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Neuroprotective effects of *Apis dorsata* forest honey on the neurons count in cerebrum and cerebellum of mice (*Mus musculus*) exposed to monosodium glutamate

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Abstract

Introduction: Monosodium glutamate or MSG can have an effect on increasing the production of reactive oxygen species (ROS) in the brain. *Apis dorsata* forest honey is known to have higher antioxidant activity than *Apis mellifera* and *Apis cerana* honey. This study aims to determine the preventive effect of *Apis dorsata* honey on the number of pyramidal cerebrum neurons and purkinje cerebellum neurons of mice (*Mus musculus*) exposed to monosodium glutamate (MSG).

Material and Methods: This study used 25 mice which were divided into 5 groups. In the K- group only aquadest was given. The K+ group was given 4mg/gBB of MSG. Groups P1, P2, and P3 given *Apis dorsata* honey at a dose of 53.82mg/20gBW, 107.64mg/20gBW, and 161.46mg/20gBW. All treatments were carried out orally for 52 days.

Results: The results showed that the average number of cerebral pyramidal neurons in the K-, K+, P1, P2, and P3 groups was 12.24±0.607; 7.24±2.875; 12.48±1.513; 15.72±0.944; and 19.28±2.827. The mean number of purkinje cerebellar neurons was 4.8±1.456; 2.08±0.807; 3.08±1.035; 3.56±0.434; and 4.68±1.390.

Discussion: The average number of pyramidal neurons and purkinje neurons in the group given preventive dose of *Apis dorsata* honey and exposed to MSG was higher and there is a significant difference compared to the group that was only exposed to MSG. It can be concluded that the administration of *Apis dorsata* honey can maintain the number of pyramidal cerebrum neurons and purkinje cerebellar neurons in mice exposed to MSG.

Keywords: *Apis dorsata* honey; MSG; Neuroprotective; Pyramid cell; Purkinje cell

1. Introduction

Monosodium glutamate or MSG is an additive widely used in various countries as a food flavoring to be served. Monosodium glutamate consists of 12% sodium, 78% glutamic acid, and 10% water [1,2]. The average MSG consumption in Europe is 0.3-0.5 g/day, and in Asia it is 1.2-1.7g/day. In general, MSG intake of 16.0 mg/kg body weight is still considered safe [3].

Based on a report from Information Handling Services (2018), Indonesia occupies the second position as an exporting and consuming country for MSG [4]. The increase in living standards, changes in diet, the development of the food processing industry, and increasing urbanization are factors that cause the growth of MSG consumption in Indonesia.

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The problem that occurs is excessive consumption of MSG is often not realized by consumers due to consumer ignorance of the amount of MSG added to fast food [5].

Excessive consumption of MSG can have an effect on increasing the production of reactive oxygen species (ROS) in the brain. Glutamate accumulation will result in overactivity and dysregulated activation of glutamate receptors, especially NMDA receptors which are the most permeable to calcium ions. The imbalance of endogenous antioxidants to neutralize the ROS formed is known as glutamate induced excitotoxicity, which will result in lipid peroxidation and degeneration of neurons [6]. To prevent this, exogenous antioxidants need to be given to help reduce the toxic effects of ROS .

Honey contains enzymatic antioxidants, namely catalase, glucose oxidase, and peroxidase, as well as non-enzymatic antioxidants, namely ascorbic acid, flavonoids, amino acids, and proteins. *Apis dorsata* forest honey is known to have higher antioxidant activity than *Apis mellifera* and *Apis cerana* honey [7,8]. Antioxidants will work by becoming an electron donor so that it will change the ROS formed to be more stable. In addition, antioxidants also act as free radical scavengers so that they can minimize the occurrence of oxidative stress [9]. This study aims to determine that the application of *Apis dorsata* forest honey can maintain the number of pyramidal cerebrum neurons and purkinje cerebellum neurons in mice (*Mus musculus*) exposed to monosodium glutamate (MSG).

2. Material and methods

This research is a kind of pure experimental research (true experimental) laboratory. In this study, 25 male mice were randomly divided into 5 groups, namely groups K-, K+, P1, P2, and P3. This research was conducted for 52 days at the Experimental Animal Cage Laboratory and the Embryology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya.

The experimental animals used in this study were male mice (*Mus musculus*) strain BALB/c aged ±8 weeks with a body weight of ±30 grams which were obtained from the Veterinary Farma Center, Jalan Ahmad Yani No.68, Surabaya. Materials for rearing mice were water given *ad libitum* and food in the form of pellets of Hi-Pro-Vite Medicated 593. Material for treatment was *Apis dorsata* forest honey (Tesso Nilo®) with three levels of preventive doses and MSG solution (Merck®) as inductor.

