EFFECT OF PROPOLIS ON BONE LOSS PREVENTION- A REVIEW

Sowmya S¹, Anil Kumar Gujjari², K. Mruthunjaya³, Sushma R⁴, Ravi M. B⁵, K. N. Raghavendra Swamy⁶

¹Lecturer, Department of Prosthodontics and Crown & Bridge, JSS Dental College and Hospital, Jagadguru Sri Shivaratheswara University, Mysore.

²Prof & Principal, Department of Prosthodontics and Crown & Bridge, JSS Dental College and Hospital, Jagadguru Sri Shivaratheswara University, Mysore.

³Professor, Department of Pharmacognosy, JSS College of Pharmacy, Jagadguru Sri Shivaratheswara University, Mysore.

⁴Lecturer, Department of Public Health Dentistry, JSS Dental College and Hospital, Jagadguru Sri Shivaratheswara University, Mysore.

⁵Reader, Department of Prosthodontics and Crown & Bridge, JSS Dental College and Hospital, Jagadguru Sri Shivaratheswara University, Mysore.

⁶Professor, Department of Prosthodontics and Crown & Bridge, JSS Dental college and Hospital, Jagadguru Sri Shivaratheswara University, Mysore.

ABSTRACT

BACKGROUND

Statement of problem- Bone loss caused by excessive osteoclast formation is the major mechanism underlying many bone diseases leading to problems in dentistry, affecting the treatment plan and patient's quality of life. Despite many treatment options, a development of novel therapies that are natural, safe, effective and economical is vital for improved strategies against bone loss. Propolis, a natural material that honeybees collect from living plants has been reported to have multiple biological effects including bone loss prevention activities. But there is no systematic review available that has evaluated the effect of propolis on bone loss prevention.

MATERIALS AND METHODS

The articles with the clinical and in vitro investigations that assessed the prevention and treatment of bone loss using propolis were selected. Electronic database was searched from 1978 to 2016 using the keywords "Propolis" or "propolis" and "bone loss" or "propolis" and "prevention of bone loss."

RESULTS

Propolis, which contains a component called Caffeic Acid Phenethyl Ester (CAPE) has an effect on prevention of bone loss. The mechanism of action of CAPE is by inhibiting NF-kappa B and NFAT activation. This in turn results in attenuation of osteoclastogenesis and bone resorption, implying that CAPE can be potential treatment for bone loss prevention.

CONCLUSION

The literature review suggests that the use of propolis can prevent bone loss. However, as most of the studies were in vitro, there is insufficient reliable evidence to truly provide a recommendation regarding this natural compound. A well-designed, randomised controlled trials are needed to provide answers to these questions.

KEYWORDS

Propolis, Bone Loss, Natural Remedies.

HOW TO CITE THIS ARTICLE: Sowmya S, Gujjari AK, Mruthunjaya K, et al. Effect of propolis on bone loss prevention- a review. J. Evolution Med. Dent. Sci. 2017;6(93):6785-6790, DOI: 10.14260/jemds/2017/1468

BACKGROUND

Bone diseases are increasing as ageing population is growing. The current treatment has various drawbacks. Over the past several years, using cell and signalling based screening techniques as well as computer-assisted small molecule docking approaches, screening regarding osteoclast inhibitors from natural compounds has continued. In this regard, propolis is one such natural compound which has gained interest of researcher.

Propolis is produced by bees that live socially. Bees collect the resin from cracks in the bark of tree and leaf buds. This resin is masticated, salivary enzymes added and partially digested.

Then it is mixed with beeswax and used in the hive to seal and protect the hive against intruders.¹ From the ethno botanical perspective, propolis is one of the few "natural remedies" that continue to be used for a long period by different civilisations.² Egyptians used propolis to embalm their cadavers. Incas used propolis as an anti-pyretic agent. Greek and Roman physicians used it as mouth disinfectant, antiseptic agent and also as healing product in wound treatment. Propolis is widely used in popular medicine, especially in communities with inadequate public health conditions.^{3,4,5}

CAPE, which is the component of propolis has many beneficial properties. Few among them are anti-mitogenic, anti-carcinogenic, anti-inflammatory and immunomodulatory properties. Literature reported that CAPE also has a property

'Financial or Other Competing Interest': None.
Submission 23-10-2017, Peer Review 23-11-2017,
Acceptance 29-11-2017, Published 11-12-2017.
Corresponding Author:
Dr. Sowmya S,
Prosthodontics and Crown & Bridge,
JSS Dental College and Hospital,
Jagadguru Sri Shivaratheswara University, Mysore.
E-mail: sowmya.neelan@gmail.com
DOI: 10.14260/jemds/2017/1468

to inhibit activation of osteoclast, thus prevents bone loss.⁶ However, till date the therapeutic potential information of CAPE for bone loss is scanty.

