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**ORIGINAL ARTICLE**

# Effect of Honey on levels of BMP-2 in post-extraction tooth sockets in Humans

MOMINA KHALID[1], SEHRISH ZAFFAR[2], MUHAMMAD MUTAHIR MEHDI[3], MOBIN RIAZ[4], FATIMA IKRAM[5], MARYAM SAQIB[6]
[1]*Senior Lecturer, Oral Biology Department, Lahore Medical and Dental College, Lahore*
[2]*Associate Professor, Pharmacology Department, CMH Lahore Medical College and Institute of Dentistry, (NUMS) Lahore*
[3]*Assistant Professor/HOD, Department of Oral Biology, Nishtar Institute of Dentistry, Multan*
[4]*Former postgraduate trainee, Department of Prosthodontics, Lahore Medical and Dental College, Lahore*
[5]*Demonstrator, Oral Biology Department, Lahore Medical and Dental College, Lshotr*
[6]*Assistant Professor, Pharmacology Department, CMH Lahore Medical College and Institute of Dentistry, (NUMS) Lahore*
*Correspondence to Dr. Momina Khalid, Email: dr.momina.khalid.92@gmail.com, Mobile No: 03244920287*

## ABSTRACT

**Background:** In Pakistan, many people undergo tooth extractions every year due to various causes such as dental caries, periodontitis, and trauma. For a healthy alveolar bone after tooth extraction, some kind of intervention is always required. Honey can possibly help to improve bone healing after tooth extractions.
**Aim:** To observe the effects of honey on bone healing of alveolar sockets after tooth extraction in humans.
**Method**: It was an experimental study, conducted at Lahore general hospital over the period of 6 months. Twenty-four participants were included in the study through simple random sampling technique. After tooth extractions in both groups, honey was injected into extracted sockets of the experimental group while the control group was left as it was. Levels of BMP-2 were tested in saliva through the ELISA technique on days 1, 3, and 7 of tooth extraction.
**Results**: In the control group, mean BMP-2 levels were 485.5± 52.63, 623.7 ±47.16 and 692.5± 11.86 while in the experimental group the mean values of BMP-2 were 494.3 ± 49.89, 703.6 ± 39.31 and 812.9 ± 34.40 at day 1, 3 and 7 respectively. The mean difference was calculated to be 8.75, 79.9, and 120.5. A significant difference (p-value <0.001) was noted in levels of BMP-2 between the experimental and control group on days 3 and 7.
**Conclusion**: Raise in levels of BMP-2 in the experimental group when compared with the control group confirmed better bone healing. Hence, honey can be used to promote bone healing in post-extraction tooth sockets. This study can be used in future to improve bone quality and minimize ridge resorption after tooth extractions.
**Keywords**: Bone morphogenetic protein-2 (BMP-2), Honey, Extracted Tooth sockets, bone healing, ELISA.

## INTRODUCTION

Honey can be defined as a concentrated aqueous solution of invert sugars, which are a complex mixture of carbohydrates mainly glucose (38%) and fructose (44%) along with other constituents like waxes, vitamins, organic acids, minerals, enzymes, proteins, amino acids, flavonoids, phenols, and pigments[1]. Honey is a viscous liquid with a density of 1.5g/cm3 with strong hygroscopic properties, relatively low heat conductivity, and low surface tension. Its color ranges from all shades of yellow to amber[2].

In bone healing, honey has been used extensively in medicine. A lot of work has been done in the past which validates the use of honey in promoting bone healing. Honey was found beneficial in enhancing bone healing in artificially introduced defects in the radius bone of rats[3]. Honey has been used to treat glucocorticoid-induced osteoporosis in postmenopausal women to enhance the quality of bone[4]. Honey was also found useful in inhibiting bone resorption in rats by the action of phenolic components present in it[5].

Bone is a heterogeneous composite material made up of a mineral phase (50-70%), an organic phase (20-40%), and water (5-10%)[6]. The inorganic phase is predominantly occupied by phosphate and calcium ions, which nucleate to form hydroxyapatite crystals (Ca10(PO4)6(OH)2)[7]. Besides this, substantial amounts of bicarbonate, sodium, potassium, magnesium, fluoride, and zinc also exist[7]. The organic phase is mainly typed I collagen (90%) and non-collagenous bone matrix proteins which are classified according to their functions, such as signaling molecules, growth factors, and enzymes[8,9]. Bone-derived factors like bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs) and osteoprotegerin are the main controllers of bone function[10].

