Effects of *Ziziphus* Honey on Bone Healing of the Extracted Tooth Socket by Evaluating Osteopontin Levels

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ABSTRACT

Objective: To evaluate the effect of *Ziziphus* honey on the healing of post-extraction alveolar sockets by estimating the levels of osteopontin (OPN) in humans.

Study Design: Randomised controlled trial.

Place and Duration of the Study: Dental section of the Lahore General Hospital, Lahore, Pakistan, from March 2020 to February 2021.

Methodology: A total of 30 patients were included in the study. The mean age was 35 ± 0.28 years. The participants were adults undergoing permanent molar extraction, randomly divided into two groups, a control group and an experimental group. After tooth extractions in both groups, 1ml of *Ziziphus* honey was administered into the extracted tooth socket of the experimental group while no intervention was done to the control group. Saliva samples were collected on day 0 before tooth extraction and on days 3 and 7 after tooth extractions. Enzyme-linked immunosorbent assay (ELISA) technique was used to measure the levels of OPN in the saliva sample. Radiographic evaluation was also done with the help of periapical radiographs using Image J® software. To find out the significance of the outcome in experimental and control groups, an unpaired t-test was applied. A p-value <0.05 was considered statistically significant.

Results: A total of 30 participants were selected for the study, of which 16 were females and 14 were males. The OPN levels between the control *vs.* experimental groups were (22.55 ± 2.45 *vs.* 23.31 ± 2.38; p = 0.4) on day 0, (30.95 ± 2.96 *vs.* 53.29 ± 4.69; p = 0.001) on day 3, and (55.33 ± 4.52 *vs.* 81.90 ± 4.49; p = 0.001) on day 7.

Conclusion: Increased salivary levels of the OPN in the experimental group with the use of *Ziziphus* honey suggests better bone healing as compared to the control group.

Key Words: *Extraction tooth, Honey, Osteopontin, Ziziphus, Bone healing.*

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INTRODUCTION

Ziziphus honey is a type of honey which has the greatest amount of minerals and energy, among more than a hundred types of honey. It is obtained from a tree named *Ziziphus Spina-Christi,* also known as *Sedr* tree as its Persian name.[1] *Ziziphus* belongs to the *Rhamnaceae* family of the plants. Dark-coloured honey has greater mineral content and polyphenol content as compared to light-coloured honey.[2] Antibacterial potential of honey is attributed to three mechanisms, the presence of hydrogen peroxide, acidic pH, and hygroscopic nature. Hydrogen peroxide is identified as a major antibacterial substance in honey, produced by an enzyme glucose oxidase which is already present in honey.[3]

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Owing to this property, studies have documented a reduction in the severity of oral mucositis with the use of *Ziziphus* honey in patients undergoing radiotherapy.[4] *Ziziphus* honey has also demonstrated antimicrobial activity against gram-negative as well as gram-positive bacteria.[5]

Osteopontin (OPN) is a non-collagenous bone matrix protein, present in bone and teeth, which is highly phosphorylated and rich in sialic acid. In humans, OPN is present in numerous biological fluids such as serum, plasma, urine, cerebrospinal fluid, and saliva.[6] OPN has the sites of serine/threonine phosphorylation that facilitate hydroxyapatite binding and a highly sustained Arginine-glycine-aspartic acid (RGD) motif that facilitates cell attachment and signalling.[7] OPN is always expressed in the process of osteogenesis, bone remodelling, and cell-mediated immune response. Along with several non-collagenous proteins, it controls the ordered deposition of minerals into the bone by modifying the quantity and dimensions of hydroxyapatite crystals. During bone mineralisation, OPN impedes the formation of hydroxyapatite crystals.[8]

OPN plays an essential role in osteogenesis, after bone drilling, it is an important factor for cell adhesion, signalling, and bone

mineralisation during new bone formation.[9] Even recombinant OPN can be used on the surface of dental implants to improve bone formation at the implant site.[10] The OPN is released from one cell during osteogenesis soon after initial bone matrix formation and continues to secrete throughout the maturation of new bone with maximum levels in the initial days.[11]

This study was designed with the objective of assessing the bone-healing effect of *Ziziphus* honey on post-extraction alveolar sockets by estimating the levels of OPN in humans.

METHODOLOGY

A randomised controlled trial was carried out in the Dental section of the Lahore General Hospital, Lahore, from March 2020 to February 2021. This study was approved by the Ethical Committee of the Post Graduate Medical Institute (PGMI), Lahore. A sample size of 30 was used as per the previous study.[12] Simple random sampling technique was used by means of computer-generated numbers using Stat Trek's random number generator.

