

## Protective Effect of Propolis against Osteoporosis in Rat Model

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### ABSTRACT

*Osteoporosis is a global problem that affects adversely the post-menopausal women and elderly men as well. It is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk. The present study aimed to evaluate the effect of Egyptian propolis in the prevention of osteoporosis induced by ovariectomy (OVX) in rats. The biochemical, bone mineral density (BMD), histopathological, morphometric and scanning electron microscopic (SEM) analyses of treated animals were performed. The increase in BMD, serum calcium (Ca), femur shaft width and decrease in serum osteocalcin, alkaline phosphatase, tartrate-resistant acid phosphatase, urinary Ca levels are higher in rats treated with propolis than those of OVX group, which indicate marked restorative action. SEM study revealed porous and erosive appearance of femur bone at the epiphyseal region, shaft and head in the OVX rats when compared with sham operated rats. Treatment with propolis decreased the resorption and maintained the intactness and integrity of the femur surface indicating its usefulness in the prevention of bone loss.*

**Key Words:** Osteoporosis, Ovariectomy, Propolis, BMD, SEM

**Abbreviations:** OVX, ovariectomized rats; BMD, bone mineral density; OC, osteocalcin; ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; SEM, Scanning Electron Microscopy; Ca, calcium.

### INTRODUCTION

Osteoporosis is a metabolic bone disease characterized by a defect in bone mineralization. After age 40, a slow process of bone loss begins in both sexes and continues until late of life. In women after menopause,

there is an accelerating rate of bone loss because of the decreasing estrogen secretion associated with aging<sup>1</sup>. Hormone replacement therapy (HRT) has been established as a regime for prevention of postmenopausal bone loss<sup>2</sup>. However, recent evidence indicates

that its long-term use is accompanied by side effects, such as increased risk of breast, ovarian and endometrial cancer<sup>3</sup>.

Bisphosphonates are currently the major drugs used to treat osteoporosis. However, they are associated with side effects as esophageal cancer and osteonecrosis of the jaw<sup>4</sup>. Thus, alternative means of proven efficacy and safety should be developed for prevention and treatment of postmenopausal osteoporosis.

**The polyphenolic compounds** (flavonoids and phenolic acids) have received some attention for their potential role in preventing osteoporosis<sup>5</sup>. Also, flavonoids have been characterized as selective estrogen receptor modulators with similar beneficial effects to raloxifene on bone<sup>6</sup>. Flavonoids have been shown to inhibit bone loss in rats, both by slowing resorption and by increasing osteoblastic activity, resulting in increased bone strength<sup>7</sup>.

**Propolis** is a resinous substance collected by the bees from various tree buds. Propolis composition is directly related to that of bud exudates collected by bees from various trees. Propolis has attracted researchers' interest in the last decades because of several biological properties, such as immunomodulatory, antitumor, antimicrobial, antiinflammatory, antioxidant, among others. More than 300 constituents have been identified in different propolis samples<sup>8</sup>. Flavonoids, aromatic acids, terpenoids, steroids and phenolic compounds appear to be the principal components responsible for the

biological activities of propolis<sup>9,10</sup>. The potential estrogenic activity of propolis was investigated *in vitro* using the MCF-7 human breast cancer cell proliferation<sup>11</sup>, human estrogen receptor binding and yeast-based steroid receptor transcription, and *in vivo* using the immature rat uterotrophic effect<sup>12</sup>.

**The aim** of the present study was to evaluate the effect of Egyptian propolis in prevention of osteoporosis induced by ovariectomy in rats (an established model for postmenopausal osteoporosis<sup>13</sup>). **To our knowledge**, the present study is the first research of the protective effects of **propolis** against high bone turnover, loss of bone Ca and reduced Ca retention associated with estrogen deficiency in OVX animals.

## MATERIALS & METHODS

This study was supported by National Research Centre Cairo Egypt [project No. 2/3/5].

