Original Article / Özgün Araştırma

The Effect of Hypericum Perforatum on Alveolar Bone Healing After Tooth Extraction

Hypericum Perforatum'un Diş Çekimi Sonrası Alveolar Kemik İyileşmesine Etkisi

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Keywords
Hypericum perforatum, Hypericaceae, St John's Wort, tooth extraction, wound healing

Anahtar Kelimeler
Hypericum perforatum, Hypericaceae, St John's Wort, diş çekimi, yara iyileşmesi

Received/Geliş Tarihi : 01.09.2020
Accepted/Kabul Tarihi : 23.04.2021

doi:10.4274/meandros.galenos.2021.37980

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Abstract

Objective: Hypericum perforatum L. (HP) (Hypericaceae) is used as a wound healing agent especially burns. Thus, the aim of this study was to evaluate the effect of an extract of HP on wound healing after tooth extraction with clinical, radiological and histopathological evaluations.

Materials and Methods: A unilateral upper central incisor was extracted from the rats. The rats were divided into four control and four test groups and were sacrificed 7, 14, 28, and 56 days after tooth extraction. HP was used in the test groups. Wound healing was evaluated clinically, radiologically and histopathologically.

Results: In clinical examinations, HP had a positive effect on wound healing. to the results of histopathological evaluations, with the use of HP, decrease in inflammation, better epithelial proliferation, connective tissue formation and cartilaginous tissue formation occurs. No effect was found on bone healing on radiological examination.

Conclusion: After tooth extraction the use HP extract result in better wound healing by clinical and histopathological evaluations. Further studies are required to evaluate the effects of oral surgery.

Öz

Amaç: Hypericum perforatum L. (HP) (Hypericaceae) özellikle yanıklarda yara iyileştirici ajan olarak kullanılmaktadır. Bu çalışmanın amacı, HP ekstraktının diş çekimi sonrası yara iyileşmesi üzerindeki etkisini klinik, radyolojik ve histopatolojik açıdan değerlendirmektir.

Gereç ve Yöntemler: Sıçanlarda tek taraflı üst orta kesici diş çekildi. Sıçanlar dört kontrol ve dört test grubuna ayrıldı ve diş çekildikten 7, 14, 28 ve 56 gün sonra sakrifiye edildi. Test gruplarında HP kullanıldı. Yara iyileşmesi klinik, radyolojik ve histopatolojik olarak değerlendirildi.

Bulgular: Klinik incelemelerde HP'nin yara iyileşmesi üzerinde olumlu etkisi vardır. Histopatolojik değerlendirme sonuçlarına göre HP kullanımı ile enflamasyonda azalma, daha iyi epitel proliferasyonu, bağ dokusu oluşumu ve kıkırdak dokusu oluşumu meydana gelir. Radyolojik incelemelerle kemik iyileşmesinde herhangi bir etki görülmedi.

Sonuç: Diş çekiminden sonra HP ekstraktının kullanılması klinik ve histopatolojik değerlendirmelere göre daha iyi yara iyileşmesi sağlamaktadır. Ağız diş ve çene cerrahisindeki etkilerin değerlendirilmesi için daha ileri çalışmalara ihtiyaç vardır.

Introduction

Successful wound healing involves initial inflammation, followed by cell proliferation, angiogenesis, epithelialization, and remodeling (1). In recent years, the use of herbal products to aid wound healing has increased; some of those products affect the various stages of healing and/or exert antimicrobial and antioxidant effects (2).

Hypericum perforatum L. (HP) has long been used as a folk medicine to accelerate the healing of wounds. Approximately 400 species of Hypericum from Europe, North America, Australia, New Zealand, Eastern Asia, and South America have been identified. Hypericum, a member of the Guttiferae family, grows in sunny areas and attains a height of 50-100 cm (3). The plant exhibits several pharmacological activities, including antimicrobial (antibacterial, antiviral, and antifungal), anti-inflammatory, and analgesic activities. Hypericum has traditionally been used to treat dermal injuries, to aid wound healing, to sterilize wounds prior to surgery (4-6), and to treat psychiatric problems like anxiety and depression due to its sedative effect (7). Recently, HP is also used to treat skin injuries and burns, especially injuries to soft tissue in modern times (8-11).