A total of 30 participants were randomly divided into two equal groups, 15 in each group, namely control and experimental groups. Participants who were included in the study, who were aged 18 years and above and advised permanent mandibular or maxillary molar extraction. Only the extraction of a single tooth was considered in an arch to standardise the area of bone healing. Participants with a history of chemotherapy or radiotherapy, comorbid conditions, chronic steroid use, or a history of antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) intake within one week of tooth extraction, and pregnant and lactating females were excluded from the study. Informed consent was taken from all the participants for both the procedure and for the publication of the study data.

Salivary sample was collected from all the participants in the control and experimental group before tooth extractions at day 0. After that, follow-up visits were conducted to collect the saliva samples on days 3 and 7 of the tooth extraction in both groups. The Spit method was used for the collection of saliva from the participants into the collection tubes according to the standard guidelines. Participants were not allowed to eat, drink, or perform oral hygiene procedures for at least one hour prior to the collection of saliva. Then, they were asked to rinse their mouth with distilled drinking water for at least one minute and spit into a 15ml sterile tube. Approximately 5ml of saliva was collected. The same guidelines were used to collect saliva on 3rd and 7th day of the study.[13]

The radiographic evaluation was also done measuring relative bone density (RBD) on days 3, 21, and 40 of tooth extraction. Periapical radiographs were taken and bone healing of the post-extraction sockets was compared in experimental and control groups with the help of ImageJ® software.

Ziziphus honey was used in this study, which was collected from the Honey Bee Research Centre at the University of Punjab, Lahore. A sample of '*Ziziphus* honey' was verified by the Botany Department of GC University, Lahore. Honey was used in its natural, undiluted, and unprocessed form.

Local anaesthesia was given for molar extraction by infiltration technique for maxillary molars and inferior alveolar nerve block technique for mandibular molars using the injection of lidocaine HCL 2% with epinephrine 1:100,000 with a maximum dose of 7 mg/kg.[14] Atraumatic extractions were done in both groups.

About 1ml of *Ziziphus* honey was administered into empty tooth sockets using a 26-gauge sterile needle syringe after tooth extractions in the experimental group. A cotton gauze was placed over the socket to retain the honey for a longer duration. In the control group, atraumatic extractions were done without the administration of honey. All the participants were asked to hold the cotton gauze for at least 30 minutes and to avoid eating, drinking, and spitting.

All the samples were processed in the Central Research Laboratory of the Lahore General Hospital. The guidelines for the processing and storage of saliva samples were followed.[13] The saliva samples were briefly vortex for approximately 20 seconds and then underwent centrifugation at the speed of 2,000 - 3,000 rpm for 20 minutes. The supernatant part without disturbing the pellet formed at the bottom of the tube. The fraction was transferred to labelled cryotubes and stored at -20 degrees centigrade.[13] A commercially available ELISA kit by Immunodiagnostics® was used for the estimation of the OPN levels. Saliva samples were collected on days 3 and 7 after tooth extractions from both experimental and control groups.

Graph pad prism 8 was used for the statistical analysis of the data. Shapiro-Wilk test was applied to test the normality of the data. Descriptive statistics were applied to numerical data of the levels of OPN i.e., mean ± SE in figures and mean ± SD in tables. To find out the significance of the outcome in experimental and control groups, an unpaired t-test was applied. The p-value of <0.05 was considered statistically significant.

RESULTS

A total of 30 participants were selected for the study of which 16 were females and 14 were males. Mean ± SD OPN levels were 22.55 ± 2.45, 30.95 ± 2.96, and 55.33 ± 4.52 in the control group and 23.31 ± 2.38, 53.29 ± 4.69, and 81.90 ± 4.49 in the experimental group at days 0, 3, and 7, respectively. The mean difference was calculated to be 0.75, 22.35, and 26.57 in both groups as shown in Table I.

Unpaired t-test was applied between the control and experimental group on days 0, 3, and 7; p-value ≤ 0.05 is considered statistically significant.

To find out the significance of the data, an unpaired t-test was applied between both study groups on days 0, 3, and 7. The mean difference of OPN levels in both groups on day 3 and day 7 was found statistically significant as the p-value was calculated to be <0.001 as shown in Figure 1.

The results of the radiographic evaluation were inconclusive due to the incomplete patient follow-up. Therefore, statistical analysis could not be generated.

Table I: Comparison of the OPN levels (ng/ml) on different days between the control and experimental group (n = 30).