**Propolis:** Propolis was collected from Gharbia province of east area of Nile Delta, Egypt. The sample was collected during March 2010.

**Propolis Extraction and Sample Preparation for HPLC:** One g propolis was cut into small pieces and extracted at room temperature with 50 ml of 70% ethanol (twice after 24 hours). The alcoholic extract was evaporated under vacuum at 50 °C until dryness. The dry extract was dissolved in methanol and filtered through a 0.45- $\mu$ m filter before HPLC analysis.

**HPLC Analysis of Propolis Polyphenolics:** The HPLC analysis was achieved with Agilent 1100

series liquid chromatography with UV detector and an auto-sampler, the method was mentioned previously<sup>10,14</sup>.

**Polyphenolics Identification and Quantification:** Polyphenolics were identified by chromatographic comparisons with authentic samples. Response factors for the authentic samples and the concentration of polyphenolics in propolis sample was calculated<sup>15</sup>.

**Preparation of propolis extract:** Propolis 70% ethanol extract was freshly prepared; solvent was evaporated under reduced pressure to a syrupy form.

**Acute toxicity (OECD guidelines 425 adoption):** Twenty healthy Wistar albino rats of either sex were randomly divided into two groups of equal size. Animals of both groups were fasted overnight before the test. The first group was given 5000 mg/kg body weight of freshly prepared ethanol extract of

propolis while the other group was given an equivalent volume of saline. The animals were observed immediately and then after 30 min, 1, 2, 4, 6 h and thereafter daily for 14 days. At the end of the fourteenth day the animals were sacrificed with excess ether anaesthesia and dissected for examination of vital organs.

**Ethics:** The study followed the regulations of Medical Research Ethics Committee, National Research Center, Egypt (i.e., Rules for the Care and Use of Laboratory Animals, no. 10-132).

**Animals:** Wistar female albino rats (150-160 g) from National Research Center animal house (Egypt) were used in the current study. Rats were fed a casein based diet prepared according to the AIN-93M diet<sup>16</sup>, (Table 1) and water *ad libitum*. The environmental conditions were standardized with respect to temperature, humidity and light.

**Table 1: Composition of casein based diet (AIN-93M diet)**

Ingredients Casein based diet	(g/kg diet)	Ingredients Casein based diet	(g/kg diet)
Corn starch	620.692	Mineral mixture (AIN-93M-MX)	35
Casein (85% protein)	140	Vitamin mixture (AIN-93M-VX)	10
Sucrose	100	L-cystine	1.8
*Corn oil	40	Choline chloride	2.5
Fiber	50	Ter-butylhydroquinone	0.008

\*Corn oil was used instead of soybean oil to eliminate any possible interference with isoflavones in soybean oil.

**Animal Experiment:** After two weeks of acclimatization, the rats were anaesthetized with sodium pentobarbitone (35 mg/kg, i.p.), and bilateral ovariectomy was done

aseptically (OVX). Sham operation (group I, n=6) was done in the same manner but only exposing the ovaries. The animals were given prophylactic ampicillin (4000 IU/kg,

i.p.) for 3 days and coloplast paste (Humlebaek, Denmark) applied locally. After 24 weeks, the OVX rats were randomly divided into four groups and orally treated with H<sub>2</sub>O (OVX group II) and 400 mg propolis / kg (group III) as daily dose for 9 weeks. The sham-operated group (I) was orally treated with H<sub>2</sub>O. The body weight of each animal was determined once a week until the final day of administration.

**Serum Chemistry:** Serum calcium (Ca), phosphorus (P), magnesium (Mg), alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) concentrations were measured by standard colorimetric methods using kits from BioDiagnostic (Cairo, Egypt). Serum Osteocalcin (OC) was measured using an enzyme linked immune sorbent assay (ELISA) kit specific for rat OC (Biomedical Technology, Staughton, IN, USA).