To our knowledge, there is no systematic review available that has evaluated the effect of propolis on bone loss prevention. Hence, this review aims at investigating the literature relating to the effectiveness of propolis (CAPE) on prevention and treatment of bone loss.

The objective of a systematic review is to provide a comprehensive and contemporary appraisal of research using transparent methods, while aiming to minimise bias. If such conditions are met, there should be greater confidence in the conclusions of the review than in other summaries of clinical evidence.

MATERIALS AND METHODS

Broad literature search was carried out over a period of three months from July to September 2016. The literature from 1978 to 2016 was considered for retrieval and discussion. Electronic databases were searched (Scopus, PubMed/Medline and Cochrane Database of Systematic Reviews) using the following keywords: "propolis" or "propolis" and "bone loss" or "propolis" and "prevention of bone loss." All of the articles that met the inclusion criteria were evaluated and selected. The inclusion criteria for the articles in this review are-

1. Studies relevant to the objective of this review.
2. In vivo, in vitro, in situ.
3. Articles published in English language.
4. Studies with outcome parameters- "propolis in prevention of bone loss."

RESULTS

The following information was recorded from each article

Sl. No.	Author and Year of Study	Study Design	Route of Administration	Duration of Therapy	Investigations Done	Conclusion/ Recommendation
1	Burcu A. Altan 2013 ⁷	Animal study model (Rat)	Systemic	17 days	Histomorphometric examination	Systemic use of propolis may hasten new bone formation at the expanded suture in rats.
2	Bushra Habeeb Al-Molla 2014 ⁸	Animal study model (Rabbit)	Systemic	4 weeks	Histological and immunohistochemical tests	Clinical application of amelogenin and propolis coating material for enhancing the osseointegration around the dental implant in short interval period.
3	Bereket et al 2014 ⁹	Animal study model (Rabbit)	Systemic	4 weeks	Dual-energy x-ray and stereologic analysis	Propolis accelerates bone formation and may shorten the consolidation phase with distraction osteogenesis.
4	Duan Wangping 2014 ¹⁰	Animal study model (OP mice)	Systemic	4 weeks	Micro-CT scan and histological analysis	With CAPE (component of propolis) treatment, the microarchitecture of the bone significantly improved with a reduction of osteoclast formation. CAPE treatment can improve bone mineral density and micro-architecture of bones and attenuate osteoclast formation.
5	Cüneyt Asım Aral et al 2015 ¹¹	Animal study model (Wistar rats)	Systemic and Local	21 days	Histomorphometric analysis	Propolis reduced fasting blood glucose levels in diabetes. In addition, propolis might be beneficial as an adjunct treatment of diabetes associated periodontitis and periodontitis without diabetes.
6	Hakki Oguz Kazancioglu 2015 ¹²	Animal study model (Sprague Dawley rats)	Systemic	15 days	Histomorphometric analysis	CAPE enhances new bone formation in midpalatal suture after RME. CAPE may decrease the time needed for retention.

Table 1. Summary of the Studies Included

5. Full text articles published in the journals and accessible online.

The literature search was carried out by 2 independent researchers. To locate additional studies, the references of the selected articles and relevant reviews were also checked. A total of 127 articles were found. Two researchers independently reviewed the abstracts. After the screening and eligibility criteria had been individually considered, if an article was included and a consensus was not reached a third author was invited to discuss the article.

The Included Articles were Reviewed in Depth and Excluded according to the following Criteria-

1. Review articles, letter to editors (not containing primary data).
2. Studies that did not use any protocol for the treatment or prevention of bone loss.