Groups	OPN levels (ng/ml) Mean ± SD		
	Day 0	Day 3	Day 7
Control group	22.55 ± 2.45	30.95 ± 2.96	55.33 ± 4.52
Experimental group	23.31 ± 2.38	53.29 ± 4.69	81.90 ± 4.49
Mean difference	0.75	22.35	26.57
p-value	0.4003	<0.001	<0.001

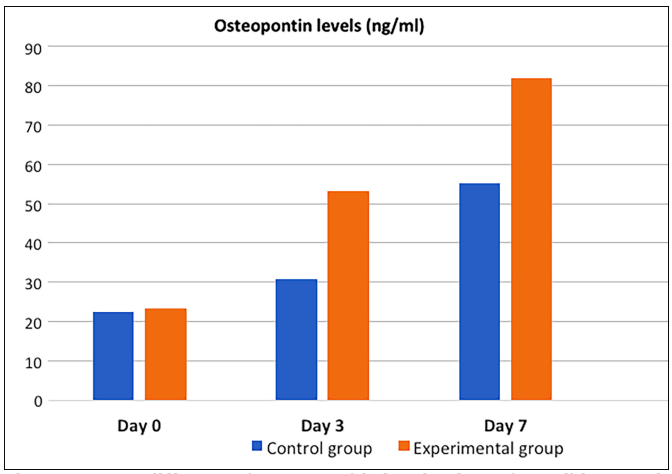

Figure 1: Mean difference (mean ± SE) in levels of OPN (ng/ml) in experimental and control groups (n = 30).

DISCUSSION

OPN levels raised significantly in the experimental group on days 3 and 7, which shows positive relation of *Ziziphus* honey with bone healing at tooth extraction sites, since levels of the OPN always increase with new bone formation. Days 3 and 7 were selected for the estimation of OPN in saliva samples because, in a previous study, an increase in levels of OPN was observed at day 3 and 7, during bone formation after tooth extraction.[11] The present study also confirms the rise in levels of the OPN during the first week of tooth extraction by the application of *Ziziphus* honey.

Many studies showed that levels of OPN significantly raised during active bone formation. In an animal study, increased expression of OPN during bone formation was observed which resulted in increased turnover and remodelling of the bone.[15] In another study, analysis of OPN levels in plasma was done after scaling and root planning, and a decline in levels was observed where active bone formation was not taking place.[16] *Ziziphus* honey has been used on alveolar sockets in an animal study where an increased number of trabeculae formation was observed in the experimental group after the tooth extractions.[12] These results corroborate the successful use of *Ziziphus* honey in tooth sockets after the extraction for better bone healing. OPN has areas of Serine/Threonine phosphorylation which aids in hydroxyapatite binding and an RGD motif which is involved in cell attachment and signalling.[17] Through this mechanism, OPN functions as a bridge between cells and hydroxyapatite and helps in bone formation. OPN also has negatively charged phosphoserines which provide a strong affinity for hydroxyapatite binding.[18]

There are many possible mechanisms by which *Ziziphus* honey can promote bone healing at tooth extraction sites. Honey can induce the markers of bone as it contains hydrogen peroxide, which can control many inflammatory mediators and consequently increase osteogenic activity.[19] Also, honey has immunomodulatory activity due to many components present in it which may influence the bone healing process.[20] The viscosity of honey allows it to establish a protective barrier which prevents infections in the wounds and thus may contribute to better bone healing.[20] Lastly, the flavonoids present in honey can increase the number of osteoprogenitor cells and inhibit osteoclastic activity, therefore, helping in bone formation.[21]

Ziziphus honey can be used to boost the natural bone healing process after tooth extraction, as better bone quality was observed in the experimental group. This intervention can be used in the future to enhance the quality of the alveolar ridge for the placement of dental implants and for the fixed or removable tooth prostheses. The present study was conducted on healthy individuals only. Any further research with immunocompromised conditions, bone disease, or other debilitating diseases can be an open avenue to evaluate the effect of honey on such patients with dental extractions. Locally applied formulations can also be prepared with *Ziziphus* honey to increase bone healing and lower the chances of infection as well.

CONCLUSION

The local application of *Ziziphus* honey on extracted tooth sockets has significantly raised the levels of OPN in saliva accounting for better bone healing in the experimental group.

ETHICAL APPROVAL:

This study was approved by the Ethical Committee of the Post Graduate Medical Institute (PGMI), Lahore, Pakistan.

PATIENTS' CONSENT:

Informed consent was taken from all the participants.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MK: Conception, design, and manuscript writing.
SZ: Final approval of the manuscript to be published.
FI, WAS: Data acquisition and analysis / interpretation.
MR: Critical revision for intellectual content.
AS: Data acquisition and critical revision.
All authors approved the final version of the manuscript to be published.

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