



BIOCHEMICAL EVALUATION OF THE THERAPEUTIC EFFECTIVENESS OF HONEY IN ORAL MUCOSAL ULCERS

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ABSTRACT

The aim of this study was to describe and quantify the therapeutic value of honey in oral mucosal ulcers healing in comparison with Glyceroloxxytriester (TGO). We also aimed to biochemically evaluate the healing effects of honey which had been collected from the Blacksea region flora on mucosal ulcers resulting in material loss.

Thirty wistar rats (240±30 g) were enrolled in this study. Excisional wounds were performed in all rats for animal oral mucosal ulcer model. They were randomly allocated to three groups: group 1 was treated with Apitherapeutic agent or honey (0,1 ml, 2x1), group 2 was treated with TGO (0,1ml, 2x1) locally, Group 3 served as the control group.

Following the surgical procedure on day 7, biopsy specimens were taken from right buccal mucosa and on day 14 biopsy specimens were taken from left buccal mucosa in all rats. Afterwards, hydroxy pyroline levels were measured. Data were analyzed statistically.

There was no statistically significant difference between Group 1 and 2, and also between Group 2 and 3, but there was statistically significant difference between Group 1 and 3 on day 7. There was no statistically significant difference between Group 1, 2 and 3 on day 14.

KEY WORDS: honey, oral ulcers, hydroxy pyroline

INTRODUCTION

Nature is the most important source for producing new medicines. Today natural honey and bee products have an important role in nutrition and treatment. Honey and honey-based products are used as sources of energy and nutrition. Also, it is important in human health care and disease treatment (1,2). Commonly aphthous ulcers and/or aphthous stomatitis are used to describe oral mucosal wound. Oral mucosal wound or mouth ulcers are sores or open lesions in the mouth which are caused by various disorders (3,4). Lesions are less common on the heavily keratinized palate or gingiva. In mild recurrent aphthous ulcers, the lesions reach a size of 0,3 to 1,0 cm and begin healing within a week (5). Most of the patients apply to dental clinics because of oral ulcers complications. During the past decade, there has been a worldwide increase in the use of traditional and complementary or natural systems of medicine (6,7). Many agents have been used to rehabilitate these patients. Mouth ulcers generally last for 10 to 14 days even if no treatment is applied. They sometimes last up to 6 weeks (3, 4, 5). Honey seems to offer considerable benefits in wound care, particularly for the treatment of chronic and infected wounds and for the treatment of burns (8). Honey is described in a number of ancient texts as a wound healing agent either on its own or in combination with other ingredients (9). Also glyceroloxytriester (TGO) is recommended for oral ulcers and for prosthetic stomatitis. TGO does not have any side effects like honey. Hydroxyproline was chosen as a potential marker of collagen because of its relevance in determining changes in tissue collagen content (10). There is an interest in hydroxyproline levels as an indicator of various pathological conditions related to collagen degradation (10,11,12). The purpose of this study was to evaluate the clinical efficiency of honey in healing oral mucosal ulcers in comparison with TGO. We also aimed to biochemically and clinically evaluate the healing effects of honey which had been collected from the Blacksea region flora on mucosal ulcers resulting in material loss.

MATERIALS AND METHODS

Thirty male wistar albino rats (average weight 240 ± 30 g) were included in this study. The animals were kept in a room which had a constant temperature of $22 \pm 1^\circ\text{C}$ with a 12-hour-light and 12-hour-darkness cycle and were fed with Standard pellet chow and wa-

ter which were available ad libitum. Rats were divided randomly into three groups as follows; group 1 was treated with honey, group 2 was treated with TGO, Group 3 was served as the control group. The type of honey used in this study was pure blossom (multi-floral) honey which was procured from apiary of local beekeepers in Blacksea Region of Turkey. Moreover, essential animal ethic report was received from Ondokuz Mayıs University Animal Ethic Commity. Excisional wounds were performed in all groups. Anesthesia was performed by i.p. Xylazine hydrochloride 0,1-0,2 mg/kg IM for 45 minutes, ketamine 10-15 mg/kg IM. 1mm thickening buccal mucosa were demarked with a punch biopsy apparatus (5 mm diameter) then taken using scalpel, in the buccal mucosa of 30 Wistar rats. When oral ulcers occurred on the first day, group 1 was treated with honey (0,1 ml, 2x1), group 2 was treated with TGO (0,1 ml, 2x1) locally. No medication was given in group 3. Following the surgical procedure biopsy specimens were taken from right buccal mucosal wound on day 7 and, the biopsy specimens were taken from left buccal mucosal wound using the same surgical procedure on day 14. Biochemical analysis: The level of hydroxy-proline (Hyp) in surgical wound was tested using alkaline hydrolysis assay on days 0, 7 and 14 after operation respectively. The samples were freeze-dried and stored at -80°C until use. Hydroxyproline was measured using Bergman's spectrophotometric method (13). Data obtained from both tests were analyzed with one-way analysis of variance, ANOVA, using statistical software (SPSS version 11.0 software) on a personal computer.

