 4mg/gBW, treatment 2 (T2)which was given with *Apis dorsata* honey 107.64 mg/20gBW and 1 hour later inducted with MSG 4 mg/gBW, and treatment 3 (T3) which was given with *Apis dorsata* honey 161.46 mg/20gBW and 1 hour later inducted with MSG 4 mg/gBW. MSG induction dose according to Widayati (2018) and *Apis dorsata* dose according to Rista and Yuziani (2014). All the treatments were given for 52 days (Figure 1).

After 52 days of treatment, all mice were euthanized using os vertebrae cervicalis dislocation and dissected to take the testes from mice. The testes were taken and put in a tissue storage pot containing 10% neutral buffer formalin and histological preparations including dehydration and staining using Hematoxylin-Eosin were made.

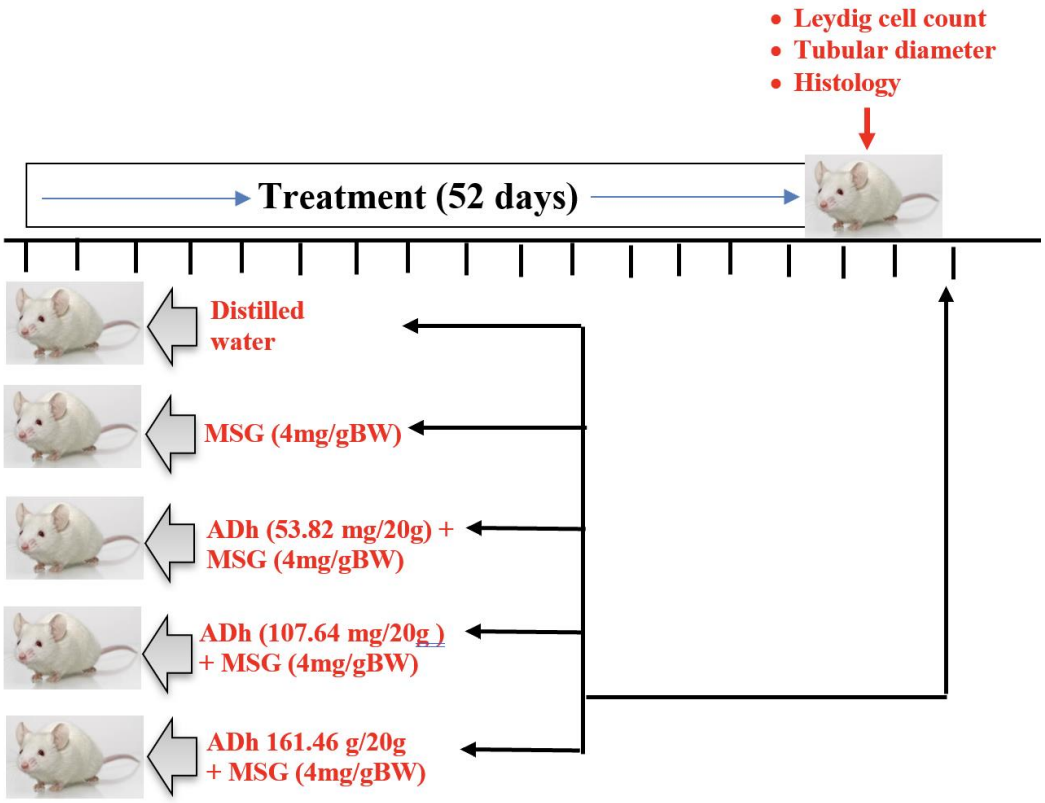

Figure 1
Experimental design and animal grouping

Histology: Histopathological slides then examined using Nikon Eclipse microscope with 100x magnification to measure the seminiferous tubules diameter and 400x magnification to count the Leydig cells in five field of view. Data analysis using ANOVA with Duncan as post-hoc test

