

Application of Propolis to Dental Sockets and Skin Wounds

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(Received 12 September and accepted 26 October 1989)

Key words: propolis, alcohol, wound healing, tooth extraction, skin

Abstract

The purpose of this study was to examine histologically the effects of propolis topical application to dental sockets and skin wounds.

After topical application of either a 10 % hydro-alcoholic solution of propolis or 10 % hydro-alcoholic solution alone, cutaneous wound healing and the socket wound after tooth extraction were examined.

The rats were sacrificed at 3, 6, 9, 15 and 21 days after the operation. The specimens were subjected to routine laboratory studies after staining with hematoxylin and eosin. It was concluded that topical application of propolis hydro-alcoholic solution accelerated epithelial repair after tooth extraction but had no effect on socket wound healing.

Introduction

Natural medicaments have been receiving an increased amount of attention recently, and popular publications and scientific reports have claimed that propolis, a compound obtained from bees, has some medicinal properties.

In a study of bacteria associated with insects, REMY CHAUVIN observed that bees (*Apis mellifica*) were more resistant to infections. This prompted him to perform some experiments, which subsequently confirmed the presence of some antibiotic on the surface of the bee's body and also in honey, wax, pollen, venom and propolis.

Propolis has been employed in orthopedics^[1], endocrinology^[2] dermatology^[3-5], pneumatology^[6] and gastroenterology^[7]. In dentistry, propolis has been used to treat aphtha^[8], *Candida albicans*^[9], acute necrotizing ulcerative gingivitis (ANUG)^[10] and periodontitis^[11]. SCHELLER et al.^[12] employed propolis as dental pulp direct dressing in dogs. GAFAR et al.^[13] presented clinico-statistical results about the use of propolis as an antiseptic in the treatment of pulp gangrene.

MAGRO FILHO et al.^[14] studied histologically the reactions of connective tissue in contact with an ointment consisting of comfrey, propolis and honey, and observed accelerated new formation of connective tissue in the first 10 postoperative days. A mild inflammatory reaction appeared on day 60, probably due to the

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vehicle, petrolatum and lanolin, which acted as a foreign material. They recommended this ointment as a therapeutic option for superficial applications.

Thus, taking into consideration the paucity of histological studies about propolis application for wound healing, the present investigation was performed to analyze the effects of topically applied propolis solution on socket wound healing after tooth extraction and on skin wounds in rats.

Materials and Methods

Forty-five male albino rats weighing 160-220 g were used in this study. After general anesthesia induced with thionembutal (Abbott), the dorsal region was shaved and disinfected. A skin fragment 5 mm in diameter was removed with a punch^[15]. In the same rats, the right maxillary incisors were extracted using specially designed instruments^[16].

Three experimental groups were prepared:

- i) Control—Fifteen animals which did not receive any treatment of dental sockets or skin wounds.
- ii) Hydro-alcoholic solution—Fifteen animals which received topical application of a 10 % hydro-alcoholic solution (1 ml of 96° grain alcohol dissolved in 9 ml of distilled water) to dental sockets immediately after tooth extraction and daily on skin wounds.
- iii) Propolis—Fifteen animals which received topical application of a 10 % hydro-alcoholic solution of propolis (1 ml of alcoholic extract of propolis dissolved in 9 ml of distilled water) to dental sockets immediately after tooth extraction and daily on skin wounds. The alcoholic extract of propolis was obtained by filtering 15 g of natural propolis with 100 ml of 96° grain alcohol. Propolis was collected *in natura* in a region of mixed flowers (*Citrus sinensis*, *Coffea arabica*, *Saccharum officinarum*, *Eucalyptus globulus*).

In each group the gingiva was sutured with No 4-0 polyester.

The animals were maintained in individual cages and received standard solid food and water *ad libitum*. The rats were killed in groups of three after postoperative periods of 3, 6, 9, 15 and 21 days. The wounds in the dorsal area, removed with marginal skin, and the right maxilla were fixed in 10 % formalin solution and subjected to routine laboratory studies after sectioning at a thickness of 6 μ m and staining with hematoxylin and eosin.

For histological evaluation of skin wounds, the superficial scars were measured and inflammatory cells were scored using a reticule adapted to the Kpl Zeiss ocular. At $\times 25.6$ magnification, the reticule was placed over the wound area and the number of small squares between epithelium levels or epithelial buds was counted. At $\times 320$ magnification, polymorphonuclear neutrophils, lymphocytes, histiocytes and plasma cells were scored in areas A, B and C (connective tissues above the epithelial buds or at level of the wound and the central area). For scoring of cells, the periphery of the reticule with 16 small squares was employed (Fig. 1). Arithmetic means of the number of small squares covering the wound areas and of the amounts of inflammatory cells were calculated after various

postoperative periods.

