Conference Paper

Utilization of Honey *Apis dorsata* as Antiosteoporosis on Requirements of Bone Calcium Ash Density on Ovariohysterectomized White Rat *(Ratus norvegicus)*

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Abstract

The purpose of this study was to determine the effect of honey bee *Apis dorsata* as anti-osteoporosis in calcium ash density (CAD) of bone in osteoporotic-induced rats. The target of this study was to know bone calcium levels after being given honey bees *Apis dorsata*. In this study, 35 female white rats (*Ratus norvegicus*) mature was used with weight 200gr. Divided into 5 groups, 2 control groups and 3 treatment groups. The negative control group (SH) was not induced by osteoporosis and was given the only aquadest of 1.5 ml/day. Whereas the positive control group was induced by osteoporosis (OH) and was given only aquadest 1.5 ml/day. T_1, T_2 and T_3 treatment groups were given bee honey with various doses including 1g / kg ad 1.5 ml aquadest, 2g / kg BB ad 1.5 ml aquadest and 3g / kg BB ad 1.5 ml aquadest. Then after 12 weeks, white rats were sacrificed for lumbar vertebrae. Furthermore, the sixth lumbar spine os vertebrae will be examined for calcium bone ash content. The data were obtained was analyzed using statistical analysis of variance (ANOVA) for the results of the calcium ash content data.

Keywords: Honey; Osteoporosis; CAD.

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1. Introduction

Osteoporosis from the word *osteo* (bone) and *porous*. Osteoporosis is a severe bone disease that increases morbidity and mortality in individuals, and it is also a serious public health concern in populations all around the world [1-2]. This disease decreases the quality of life from reduced independence and hindered physical, mental, and

social well-being [3] as well as increased frailty, morbidity, and mortality in individuals diagnosed with it [4-5].

The most common cause of osteoporosis in Indonesia is the age factor, bone metabolic disorders, lack of activity, lack of protein, and a lack of vitamin D. According to the data of Health Ministry of Indonesia (2015) the prevalence of osteoporosis in women is up to 4 times higher than men and increased as the increase of age. The occurrence of osteoporosis can also occur in pets due to the treatment of Ovariohysterectomy (OH) that has long-term effects on the deficiency of ovarian hormones, particularly estrogens following the surgery [6-7].

Osteoporosis can only be prevented by eating a high calcium diet, regular exercise, taking adequate vitamin D, and minimize the cause of osteoporosis. Dietary consumption with high calcium, in general, is using milk as a source of calcium, but the current price of milk is quite expensive. The use of vitamin D supplementation would require advice from a nutritionist doctor in order not wrong in the doses which are used. This is become worse by the high hours of work or business of household activity can certainly obstruct regular sports activities [8].

Another variable used is honey as anti-osteoporosis. Honey is a natural product that is widely used for a variety of therapeutic effects. Honey contains 200 mixture of sugars (fructose, glucose, maltose and sucrose), ascorbic acid, protein and carotenoids as well as other ingredients with low levels such as minerals, protein, vitamins, organic acids, flavonoids, acid phenol, Enzymes and other chemical content [9-10]. Honey has been shown to stimulate the growth of granulation tissue and epithelial tissue so it can accelerate wound healing, effective as an antioxidant and anti-inflammatory agent [11-13]. Honey composed of major sugars content such as monosaccharides, disaccharides, oligosaccharides and polysaccharides as well as containing enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase [12, 14].

One species of honey in Indonesia is *Apis dorsata* that can be found almost in the entire archipelago of Indonesia except Maluku and Irian Jaya [15]. Of the several species of honey bees in Indonesia, Apis dorsata species are shown to contain higher phenols and flavonoids indicating that Apis dorsata honey has a high antioxidant potential [16].

Based on research, the content of Apis dorsata honey through the mechanism of flavanols on honey will interact directly with the estrogen through receptor ER-β and ER-α, the content of gluconic acid that can increase calcium absorption in the intestine and Flavonoids in honey induced RANKL in NF-κB ligand and osteoprotegerin (OPG) receptors to prevent bone loss [17-18].

Phenol content in Apis dorsata bee honey can play a role in the metabolism of bone, especially on osteoblast, some polyphenols such as EGCG (epigallocatechin-3-gallate), resveratrol and icariin can modulate several transcription factors that activate genes linked osteoblast [19]. From these two variables, it can be said that the potential of honey that can maintain the density and function of the bone organ is big, as well as the availability of materials that have anti-osteoporosis potential.

2. Materials and methods

The present study has approved by Animal Care and Use Committee Faculty of Veterinary Medicine University of Airlangga with ethical clearance certificate number 655-KE.

The honey sample was collected from tropical forest of Batulanteh Sumbawa Island. Honey was obtained from Indonesia Forest Honey Network and confirmed for the bioactive compound in Assessment Service Unit Faculty of the Pharmacy University of Airlangga and the giant honey bees which produced the honey product were taken to make species confirmation in Laboratory of the Entomology Tropical Disease Centre University of Airlangga.

