Protective Effects of *Apis Dorsata* Honey Supplementation on Kidney Histopathology in Mice with Monosodium Glutamate Exposure

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Abstract

This study was conducted to investigate the protective effects of *Apis dorsata* honey on the histopathological changes of the kidney in mice exposed to monosodium glutamate. This study used 25 male mice as the experimental animal which were divided into five groups with five replication, respectively i.e. (C-) was administered basal diet, (C+) was administered 4 mg/g BW MSG, (T1) was administered *A. dorsata* honey of 53.82 mg/20g BW followed by MSG 4 mg/g BW, (T2) was administered *A. dorsata* honey of 107.64 mg/20g BW followed by MSG 4 mg/g BW, and (T3) was administered *A. dorsata* honey of 161.46 g/20g BW followed by MSG 4 mg/g BW. All treatments were carried out orally for 52 days. This study was observed tubular epithelial hydropic degeneration, epithelial necrosis, and glomerular necrosis. Data were analyzed using Kruskal-Wallis test followed by the Mann-Whitney test. As a result, for the epithelial and glomerular necrosis variables, the T3 group showed significant results when compared with C+, T1, and T2 groups. Moreover, T3 was not significantly different from the C- group. It can be concluded that *A. dorsata* honey in T3 group could protect mice kidneys from the damaging effect of MSG.

Keywords: *Apis dorsata*, health, honey, kidney, monosodium glutamate

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INTRODUCTION

Umami is the fifth core taste of our tongue alongside the four basic tastes which are; bitter, salty, sweet, and sour. We owe the taste of umami to monosodium glutamate (MSG). It is a common food additive that is commonly used in Asian delicacies and fast food. MSG consumption is growing worldwide with an average daily intake estimated at 3-4 g per day (Sharma *et al.*, 2013). The use of MSG has increased throughout the world in recent years as flavoring enhances palatability and food selection in a meal. In recent years, many studies have been made on the adverse effects of consuming MSG. The excessive consumption of MSG is an alarming issue among people from various parts of the world. There are multiple scientific studies evidence supporting MSG has degenerative effects on the body when consumed extensively. The excessive use of MSG has been shown to increase oxidative stress in different organ systems and cause glucose metabolism disorders, obesity, and coronary diseases (Weliyani *et al.*, 2021).

Kidneys are highly sensitive organs to chemicals like MSG. Chronic oral MSG intake in rats leads to changes in antioxidant systems and renal markers including lipid peroxidation byproducts, in agreement with what was observed in rats injected with MSG. Moreover, dietary MSG increases the urinary pH in rats and causes alkaline urine which influences the kidney's

capacity to secrete or reabsorb metabolites that contribute to stone formation (Sharma, 2015).

The effect of MSG toxicity on tissues and organs is influenced by the production of exogenous Reactive Oxygen Species (ROS) (Anbarkeh *et al*., 2019). Excessive production of ROS will affect cell membrane activity cause lipid membrane peroxidation and ultimately cause cells to experience necrosis caused by oxidative stress (Liwikasari, 2018). Antioxidants are natural ingredients that can protect lipid membranes from oxidation (Himawan *et al*., 2021). Honey consists of antioxidant properties like phenolic acid and flavonoids that can suppress oxidative stress (Lobo *et al*., 2010). Honey produced by the *A. dorsata* honey bee has antioxidant properties and they act as free radical scavengers. Based on a study carried out by Monirruzaman *et al*. (2013), *A. dorsata* honey has the highest phenolic compound and flavonoid content when compared to other honey like Acacia honey, Borneo honey, and Pineapple honey. This study aimed to investigate the protective effects of *A. dorsata* honey on the histopathological changes of the kidney in mice with MSG exposure.

MATERIALS AND METHODS

Ethical Approval

This study has been approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, and was declared ethically worthy with code number 1.KE.075.08.2020.

Study Period and Location

This study was conducted in February–June 2020. The locations of this study were Animal Laboratory and Clinic, Faculty of Veterinary Medicine, Universitas Airlangga.