From the studies included in the search strategy, 11 articles were included in the review. In spite of an attempt to include clinical studies, there were no evidence of clinical studies on bone loss prevention by propolis. After carefully reading the abstracts, 9 articles were selected for full-text evaluations; 3 of the 9 articles could not be found despite several attempts to contact journals and libraries. The study design, the type of application of propolis and the material described in the articles were recorded. Because of the large variability in the data regarding the methodologies that were used to measure the effect of propolis on prevention of bone loss, only a qualitative investigation was possible and various comparisons were made between studies in an attempt to analyse the effect of propolis on the prevention of bone loss

Burcu A et al (2013) investigated the effects of systemically given propolis on the expanded premaxillary suture in a rat study model. A total of 24 rats were utilised. They were randomly divided into three groups as only expansion (OE), expansion plus propolis (PRO) and non-expansion (control) groups. Once the 5-day expansion period was completed, the OE and PRO groups underwent 12 days of mechanical retention. Next histomorphometric examination was performed to know the number of osteoclasts and capillaries. Along with this the intensity of inflammatory cells and amount of new bone formation were also determined. Results showed a definitive increase in bone formation in the PRO group relative to the control group. "Systemic use of propolis will hasten new bone formation at the expanded suture" in rats was the conclusion of the study.⁷

Bushra Habeeb Al-Molla (2014) aimed to evaluate the effect of osteocalcin as bone formation markers in amelogenin/ propolis coated and uncoated implant. This was carried out in different interval periods like 1, 2 and 4 weeks. 30 New Zealand white rabbits were used. Commercially, pure Titanium (cpTi) implants which were coated with amelogenin/ propolis were placed in the tibias. Histological and immunohistochemical tests for detection of effect of osteocalcin were performed. An early bone formation, mineralisation and maturation in comparison were noted in amelogenin/ propolis coated titanium implant than the control group. Immunohistochemical results showed positive reaction for osteocalcin. It was expressed by osteoblast cells (OB) at implants coated with amelogenin/ propolis. This indicated that bone formation and maturation was accelerated by adding biological materials to the implant surface. The study concluded with the information that "coating of implants with amelogenin/ propolis showed increment in osseointegration in short interval period."⁸

Bereket C et al (2014) recorded the effect of propolis on new bone formation after distraction osteogenesis (DO). The study consisted of 3 groups: control group, P100 and P200. DO of the left mandible of rabbits was carried out after an osteotomy between the first molar and the mental foramen. Dual energy x-ray absorption was used to know the bone mineral content and bone mineral density at 1 and 4 weeks after the procedure. After the subjects were sacrificed, the volume of connective tissue, new bone formation and the number of capillaries were measured using stereologic analysis. After 4 weeks through the dual-energy x-ray absorption, it was noted that bone mineral content and bone mineral density were higher in the groups treated with propolis. There were no significant differences in connective tissue volume and number of capillaries among the groups when analysed with stereologic analysis. New bone volume was lowest in the P200 group. Authors concluded that propolis accelerates bone formation and may shorten the consolidation phase with DO.⁹

Duan Wangping 2014 evaluated the effect of CAPE on bone loss in OP mice using micro-computed tomography (CT) and histology. A total of 18 mice were used and divided into three groups. The 6 mice in the sham + PBS group which were intraperitoneally injected with PBS during the curing period and did not undergo ovariectomy. Twelve mice were ovariectomised (OVX) to induce OP. Out of them, 6 in the

OVX+ CAPE group were intraperitoneally injected with 0.5 mg/kg CAPE twice per week for 4 weeks after ovariectomy.