Results

Skin wounds

Control: After 3 days, a mild degree of epithelial bud growth had occurred between newly formed connective tissue and the scab. This connective tissue presented areas of edema and degeneration (Fig. 2). On day 6, the epithelial bud covered 2/3 of the wound area, but the newly formed connective tissue was still edematous and exhibited polymorphonuclear neutrophils and young fibroblasts. By day 9, epithelium with a thin layer of keratin covered the wound. The connective tissue contained many collagen fibers and newly formed capillaries. After 15 days, the epithelium was thicker and showed some papillary crests, the adjacent connective tissue contained numerous fibroblasts and collagen fibers. On day 21, keratinized stratified squamous epithelial tissue covered the connective tissue formed from fibroblasts, collagen fibers and some fibrocytes.

Hydro-alcoholic solution: In comparison with the control group, there was more pronounced edema with polymorphonuclear neutrophils and lymphocyte infiltration, amorphous areas and some macrophages (Fig. 3). Epithelial tissue showed extensive covering of the wound on days 6 and 9. On day 15, epithelial tissue with some papillary crests was present and the subjacent connective tissue exhibited some lymphocytes and histiocytes and a few polymorphonuclear neutrophils. In the later post-operative period, keratinized stratified squamous epithelial tissue was seen.

Propolis: On day 3, pronounced epithelial buds had grown from the wound level (Fig. 4) and epithelial tissue covered a large area of the wound. The epithelial tissue was thicker, presenting papillary crests, on day 15 and in the later period this tissue was keratinized and stratified.

The inflammatory cell scores and data of wound measurements are presented in Figs. 5 and 6.

Dental socket healing

To determine the results of healing, we established three equal areas in the dental socket designated as the cervical, middle and apical thirds, respectively, from the gingival margin to the alveolar fundus.

Control: After 3 days, the alveolus was filled with a blood clot and the remnants of the periodontal ligament near the cortical bone. In the apical and the middle thirds there were newly formed fibroblasts and capillaries invading the blood clot. On day 6, the alveolus was partly filled with newly formed connective tissue and showed thin osseous trabeculae principally in the apical third. On day 9, the alveolus was filled with connective tissue and exhibited areas of newly formed osseous trabeculae, which were thicker in the apical third. After 15 days these osseous trabeculae were thicker at the periphery of the cortical bone (Fig. 7). On day 21, the alveolus exhibited thick osseous trabeculae and some areas of

mature connective tissue.

Hydro-alcoholic solution: On day 3, only the apical third of the alveolus was filled with a blood clot and newly formed fibroblasts and capillaries showed peripheral invasion. At the middle and cervical thirds, there was predominance of fibrin with an amorphous appearance and a few red cells. On day 6, the apical third contained newly formed connective tissue and some thin osseous trabeculae, near the cortical bone. The middle and cervical thirds contained fibrin, a few cells and polymorphonuclear neutrophil infiltration. After 9 days, the apical third showed newly formed connective tissue and osseous trabeculae surrounding some areas filled with either blood clots or the irrigation solution. In the cervical third, inflammatory infiltration and some necrotic cortical bone fragments were seen. On day 15, there was more intense formation of new osseous trabeculae near the apical cortical bone and persistent areas of intercellular edema (Fig. 8). In the late period, thick or thin osseous trabeculae were present at the apical and remaining thirds of the alveolus, respectively.

Propolis: By day 3, there were blood clots, remnants of the periodontal ligament and newly formed fibroblasts and capillaries in the apical third along with a certain amount of osteoid tissue. On day 6, osteoid tissue and thin osseous trabeculae were present at the apical third. At the middle third level, there were newly formed osseous trabeculae. On day 9, at the apical and middle thirds, there were osseous trabeculae or osteoid tissue near the cortical bone. These newly formed osseous trabeculae (Fig. 9) were thicker at the same levels on day 15, but there were some areas filled with connective tissue. The middle and cervical levels showed polymorphonuclear neutrophil infiltration. Thick osseous trabeculae were present at the apical and middle thirds in the late postoperative period. In the cervical third, these osseous trabeculae were thin and there were persistent areas of polymorphonuclear neutrophils.

Discussion

Scientific reports on propolis have not standardized its concentration, vehicles or indications. It has been apparent that some users and even professionals have adopted empirical procedures with this apitherapeutic. We therefore used a common commercial product, making a 10 % aqueous solution^[1]. BECKEMEIER et al.^[17] observed a more efficacious antibacterial effect of an ethanol extract of propolis in comparison with other means of active-principle extraction.