Beneficial effects of topical application of HP have been reported in both animal (12) and human (8) studies. However, research on HP in the field of dentistry is limited. Paterniti et al. (13) investigated the effect of HP on periodontitis in rats, Raak et al. (3) performed a systematic review on the use of HP for pain conditions, and Mendi et al. (14) evaluated the effect of HP on the differentiation of dental pulp *in vivo*. In this study, we evaluated the effect of HP on wound healing after tooth extraction by performing clinical, radiological, and histological analyses.

Materials and Methods

Preparation of HP Extract

Aerial parts of HP were collected around Kayseri/Turkey, washed, dried at room temperature, and stored in the Herbarium of the Faculty of Pharmacy, Erciyes University, Kayseri, Turkey. A total of 25 g of dried and crushed material was extracted into 500 mL methanol for 8 h using a Soxhlet extractor, evaporated to dryness at 40 °C in a rotary evaporator, and lyophilized.

Animals and Tooth Extraction

The experimental protocols were approved by the Erciyes University Animal Ethical Committee, Kayseri, Turkey (approval number: 11/124, date: 23.11.2011). We used 48 Wistar Albino rats (mean age, 1 year; weight, 250 g). The rats were housed in a temperature-controlled room (22±2 °C) under a 12/12 h light/dark cycle with free access to food and water, and were randomly divided into eight groups of six animals each (four test and four control groups).

Test Groups

Test group 1: The unilateral upper central incisor was extracted from rats. hp extract (0.2 cc) was inserted into the extraction sockets and the mucosae were sutured using 3.0 resorbable sutures. the rats were sacrificed 7 days after tooth extraction. the surgeries were performed under general and topical anesthesia. ketamine (ketalar; Pfizer, New York, NY, USA) (50 mg/kg) and xylazine (Rompun, Bayer, Leverkusen, Germany) (8 mg/kg) were used for general anesthesia, and articaine (Ultracain DS; Hoechst AG, Frankfurt, Germany) (0.2 mL) for local anesthesia and hemostasis.

Test group 2: Same as test group 1, but rats were sacrificed 14 days after tooth extraction.

Test group 3: Same as test group 1, but rats were sacrificed 28 days after tooth extraction.

Test group 4: Same as test group 1, but rats were sacrificed 56 days after tooth extraction.

Control groups

Control group 1: Same as test group 1, but HP extract was not used.

Control group 2: Same as test group 2, but HP extract was not used.

Control group 3: Same as test group 3, but HP extract was not used.

Control group 4: Same as test group 4, but HP extract was not used.

All surgeries were performed by the same operator. The surgical area was evaluated for any sign of infection. Animals with ≥15% weight loss or wound infection were excluded. There were animal losses in control groups 1 and 4 (one in each group).

Clinical Observation

Extraction socket healing was evaluated, and mucosal closure was graded as none (-), partial (+), or complete (++). Complete mucosal closure was graded as "complete", partial closure without complete

primer mucosal healing as "partial closure", and no closure of the socket or sign of alveolar osteitis as "none."

Radiological Examination

The maxillae were dissected out and fixed in 10% (v/v) formalin. All maxillae without decalcification were evaluated by micro-computed tomography (CT) (SkyScan 1172; SkyScan, Kontich, Belgium).

Sections were scanned using a 0.5 mm aluminum and copper filter (80 kV, 124 mA, rotation of 360° rotation step of 0.40°). The approximate scanning time per section was 70 minutes. Images with a resolution of 2,000×2,000 and pixel size of 13.68 µm were obtained. NRecon 1.6.9.4 (SkyScan) software was used to eliminate noise and artifacts. SkyScan DataViewer 1.5.0 (SkyScan) was used to eliminate positional errors in the sagittal, transverse, and vertical directions. The examination areas were limited by determining the first and last transversal section in which the extraction socket was seen using CTAn 1.13.5.1 software (SkyScan). Next, the extraction socket was isolated from the surrounding tissues and air spaces, and the optimal thresholding segmentation was determined.

