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The Effect of *Apis dorsata* Honey Treatment on Calcium Levels of Mandibular Bone in Ovariohysterectomized Wistar Rats

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Abstract

This study aimed to evaluate the efficacy of *Apis dorsata* honey on calcium levels in the mandibular bone of Wistar rats after ovariohysterectomy as an animal osteoporosis model for treating cases of osteoporosis. This study used 20 female rats, which were divided into five treatment groups with four replications. SHAM was the negative control group; OH was the ovariohysterectomized group without treatment as a positive control; the AD1, AD2, and AD3 groups were ovariohysterectomized rats with *A. dorsata* honey at doses of 1 g/kg bw, 2 g/kg bw, and 4 g/kg bw for 84 days. After 84 days of treatment, the left mandibular bones of the rats were collected, weighed, and stored in 10% neutral buffered formalin. Calcium levels were calculated using proximate analysis. The results showed a decrease in bone calcium levels in the ovariohysterectomy group, the highest results were in the SHAM group, and the lowest results were in the AD3 group. It can be concluded that the administration of *A. dorsata* honey could not maintain the calcium level of the mandibular bone in the ovariohysterectomized Wistar rats.

Keywords: *Apis dorsata*, calcium levels, health, osteoporosis, ovariohysterectomy

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INTRODUCTION

Osteoporosis is a systemic disorder characterized by a decrease in bone mass with structural damage to bone tissue, resulting in a decrease in bone mineral density (BMD) and a predisposing factor for fracture events (Chan *et al*., 2016). Osteoporosis is a major health problem, especially in middle-aged and elderly women (Hwang *et al*., 2015), this is related to estrogen hormone deficiency due to decreased ovarian function (Laswati *et al*., 2015). Individuals who have had an ovariohysterectomy will also have estrogen deficiency (Komori, 2015). Estrogen deficiency results in an imbalance in the processes of bone formation and dismantling (Laswati *et al*., 2015), which can affect bone mass and strength (Curtis *et al*., 2015).

The main cause of osteoporosis cases is estrogen hormone deficiency, so the proper therapy is to give a replacement preparation (Yudaniayanti *et al*., 2019). Maintaining the body's calcium balance is very important to prevent bone loss and fracture (Gupta and March, 2016). Therapy using antiresorptive drugs aims to replace the function of the hormone estrogen in regulating bone metabolism (Chan *et al*., 2016), but calcium supplementation is still needed to optimize osteoporosis drug therapy (Gupta and March, 2016). The consumption of calcium supplements, vitamin D, and osteoporosis medications requires high costs. Some synthetic drugs cause side effects such as breast cancer, osteonecrosis of the jaw, hypercalcemia, and hypertension (Perricone and Madison, 2017).

The use of natural ingredients as alternative ingredients for osteoporosis therapy has been proven to improve bone structure. This is following the study of Zaid *et al*. (2012), who conducted study on ovariectomized rats by administration honey produced by *Apis dorsata* bees was an increase in trabecular bone structure. Honey is a natural sweet substance produced by bees with nectar as the raw material. *A. dorsata* is

the largest and most productive forest honey, and the honey produced is believed to be more efficacious than *A. melifera* (Muslim, 2014). Honey contains about 200 substances, including sugars, minerals, proteins, vitamins, enzymes, organic acids, phenolic compounds, and volatile compounds (da Silva *et al*., 2016).

Tualang honey produced by *A. dorsata* bees has a higher flavonoid content than the other honey. Flavonols can bind to ER-α and ER-β (Zaid *et al*., 2012). ER-α stimulation activates the process of bone formation in osteoblasts, inhibits bone resorption in osteoclasts, and inhibits osteoclastogenesis in osteoclast progenitor cells (Laswati *et al*., 2015). Honey contains gluconic acid, which can increase calcium absorption in the intestine (Zaid *et al*., 2012). Honey also contains several minerals in the form of potassium, calcium, copper, iron, magnesium, manganese, phosphorus, sodium, zinc, and selenium (Cianciosi *et al*., 2018).

Honey has a complete composition, so it is effective in the treatment of osteoporosis, so study is needed to determine the effect of *Apis dorsata* honey on calcium levels of the mandibular bone of ovariohysterectomized female Wistar rats based on analysis of calcium levels.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Animal Care and Use Committee (ACUC) Ethical Clearance, Faculty of Veterinary Medicine, Universitas Airlangga No: 665-KE.

Study Period and Location

This study was conducted in February–June 2017. The locations of this study were Animal Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga as a place of treatment; Clinical Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga for the induction of osteoporosis and mandibular extraction; and the Animal Nutrition Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga for the analysis of mandibular bone calcium levels.