Experimental Design

The study design that was used a completely randomized design (CRD). A total of 25 male mice, aged 10–12 weeks old, weighed ±30 grams body weight were used for this study. After the adaptation procedure of the experimental animals ended, a randomized sample was performed to put each mice into the treatment group. The treatment group in this study consisted of five groups, i.e. (C-) was administered basal diet, (C+) was administered 4 mg/g BW MSG, (T1) was administered A. dorsata honey of 53.82 mg/20g BW followed by MSG 4 mg/g BW, (T2) was administered A. dorsata honey of 107.64 mg/20g BW followed by MSG 4 mg/g BW, and (T3) was administered A. dorsata honey of 161.46 g/20g BW followed by MSG 4 mg/g BW. *A. dorsata* honey orally administered followed by MSG solution an hour later administrated orally from day 1 until day 52.

MSG and *A. dorsata* Honey Preparation

The MSG suspension was prepared from MSG crystals that were diluted in distilled water. The dosage used in this study was 4 mg/g BW.

Meanwhile, *A. dorsata* honey that was used in this study was made of Tesso Nilo brand. The honey was diluted with distilled water to obtain 3 different preventive dosages.

Kidney Histopathological Sample Preparation

After 52 days of treatment, all the mice were anesthetized with a combination of ketamine (100 mg/kg BW) and xylazine (10 mg/kg BW) intraperitoneally and sacrificed by cervical dislocation (Flecknell, 2016). The abdominal wall was dissected with minor surgical equipment to collect the kidneys which were stored in a container that was filled with 10% formalin liquid. The histopathological changes in the kidney were observed using Hematoxylin-eosin (HE) and a microscope with 400x magnification (Hamid *et al*., 2022).

Data Analysis

The data was analyzed using the Kruskal-Wallis test and continued with Mann-Whitney if there was a significant ($p < 0.05$) to determine the differences in each group.

RESULTS AND DISCUSSION

Based on Table 1, it was reported that the C- group had the lowest number of tubular epithelial

Table 1. Hydropic degeneration, tubular epithelial necrosis, and glomerular necrosis in mice kidneys

Treatment	Degeneration (Mean ± SD)	Necrosis (Mean ± SD)	Glomerular Necrosis (Mean ± SD)
C-	$0.60^{a} \pm 0.548$	$0.80^{ab} \pm 1.095$	$0.00^{a} \pm 0.000$
C+	$1.60^{a} \pm 0.548$	$2.80^{c} \pm 1.095$	$3.80^{d} \pm 1.095$
T1	$1.40^{a} \pm 0.894$	$2.40^{c} \pm 0.894$	$2.40^{cd} \pm 1.342$
T2	$1.80^{a} \pm 0.447$	$2.00^{bc} \pm 1.414$	$1.80^{bc} \pm 1.643$
T3	$1.40^{a} \pm 0.548$	$0.00^{a} \pm 0.000$	$0.60^{ab} \pm 1.342$

Different superscripts in the same column showed significant differences ($p < 0.05$).