Bone mineral density, tissue mineral density, formed bone volume (object volume; OV), formed bone percentage (percentage object volume; POV), formed bone intersection surface, formed bone structural thickness, and the "percentage of newly formed bone" (BVC) were evaluated.

Histopathological Evaluation

After micro-CT evaluation, tissue sections containing an extraction socket and surrounding mucosae were prepared for histopathological evaluation. The sections were fixed in 4% formalin solution. Decalcified bone sections were prepared using a cutting/grinding instrument (Exakt 300 CL; Exakt Apparatebau, Norderstedt, Germany) by cutting from the center of the extraction socket in the sagittal plane. Sections were thinned to 70 µm using a micro-abrasion instrument (Exakt 400 CS; Exakt Apparatebau) with 1,000-, 1,200-, and 2,500-mesh abrasion tools. Sections were stained with hematoxylin-eosin and examined under a light microscope (BX50; Olympus, Tokyo, Japan). Epithelial proliferation, inflammatory cell density (number of polymorphonuclear leukocytes), cartilage and bone formation, and collagen fiber density status was classified as none (-), moderate (+), or intense (++).

The clinical, radiological, and histological evaluations were performed independently by two blinded investigators. Any discrepancies were resolved by discussion and consensus.

Statistical Analysis

MINITAB 14 (Minitab Inc., State College, PA, USA) power analysis software was used for sample size determination. The required sample size was determined as five animals per group (α=0.05, 80% power). However, six animals were included in each group to offset any losses. SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Fisher's Exact test, the chi-squared test, or the Mann-Whitney U test were used for between-group comparisons, as appropriate, and $p<0.05$ was taken to indicate statistical significance.

Results

Clinical Observations

At 7 days after extraction, mucosal closure was significantly higher in the test groups than the control groups ($p<0.05$), but not at 14, 28, or 56 days ($p>0.05$).

Radiological Examination

At 7 days after extraction, the OV, POV and BVC values were significantly higher in the control groups than the test groups ($p<0.05$, Figures 1, 2).

Histopathological Evaluation

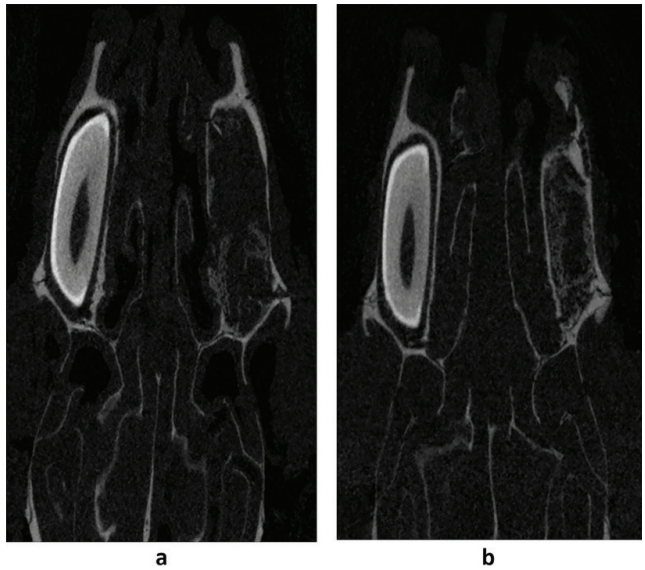

Figure 1. Horizontally section of micro-CT images. (a) 14 days control group. (b) 14 days test group
Micro-CT: Micro-computed tomography