RESULTS

The results of Leydig cell count and seminiferous tubules diameter can be seen on Figures 2 and 3. The data was processed using One-Way ANOVA and Duncan post-hoc test. In Figure 2, Leydig cells count in the C- group which was only given aquadest was 44 ± 1.64, which showed significant result ($p < 0.05$) when compared to the C+ group given with MSG 4mg /gBW which was 25.24 ± 2.24. The T1 group with the Leydig cells count 28.56 ± 1.47 showed significant results ($p<0.05$) with the T2 and T3 groups with the number of Leydig cells count 38.28 ± 1.37 and 42.68 ± 1.39. The T3 group showed results that were not significantly different ($p>0.05$) from the C- group.

The diameter of the Seminiferous tubules in the C- group which was only given aquadest was 199.13 ± 4.78 showed significant results ($p < 0.05$) when compared to the C+ group given with MSG PO 4mg / gBW treatment which was 158, 53 ± 5.21. Treatment groups T1 and T2 with seminiferous tubule diameter 173.36 ± 1.73 and 183.99 ± 2.58, respectively, showed significant differences ($p < 0.05$) with C- group 284.04a ± 3.60. The T3 group with a diameter of 195.66 ± 3.57 showed no significant difference ($p > 0.05$) with the C- group. (Figure 3).

On average, all treatment groups were given certain preventive doses of *Apis dorsata* honey before being given MSG PO 4mg/gBW (TI with a dose of 53.82mg / 20g, T2 with a dose of 107.64 mg/20g, and T3 with a dose of 161.46 mg/20g) showed a significant result ($p < 0.05$) compared to the C+ group which was only given MSG PO 4mg / gBW.

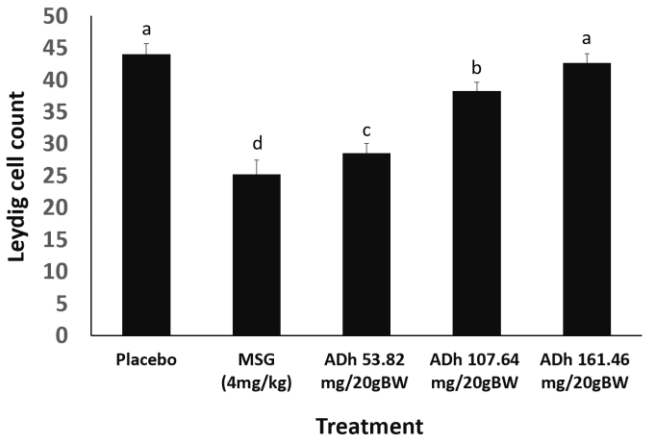

Figure 2:
The average Leydig cells count in each treatment group. Each bar represents
Different superscripts in one column showed significant differences ($p < 0.05$)

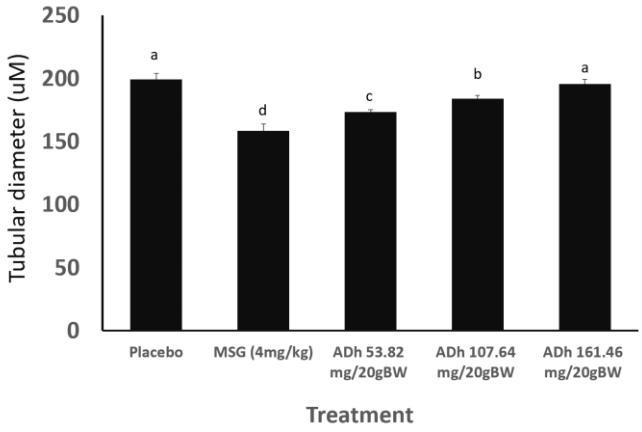

Figure 3:
Seminiferous tubule diameter mean graph in each treatment group.
Different superscripts in one column showed significant differences ($p < 0.05$)