The remaining six OVX mice in the OVX + PBS group were treated with PBS. 4 weeks after ovariectomy, all the mice were sacrificed. Micro-CT scan and histological analysis was carried out after the tibias were bilaterally excised. A significant decrease in bone mineral density (BMD, $P < 0.05$) was noted with mice in the OVX + PBS group compared with the sham + PBS group, bone volume fraction (BV/TV, $P < 0.01$), trabecular thickness (Tb.Th, $P < 0.05$) and trabecular number (Tb.N, $P < 0.01$) as well as a non-insignificant increase in the number of osteoclasts (N.Oc/ B.Pm). With CAPE treatment, there was significant improvement in the micro-architecture of the tibial metaphyses with a reduction of osteoclast formation, BV/ TV in the OVX + CAPE group was significantly increased by 33.9% ($P < 0.05$) when compared with the OVX+PBS group. This reports that CAPE therapy results in the protection of bone loss induced by OVX.¹⁰

Cüneyt Asım Aral et al 2015 aimed to examine the anti-inflammatory effects of propolis with the systemic and local effects on experimental periodontitis and diabetes. Fifty-six Wistar rats were divided into seven groups: they were (1) negative-control (NC), (2) periodontitis (P), (3) diabetes (D), (4) diabetes + periodontitis (DP), (5) periodontitis + propolis (P-Pro), (6) diabetes + propolis (D-Pro) and (7) diabetes + periodontitis + propolis (DP-Pro). With the placement of ligature periodontitis was induced and diabetes was induced by streptozotocin injection. Oral gavage (100 mg/kg/day) of Propolis (Pro) was administrated. Plasma was obtained for analysis and alveolar bone level was evaluated using histomorphometric analysis. On the 21st day however the final blood glucose levels for D, DP and DP-Pro were significantly different, but compared to NC D-Pro was not significantly different ($P = .052$). There were no statistically significant differences in blood glucose concentrations between P and P-Pro between D and D-Pro and between DP and DP-Pro. It was noted that all groups showed significantly more alveolar bone loss compared with NC. In DP and DP-Pro and D and D-Pro, there was no difference. However, a significant difference in bone loss was found between P and P-Pro, tumour necrosis factor- α (TNF- α), matrix metalloproteinase-8 (MMP-8) levels and plasma interleukin 1 beta (IL-1 β) were not significantly different among groups. Study concluded by reporting that in diabetes, propolis reduced fasting blood glucose levels. In addition, propolis can be used as an adjunct treatment of diabetes associated periodontitis and periodontitis without diabetes.¹¹

Hakki Oguz Kazancioglu 2015 studied the possible effectiveness of caffeic acid phenethyl ester (CAPE) on new bone formation after RME in rat mid palatal suture. 20 Dawley rats were used in this study. The animals were divided into 2 groups as CAPE and control group. CAPE was administered systemically via intraperitoneal injection. On all animals, RME procedure was performed. Hence, the springs were placed on the maxillary incisors of rats and activated for 5 days. Later the springs were removed and replaced with short lengths of rectangular retaining wire for consolidation period of 15 days. Histomorphometric analysis was carried out to know the new bone formation. There was new bone formation in the CAPE group than the control group ($P < 0.05$). Hence, it was reported that CAPE enhances new bone

formation after RME in midpalatal suture. The author concluded the study by reporting that CAPE may decrease the time needed for retention.¹²

DISCUSSION

A limited number of eligible studies were identified in this review. When clinical studies are not available, animal experimental studies are helpful to prove cause and effect relationships and to test the potential of novel therapeutics.¹³ Various animal species are used for evaluation of bone healing in the studies. However, small animal models are more frequently used than larger animals, because they are more cost efficient and easier to handle due to ethical issues.

There are various techniques such as histomorphometry, immunohistochemical and radiological methods to investigate bone formation. Histomorphometric analysis considered the gold standard to evaluate bone formation and is the only technique that provides the opportunity to perform an in-situ analysis of bone metabolism and cellular activity.^{14,15}

From the result of our review, it is evident that propolis play a significant role in prevention of bone loss. Propolis is one of the few treatments, which has drawn the attention over a long period of time. The potential of this beehive product as a natural antibiotic has long attracted interest. It has recently found use in various formulations for dermatology, otorhinolaryngology, gynaecology, odontology, and veterinary medicine.