The treatment groups in this study were: group K- only given aquadest, group K+ were given MSG 4mg/gBW, groups P1, P2, and P3 were given MSG 4mg/gBW and one hour later they were given *Apis dorsata* honey with sequential doses of 53.82 mg/g20gBW, 107.64mg/20g BW, and 161.46 mg/20gBW for 52 days. On day 53 the mice were sacrificed by cervical vertebrae dislocation. Furthermore, the brain organs were taken to make histological preparations with Hematoxylin-Eosin staining.

Histological preparations were observed using a Nikon Eclipse light microscope with a magnification of 400x for 5 fields of view to count the number of normal cerebellar pyramidal neurons and purkinje cerebellar neurons. The data obtained were analyzed using One Way ANOVA test and Duncan's test as a post-hoc test.

3. Results

Based on table 1, it can be seen that there was a significant difference ($p<0.05$) in the form of a decrease in the average number of normal pyramidal neurons in the cerebrum in the K+ group given the MSG PO 4mg/gBB solution with an average of 7.24 ± 2.875 compared to the K- group given aquadest PO with an average of 12.24 ± 0.607. All treatment groups that were given a preventive dose of *Apis dorsata* forest honey (Groups P1, P2, and P3) before MSG administration showed significant differences with Group K+ which was only given MSG solution without a preventive dose of *Apis dorsata* forest honey. The P1 group with a mean of 12.48 ± 1.513 there was no significant difference ($p>0.05$) against the K- group. Groups P2 and P3 with a mean of 15.72 ± 0.944 and 19.28 ± 2.827 were significantly different ($p<0.05$) when compared to groups K- and P1.

Based on table 1, it can be seen that there was a significant difference ($p<0.05$) between the K- group given aquadest PO with an average number of normal Purkinje neurons 4.8 ± 1.456 compared to the K+ group given MSG PO solution of 4 mg/gBW with a mean of 2.08 ± 0.807. The results of data processing statistically showed a significant difference ($p<0.05$) in groups P2 and P3 who were given a preventive dose of *Apis dorsata* forest honey with an average of 3.56 ± 0.434 and 4.68 ± 1.390 when compared to the K+ group which was only given MSG solution without giving a preventive dose. *Apis dorsata* forest honey. It can be seen in table 1 that the P1 group with a mean of 3.08 ± 1.035 was not significantly different ($p>0.05$) with the K+ and P2 groups. The P3 group with the highest preventive dose of *Apis dorsata*

forest honey was significantly different ($p<0.05$) compared to the K+, P1, and P2 groups, but not significantly different when compared to the K- group.

Table 1 Average calculation results of pyramidal neurons in the cerebrum and Purkinje neurons in the cerebellum in each treatment group (Mean ± SD)

Group	Pyramidal cerebral neurons (Mean ± SD)	Purkinje cerebellar neurons (Mean ± SD)
K-	$12.24^{b} \pm 0.607$	$4.80^{c} \pm 1.456$
K+	$7.24^{a} \pm 2.875$	$2.08^{a} \pm 0.807$
P1	$12.48^{b} \pm 1.513$	$3.08^{ab} \pm 1.035$
P2	$15.72^{c} \pm 0.944$	$3.56^{b} \pm 0.434$
P3	$19.28^{d} \pm 2.827$	$4.68^{c} \pm 1.390$

Note: The difference in superscripts in the same column shows a significant difference between treatments ($p<0.05$)