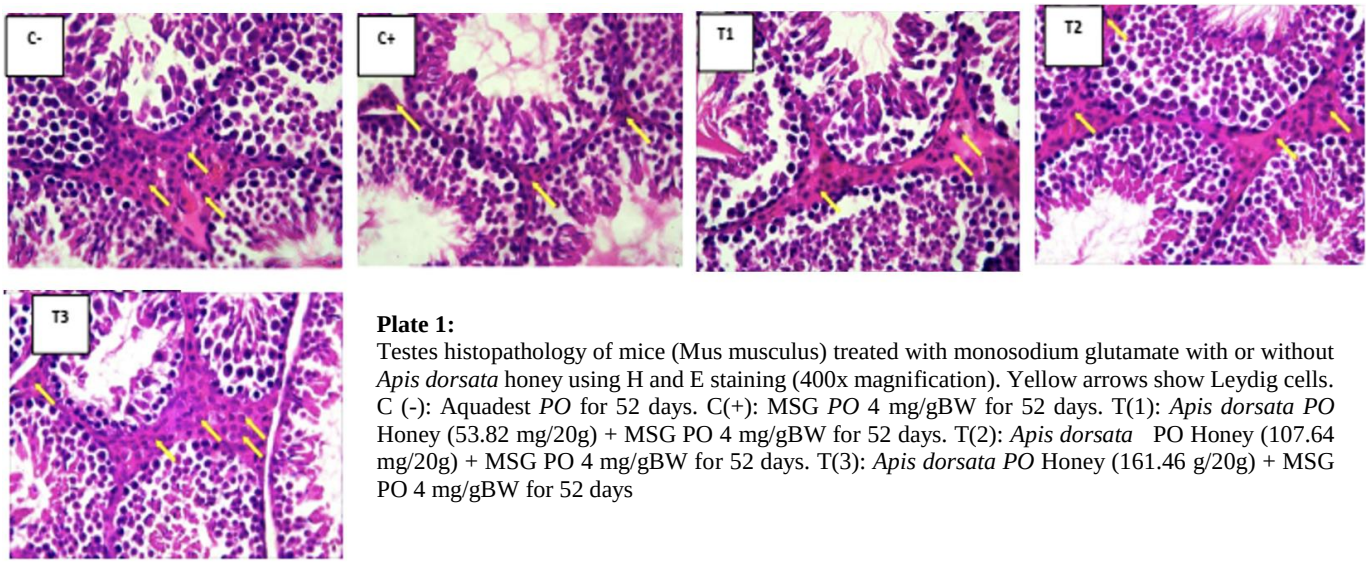

Plate 1:
Testes histopathology of mice (Mus musculus) treated with monosodium glutamate with or without *Apis dorsata* honey using H and E staining (400x magnification). Yellow arrows show Leydig cells. C (-): Aquadest *PO* for 52 days. C(+): MSG *PO* 4 mg/gBW for 52 days. T(1): *Apis dorsata PO* Honey (53.82 mg/20g) + MSG PO 4 mg/gBW for 52 days. T(2): *Apis dorsata* PO Honey (107.64 mg/20g) + MSG PO 4 mg/gBW for 52 days. T(3): *Apis dorsata PO* Honey (161.46 g/20g) + MSG PO 4 mg/gBW for 52 days