**Urine Chemistry:** Urine Ca, P and creatinine (Cr) values were analyzed by the same method used for the serum samples.

**Measurement of bone mineral content (BMC) and bone mineral density (BMD):** The BMC and BMD were made at the proximal, distal and total femur by dual energy X-ray absorptiometry PIXImus (GE Lunar Co, Wisconsin, USA).

**Histopathological Studies of Bone** Histopathological analysis was carried out on femur of rats. After the rats were sacrificed the right femurs were removed, dissected free of soft tissue and fixed in 10 % buffered formal saline. The specimens were then decalcified in EDTA solution for 10-14 days at 5°C. Tissues were

dehydrated in graded alcohols and embedded in paraffin. 5µm sections were cut and stained with hematoxylin and eosin (H & E) for morphological study<sup>17</sup>.

**Morphometric Measurement:** Quantitative analysis measurement was achieved by using computerized image analyzer (Leica Qwin 500 image). The thickness of the shaft of the femur was measured in longitudinal sections of all groups (10 fields per section at power magnification x 40). Mean shaft thickness for each group was obtained and subjected for statistical analysis.

**Scanning Electron Microscopy (SEM)** The frozen femurs were placed in 5% sodium hypochlorite solution (Commercial Bleach) for 4 h. The bones were then dehydrated in ethanol and dried, mounted on stubs and coated with gold using a sputter coater.<sup>18</sup> The bones were examined on JEOL JXA-840A electron Probe Microanalyzer, for the observation of qualitative bone resorption at the epiphyseal edges, shaft and head of femur.

**Statistical analysis:** The data was analyzed using one way ANOVA followed by post hoc Sheffe's Test using SPSS computer software Version 7.5. Level of significance was measured at 0.05 and 0.01. t paired test was used for the analysis of weekly weight gain.

## RESULTS

### HPLC Quantitative Analysis of Propolis Polyphenolics:

The polyphenolics present in propolis sample was studied by HPLC

analysis. A total of 27 compounds were detected in propolis sample, from which 3 isoflavones (formononetin, genistein, prunetin) were identified. Naringenin,

quercetin-3,3'-dimethylether and pinocembrin were highly detected in propolis sample (Table 2).

**Table 2.HPLC Quantitative Analysis of Propolis Polyphenolics**

No	Name	mg/g propolis	No	Name	mg/g propolis	No	Name	mg/g propolis
1)	Hydrocinnamic acid	18.1	10)	Quercetin-7-methylether	4.6	19)	Hesperetin	3.9
2)	Hydrocaffeic acid	15.7	11)	Dimethylallyl caffeate	2.4	20)	8-Methoxy-kaempferol	0.75
3)	Coniferyl alcohol	2.8	12)	Pinocembrin	39.4	21)	Apigenin	1.02
4)	Caffeic acid	1.6	13)	Luteolin	3.7	22)	Luteolin-3'-methylether	5.4
5)	Eriodictyol	4.7	14)	Quercetin	1.4	23)	Prunetin	3.0
6)	Liquiritigenin	2.0	15)	Naringenin	14.5	24)	Formononetin	1.8
7)	Myricetin	5.6	16)	Pinobankasin	0.83	25)	Acacetin	7.0
8)	Quercetin-3,3'-dimethylether	22.8	17)	Quercetin-3-methylether	1.3	26)	Biochanin A	10.2
9)	Formononetin	10.1	18)	Genistein	1.9	27)	Pinostrobin	1.2
							<i>Total</i>	187.7

**Animal Weight:** The results of the present study indicate that body weight gain was higher in OVX group than that in sham group ( $p < 0.01$ ). The weight gain for the OVX rats with control diet was not statistically different from the OVX treated group (Table 3).