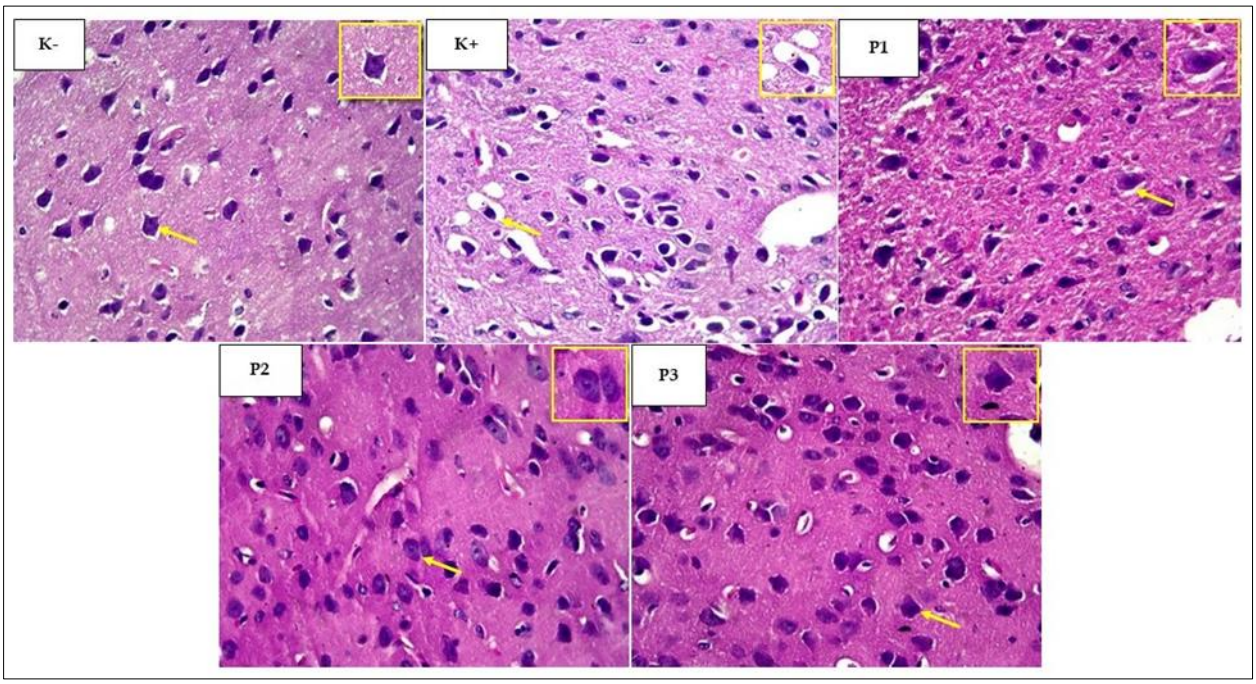

Figure 1 Histology of the cerebrum of mice (*Mus musculus*) that were given *Apis dorsata* forest honey preventively and exposed to MSG for 52 days. Yellow arrows indicate pyramidal neurons. The photo above was taken using a Nikon Eclipse E-100 light microscope with HE staining and 400x magnification

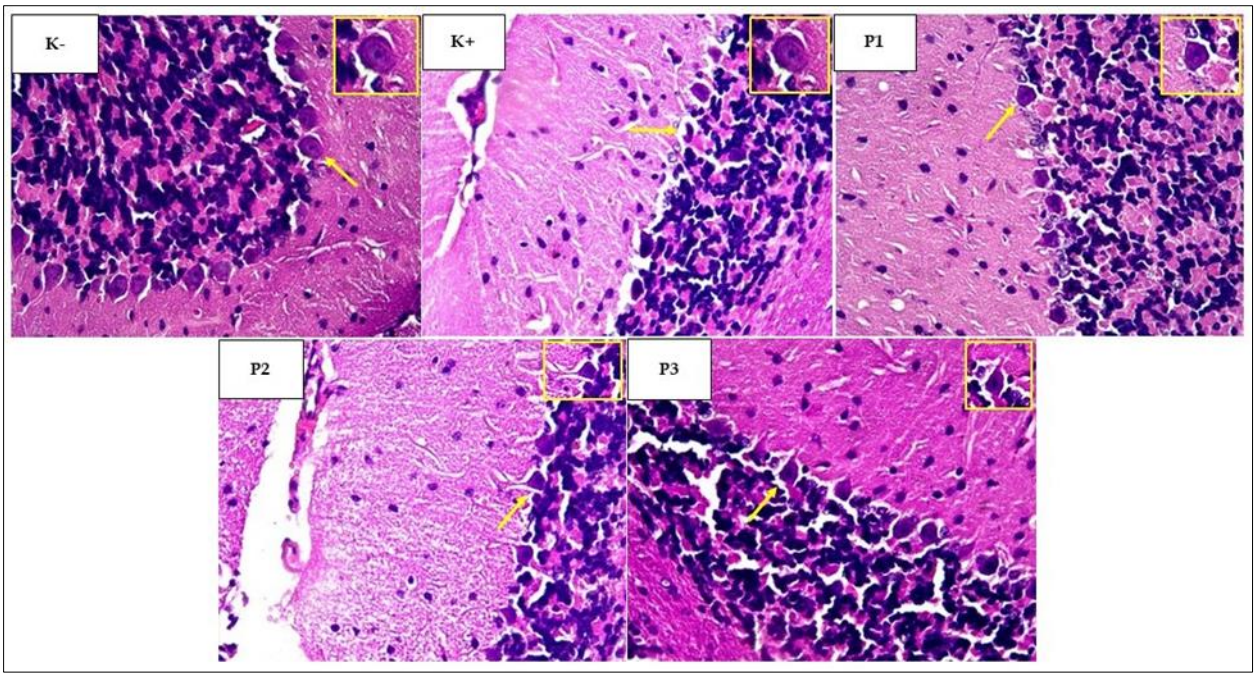

Figure 2 Histology of the cerebellum of mice (*Mus musculus*) treated with *Apis dorsata* forest honey preventively and exposed to MSG for 52 days. Yellow arrows indicate Purkinje neurons. The photo above was taken using a Nikon Eclipse E-100 light microscope with HE staining and 400x magnification

4. Discussion

Giving MSG 4mg/gBW for 52 days in mice (*Mus musculus*) can reduce the number of normal cerebrum pyramidal neurons and purkinje cerebellar neurons. Preventive administration of *Apis dorsata* forest honey has been shown to maintain a normal number of pyramidal cerebrum and purkinje cerebellum neurons.