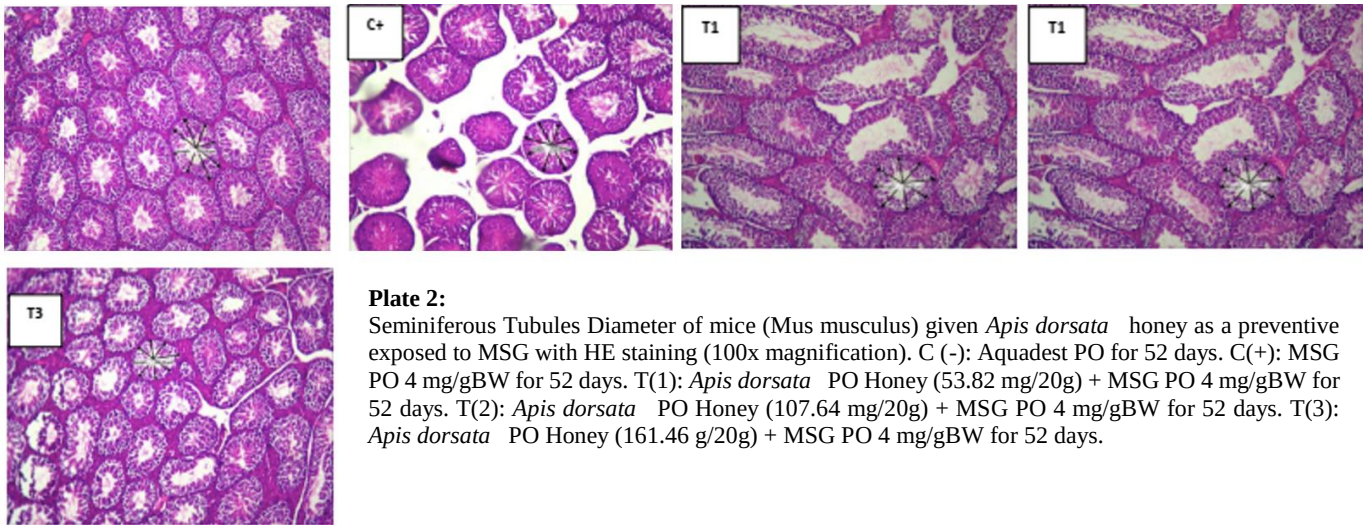

Plate 2:
Seminiferous Tubules Diameter of mice (Mus musculus) given *Apis dorsata* honey as a preventive exposed to MSG with HE staining (100x magnification). C (-): Aquadest PO for 52 days. C(+): MSG PO 4 mg/gBW for 52 days. T(1): *Apis dorsata* PO Honey (53.82 mg/20g) + MSG PO 4 mg/gBW for 52 days. T(2): *Apis dorsata* PO Honey (107.64 mg/20g) + MSG PO 4 mg/gBW for 52 days. T(3): *Apis dorsata* PO Honey (161.46 g/20g) + MSG PO 4 mg/gBW for 52 days.

DISCUSSION

Administering MSG at a dose of 4mg/gBW for 52 consecutive days reduced Leydig cells count and the diameter of the seminiferous tubules of mice (*Mus musculus*). Giving *Apis dorsata* honey a preventive dose has been proven to be able to maintain the Leydig cells count and the diameter of the seminiferous tubules.

MSG has a toxic effect on the reproductive system through the pre-testicular and testicular mechanisms. Activation of the mGluR and iGluR receptors which will have a synergistic effect with the NMDAR receptor facilitates the calcium ion gate channel so that the intracellular Ca2+ levels increase significantly. The increase in ionic levels increases the production of ROS, activates pro-apoptotic proteins such as caspase-3, activates the proteolytic system, increases NO expression due to aNOS activation, and decreases levels of endogenous antioxidants. This series of processes is known as excitotoxicity (Kritis *et al*, 2015).

This situation causes oxidative stress and death of nerve cells in the hypothalamus which will interfere with the function of the hypothalamus-pituitary-gonad axis, thereby disrupting the secretion of FSH and ICSH hormones (Kamalah, 2019).

Impaired ICSH secretion will have an impact on decreasing Leydig cell count due to a lack of hormonal stimulation (Malini, 2020). This can be seen in Leydig cells count in the C+ group which was given 4mg / gBW of MSG which decreased significantly ($p < 0.05$) when compared to the C- group which was only given aquadest. This finding is also in line with the research conducted by Edward (2015) that MSG can significantly reduce FSH and ICSH levels.