In our study there was a delay of wound healing in the group treated with a hydro-alcoholic solution. The more intense intercellular edema seen on day 3 may explain the decrease of inflammatory cells in this group. Naturally, grain alcohol dissolved in distilled water provoked a less intense reaction than a solution of 96° alcohol and xylocaine without vasoconstrictor^[18]. It is recognized that grain alcohol is less injurious because it has fewer chemical contaminants than alcohol derived from sugar cane. Therefore, we think that the hydro-alcoholic solution

provoked less toxic effects and improved the humidity of the wounds.

In the skin wounds treated with propolis solution, epithelization and connective tissue maturation were accelerated. This could be due to some environmental factors, as well as an antibacterial effect, such as humidity, the presence of Zn, Fe and Ca in the ethanol extract of propolis^[19], the activation of cell metabolism *in vitro*^[20] and the probable action of propolis as a mitotic inhibitor of the chalone-adrenalin complex in rat skin^[21]. However, our present study was unable to indicate which of these factors favored the observed epithelization.

The arithmetic means of inflammatory cell scores in skin wounds treated with propolis solution, although indicating more pronounced irritation on postoperative day 3, showed a continuous decrease of inflammatory cells until day 21. On the other hand, the hydro-alcoholic solution produced a non-uniform result, and in the late postoperative period this group showed a higher inflammatory cell score than the propolis group.

Wound quantification using a reticule of small squares also suggested an increase of epithelization in the animals treated with propolis solution.

Dental socket irrigation with hydro-alcoholic solution was reported to delay the process of wound healing after tooth extraction more markedly than irrigation with physiological saline solution^[22]. However, our present result was similar to that obtained in a group treated by SAAD NETO et al.^[23], in which the dental socket was irrigated with xylocaine without vasoconstrictor.

In our study, we observed intercellular edema and amorphous tissue in the newly formed connective tissue. We think that the hydro-alcoholic solution did not provoke a more intense reaction in view of its absorption. Generally, liquid substances produce different reactions from those induced by slow-release products or solid materials^[24]. On postoperative day 3, in the group treated with propolis solution, there was an acceleration of wound healing. After this period, however, this group always showed a delay in comparison with the control group. Only on postoperative day 21 there was more acceleration than in the hydro-alcoholic group. For this reason, we believe that the presence of resinous remnants of propolis may explain the altered wound healing. The dental socket is known to have some particular aspects of wound healing that differ from other bone healing processes^[24].

The concentration of propolis employed in this study did not provoke any marked alteration of alveolar socket healing, even though a favorable influence was noted during the initial postoperative period. Therefore, we consider that a hydro-alcoholic solution of propolis has no advantageous effect on wound healing after tooth extraction. However, we think that further studies are justified in order to fully evaluate the efficacy of propolis in infection.

Conclusions

It was concluded that application of a 10% hydro-alcoholic solution of propolis accelerated the epithelization of skin wounds but did not accelerate wound healing after tooth extraction.

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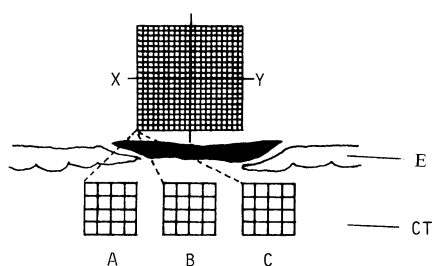


Fig. 1 Reticule that was adapted to the ocular of the optical microscope. First position: the x and y points were located over the wound area and the number of small squares between epithelial levels or buds was counted. 2nd position: Inflammatory cells were scored at areas A, B and C, employing only 16 small squares.
(E=epithelium; CT=connective tissue)



Fig. 2 Skin wound. Control. Day 3. Epithelium level and connective tissue with inflammatory cell infiltration and areas of edema are seen (H. & E., x63).

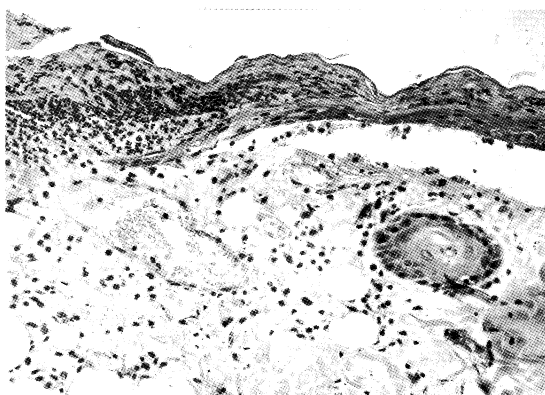


Fig. 3 Skin wound. Hydro-alcoholic solution. Day 3. Epithelial bud between the scab and connective tissue with pronounced edema and inflammatory cell infiltration are seen (H. & E., x160).