RESULTS

There was no infection throughout the wound healing process. Reepithelialisation was started on the first day for all groups. The wounds of all groups were covered by new mucosa epithelium and were similar to the normal one on day 7 and 14. The means and standard deviations were recorded for each group, and Tukey's HSD test was used to determine significant differences between the groups. One-way ANOVA was used to compare the differences of the means of the groups. Variances of the groups were homogeneous. The means of the groups were as follows respectively: for Group 1, it was 18,2722 (ranged between 16,3172- 20,2273, Confidence intervals %95); for Group 2 it was 17,8520 (ranged between 16,2047-19,4993, Confidence intervals %95) and for

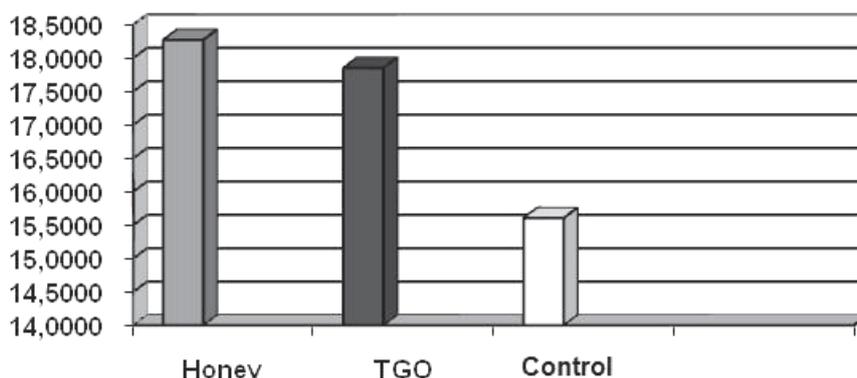


FIGURE 1. The mean of the Groups on day 7.

Group 3 it was 15,6044 (ranged between 14,6742-16,5347, Confidence intervals %95) on day 7 (Figure 1). Tukey's test was applied for the differences of the means of the groups. According to the Tukey's, test there was no statistically significant difference between Group 1 and 2 ($p=0,902>0,05$), and also between Group 2 and 3 ($p=0,071>0,05$), but there was statistically significant difference between Group 1 and 3 ($p=0,033<0,05$). Additionally, the mean of the Group 1 was 15,2425 (ranged between 13,4616- 17,0234, Confidence intervals %95); the mean of the Group 2 was 15,5410 (ranged between 14,9642- 16,1178, Confidence intervals %95) and the mean of the Group 3 was 14,0450 (ranged between 12,9739- 15,1161, Confidence intervals %95), on day 14 (Figure 2). According to the results, there was no statistically significant difference between Group 1 and 2 ($p=0,910>0,05$). Similarly, there was no statistically significant difference between Group 2 and 3 ($p=0,091>0,05$). Finally, there was no statistically significant difference between Group 1 and 3 ($p=0,240>0,05$).

DISCUSSION

Commonly aphthous ulcers and/or aphthous stomatitis are used to describe oral mucosal wound. Oral mucosal wound or mouth ulcers are open lesions within the mouth, caused by various disorders. An ulcer is a crater-like lesion on the mucous membrane caused by an inflammatory, infectious, or malignant condition. Various types of wound can appear anywhere within the mouth, including the inner cheeks, gums, tongue, lips, or palate (3, 4). The early lesions are vesicles which can affect any part of the oral mucosa. The vesicles are domeshaped and usually 2-3 mm in diameter. Rupture of vesicles leaves circular, sharply defined, shallow ulcers with yellowish or greyish floors and red margins. The ulcers are painful and may interfere with eating. The gingival margins are frequently swollen and red, particularly in children, and the regional lymph nodes are enlarged and tender (14). Most mouth wounds are cold sores (also called fever blisters), canker sores, or other irritations caused