Rats were obtained from Faculty of the Medicine University of Airlangga. Rats which used in this study were female wistar rat with range 195-220 gr of BW. Animal Laboratory was fed by ration 16% of crude protein and 2657 Kcal/Kg of ME which mixed and grinded in Animal Feed and Nutrition Laboratory of Faculty of the Veterinary Medicine University of Airlangga.

Twenty female rats were randomly divided into 5 groups and adapted for 3 weeks in Laboratory of Animal Laboratory Faculty of the Veterinary Medicine University of Airlangga. The groups consist of SH (Sham-Operated, which was the only laparotomy without ovarium resection), OH (Control positive as post menopause osteoporotic), and 3 gradually increased dosage T_1(1 gr/Kg BW), T_2 (2 gr/Kg BW), T_3(3 gr/Kg BW). Honey sample was solved by aquadest ad 1.5 ml. At the end of adaptation period, five groups of rats were operated under a surgical procedure with ketamine HCL 50mg/Kg BW and 10 mg/Kg BW [20] in Animal Hospital University of Airlangga. The surgery was conducted in SH was explorative laparotomy without ovarium resection. The 4 groups were resected for ovarium and its uteri. Every rat was gavaged with 1.5 ml honey solution for groups T_1, T_2, T_3 and 1.5 ml aquadest for SH and OH at post-surgery for 12 weeks. At the end of week 12, the rats were sacrificed with ketamine with dosage 50 mg/Kg BW intraperitoneal [20]. The os vertebrae lumbal 6 were dissected, cleaned and stored in formalin 10%.

Ca ash density was measured by ash density calcium analysis procedure. With 3 steps, among other ash content analysis, HCl extraction and Ca analysis. Os vertebrae lumbal 6 was dried in 105°C for 2 hours. Ash analysis procedure done with sample were burned until there was no smoke anymore, put into a furnace at 700°C for 1-5 hours, cooled sample put into exicator for 10-15 minutes and weighed. Ash analysis used :

HCl extraction added with Chapman solution (boiled) for 30 minutes, NH_4OH concentrated (25%-27%) added until it turns green colour stored for 1 night, filtered and added with $H2SO4$ 25% boiled at 80-90°C, titration with $KMnO4$ 0.02N. Blanco was made by 2.5cc HCl 2.5% diluted with 8.5cc concentrated HCl.

$$\text{CaO density} = \frac{(\text{V. Sample Titration} - \text{V. blanco titration}) \times \text{Titer } KMnO_4 \times C \times 28}{\text{mg Sample}} \times 100\%$$

$$\text{Ca density} = \frac{\text{Atom Weigh Ca} \times \%CaO}{\text{Molecule Weigh CaO}}$$

With:

Atom Weigh Ca = 40

Molecule Weigh CaO = 56

Statistical analysis was carried out using SPSS for ANOVA.

3. Results

The effect of honey on the bone has increased the density of trabecular histopahology [17]. in detail, the result of this study was based on, Ca ash density as follow. According to [21] significantly bone loss in os vertebrae lumbal occurred after 60 days. CAD of os femur with low calcium feed intake was highly decreased for 8 weeks compared with normal bone [22]. In contrast, the result shows different significance. The higher dosage of honey solution, the smaller CAD (**Table 1**).

Based on research of [22], ash density ovariohysterectomized of os femur significantly loss compared with SHAM operated. From the result that rats which given sham-operated (SH) has almost the same result with rats which induced using ovariohysterectomy (OH). It was could be caused by remodeling of the bone. The term bone remodeling refers to a process characterized by the demolition and subsequent reconstruction of microscopic portions of the calcified bone matrix [21]. Rats which induced osteoporosis and given treatment (T1, T2, T3) were significantly osteoporosis. In contrast, the result of this study contradicts with the report of [23]. Honey gave negative effect for treatment of osteoporosis its means using honey of Apis dorsata from Sumbawa island can't be used as alternative medicine in a postmenopausal woman.

Table 1: Analysis of Average Ca Ash Density.

Treatment	X ± SD
SH	6.16 ± 1.15^{a}
OH	5.64 ± 0.90^{a}
T_1	4.68 ± 0.67^{ab}
T_2	3.87 ± 0.51^{b}
T_3	3.72 ± 0.53^{b}
The different superscript alphabet in the same column indicate significantly different (P<0.05)	

From this study, we can conclude that ovariohysterectomized white rats which given Apis dorsata honey/ as the treatment of this experiment did not increase CAD. Between SH and OH have a similar result with T1 CAD. Between SH and T3 have a significantly different result. Sixth lumbar vertebrae which induced osteoporosis by ovariohysterectomy treatment for 3 months did not show any alteration. At last, Apis dorsata Sumbawa honey showed pro-osteoporosis medicine.

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