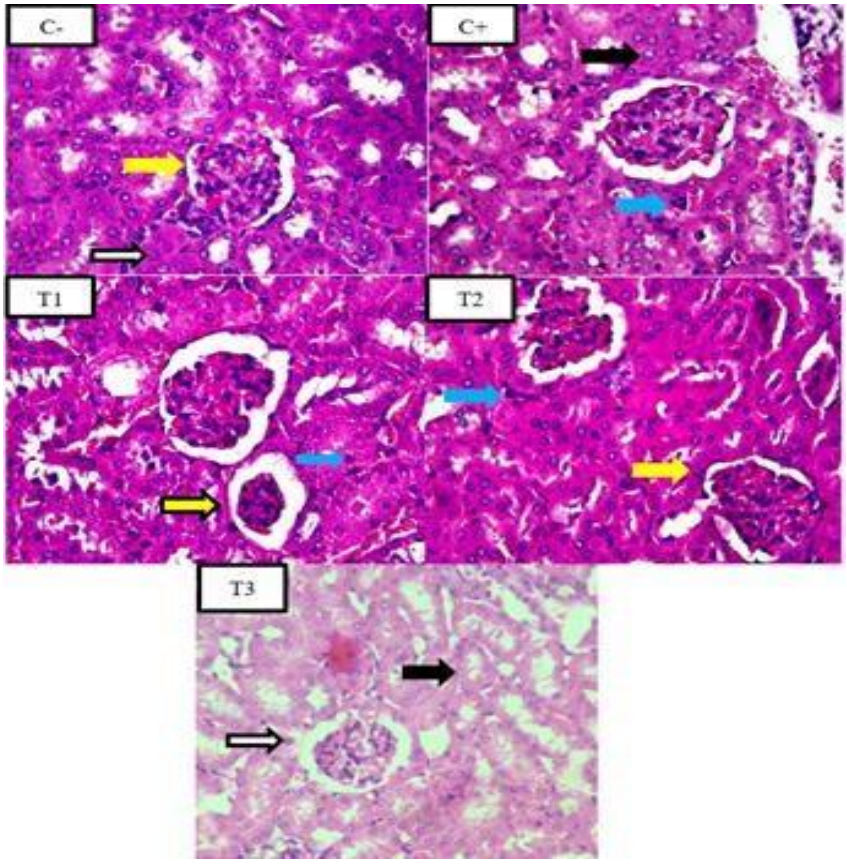

Figure 1. Histopathological features of the kidney in mice using HE staining, 400x magnification. Normal tubular cells (→), hydropic degeneration on tubular epithelial (→), tubular epithelial necrosis (→), normal glomerulus (→), and glomerular necrosis (→).

hydropic degeneration, tubular epithelial necrosis, and glomerular necrosis as in this treatment group only distilled water was induced. In treatment group C+, tubular epithelial necrosis and glomerular necrosis were the highest as in this treatment group only MSG was induced at the dosage of 4 mg /g BW for 52 consecutive days. It can be seen in Table 1 that from treatment groups T1 until T3, the number of tubular epithelial necrosis and glomerular necrosis significantly decreased as the preventive dosage of *A. dorsata* honey in each treatment group; T1, T2, and T3 increased respectively. It can also be seen that C- and T3 for tubular epithelial necrosis and glomerular necrosis showed no significant differences.

However, this was not the same for tubular epithelial hydropic degeneration. The T2 group had a higher number of hydropic degenerations, followed by the C+ group. Hydropic degeneration can be seen from the renal tubules that showed swelling of renal tubular epithelial cells, accumulation of water, part of the cytoplasm cloudy, and start to form a space around the cell nucleus (Figure 1).

This was due to the administration of MSG at 4 mg/g BW for 52 days and the lower dosage of preventive dose 107.64 mg/20g BW *A. dorsata* honey in the T2 group. Hydropic degeneration happens as a result of the glutamic acid contained in MSG is the source of free radicals that will activate the glutamate receptors in the kidneys in excess and hence, causing a decrease in endogenous antioxidant levels (Werdhasari, 2014). This imbalance triggers an increase in ROS which results in the damage of DNA and RNA, increased protein peroxidation, and increased lipid peroxidation (Sayuti and Rina, 2015). Damages like these cause problems at the cellular level, such as mitochondrial dysfunction. Mitochondria are the site for the formation of ATP. Therefore, damage to mitochondria results in a decrease in the production of ATP which is needed by the body (Safitri *et al*., 2022). The decrease in ATP production induces the programmed cell death or apoptosis of the cells that make up the renal tubules, resulting in cell injury (Suarjaya *et al*., 2012; Al-Anshori *et al*., 2023). Cell injury causes a shift of extracellular fluid into the cell, resulting in the loosening of the epithelium that makes up the proximal tubule and it later facilitates the entry of various components into the constituent cells tubules. This shift causes swelling of the convoluted tubular epithelial cells or hydropic degeneration (Fikri *et al*., 2023). In addition to that, tubular epithelial hydropic degeneration is the early sign of cellular degeneration in response to injury and this condition is often reversible. With a higher preventive dosage of *A. dorsata* honey, this condition is reversible due to the higher antioxidant properties in the honey.