On the other hand, oxidative stress, membrane lipid peroxidation, and decreased FSH secretion affect the production of spermatogenic cells thus affect the diameter of the seminiferous tubules (Kalsum, 2013). In this study, it was found that the seminiferous tubules diameter between C+ group that was given 4mg / gBW MSG significantly different ($p > 0.05$) compared to the C- group which was only given aquadest. This finding is in line with research conducted by Kalsum (2013) that MSG can reduce the diameter of the seminiferous tubules.

In the T1 treatment group with a dose of 53.82 mg / 20g, T2 with a dose of 107.64 mg / 20g, and T3 with a dose of 161.46g / 20g, there was a significant increase between the Leydig cells count compared to C+ ($p < 0.05$). The T3 group showed an effective preventive dose and there was no significant difference with the C- group ($p > 0.05$). These results indicate that giving honey with a minimum preventive dose of 53.82mg / 20g in mice exposed to MSG can maintain Leydig cells count and the preventive dose of T3 of 161.46g / 20g is the optimal dose because there was no significant difference with the C- group which is only given aquadest ($p>0.05$). A positive correlation between preventive honey doses on Leydig cell count was also found in a study conducted by Fitri (2019).

Meanwhile in seminiferous tubules diameter, among treatment group with a dose of 53.82 mg / 20g for T1, T2 with a dose of 107.64 mg / 20g, and T3 with a dose of 161.46g / 20g there was a significant increase between seminiferous tubule diameter compared to C+ ($p < 0.05$). The T3 group showed an effective preventive dose and there was no significant difference with the C- group ($p > 0.05$). These results indicate that giving honey with a minimum preventive dose of 53.82mg / 20g in mice exposed to MSG can maintain the seminiferous tubule diameter and the preventive dose T3 of 161.46g / 20g is the optimal preventive dose.

The honey mechanism important in maintaining the Leydig cells counts against MSG stressors located in its content. *Apis dorsata* honey contains a lot of polyphenols, flavonoids, vitamin C, and also some enzymatic antioxidants such as catalase and peroxidase, and glucose oxidase (Saputri, 2017). *Apis dorsata* honey also contains higher phenolic compounds, flavonoids, and antioxidants than Apis mellifera and *Apis cerana* honey (Moniruzzaman *et al*., 2013). The high antioxidant content will inhibit the formation of ROS which will prevent hypothalamic ablation, thereby normalizing the function of the hypothalamus-pituitary-gonad axis (Muawanah *et al*, 2015).

The main phenolic compounds of honey Anthraquione play a role in donating electrons and hydrogen atoms from the

hydroxyl groups of phenolics and function to stabilize free radical compounds. This compound also functions as a scavenger of oxygen and helps regenerate endogenous antioxidants. On the other hand, compounds in honey have a role in inhibiting excess NO production by inhibiting the NOS enzyme and reducing inflammation that occurs (Owoyele *et al*, 2011). Enzymatic antioxidants in honey and vitamins also play an important role in preventing the formation of ROS by donating electrons through enzymatic reactions (Muawanah *et al*, 2015; Saputri, 2017). The combination mechanism between several compounds in *Apis dorsata* honey works together to overcome the formation of ROS and prevent ablation of the hypothalamus so that ICSH production remains normal even though it is given a stressor in the form of MSG. Antioxidants in *Apis dorsata* honey also inhibit the occurrence of cell membranes peroxidation of spermatogenic in the presence of phenolic compounds such as anthraquinone which scavenger oxygen from reactive compounds to minimize ROS encounters with PUFAs (Moniruzzaman *et al*, 2013). Honey also increase the regeneration of seminiferous tubules testicle thus lead to protection in seminiferous tubules diameter (Safitri *et al*, 2016). *Apis dorsata* honey can maintain the Leydig cells count and seminiferous tubules diameter in mice (Mus musculus) exposed to monosodium glutamate.

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