**Table 3.** Body weight of sham group, ovariectomized group and Propolis group.

	Week 0	Week 24	Week 33
<b>Sham</b>	121.50±0.38	232.23±0.48 <sup>b,d</sup>	245.10±0.14 <sup>b,d</sup>
<b>OVX</b>	121.08±0.61	305.78±0.27 <sup>a,c</sup>	310.70±0.25 <sup>a,c</sup>
<b>Propolis</b>	120.95±0.17	307.97±0.17 <sup>a,c</sup>	309.05±0.16 <sup>a,c</sup>

Values are given as mean ± SE for six rat in each group. **a**, significant difference at  $P < 0.05$  compared to sham; **b**, significant difference at  $P < 0.05$  compared to ovx; **c**, significant difference at  $P < 0.01$  compared to sham.; **d**, significant difference at  $P < 0.01$  compared to ovx.

**Serum Chemistry:** The serum alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP) and osteocalcin (OC) activity of sham, OVX and OVX treated group are shown in Table 4. Rats of OVX group showed a significant increase in serum ALP, TRAP and OC activity when compared to animals of sham group,  $P < 0.01$ . This increase was significantly lowered ( $P < 0.01$ ) in OVX treated group. Likewise, the significant decrease in serum calcium (Ca), phosphorus (P) and magnesium (Mg), in ovariectomized animals, compared to sham, was effectively

increased in rats on OVX treated group ( $P < 0.05$ ), Table 4.

**Urine Chemistry:** The urinary calcium, phosphorus and creatinine concentration together with Ca:Cr ratio of sham group, ovariectomized and propolis treated group are shown in Table 5. Compared with the sham-operated group, OVX animals showed a significant increase in all the urinary parameters studied. Elevated responses of all these parameters were significantly decreased in treated group at  $P < 0.01$ .

**Table 4:** The serum alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), osteocalcin (OC), calcium (Ca), phosphorus (P) and magnesium (Mg) concentration in the different studied groups.

parameter	Sham	OVX	Propolis
ALP (U/l)	15 <sup>^</sup> .42±15.87 <sup>b,d</sup>	275.14±3.35 <sup>a,c</sup>	156.14±11.27 <sup>b,d</sup>
TRAP (U/l)	48.99±0.866 <sup>b,d</sup>	61.9±0.50 <sup>a,c</sup>	40.8±1.43 <sup>a,b,d</sup>
OC (ng/ml)	0.55±0.18 <sup>b,d</sup>	3.1±0.71 <sup>a,c</sup>	0.45 ±0.08 <sup>b,d</sup>
Ca (mg/dl)	9.70±1.59 <sup>b,d</sup>	8.72±1.43 <sup>a,c</sup>	9.11±1.49 <sup>a,b,c</sup>
P (mg/dl)	5.74±0.11	5.4 ± 0.13	6.01± 0.07 <sup>b,d</sup>
Mg (mg/dl)	2.53±0.41 <sup>b</sup>	2.38±0.38 <sup>a</sup>	2.44±0.38

Values are given as mean±SE for six rat in each group. **a**, significant difference at  $P < 0.05$  compared to sham; **b**, significant difference at  $P < 0.05$  compared to ovx; **c**, significant difference at  $P < 0.01$  compared to sham.; **d**, significant difference at  $P < 0.01$  compared to ovx.

**Table 5.** Urinary Ca, P, and creatinine excretion values in the different studied groups.

	SHAM	OVX	Propolis
Ca (mg/24 h)	0.078±0.049 <sup>b,d</sup>	0.47± 0.105 <sup>a,c</sup>	0.081±0.018 <sup>a,b,c,d</sup>
P (mg/24 h)	0.54 ± 1.83 <sup>b,d</sup>	0.931±1.07 <sup>a,c</sup>	0.825 ± 1.61 <sup>a,b,c,d</sup>
Cr (mg/24 h)	0.28±0.48 <sup>b,d</sup>	0.532±2.041 <sup>a,c</sup>	0.360±0.06 <sup>a,b,c,d</sup>
Ca/Cr ratio	0.28±0.005 <sup>b,d</sup>	0.883±0.005 <sup>a,c</sup>	0.225±0.001 <sup>b,d</sup>

Values are given as mean±SE for six rat in each group. **a**, significant difference at  $P < 0.05$  compared to sham; **b**, significant difference at  $P < 0.05$  compared to ovx; **c**, significant difference at  $P < 0.01$  compared to sham.; **d**, significant difference at  $P < 0.01$  compared to ovx.