Experimental Design

The experimental animals used were 20 female Wistar rats, three months old, weighed 200–220 g, and in good health. All rats were placed in the same environmental conditions, given a standard diet and drinking water ad libitum. Rats were adapted for seven days (Yudaniayanti *et al*., 2017).

A. dorsata honey was collected from the forests of Batulanteh, Sumbawa, and West Nusa Tenggara. Based on the study of Zaid *et al*. (2010), the effective dose of honey for antiresorptive was 2 g/kg bw. In this study, the doses used were 1 g/kg bw as a low dose, 2 g/kg bw as a medium or effective dose, and 4 g/kg bw as a high dose.

Rats were randomly selected and divided into five treatment groups with four replications. SHAM was the negative control performed with surgery and administered 1 ml of aquadest orally per day; OH was a positive control performed with ovariohysterectomy and administered 1 ml of aquadest orally per day; AD1, AD2, and AD3 groups were ovariohysterectomy rats administered *A. dorsata* honey at 1 g/kg bw, 2 g/kg bw, and 4 g/kg bw + 1 ml aquadest orally. The treatment was administered using a spuit and gavage needle a day after surgery for 84 days.

Ovariohysterectomy

Modeling of rats estrogen deficiency in OH, AD1, AD2, and AD3 groups was performed by ovariohysterectomy. Rats were anesthetized using a combination of ketamine HCl 50 mg/kg bw and xylazine HCl 10 mg/kg bw intramuscularly (Yudaniayanti *et al*., 2017). A midventral abdominal incision is made from under the umbilicus (Sardjana and Kusumawati, 2011).

Using the spay hook, the corpus uteri was identified at the bottom of the dorsal bladder and traced towards the left cranial uterine cavity until the left ovary was found. The ligament and blood vessels were clamped with a hemostat right next to the cranial ovary, and another hemostat was attached next to the first. The blood vessels were then cut between the two hemostats. The same procedure was done on the right ovary. On the

area between the bifurcation and the cervix uteri, two clamps with hemostats were performed. The left and right uterine arteries were ligated, and the uterine body was cut between the two hemostats. After confirmation of no bleeding, the hemostats were removed, and the remaining tissue of the reproductive organs was put back into the abdominal cavity (Yudaniayanti *et al*., 2017).

After the surgery, the abdominal cavity was irrigated to prevent infection. Then suturing was done to close the incision wound on the peritoneum, musculature, and skin. For postoperative care, experimental animals were injected with enrofloxacin 10 mg/kg bw intramuscularly for two days. The stitched skin was administered iodine on the seam surface and given sterile gauze, covered with Hypafix and surgical plaster tape (Yudaniayanti *et al*., 2017).

Sample Evaluation

On the 85th day, rats were euthanized using Ketamine HCl 50 mg/kg bw to collect sinister mandibular bone samples. The sinister mandibular bone rinsed using 0.9% NaCl solution and then inserted into an organ container. The sinister mandibular bone was immersed in 10% neutral buffered formalin.

Analysis of bone calcium levels was performed by burning the bone completely at a temperature of 500–700°C for several hours until white ash appears. Ash was added with concentrate HCl to form HCl extract, and then calcium analysis was performed according to the procedure. Then calcium levels were calculated based on the formula for analysis of calcium levels (Al-Arif *et al*., 2016).

Data Analysis

Data obtained from the examination of calcium levels were analyzed using ANOVA and followed by Duncan's multiple range test ($p < 0.05$). All data were analyzed statistically using SPSS 20.0 for Windows.

RESULTS AND DISCUSSION

Evaluation of calcium levels of the mandible in Wistar rats ovariohysterectomy after administration of *A. dorsata* honey can be seen in Table 1. Based on analysis showed that there were significant differences in calcium levels with a significant result of $p < 0.05$, followed by Duncan's multiple range test with a significant level of 5%. The results showed that SHAM group was significantly different from AD1, AD2, and AD3 but had not significantly different from the OH group. The OH group was significantly different from AD1, AD2, and AD3 but had not significantly different from SHAM. AD1 had significantly different from SHAM, OH, and AD3, but had not significantly different from the AD2 group. The AD2 group different significantly from the SHAM, OH, and AD3 groups but had not significantly different from the AD1 group. The AD3 group was significantly different from SHAM, OH, AD1, and AD2.

Table 1. Calcium levels in all treatment groups

Treatment Group	Calcium Levels (%) Mean ± SD
SHAM	$36.48^{c} \pm 0.95$
OH	$35.61^{c} \pm 0.72$
AD1	$33.01^{b} \pm 1.84$
AD2	$32.62^{b} \pm 0.85$
AD3	$28.31^{a} \pm 0.85$

The same superscript in the same column showed not significant difference ($p > 0.05$).