The examination results of tubular epithelial necrosis can be seen in Table 1. Treatment group C- showed a significant difference with treatment groups C+, T1, and T2 but showed no significant difference between treatment group T3. Treatment group C+ showed a significant difference between treatment groups C- and T3 and showed no significant difference with treatment groups T1 and T2. Tubular epithelial necrosis is the result of cell death following irreversible injury by hypoxia, ischemia, and membrane injury that is caused by toxins and other substances and mechanisms (Arimbi *et al*., 2015). The microscopic changes of necrosis were characterized by pyknosis, karyorrhexis, or karyolysis. Pyknosis can be observed by nuclear condensation with shrinkage and intense basophilia, karyorrhexis is characterized by nuclear fragmentation, while karyolysis is characterized by nuclear dissolution or loss (Erlangga *et al*., 2023). Dead cells also have intense cytoplasmic pallor and become swollen, rounded, and detached from the basement membrane or neighboring cells (Miller *et al*., 2017).

The examination results of glomerular necrosis can be seen in Table 1. Treatment group C- showed a significant difference with treatment group C+, T1, and T2, but showed no significant difference between treatment group T3. The treatment group C+ showed a significant difference between treatment groups C-, T2, and T3, but showed no significant difference between treatment group T1. Glomerular necrosis is demonstrated by the extensively atrophied or shrinking of glomerulus cells, the space of the glomerular cells that have been lysed by phagocyte cell activity (Plessis *et al*., 2011).

Based on this study, in the T1 treatment group with a dose of 53.82 mg/20g BW, T2 with a dose of 107.64 mg/20g BW, and T3 with a dose of 161.46 g/20g BW there was a significant difference between histopathological changes compared to C+ ($p < 0.05$). The treatment group T3 showed an effective preventive dose and there was no significant difference with the C- group. These results indicate that giving honey with a minimum preventive dose of 53.82 mg/20g BW in mice exposed to MSG has protective effects on the histopathological structures of the kidney and the preventive dose of 161.46 g/20g BW is the optimal preventive dose. It is consistent with the study carried out by Fukuda *et al.* (2011) that jungle honey has antioxidant and antitumor activities in dosage as low as 1 mg/mouse/day. This is following the opinion, that honey will cause the stem cells to develop rapidly and differentiate into cells that are needed as a

response to the defect and enhancement of the immune response (Prasetyo *et al*., 2016).

Honey has nephroprotective potency. The mechanism of honey is significant in protecting the histopathological structures of the kidney in mice against exposure to MSG. *A. dorsata* honey consists of various natural compounds that are rich in flavonoids, phenolic acids, Vitamin C polyphenols, and also some enzymatic antioxidants such as catalase and peroxidase, and glucose oxidase which act as a natural antioxidant (Saputri *et al*., 2017). *A. dorsata* honey also contains higher phenolic compounds, flavonoids, and antioxidants than *A. mellifera* and *A. cerana* honey (Moniruzzaman *et al*., 2013). The high antioxidant content will inhibit the formation of ROS which will prevent lipid membrane peroxidation. Flavonoids fight off free radicals by donating their electrons to molecules that have unpaired electrons such as free radicals while remaining stable and also making the reactive molecules achieve stability hence, flavonoids play an important role in inhibiting the increase of free radicals in the body (Weliyani *et al*., 2021; Listyorini *et al*., 2021). The phenolic compounds of honey play an important role in donating electrons and hydrogen atoms from the hydroxyl groups of phenolics and function to stabilize free radical compounds (Al-Zuhroh *et al*., 2021). This compound also functions as a scavenger of oxygen and helps regenerate endogenous antioxidants. Enzymatic antioxidants in honey such as catalase and peroxidase, and glucose oxidase helps prevent the ROS formation by inhibiting enzymes that play a role in the ROS formation such as xanthine oxidase, lipoxygenase, protein kinase, and NADPH oxidase (Saputri *et al*., 2017) and vitamins also play an important role in preventing the formation of ROS by donating electrons through enzymatic reactions (Muawanah *et al*., 2015).

CONCLUSION

We can conclude that *A. dorsata* honey in T3 group could protect mice kidneys from the damaging effect of MSG. These antioxidant constituents in *A. dorsata* honey play a vital role in inhibiting structural changes in the kidneys as well as protecting the kidney from kidney dysfunction that results in kidney damage.

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AUTHORS' CONTRIBUTIONS

W: Conceptualization and drafted the manuscript. SBSR, WPL, ES, and NT: Treated the animal laboratory. SK and AP : Validation, supervision, and formal analysis. AP: Performed the statistical analysis and the preparation of table and figure. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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