**Table 6:** Bone mineral content (g), bone mineral density (g/ cm<sup>2</sup>) in proximal, distal and total regions for femur in the different studied groups.

Parameters	GROUPS		
	SHAM	OVX	Propolis (P)
<b>BMD (Proximal)</b>	0.113±0.012 <sup>b,d</sup>	0.104±0.015 <sup>a,c</sup>	0.122±0.09 <sup>a,b,c,d</sup>
<b>BMC (proximal)</b>	0.094±0.004 <sup>b,d</sup>	0.072±0.036 <sup>a,c</sup>	0.090±0.039 <sup>a,b,c,d</sup>
<b>BMD ( Distal )</b>	0.106±0.046 <sup>b,d</sup>	0.099±0.028 <sup>a,c</sup>	0.115±0.037 <sup>a,b,c</sup>
<b>BMC( Distal )</b>	0.103±0.009 <sup>b,d</sup>	0.056±0.032 <sup>a,c</sup>	0.093±0.033 <sup>a,b,c,d</sup>
<b>BMD (Total )</b>	0.119±0.003 <sup>b,d</sup>	0.105±0.005 <sup>a,c</sup>	0.125±0.058 <sup>a,b,c,d</sup>
<b>BMC (Total )</b>	0.256±0.021 <sup>b,d</sup>	0.188±0.092 <sup>a,c</sup>	0.279±0.012 <sup>a,b,c,d</sup>

Values are given as mean±SE for six rat in each group. **a**, significant difference at  $P < 0.05$  compared to sham; **b**, significant difference at  $P < 0.05$  compared to ovx; **c**, significant difference at  $P < 0.01$  compared to sham.; **d**, significant difference at  $P < 0.01$  compared to ovx.

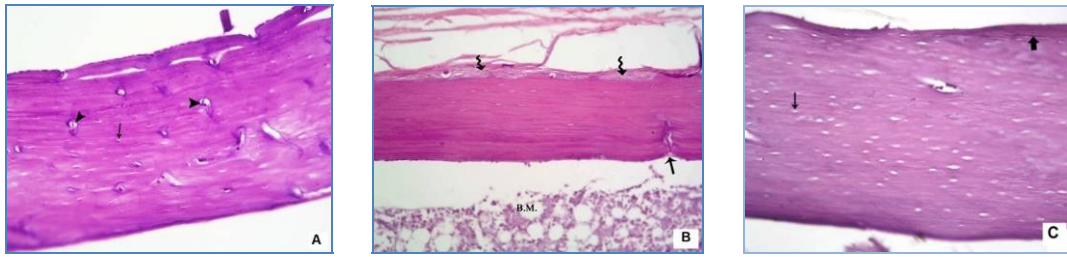
**BMC and BMD:** Animals in the OVX group had significantly lower density and content of the proximal, distal and total femur ( $P < 0.01$ ) compared with the sham group. propolis group was seen to recover the density of right femur bones significantly, ( $P < 0.01$ ) (Table 6).