Two factors in bone metabolism are bone formation and bone resorption, in which osteoblasts and osteoclasts play a key role respectively. The intercellular communication between osteoblasts and osteoclasts is crucial to bone homeostasis.^{16,17} If there is no positive balance between new bone formation and bone resorption, then it induces metabolic bone diseases.¹⁸ The bone diseases such as rheumatoid arthritis, osteoporosis, Paget's disease and periodontitis related bone loss are mainly related to a relative increase in the activity and number of bone-resorbing osteoclasts and the activation of NF- κ B.^{19,20}

Osteoclastic cells are derivatives of the monocyte-macrophage lineage.²¹ A member of the tumour necrosis factor (TNF) receptor-ligand family is called RANKL. It is the most important cytokine in the differentiation of osteoclasts. It is directly involved in the differentiation of monocyte-macrophages into osteoclasts.²¹⁻²³ It is also essential for osteoclast formation, activation and survival. When there is an excessive RANKL signalling, it results in increased osteoclast formation and hence bone resorption. Thus, it has been proposed that if targeting RANKL-induced osteoclast formation and signalling then it can be used as a therapeutic approach in reducing osteoclast formation.

The report of one of the study suggest that natural compounds such as parthenolide,²⁴ naringin,²⁵ mangiferin²⁶ and CAPE have inhibitory effects on osteoclast formation. CAPE was extracted from propolis by Grunberger et al²⁷ in 1988 for the first time in its history. It has been stated that CAPE may accelerate wound healing. CAPE which has anti-tumour and anti-inflammatory properties is a natural NF- κ B inhibitor from honeybee propolis. Activation of CAPE results in the attenuation of osteoclastogenesis and bone resorption by inhibition of NF- κ B and nuclear factor of activated T cells.^{7,28}

It has also been reported that CAPE induces osteoclast apoptosis.^{29,30} Ha et al reported that bone destructive diseases can be treated using CAPE, due to its inhibition effect on receptor activator nuclear factor- κ B ligand that are key molecules for activation of osteoclasts.²⁸ Ang et al in his study stated that CAPE induces apoptosis and inhibits osteoclastogenesis through the suppression of nuclear factor of activated T-cells activation and receptor activator nuclear factor- κ B ligand-induced osteoclast formation. With all these evidences, researchers suggest that CAPE may be used in pathological bone diseases.^{28,30}

Fibroblasts have an important role in wound healing process, hence increasing the number of fibroblasts improves tissue repair. It is stated that CAPE enhances fibroblast activity; in this way increases the wound healing process.³¹ Song et al reported that CAPE increase collagen-like polymer levels secreted by fibroblasts.³²

Tanimoto et al (2012) and Bushra Habeeb Al-Molla (2014) found that propolis enhanced the bone formation in one week and stated that amelogenins increases the mineralisation accompanied by the up-regulation of bone markers in human bone marrow MSCs during osteogenic differentiation. This suggests a certain role of amelogenin and propolis in the modulation of osteogenic differentiation of MSCs.^{9,33}

Studies done by Al-Molla 2007 and Altan et al 2013, found that the use of propolis accelerated the new bone formation at the expanded suture in rats after 12 days of mechanical retention.^{7,34} Novaes et al 2010 and Bushra Habeeb Al-Molla (2014) reported that osteocalcin as one of the important indicators of osteogenic differentiation and bone tissue formation.^{8,35} Mixing of bioinert propolis with a biological material (amelogenin protein) proved to increase the bioactivity of the product and to promote mechanical properties of the implant and enhanced the osseointegration during healing period.

Studies by Toker et al and Cüneyt Asım Aral et al 2015 showed significantly reduced alveolar bone loss in the periodontitis patients when Propolis was administered.^{11,36} Another study on Propolis reported that in cell cultures it can prevent bone loss with the effects of one of its active components, Caffeic acid phenethyl ester (CAPE) through the suppression of cell signalling pathways of RANKL induced NF- κ B and NFAT activity.⁶

Worldwide Usage of Propolis

Today, various forms of propolis are available for different purpose. The forms in which they are available includes tablets, capsules, chewing granules, soluble granules, throat pastilles and chewing gums, sprays, mouthwash (both with and without alcohol), toothpastes etc.

