Effect of Honey on Healing Process of Extraction Socket in Rabbits

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ABSTRACT

Background

Honey is regarded as the oldest wound dressing. It accelerates wound healing in burn, infected and open wounds. Its effect on wound healing process in extraction socket is not fully established till today.

Objective

To evaluate the effect of honey on the healing process of socket after tooth extraction in New Zealand White rabbits.

Method

Extraction of first premolar tooth on both sides of lower jaw in six rabbits was done under general anesthesia produced by ketamine and Xylazine followed by local application of honey on one socket (honey group) and normal saline (control group) in the opposite socket. On 7th day, biopsy was taken from the extraction site and histopathological examination was done. Student's t-test was used for comparison between the groups and the differences were considered to be statistically significant at P-value less than 0.05.

Result

There was a significant difference between control group and honey group in terms of fibroblast proliferation ($p = 0.0019$) and bony trabeculae formation ($p=0.0003$). Inflammatory cells were also observed in both groups and it was statistically not significant ($p=1.0000$). Overlying epithelium was hyperplastic in the both groups.

Conclusion

Local application of honey promoted the healing process by increasing fibroblast proliferation and bony trabeculae formation. Further studies in larger animals and human should be conducted to confirm the efficacy of honey in extraction socket healing.

KEY WORDS

Extraction socket, Honey, Rabbit, Wound healing

INTRODUCTION

Honey is a natural product produced by honeybee from the nectars of various plants.[1] It is regarded as one of the oldest known natural drug since ancient times. Its composition varies depending on the plants on which the bees feed and hence its medicinal property differs in different areas of the world.[2] It is a nontoxic and nonirritating substance and has been used as medicine to treat infantile gastroenteritis, as intra-canal medicament during root canal therapy of tooth, in dry-socket and periodontitis.[3-7] Honey has also antibacterial effect against Staphylococcus aureus and is effective in burns, infected wounds and skin ulcers.[8-9] It also reduces duration of wound healing in rats.[10] Its high viscosity helps to provide a protective barrier to prevent the infection and reduce inflammation and edema, stimulate epithelialization and tissue regeneration.[11,12]

Dry socket, fascial space infections, soft tissue infections, postoperative pain and paresthesia are the common complications after tooth extraction.[13-14] Prescription of antibiotics is common after tooth extractions to prevent or minimize such complications. Use of antibiotics may result in an increase in adverse effects, cost and antimicrobial resistance.[15] It is also important to explore natural compounds as promotor of wound healing. The early healing of extraction socket is generally required to place denture or dental implants in case of tooth extraction. Several changes can occur in the alveolar process after tooth extraction that may prevent or delay prosthodontic and other dental treatments.[16] Maintenance of adequate alveolar ridge volume is also important to achieve a long-term esthetically acceptable intraoral prosthesis or implants.[17] Therefore, it is of utmost importance that the extraction socket healing process should form an alveolar ridge with a sufficient volume of hard and soft tissues at a faster rate to allow esthetic outcome.[18]

Honey is becoming more important as antimicrobial resistance to antibiotics is increasing.[10] There is not enough reports on effect of honey in wound healing process after tooth extraction. Animal experimentation is often a necessary step for developing therapies applicable to man. The rabbit is one of the most widely used models for studying bone remodeling and wound healing.[19] Bone mineral density and fracture toughness is similar between rabbits and human.[20] The Rabbit model is an appropriate animal model for extraction socket healing studies to properly simulate human in vivo environment as it is reproducible, accurate, non-aggressive and easy to house and handle.[21] The objective of our study was to evaluate the effect of Nepalese honey on healing process of socket after tooth extraction.

METHODS

It was an experimental study in six New Zealand White rabbits, Oryctolagus cuniculus (age group of 3-5 months, weight 2-2.5 kg) conducted in the department of Clinical Pharmacology and Therapeutics, BP Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal between June to November, 2017. It was a pilot study. Resource equation method was used to calculate the sample size of the rabbits.[22] They were purchased from New Delhi, India. The animals were fed Bengal gram, cabbage, dry bread, lettuce, and tap water ad libitum. Prior to beginning of the study, the animals were kept in stainless steel cage (1 m^2) under standard condition of animal house for acclimatization for 15 days. Utmost care was taken to avoid unnecessary stress and discomfort to the animals throughout the experiment according to the guidelines given by the Committee for the Purpose of Control and Supervision of Experimentation on Animals, India.[23-24] A veterinarian staff monitor the general health status of each animal throughout the study.