#### **Histopathological Studies in Bone:**

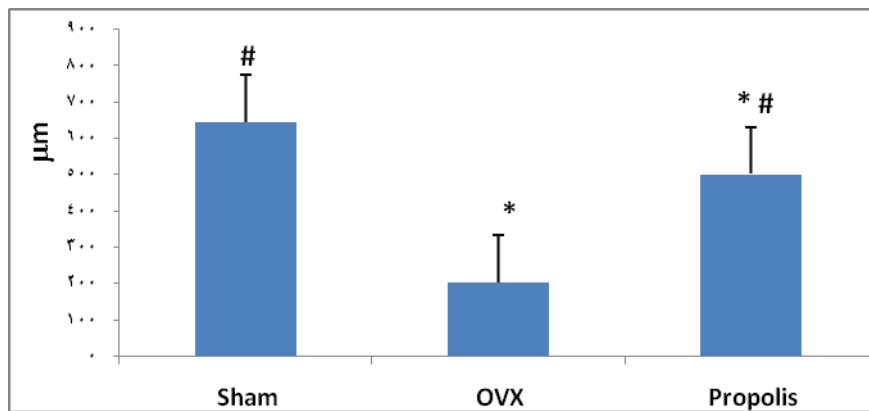
The histopathological sections of the middle shaft of the femur bone of a sham group showed the normal architecture of the bone tissue (Fig. 1 A). The ovariectomized group shows irregular resorption areas under the endostium and under the periostium (Fig. 1 B). Examination of the middle shaft of the femur bone of a rat treated with propolis extract showed improvement of bone tissue architecture in the form of regularity of both inner and outer surfaces of the bone tissue although the Haversian canals are still widened (Fig. 1C).

**Morphometric results:** The femur shaft of animals in the **sham group** showed a mean total thickness 643.18 µm, in the **ovariectomized group** the thickness decreased to 203.08 µm. With propolis treatment, the shaft thickness returned more or less back near to the sham group value (501.24 µm) ( $P < 0.01$ ), (Fig. 2).

**Scanning electron microscopy:** The epiphyseal edges of the distal part of the femur bone, its shaft and head were observed to study the resorption pattern in all groups. Extensive resorption was detected in ovariectomized animals (Fig.3; b1,b2 and b3) when compared with sham operated animals in which the bony surfaces lacked any resorption pits (Fig.3; a1,a2 and a3). In propolis group the surfaces of the epiphyseal edges, the shaft and the head of the femur were almost similar to the sham group (Fig.3; c1,c2 and c3).

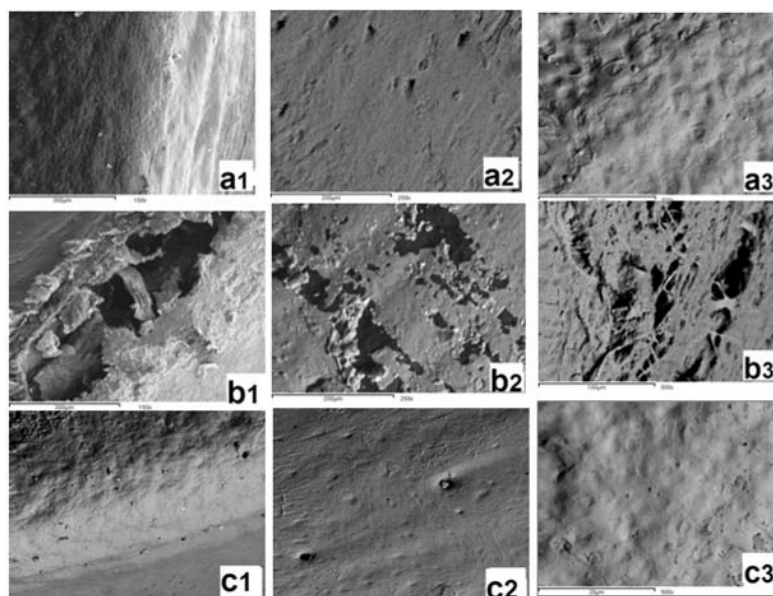


**Fig. 1:** Photomicrographs of a longitudinal section in the shaft of the femurs of a female albino rat, **A:** of the **shamgroup** showing Volkmann's canals (arrow head) and osteocytes inside their lacunae (arrow). **B:** of the **ovariectomized group** showing irregular resorption areas under the endostium (arrow) and under the periostium (zigzag arrow). Note the medullary cavity with the bone marrow inside (B.M.). **C:** of the **propolis group**, normal bone architecture is noted; cement lines can be seen (thick arrow) with no separation of the bone lamellae. The osteocytes are seen inside their lacunae (thin arrow).