In most countries of the world, the propolis use is not regulated. In some countries, e.g. Austria, France, Spain, Japan, Taiwan, Korea, USA and Brazil propolis is considered as food supplement together as the bee products, bee pollen and royal jelly. In others like Switzerland and Germany, it is considered a medicine.³⁷

Allergy and Toxic Effects of Propolis

Most allergy studies are conducted with poplar propolis. There are no reports on allergy cases of other propolis types. Poplar propolis can cause contact dermatitis.³⁸ Propolis

allergy upon ingestion seems to be less frequent than contact allergy, due probably to its anti-allergenic and anti-inflammatory properties. No data on the allergy frequency of the population is available. Reports on individual cases of people allergic to propolis ingestion have been published. On the other effect, propolis ingestions have been shown to have an anti-allergenic effects due to the flavonoid.³⁹

CONCLUSION

The literature suggests that the use of propolis can be one of the best natural products to prevent bone loss. Patients may be more willing to use propolis than the other bone-inducing materials or methods, because propolis is an entirely natural and inexpensive product and has no known side effects other than mild allergic reactions. However, more clinical studies evaluating regarding the propolis is worthwhile. Well-designed randomised controlled trials are needed in future studies.

REFERENCES

- [1] Souza BM, Palma MS. Peptides from Hymenoptera venoms. In: Lima MH. edr. *Animal toxins: State of Art - Perspectives in Health and Biotechnology*. Editora UFMG 2009;345-67.
- [2] Bankova VS, de Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. *Apidologie* 2000;31(1):3-15.
- [3] Farooqui T, Farooqui AA. Beneficial effects of propolis on human health and neurological diseases. *Frontiers in Bioscience (Elite Edn)* 2012;4(1):779-93.
- [4] Aliyazicioglu Y, Demir S, Turan I, et al. Preventive and protective effects of Turkish propolis on H₂O₂-induced DNA damage in foreskin fibroblast cell lines. *Acta Biologica Hungarica* 2011;62(4):388-96.
- [5] Ang ES, Pavlos NJ, Chai LY, et al. Light microscope images of TRAP staining at ROI in tibialmetaphyses. *Chin Med J* 2014;127(22):393-6.
- [6] Ang ES, Pavlos NJ, Chai LY, et al. Caffeic acid phenethyl ester, an active component of honeybee propolis attenuates osteoclastogenesis and bone resorption via the suppression of RANKL-induced NF-kappaB and NFAT activity. *J Cell Physiol* 2009;221(3):642-9.
- [7] Altan BA, Kara IM, Nalcaci R, et al. Systemic propolis stimulates new bone formation at the expanded suture: a histomorphometric study. *Angle Orthod* 2013;83(2):286-91.
- [8] Al-Molla BH, Al-Ghaban N. Localization of osteocalcin in amelogenin-propolis coated dental implants in Rabbits. *Journal of Natural Sciences Research* 2014;4:9.
- [9] Bereket C, Özan F, Şener I, et al. Propolis accelerates the consolidation phase in distraction osteogenesis. *J Craniofac Surg* 2014;25(5):1912-6.
- [10] Duan W, Wang Q, Li F, et al. Anti-catabolic effect of caffeic acid phenethyl ester, an active component of honeybee propolis on bone loss in ovariectomized mice: a micro-computed tomography study and histological analysis. *Chin Med J (Engl)* 2014;127(22):3932-6.
- [11] Aral CA, Kesim S, Greenwell H, et al. Alveolar bone protective and hypoglycemic effects of systemic propolis treatment in experimental periodontitis and diabetes mellitus. *J Med Food* 2015;18(2):195-201.
- [12] Kazancioglu HO, Aksakalli S, Ezirganli S, et al. Effect of caffeic acid phenethyl ester on bone formation in the expanded inter-premaxillary suture. *Drug Des Devel Ther* 2015;9:6483-8.
- [13] Graves DT, Fine D, Teng YTA, et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. *J Clin Periodontol* 2008;35(2):89-105.
- [14] Iwaniec UT, Wronski TJ, Turner RT. Histological analysis of bone. *Methods Mol Biol* 2008;447:325-41.
- [15] Kulak CA, Dempster DW. Bone histomorphometry: a concise review for endocrinologists and clinicians. *Arq Bras Endocrinol Metabol* 2010;54(2):87-98.
- [16] Guney A, Karaman I, Oner M, et al. Effects of propolis on fracture healing: an experimental study. *Phytother Res* 2011;25(11):1648-52.
- [17] Kular J, Tickner J, Chim SM, et al. An overview of the regulation of bone remodelling at the cellular level. *Clin Biochem* 2012;45(12):863-73.
- [18] Phan TC, Xu J, Zheng MH. Interaction between osteoblast and osteoclast: impact in bone disease. *Histol Histopathol* 2004;19(4):1325-44.
- [19] Xu J, Wu HF, Ang ESM, et al. NF-kappaB modulators in osteolytic bone diseases. *Cytokine Growth Factor Rev* 2009;20(1):7-17.
- [20] Rea SL, Walsh JP, Layfield R, et al. New insights into the role of Sequestosome 1/p62 mutant proteins in the pathogenesis of Paget's disease of bone. *Endocr Rev* 2013;34(4):501-24.
- [21] Teitelbaum SL. Bone resorption by osteoclasts. *Science* 2000;289(5484):1504-8.
- [22] Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;93(2):165-76.
- [23] Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998;95(7):3597-602.
- [24] Yip KH, Zheng MH, Feng HT, et al. Sesquiterpene lactone parthenolide blocks lipopolysaccharide induced osteolysis through the suppression of NF-kappaB activity. *J Bone Miner Res* 2004;19(11):1905-16.
- [25] Ang E, Yang X, Chen H, et al. Naringin abrogates osteoclastogenesis and bone resorption via the inhibition of RANKL-induced NF-kB and ERK activation. *FEBS Lett* 2011;585(17):2755-62.
- [26] Ang E, Liu Q, Qi M, et al. Mangiferin attenuates osteoclastogenesis, bone resorption and RANKL induced activation of NF-kB and ERK. *J Cell Biochem* 2011;112(1):89-97.
- [27] Grunberger D, Banerjee R, Eisinger K, et al. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia* 1988;44(3):230-2.
- [28] Ha J, Choi HS, Lee Y, et al. Caffeic acid phenethyl ester inhibits osteoclastogenesis by suppressing NF kappaB and downregulating NFATc1 and c-Fos. *Int Immunopharmacol* 2009;9(6):774-80.