The ethical clearance was taken from the Institutional Review Committee, BPKIHS (IRC/497/015). Honey was purchased from local merchants of Rajarani Bazar, Dhankuta, Nepal. It was dark amber colored, clear with high viscosity and fruity smell. It was unprocessed. It was used in its natural and undiluted form. All the experiments were carried out between 8.30 am to 3 pm. Split mouth design was used for the intervention; one side of the mandibular socket was used as control and other side in the same rabbit as experimental. The same rabbit served as control and experimental group. General anesthesia was produced by intraperitoneal injection of ketamine 25 mg/kg and Xylazine 5 mg/kg body weight. Local infiltration of lidocaine (2%) with epinephrine (1:200,000) was also administered for hemostasis and to reduce postoperative pain. Extraction of lower 1st premolar teeth were done atraumatically using periosteal elevator and pediatric forceps. The sockets were curetted and abundantly irrigated with normal saline. All radicular tissue were also removed from the sockets. Then 0.1 ml honey was locally applied in the one of extraction socket and normal saline (as control) in the other socket (using a 26G needle). The socket was sutured with 5-0 silk suture. Analgesic (Paracetamol 20 mg/kg) and antibiotic (Enrofloxacin 10 mg/kg) were also administered.

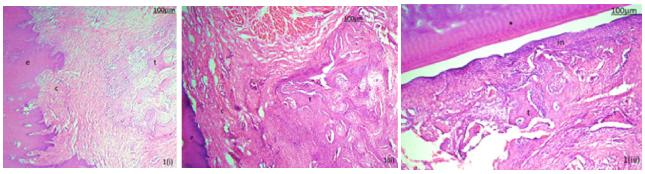
The animals were sacrificed on day 7 with intravenous administration of pentobarbital overdose (100 mg/kg). The access to the surgical site was made through an extra-oral approach by placing an incision from the labial commissure extending distally for about 1.5 cm. An incision was then placed bucally along mandibular vestibule apically in the region of previously extracted premolar connecting it with two vertical incisions medially and distally along the attached gingiva. A similar fashion incision was also made along the lingual aspect of the extracted premolar tooth connecting both at the ridge. Gingiva was elevated along the incision lines apically, medially and distally to expose the underlying bone, while still keeping the gingiva attached to the premolar alveolar bone. Otseotomy cuts were then made to remove the premolar alveolar segment in an enbloc fashion. The specimen was placed in 10% buffered formalin

solution for fixation for two days and demineralized in 10% nitric acid solution for 6-10 days till decalcification. Five µm-thick longitudinal sections were cut and stained with hematoxylin and eosin (H & E) for histologic examination using a light microscope and photographs were taken with digital camera. The biopsy samples were coded and the pathologist was blinded whether the samples belonged to honey treated or control group.

The parameters like epithelialization, infiltration of inflammatory cells, fibroblast proliferation (collagen density) and extent of bony trabeculae formation were used for the evaluation of healing process. The methods given by Habiboallah et al was used with modification.[25] The parameters like inflammatory cells count, fibroblast proliferation and bony trabeculae were scored from 1 to 3, with 1 as mild increase, 2 as moderate increase, and 3 as marked increase. Sparsely scattered neutrophils and arranged in a random fashion were considered mild increase, localization of 3-10 and >11 cells in the wound tissue per 400X magnification considered a moderate and marked increased neutrophils respectively. Fibroblasts organized in a random pattern were considered as mild increase. Localization of fibroblasts in the wound tissue in number of 3 - 10 and >11 per 400X magnification were considered a moderate and a marked increase in fibroblast respectively. Similarly bony trabeculae were graded as mild increase if the bony trabeculae were sparse, individual and filling less than one third of the socket, moderate if the bony trabeculae were dense, individual and filling one third to two third of the socket, and marked if the bony trabeculae were dense, interconnected and filling more than two third of the socket. Mean and standard deviation were calculated. Student's t test was used to analyze inflammatory cell count, fibroblast proliferation and bony trabeculae between control group and honey group using SPSS version 11.0 software. P value less than 0.05 was considered statistically significant.