**Fig. 2:** The thickness of the shaft of the femur of all groups. Values are given as mean  $\pm$  SE for six rats in each group. \*, Significant difference at  $P < 0.01$  compared to Sham group. #, significant difference at  $P < 0.01$  compared to OVX group.





**Fig. 3:** Scanning electron photomicrographs (150X) demonstrating resorptive surface in remodeling sites at the epiphyseal edges of femur from rats of sham operated (**a1**), Ovariectomized (**b1**) and **propolis** group (**c1**). Scanning electron photomicrographs for Shaft (**a2**, **b2** & **c2**, 250X) and for femur head (**a3**, **b3** & **c3**, 500X). Note the increased number of resorption pits in OVX group compared with sham and treated groups.

## DISCUSSION

Ovarian hormone deficiency associated osteoporosis following menopause is the most common cause of age-related bone loss. This disorder is characterized by reduced amount of bone leading to diminished physical strength of the skeleton and an increased susceptibility to fracture<sup>5</sup>.

Phytoestrogens are a group of biologically active plant substances with a chemical structure similar to that of estradiol, an endogenous estrogen. This structural similarity accounts for the ability of these compounds to bind to estrogen

receptors (ERs) and exert various estrogenic or antiestrogenic effects<sup>19</sup>.

**To our knowledge**, the present study is the first demonstration of the protective effects of **propolis extract** against high bone turnover, loss of bone Ca and reduced Ca retention associated with estrogen deficiency in OVX animals.

As expected, OVX animals in the present study exhibited all the characteristics associated with estrogen deficiency, such as weight gain, negative calcium balance, high bone turnover and uterine atrophy<sup>13</sup>.

The results of this study indicate that body weight gain was higher in OVX group than in sham group

( $p < 0.01$ ) and the weight gain for the OVX rats was not different from that of **propolis treated** group; a positive association between bone density and body weight has been well documented<sup>20</sup>. It also was devoid of any uterotrophic activity because uterine weight was not different in OVX and propolis treated group (data not shown). The results suggest that propolis extract at the given dose did not behave like estrogen in the regulation of body weight and uterine tissue growth in the OVX rats.

The serum activity of alkaline phosphatase (ALP) and osteocalcin (OC), an index of bone formation<sup>21</sup>, has been reported to be significantly greater in an OVX group than in a sham group. Tartarate-resistant acid phosphatase (TRAP) is released during the bone-resorbing activity of osteoclasts<sup>22</sup>, a similar change was observed in this study. **Propolis treatment significantly decreased** the activity of serum (ALP, TRAP and OC).

Fasting urinary calcium excretion and **calcium to creatinine ratio** could also be used as an important variable for estimating net bone resorption. Again, the treatment with propolis reduced the urinary excretion of calcium and **calcium to creatinine ratio** to a lower level than that of OVX group. Urinary excretion of Ca was elevated in the OVX rats<sup>23</sup>. **Since a rise in serum alkaline phosphatase and the urinary calcium to creatinine ratio have been linked with collagen degradation, bone resorption and osteoporosis**<sup>24</sup>.

As expected, OVX resulted in significant decrease in the **femur**

**BMD** after **33** weeks. This loss of bone mass was accompanied by a significant increase in bone remodeling, as was evidenced by the increased levels of the bone turnover markers (serum OC, ALP and TRAP). Treatment with propolis prevented the decreases in BMD, which was reflected by the decreases in serum OC, ALP and TRAP levels, indicating a reduction in bone turnover. A decrease in urinary calcium excretion and increase in serum calcium might contribute to the increase in BMD<sup>25</sup>.