- [29] Hung MW, Shiao MS, Tsai LC, et al. Apoptotic effect of caffeic acid phenethyl ester and its ester and amide analogues in human cervical cancer ME180 cells. *Anticancer Res* 2003;23(6c):4773-80.
- [30] McEleny K, Coffey R, Morrissey C, et al. Caffeic acid phenethyl ester-induced PC-3 cell apoptosis is caspase-dependent and mediated through the loss of inhibitors of apoptosis proteins. *BJU Int* 2004;94(3):402-6.
- [31] Lamme EN, Van Leeuwen RTJ, Brandsma K, et al. Higher numbers of autologous fibroblasts in an artificial dermal substitute improves tissue regeneration and modulates scar tissue formation. *J Pathol* 2000;190(5):595-603.
- [32] Song HS, Park TW, Sohn UD, et al. The effect of caffeic acid on wound healing in skin-incised mice. *Korean J Physiol Pharmacol* 2008;12(6):343-8.
- [33] Tanimoto K, Huang YC, Tanne Y, et al. Amelogenin enhances the osteogenic differentiation of mesenchymal stem cells derived from bone marrow. *Journal of Cells Tissues Organs* 2012;196(5):411-9.
- [34] Al-Molla B. The effects of the Iraqi propolis on the artificial bony defects on the rabbit's mandible. Msc thesis, college of Dentistry, University of Baghdad. 2007.
- [35] Novaes AB, de Souza SL, de Barros RR, et al. Influence of implant surfaces on osseointegration. *Braz J Oral Maxillofac Surg* 2010;21(6):471-81.
- [36] Toker H, Ozan F, Ozer H, et al. A morphometric and histopathologic evaluation of the effects of propolis on alveolar bone loss in experimental periodontitis in rats. *J Periodontol* 2008;79(6):1089-94.
- [37] Bee Product Science. www.bee-hexagon.net 2016.
- [38] Walgrave SE, Warshaw EM, Glesne LA. Allergic contact dermatitis from propolis. *Dermatitis* 2005;16(4):209-15.
- [39] Hay KD, Greig DE. Propolis allergy: a cause of oral mucositis with ulceration. *Oral Surgery Oral Medicine and Oral Pathology* 1990;70(5):584-6.