RESULTS

All rabbits remained in good health throughout the study. The animals did not present any complications during the postoperative period. The control group and the honey treated group showed a similar clinical wound healing sequence over time. The extraction sockets had a visible stable clot underneath the suture on postoperative day (POD) one. POD two and three showed the beginning of the inflammatory phase marked by hyperemic soft tissue surrounding the socket wall with later development of some edematous soft tissue. However, from POD four onwards there was reduction of the inflammation with red to black clot underneath being gradually replaced to pink color. By the end of POD seven, the socket was completely covered by the soft tissue in regards to the axial plane with no signs of any active infection/inflammation. However, vertically the socket appeared to be two third healed from the base. The extraction socket wounds showed healthy soft tissue covering the extraction sockets at day POD seven. Histological analysis of the extraction socket wounds of the control group (CG) revealed abundant infiltration of inflammatory cells (Neutrophils) in the connective tissue, hyperplastic epithelium, limited amount of blood vessels and less bony trabeculae (fig. 1).

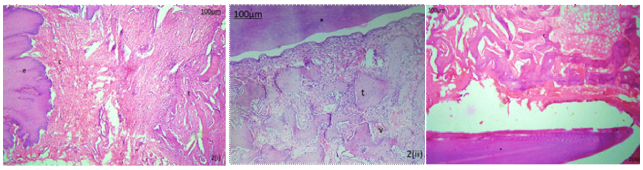


[e]Epithelium, [c]Collagen fibers, [t]Bony trabeculae, [in]Inflammatory cells, [*]Adjacent tooth

Figure 1. Photomicrograph of hematoxylin and eosin stained sections of extraction wound of control group (magnification 4X).

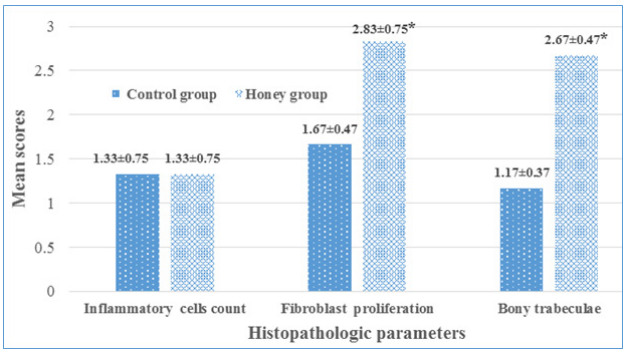
We also observed abundant inflammatory cells in connective tissue, abundant new collagen synthesis with irregular orientation, moderate neovascularization, hyperplastic epithelium and more dense bony trabeculae in extraction socket wounds of honey group (HG) (fig. 2).

We also found that honey lead to formation of bony trabeculae throughout the socket in the HG compared to the CG. In each socket of the CG, there were abundant bony trabeculae formation in lower third only and less in middle and upper third [Figure 1(i) and (ii)]; but it was also present in middle and upper third of the socket in the HG [fig. 2(ii) and (iii)].



[e]Epithelium, [c]Collagen fibers, [t]Bony trabeculae, [in]Inflammatory cells, [v]Blood Vessel, [*]Adjacent tooth

Figure 2. Photomicrograph of hematoxylin and eosin stained sections of extraction wound of Honey group (magnification 4X)



*Statistically significant (P value <0.01)

Figure 3. Histopathologic parameters of extraction socket wound (n=6)

Our study showed that the local application of honey promote the healing process remarkably in the HG compared to the CG. The HG presented more collagen

fibers, abundant neutrophils but that was not statistically significant. There was a statistically significant difference between CG and HG in terms of fibroblast proliferation (p= 0.0019) and bony trabeculae formation (p=0.0003) (fig. 3).