Fig. 4 Skin wound. Propolis. Day 3. Epithelial bud has grown between the scab and newly formed connective tissue (H. & E., x160).

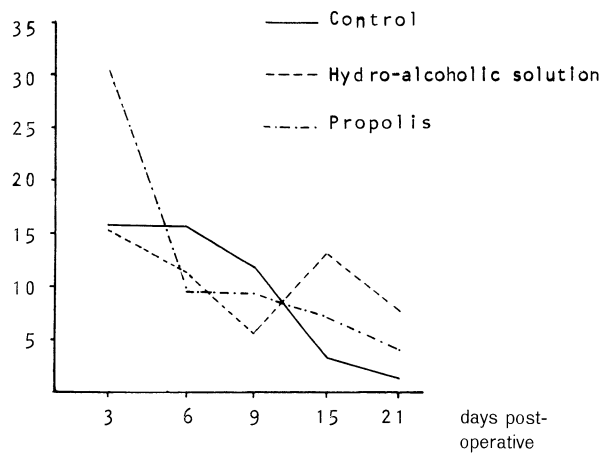


Fig. 5 Means of inflammatory cells

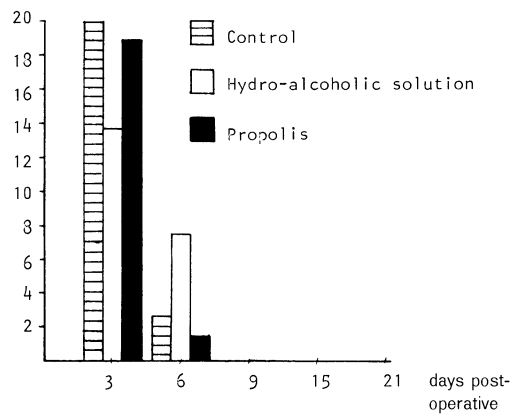


Fig. 6 Means of numbers of small squares over non-epithelized wound areas

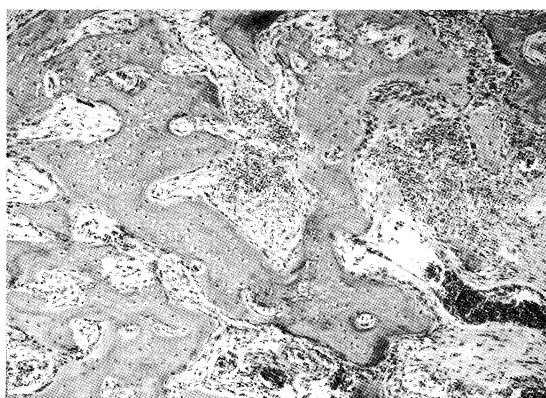


Fig. 7 Dental socket. Control. Day 15. Thick osseous trabeculae are evident (H. & E., x63).

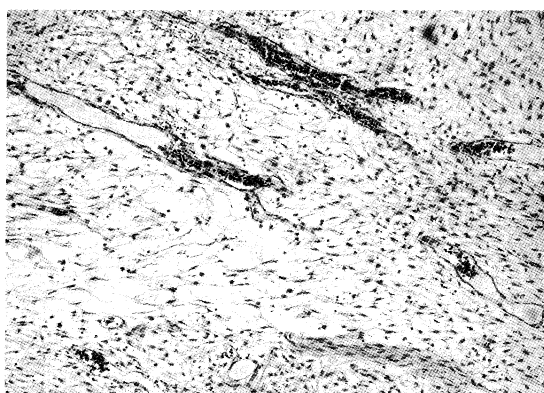


Fig. 8 Dental socket. Hydro-alcoholic solution. Day 15. Connective tissue with areas of intercellular edema are evident (H. & E., x63).

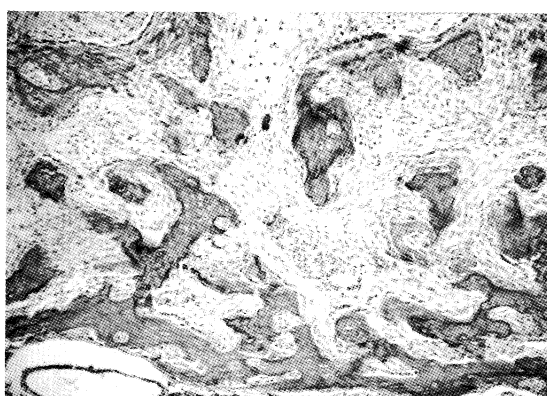


Fig. 9 Dental socket. Propolis. Day 15. Newly formed osseous trabeculae are seen in the middle third (H. & E., x63).