The brain is very susceptible to damage and oxidative stress due to its high oxygen demand reaching 20% and the amount of polyunsaturated fatty acids Polyunsaturated Fatty Acids. (PUFAs) are high in neuronal membranes [10]. Administration of MSG in the long term and excessive can quickly increase blood plasma glutamate levels which are the main neurotransmitters in the central nervous system [11].

Glutamate accumulation in the synaptic cleft will activate mGluR and iGluR receptors, especially NMDA receptors which are the most permeable to calcium ions. Increased calcium (Ca^{2+}) Intracellular sources from both intracellular and extracellular sources contribute to glutamate-mediated cell death. Activation of mGluR by gutamate will activate phospholipase C which increases the production of 1,4,5-inositol triphosphate (IP3) and triggers the release of calcium from intracellular Ca^{2+} stores [12]. This activity will increase the influx of calcium ions which will trigger the formation of free radicals and proteolytic enzymes in neurons and decrease the level of endogenous antioxidants in the brain. This mechanism is known as glutamate induced excitotoxicity [6].

Oxidative stress that occurs continuously will cause damage to neuronal membranes, cytoskeleton, and DNA which ultimately results in neuronal death [13]. This can be seen in the number of normal pyramid and purkinje neurons in the K+ group significantly decreased ($p<0.05$) compared to the K- group. These results are also in accordance with research conducted that MSG administration can significantly reduce the number of pyramidal cerebrum neurons and purkinje cerebellar neurons [14,15].

The mean number of cerebral neurons in the P2 and P3 groups was higher and significantly different compared to K-. In several research reports, polyphenolic compounds which are also present in *Apis dorsata* forest honey have shown a neuroprotective effect [16]. Neuroprotective effects refer to strategies and mechanisms in defending the central nervous system from nerve injury and acute and chronic neurodegenerative disorders [17]. Neuroprotection in mammals is regulated by neurotrophic factor which is a protein that stimulates the survival, development, and function of neurons. Nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) are one of the most studied neurotrophic factors. The main receptors for NGF and BDNF are TrkA and TrkB. Neurotrophic factor works by

preventing neurons from starting the apoptotic process and also inducing differentiation of progenitor cells to form neurons (neurogenesis) [16].

Experimental research using a mouse model conducted that polyphenols extracted from olive pomace mostly contain hydroxytyrosol, which can significantly increase levels of NGF and BDNF in brain areas such as the hippocampus and olfactory lobes and an increase in the expression of the main receptors, namely TrkA and TrkB [17].

The mean number of pyramidal cerebrum neurons in the P3 group was higher and significantly different than K+ but not significantly different from K-. Based on research conducted by Oyefuga (2012) giving honey at a dose of 250mg/kgBB both in the long and short term significantly reduced lipid peroxidation in brain tissue and was accompanied by an increase in the activity of superoxide dismutase (SOD) and glutathione reductase, thereby reducing free radical damage [18].

Consumption of exogenous antioxidants such as honey can overcome oxidative stress, prevent free radicals from attacking neuron membrane components, and prevent lipid peroxidation in neuronal membranes. *Apis dorsata* honey has been shown to contain phenolic compounds, flavonoids and several enzymes such as glucose oxidase and peroxidase and has higher antioxidant activity than *Apis mellifera* and *Apis cerana* honey [8]. Antioxidant mechanisms in preventing oxidative stress include: elimination of O^2, scavenging ROS/RNS and their precursors, inhibiting ROS formation, binding of metal ions needed as catalysts for ROS formation, and increasing the effectiveness of endogenous antioxidants [19].

5. Conclusion

The mean number of pyramidal cerebrum neurons and purkinje cerebellum neurons in the group that was given *Apis dorsata* forest honey was higher and significantly different ($p<0.05$) when compared to the group that was only exposed to MSG without being given *Apis dorsata* forest honey. The conclusion of this study is that the administration of *Apis dorsata* forest honey can maintain the number of pyramidal cerebrum neurons and purkinje cerebellum neurons in mice (*Mus musculus*) exposed to MSG.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have not declared any conflict of interest. The authors alone are responsible for the content and writing of the paper.

Statement of ethical approval

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.

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