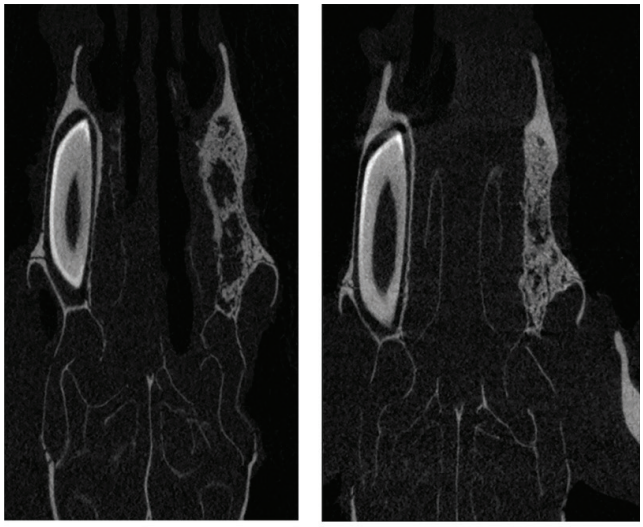

Figure 2. Horizontally section of Micro-CT images. (a) 56 days control group. (b) 56 days test group
Micro-CT: Micro-computed tomography

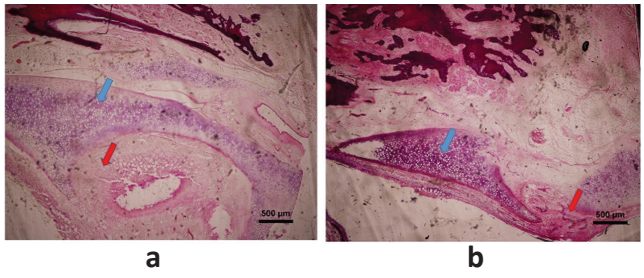

Figure 3. Connective tissue (red arrow) and bone (blue arrow) formation. H&E 500 µm. (a) 28 days control group. (b) 28 days test group
H&E: Hematoxylin and eosin

At 7 days after extraction, epithelial proliferation was significantly greater in the test groups and inflammatory cell infiltration was significantly greater in the control groups ($p<0.05$, Table 1). At 14 days, epithelial proliferation and connective tissue formation were significantly greater in the test groups and inflammatory cell infiltration was significantly greater in the control groups ($p<0.05$, Table 2). At 28 days, connective and cartilaginous tissue formation rates were significantly higher in the test groups ($p<0.05$, Figure 3). At 56 days, the cartilaginous tissue formation rate was significantly higher in the test groups ($p<0.05$).

Discussion

This study assessed the effect of HP extract on wound healing after tooth extraction based on radiological, and histopathological analyses. HP extract promoted wound healing according to the clinical and histopathologic evaluations; however, it did not significantly affect bone healing according to the radiologic evaluation. Therefore, HP may promote wound healing after tooth extraction.

HP increased mucosal closure, but the effect was significant only at 7 days after extraction. Previously, it was reported that HP significantly accelerates wound healing (8-12,15). However, those studies applied HP extract topically to the wound, whereas we delivered the extract directly to the extraction socket. This difference may explain the lower wound healing efficacy of HP in this study.

Histologically, wound healing involves an inflammatory phase, a proliferative phase, and a remodeling phase, all of which can be accelerated by treatment. The inflammatory phase is essential for wound healing, but should not be prolonged because this delays healing (16). In this study, HP extract reduced the severity and duration of the inflammatory phase. The test groups showed significantly lower inflammatory cell infiltration at 7 and 14 days, consistent with prior reports of the anti-inflammatory effect of HP, particularly during early phases of inflammation (10,12,15,17).

In this study, the HP extract promoted epithelial proliferation, and the formation of connective and cartilaginous tissue. Granulation tissue is primarily composed of fibroblasts, collagen, edema, and vessels (11). In the proliferative phase of wound healing, fibroblasts promote scar formation and collagen synthesis. The flavonoids in HP promoted epithelial cell proliferation and migration, and collagen synthesis, and increased the proportion of polygonal fibroblasts (9).

In this study, connective tissue formation peaked at day 14, and decreased at days 28 and 56, in both the test and control groups. During wound healing, collagen density peaks within the first 14 days and decreases gradually thereafter as bone tissue forms (18); this may explain our results. Also, HP reportedly accelerates epithelization in the early stages of primary and secondary wound healing (12,19,20).