The rate of bone turnover after ovariectomy can be attributed to the absence of estrogen. Estrogen has the ability to decrease the differentiation of the bone resorbing osteoclast progenitor cells<sup>26</sup>, inhibit the bone resorbing activity of terminally differentiated osteoclasts<sup>25</sup>. That is why estrogen deprivation can cause repeated activation of the bone remodeling mechanism and elevate the rate of bone turnover<sup>27</sup>.

The mechanisms by which propolis flavonoids positively affect bone turnover rate may be directly by interacting with estrogen receptors ER- $\beta$ . The rat and human ER exists as two subtypes ER $\alpha$  and ER $\beta$ . ER $\beta$  is more abundant than ER $\alpha$  in bone tissue while ER $\alpha$  is mainly distributed in reproductive system, especially the breast and uterus. Thus, propolis flavonoids, as naturally occurring Selective Estrogen Receptor Modulators, might show higher affinity for ER $\beta$  than for ER $\alpha$  that produces optimal action in preventing bone loss without stimulating an unwanted proliferation of the uterine tissues<sup>20</sup>.

Some sterols have close structural relationship to estradiol and bind to estrogen receptors alpha and beta<sup>28</sup>. In previous studies, propolis contained beside flavonoids, some steroids and terpenoids, which may contribute to this effect<sup>10</sup>. Another possibility would be that the polyphenolic compounds such as flavonoids in propolis extract affect bone, at least in part, through estrogen receptors (ER) as phytoestrogens do<sup>26</sup>.

Our results were confirmed by **histopathological** examination of the femurs. Propolis treated group revealed increase in shaft width than that with OVX group, which indicate marked restorative action, thus suggesting that the protective action of the propolis treatment may be due to an increase in bone formation with a reduction in bone resorption.

**SEM** is used to determine the pattern of bone resorption at the epiphyseal region of distal femur, shaft and head which predominantly contain the areas of bone resorption. Porous and erosive appearance of femur at the epiphyseal edges was more pronounced and prevalent in OVX animals when compared with shamoperated. Treatment with propolis decreased the resorption and maintained the intactness and integrity of the surface.

## CONCLUSION

The present study clearly demonstrates that daily oral administration of propolis over nine weeks period in the adult OVX rat could ameliorate the estrogen

deficiency-induced bone loss in ovariectomized rats.

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## التأثير العلاجي لصمغ النحل على نموذج لهشاشة العظام فى حيوانات التجارب

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هشاشة العظام مشكلة عالمية تؤثر على النساء فى مرحلة انقطاع الطمث وكذلك تؤثر على الرجال مع تقدم العمر، وهذا المرض يتميز بانخفاض فى كثافة العظام و تدهور فى شكل النسيج العظمى مما يؤدى الى سهولة كسر العظام.

اجرى هذا البحث لدراسة التأثير العلاجي لصمغ النحل على نموذج لهشاشة العظام فى حيوانات التجارب. اوضحت نتائج القياسات أن صمغ النحل له تأثير علاجي واضح لنموذج هشاشة العظام (الفئران التى أزيل الرحم لها). إذ أن كثافة العظام قد أنخفضت الى حد كبير بعد ٣٣ أسبوع وهذا الانخفاض مؤكد بوجود زيادة فى الدلائل الموجودة فى الدم (أوستيوكلستين، الفوسفاتاز القلوى والفوسفاتاز الحمضى) والدلائل الموجودة فى البول. أما فى الفئران التى تم معالجتها بصمغ النحل وجد أن هذه الدلائل قد أنخفضت وان كثافة العظام قد زادت تقريبا الى المستوى الطبيعى. وقد دُعمت النتائج بالدراسة الشكلية لفخذ الفئران حيث وجد أن عرض الساق قد زاد فى المعالجة بصمغ النحل وذلك نتيجة لأن عملية بناء العظم أكبر من عملية الهدم وأكدت النتائج بالفحص بالميكروسكوب الألكترونى اذ ظهرت هشاشة واضحة فى مجموعة نموذج هشاشة العظام وقد ادى العلاج الى تحسن واضح فى المجموعه المعالجة.