Bone morphogenetic proteins (BMPs) belong to the transforming growth factors (TGFs) superfamily of growth factors[11]. Almost 20 different BMPs have been recognized so far, of which bone morphogenetic proteins 2, 4, 5, 6, and 7 (BMP-2, BMP-4, BMP-5, BMP-6, BMP-7) have notable osteoinductive properties[12,13]. These proteins have a significant role in several phases of osteogenesis, including bone formation, bone induction, and bone regeneration[14].

BMP-2 was first isolated by Urist et al in 1965[15]. BMP-2 plays a substantial role in promoting the differentiation of mesenchymal cells to osteoblasts completed by the Smad signal pathway thus regulating the transcription of encoding enzymes and proteins such as alkaline phosphatase, osteocalcin, and bone sialoprotein which are crucial for osteogenesis[16]. BMP-2 is considered the most powerful growth factor encouraging bone regeneration.[17] Moreover, it is one of the most crucial factors required for the differentiation of mesenchymal cells to osteogenic lineage in the early phase of osteogenesis[18].

Currently no work has been done to study the effect of honey on the markers of bone formation such as BMP-2. By the help of this study, the quality and quantity of bone can be improved after tooth extraction and there will be minimum ridge resorption which is a crucial for implants and fixed as well removeable tooth prosthesis.

## METHODOLOGY

It was an experimental study carried out at Lahore General hospital over a period of one year. Twenty-four participants were equally divided into two groups by simple random sampling. Twelve participants were included in each group, named experimental and control groups.

The study was approved by the ethical review board of the Post Graduate Medical Institute, Lahore. In inclusion criteria, the participants included were healthy, of either gender with adult age (18 years and above) and all permanent maxillary and mandibular molars were included. Those participants were excluded which had a history of radiotherapy, chemotherapy, diabetes, or hypertension, Pregnant and lactating females, Patients on oral contraceptives or steroid therapy or taking antibiotics or NSAIDs within one week. Informed consent was taken from all participants.

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Salivary samples were collected from all the participants according to the standard guidelines[19]. Participants were not allowed to eat, drink or perform oral hygiene procedures for at least one hour before the collection of saliva. Then, they were asked to rinse their mouth with distilled drinking water for at least one minute and to spit into a 15ml sterile tube. Approximately 5ml of saliva was collected. The same guidelines were used to collect saliva on the 3rd and 7th days of the study.

Honey was obtained from *Apis Mellifera* colonies from the University of Punjab, Lahore. In the experimental group, immediately after atraumatic tooth extraction, 100 mg/kg/BW 20 of honey was administered into the socket with the help of a 26-gauge sterile needle syringe, and the socket was secured with the help of cotton gauze. In the control group, only atraumatic extractions were done without any other intervention.

Postoperatively, participants were asked to retain cotton gauze in place for at least 30 minutes, to avoid spitting or eating/drinking anything during this duration and to remove the gauze after 30 minutes. No postoperative antibiotics were prescribed. For pain suppression, Paracetamol (1000 mg) was prescribed.

After the collection of saliva, the sample was briefly vortexed for approximately 20 seconds so that it whirls up the side of the tube. Each sample was centrifuged at the speed of 2000-3000 rpm for 20 minutes. The supernatant part was removed taking care not to disturb the pellet formed at the bottom of the tube. The fraction was then transferred to labelled cryotubes. All the fractions were stored at -20 degree centigrade. After the collection and storage of saliva samples, the ELISA technique was carried out using a commercially available ELISA kit for BMP-2 according to the manufacturer's instructions. Saliva was collected at 3 intervals during this study, on day 1, day 3, and day 7. Levels of BMP-2 were measured by the ELISA technique.

Data was entered into Graph Pad Prism 8 for statistical analysis. The numerical data for relative bone density (RBD) was presented as descriptive statistics, i.e., Mean ± SD in tables and mean ± SE in graphs. An unpaired T-test was applied to find the significance of the outcome in both study groups. The p-value of <0.05 was considered statistically significant.