Table 1. Analysis of histopathological evaluation results of control and test groups at 7 days time point

	Control group 1			Test group 1		
	-	+	++	-	+	++
Epithelial proliferation*	4 (80%)	1 (20%)	-	1 (16.7%)	4 (66.7%)	1 (16.7%)
Inflammatory cell infiltration†	-	1 (20%)	4 (80%)	-	3 (50%)	3 (50%)
Connective tissue formation	-	4 (80%)	1 (20%)	-	4 (66.7%)	2 (33.3%)
Cartilaginous tissue formation	-	3 (60%)	2 (40%)	-	4 (66.7%)	2 (33.3%)
Bone formation	-	-	-	-	-	-

Percentage of different histologic findings in the control and test groups. * and † indicates statistically significant difference between the control and test groups ($p<0.05$). (-): None, (+): Moderate, (++): Intense

Table 2. Analysis of histopathological evaluation results of control and test groups at 14 days time point

	Control group 2			Test group 2		
	-	+	++	-	+	++
Epithelial proliferation*	2 (33.3%)	3 (50%)	1 (16.7%)	-	3 (50%)	3 (50%)
Inflammatory cell infiltration†	-	2 (33.3%)	4 (66.7%)	-	4 (66.7%)	2 (33.3%)
Connective tissue formation‡	-	4 (66.7%)	2 (33.3%)	-	2 (33.3%)	4 (66.7%)
Cartilaginous tissue formation	-	4 (66.7%)	2 (33.3%)	-	3 (50%)	3 (50%)
Bone formation	-	6 (100%)	-	-	5 (83.3%)	1 (16.7%)

Percentage of different histologic findings in the control and test groups. *, † and ‡ indicates statistically significant difference between the control and test groups ($p<0.05$). (-): None, (+): Moderate, (++): Intense

The flavonoids in HP slow or prevent necrosis and improve vascularity, which reduces lipid peroxidation, increases wound circulation and collagen fiber strength, increases DNA synthesis, and prevents cell injury (11).

Collagen, which is the main structural protein of connective tissue, promotes healing by enhancing connective tissue formation and increasing tissue vascularization (21). Motta et al. (22) reported that collagen has a direct role in fibroblast metabolism and promotes the aggregation of fibroblasts, which in turn promotes collagen lysis and fibroblast proliferation. Furthermore, Öztürk et al. (9) and Hostanska et al. (23) reported that HP promotes wound healing by stimulating collagen synthesis and fibroblast migration, rather than cell proliferation. However, Süntar et al. (10) showed that the promotion of epithelialization and healing by HP is not related to fibroblast proliferation or angiogenesis.

HP did not significantly modulate bone formation in this study, in contrast to several previous studies; the difference may be due to use of differences in study methodologies. Halicioglu et al. (24) reported that systemic use of HP accelerated the formation of new bone in orthopedically expanded premaxillary sutures in rats. Damlar et al. (25) reported that, in rabbits, oil extracts of HP improved bone healing in defects filled with bovine-derived xenograft. Paterniti et al. (13) evaluated the effect of HP extracts on active inflammatory periodontal disease; HP significantly inhibited plasma extravasation and bone resorption during periodontitis.

The presence of an infection may interrupt or slow wound healing. Hyperforin shows antibacterial activity against Gram-positive bacteria (4). The wound healing effect of HP might be related to its antibacterial activity (20).

Conclusion

Within the limitations of this study, HP extract promoted wound healing after tooth extraction, according to clinical and histopathological evaluations. Because HP extract is infrequently used in dentistry and no study has assessed its utility for oral surgery wounds, including tooth extractions, our findings will guide future research on the utility of HP extract in oral surgery.

Ethics

Ethics Committee Approval: The experimental protocols were approved by the Erciyes University Animal Ethical Committee, Kayseri, Turkey (approval number: 11/124, date: 23.11.2011).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.G.Ç., Concept: O.E., Design: O.E., Data Collection or Processing: F.G.Ç., Analysis or Interpretation: F.G.Ç., Literature Search: F.G.Ç., Writing: F.G.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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