The results showed that the OH group calcium levels were lower than the SHAM group. Ovariectomy can increase bone turnover and accelerate bone loss, this is related to estrogen hormone deficiency (Komori, 2015). The highest levels of bone calcium were found in the SHAM treatment group, owing to calcium levels in the blood being balanced so that there is no need to absorb bone for the process of homeostasis (Erlangga *et al*., 2023). The condition also does not reduce the composition of ash such that the weight of ash is proportional to bone weight. Calcium levels in the treatment group that was given honey were lower than those in the SHAM and OH groups; the higher the dose given, the lower the calcium levels in the mandibular bone. This can be related to the analysis used. In this study, the calculation of calcium levels was done by proximate analysis. Bones are burned at high

temperatures to ash, extracted with HCl, and then analyzed for calcium levels (Al-Arif *et al*., 2016).

When a material is completely burned at 500–700°C for several hours, it produces white ash containing inorganic minerals. Ash content analysis describes the mineral content roughly but does not accurately describe the mineral content. Materials with a high ash content are identical to materials with a high mineral content, but not to specific mineral content, such as calcium content. Ash content was calculated by comparing ash weight and bone weight (Al-Arif *et al*., 2016). Lukmandaru and Hidayah (2017) stated that calcium levels were negatively quadratically correlated with ash levels, which means when calcium levels are high, it is likely to have low ash levels.

The escalation in mandibular bone weight was shown in the AD2 and AD3 treatment groups. This finding follows the study of Zaid *et al*. (2010), which found that the tibia bone given to rats receiving honey from *A. dorsata* was heavier than the tibia bone of the control group after ovariectomy in Wistar rats. Per the study of Muhammad (2018), the administration of *A. dorsata* honey can increase the weight of the femur bone in an ovariohysterectomy rat.

This study found an increase in bone weight but had not an increase in calcium levels. Bone is composed of inorganic material in the form of bone mineral (hydroxyapatite) and organic material in the form of collagen type 1, non-collagen protein, fat, and water (Boskey, 2013). Wardati (2018), stated that the administration of *A. dorsata* honey increases the number of osteoblasts and decreases the number of osteoclasts. Osteoblasts are bone cells that secrete bone matrix. The bone organic matrix consists of collagen type 1 and a small number of other proteins in the form of osteopontin and osteocalcin, which play an important role in regulating the organic matrix and bone minerals (Blair *et al*., 2017; Safitri *et al*., 2023). Increasing the number of osteoblast cells will increase the bone's organic matrix. Based on these statements, it can be said that bone mass increases because it contains more organic matrices than inorganic matrices (Fikri *et al*., 2023).

The absence of an increase in calcium levels in the bones does not imply that they are of poor quality (Weliyani *et al*., 2021). Many factors determine the quality and quantity of bone. Bone quality is determined based on cortical bone size, bone turnover, and bone microarchitecture. Bone quantity is determined based on bone density, i.e., the amount of minerals in the bone (Al-Zuhroh *et al*., 2021). A decrease in bone density, as measured by the amount of bone mineral, indicates a decrease in bone mass and affects the level of bone strength (Safira *et al*., 2023). The size, shape, and composition of bone architecture also determine bone strength (Yudaniayanti *et al*., 2019).

Zaid *et al*. (2012) discovered that *A. dorsata* honey administration had a positive effect on the structure of bone trabecular but not on the body weight of ovariectomy Wistar rats. According to Yordan *et al*. (2018), administering *A. dorsata* honey can prevent decreased femur bone density in ovariohysterectomized Wistar rats. *A. dorsata* honey is effective in increasing bone strength in ovariectomy mice because honey contains high levels of polyphenols, which act as antioxidants (Yudaniayanti *et al*., 2018; Prastiya *et al*., 2019). The condition of osteoporosis increases the porosity of the trabecular bone matrix and decreases cortical bone thickness due to resorption in the endosteum. As in the study of Yudaniayanti *et al*. (2019), the administration of honey can increase cortical bone thickness, reduce the area of damage due to osteoclast activity, and increase bone strength. Based on some of the statements above, the administration of *A. dorsata* honey can improve bone structure but cannot maintain calcium levels in bones (Listyorini *et al*., 2021). Giving *A. dorsata* honey can still be used as an anti-osteoporosis therapy because honey contains other components besides calcium that can improve bone structure.

CONCLUSION

Based on study results from the administration of *A. dorsata* honey to the calcium levels of the mandibular Wistar rat, which underwent ovariohysterectomy as an osteoporosis

model, it can be concluded that *A. dorsata* honey cannot maintain bone calcium levels.

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AUTHORS' CONTRIBUTIONS

ISY: Conceptualization and drafted the manuscript. SNR, HP, and PH: Treated the animal laboratory. ISY: Validation, supervision, and formal analysis. PH: Performed the statistical analysis and the preparation of table. HP and PH: Performed sample evaluation. 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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