DISCUSSION

In this experimental study, effect of honey on wound healing process after tooth extraction was evaluated histologically in rabbits. To the best of our knowledge, this is the first report on the effect of honey on healing process of extraction socket in rabbits. Split-mouth design led us to use the single animal as both control and test. The CG and the HG showed a similar clinical wound healing sequence over time. New epithelial cells developed from the adjacent normal epithelium which covered most of the extraction socket surface. Almost all of the extraction sockets were filled with a mixture of granulation tissue, fibrous tissue and bony trabeculae. Honey promotes the formation of healthy granulation tissues by reducing inflammation, hyperemia, edema and exudation.[26] The socket healing was histologically different between the two groups in term of bony trabeculae formation which was more extensive for those sockets in which honey was locally applied. Hygroscopic nature, acidic pH and presence of enzymes, minerals and vitamins are important properties of honey which prevents bacterial growth and help wound repair.[27] It was interesting to find out that in our study the rate of bony trabeculae formation was rapid in HG. More than one third of the extraction socket in HG was filled with bony trabeculae on 7th day of tooth extraction. Similar finding was also reported by Peimani et al. in which extent of bony trabeculae was more pronounced after honey application in rat.[28] However, new mineralized bone occupied half of the extraction socket on 14th day as reported by Cardaropoli et al.[29] Our finding was also in contrast to that of Ilyas et al. in which bony trabeculae formation was not seen on day 7 in rats after honey application.[30] In another reports, new bone formation was observed in the extraction socket in the first and second weeks in rats after systemic administration of doxycycline and erythromycin.[31] Hajizadeh et al. also reported that honey could accentuate bone healing of mandibular small defects in rats.[32] The healing process of the extraction socket begins with the formation of woven bone which ultimately remodels and restores the defect.[33] The osteoblasts produces new bone that starts from the apical and lateral walls toward the center and the healing process culminates with filling of the socket by trabecular bone.[34] It is reported that bone formation in the socket in human begin as early as 10 days after extraction and it is completely restored with bone within 10-20 weeks.[35-36] In our study bony trabeculae formation was evident at day seven after tooth extraction. Biologic differences among individuals, alveolar socket size and the extent of surgical trauma induced during the extraction procedure influences the healing of extraction socket.[18] Due to its modulatory effect on inflammation, honey may have promoted wound healing.[37] Various growth factors and cytokines initiate a series of events via ligand-receptor interactions, including signal transduction, gene transcription, mRNA-directed protein biosynthesis and secretion of post-translational proteins and act as mitogenic and angiogenic signals at the early stage of bone healing.[38] Honey has anti-bacterial, anti-oxidant, anti-viral and anti-inflammatory properties. High sugar content of the honey produces osmotic effect which preclude the bacterial growth and boost healing. The pH of honey is 4.4 which causes acidification of wound and promote healing along with decrease colonization by microorganism. Numerous phytochemicals like flavonoids, monophenolics, polyphenolics and vitamin C are present in honey that act as antioxidants. Honey also encompasses aqueous and lipophilic antioxidants which can act at different cellular levels as an ideal natural antioxidant which helps in decreasing inflammation and oxidative stress which causes further healing boost.[39] Further studies are needed to confirm the exact mechanism involved and the role of topical honey on extraction socket healing.

Honey application increased fibroblast proliferation in the socket compared to the control group. Similar finding was also reported by Ilyas et al in rat.[30] Signaling molecules like platelet-derived growth factor, insulin-like growth factors, transforming growth factor-beta and fibroblastic growth factors regulates initial healing responses in a wound by initiating cell migration, differentiation and proliferation.[40] Beneficial effect of honey in acceleration of wound healing is thought to be due to stimulation of cytokine production, tissue regeneration, angiogenesis and fibroblast and osteoblast growth.[5] Catalase present in honey minimizes excessive collagen content and hence it accelerates the wound healing process.[41]

Honey is cheaper than oral antibiotics and there is also no threat of antibiotic resistance with use its use on extraction socket. Our study findings suggests that honey application promoted wound healing in extraction socket in rabbits and hence it may be used to promote wound healing in human extraction socket in normal healthy patients as well as in diabetic and irradiated patients and also in alveolar osteitis; however further clinical studies should be performed in future to explore the efficacy of honey on healing of extraction socket in human. Our study had some limitations. The duration of the study was short as it was a pilot study. The chromatographic analysis of the honey was not done. Other parameters of wound healing like levels of cytokines and various growth factors, wound contraction and remodeling were not evaluated in the study. Bone density could not be measured through digital X-ray images. Since the study was conducted on small animal, further studies in larger animals like dogs, small ruminants, pigs, non-human primates could be conducted before extrapolating the study findings to the human. The comparison of the effect of honey with other already known anti-inflammatory drugs or anti-bacterial drugs was not performed due to some logistic problems.

CONCLUSION

With the limitations of the present study, it showed that local application of honey promoted the healing process after tooth extraction particularly by increasing fibroblast proliferation and amount of bone trabeculae formation. Additional studies are needed to investigate more extensively the effects of honey on healing of extraction socket in animals. Further studies in human should be conducted to establish the wound healing efficacy of honey and to sustain our results.

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