## RESULTS

Total number of 24 participants, 14 female and 10 males with a mean age of 33±0.48 years were equally divided into an experimental group and a control groups. The mean levels of BMP-2 in the control and experimental group were expressed as Mean±SD. In the control group, mean BMP-2 levels were 485.5±52.63, 623.7±47.16 and 692.5±11.86 while in the experimental group the mean values of BMP-2 were 494.3±49.89, 703.6±39.31 and 812.9±34.40 at day 1, 3 and 7 respectively. The mean difference was calculated to be 8.75, 79.9, and 120.5 respectively in both groups. A T-test was applied to find out whether the mean difference was statistically significant between the control and experimental group. Statistically, a significant difference was observed on day 3 and day 7 in both groups with a p-value <0.001.

Table 1: Comparison of BMP-2 levels on different days between experimental and control groups.

| | **BMP-2 levels at (pg./ml)** | | |
|---|---|---|---|
| | **Day 1** | **Day 3** | **Day 7** |
| Control Group | 485.5±52.3 | 623.7±47.16 | 692.5±11.86 |
| Experimental Group | 494.3±49.9 | 703.6±39.31 | 812.9±34.0 |
| Mean difference calculated in both groups | 8.175 | 79.9 | 120.5 |
| *p*-value | 0.6438 | <0.0001 | <0.0001 |

## DISCUSSION

In this era of implant dentistry, an intervention is always required to get better bone quality during post-extraction socket healing.[21]There is always a chance of wound infection or delayed socket healing after tooth extractions.[22] It was observed that 89% of sites after tooth extraction had uneventful healing and 11% had some kind of complications[23].

The quality of bone and its volume has a substantial impact on implant therapy after tooth extraction.[24] To have an ideal aesthetic outcome using an implant or conventional prosthesis, sufficient ridge volume is required which depends on the healing pattern of bone after tooth extraction.[25] Implant placement sites with higher cortical bone density and greater bone thickness are perfect to achieve high success rates after implant therapy therefore a lack of ideal bone density or thickness can result in poor implant stability[26].

In this study the peak levels of BMP-2 were noted at the seventh day of the tooth extractions. Our results coincide with other studies[25,27] where peak levels of BMP-2 were found at seventh day of tooth extractions. Also, It was observed in another study that the expression of BMP-2 was increased in osteoblasts and chondrocytes during the early phases of bone healing.[28]Intra-cytoplasmic staining of BMP-2 in osteoprogenitor cells, osteoblasts, and osteocytes were present in the areas undergoing revascularization and osteogenesis[29].

In an animal study[30] the effect of sodium hyaluronate was observed on extraction socket healing and the progress of bone healing was observed through measuring the expression of BMP-2 in control and experimental groups. Similar bone marker is used in present study to measure the difference in bone healing in both groups by the use of Honey.

BMPs bind to type I and type II serine-threonine receptors to form specific complexes which are involved in the regulation of phosphorylation of smad1/smad5/smad[8] and then these complexes associate with smad[4] and translocate into the nucleus[31]. BMPs then persuade the differentiation of mesenchymal stem cells of bone marrow to osteoblasts and chondrocytes. The direct effect of phenolic compounds present in honey was also observed in raising BMP-2 levels thus promoting the differentiation of osteoblasts which helps in the formation of new bone[32].

Hydrogen peroxide ($H_2O_2$) present in honey acts as a messenger that modulates various cell signalling pathways thus regulating the expression of many genes encoding proinflammatory mediators such as cytokines and growth factors. Therefore, it can induce bone markers directly and increase osteogenic activity[5]. The high content of sugars present in honey can improve the local nutrition of the damaged areas[33] All these mechanisms may have a direct effect in increasing the levels of BMP-2 by the application of honey at tooth extraction sites. Hence, the antioxidants and anti-inflammatory properties of honey strongly support the argument that honey can hasten bone healing as evident by the increase in the bone markers.

## CONCLUSION

Honey raises the salivary levels of BMP-2, indicating enhanced bone healing. Therefore, it can be used as an adjunct in bone healing to fasten the recovery after the tooth extractions.

**Recommendation:** In the future, by using this intervention, there would be minimal ridge resorption and preservation of the quality of the alveolar socket after tooth extraction due to better bone healing. Adequate bone support will be present for implant placement as well as for removable or fixed prostheses.

**Conflict of interest:** Nothing to declare

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