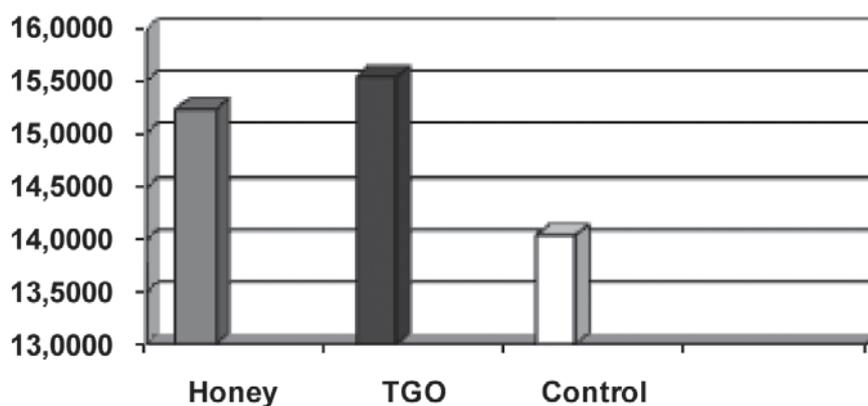


FIGURE 2. The mean of the Groups on day 14.

by several factors such as biting the cheek, a sharp or broken tooth or poorly fitting dentures, burning mouth from hot food or drinks etc (3,4,15). A smear showing virus-damaged cells is additional diagnostic evidence (14). The herpes virus ulcers can be provoked by other pathologies, especially if there is fever, stress, hormonal changes (such as menstruation), and sun exposure (16). The cause of some oral mucosal ulcers may be related to a temporary weakness in immune system (for example, from cold or flu), hormonal changes, mechanical irritation, stress, low levels of vitamin B12, folate, iron and ferritin (5,16). Oral lesions usually resolve within a week to ten days, but malaise can persist so long that an adult may not recover fully for several weeks (14). Less commonly, oral mucosal ulcers can be a sign of an underlying illness, tumour, or reaction to a medication; infection, autoimmune diseases, malignancy, bleeding disorders and immunosuppression to name some (3,4). Drugs that might cause oral mucosal ulcers include chemotherapeutic agents for cancer, aspirin, barbiturates, penicillin, phenytoin, streptomycin, sulfonamides (15).

In the literature, a lot of methods were described in creating ulcers on the oral mucosa. Fujisawa et al described that oral mucosal ulcers can create on the gingiva of the rabbits by chemical injury with acetic acid. Chen et al. (17) established a rat model of oral mucositis using busulfan (6.0 mg.kg⁻¹.d⁻¹) x 4 d and cyclophosphamide (120 mg.kg⁻¹.d⁻¹) x 2 d were administered by intrastomach perfusion and intraperitoneal injection, respectively. Then cheek mucosa was irritated by superficial scratching on day 6. Lara et al. (18) presented an animal model for mucositis induced by fluorouracil in rats then mucosa was irritated by superficial scratching with an 18-gauge needle. Mitsuhashi et al (19) performed an animal model for mucositis induced in hamsters through a combination treatment of 5- fluorouracil and mild abrasion of the cheek pouch. In the present study, oral mucosal ulcers were created by a 1mm-thickening of the buccal mucosa which was then demarked with a punch biopsy apparatus (5 mm diameter) before being removed with a scalpel from the rat's buccal mucosa. This method is very useful for creating uniform ulcer diameters. Application of the apparatus is very easy. Surgical time is shorter than the other methods mentioned above. Consequently this method reduced the duration of anesthesia. Furthermore, the anesthesia period is reduced. This is important for the health of the rats. In addition, there

are no side effects in comparison with other methods. Treatment depends on the underlying cause of the oral mucosal ulcers. A topical anesthetic (applied to a localized area of the skin) such as lidocaine or xylocaine may be used to relieve pain. An antifungal medication may be prescribed for oral thrush (a yeast infection). An antiviral medication may be prescribed for herpes lesions. Antibiotics may be prescribed for secondary infection. Taking folate, vitamin B12, L-lysine tablets or increasing lysine in diet (found in fish, chicken, eggs, and potatoes) may reduce outbreaks (3, 4).

Oral ulcers generally last for 10 to 14 days, even if no treatment is applied. They sometimes last up to 6 weeks. The patient should avoid hot beverages and foods, spicy and salty foods, and citrus, and should take pain relievers like acetaminophen which makes him or her better (3, 4, 5, 14). Medication prescribed should be relevant to the severity of the disease. In mild cases with two or three small lesions, a protective emollient such as Orabase (Bristol-Myers Squibb, Princeton, NJ) or Zilactin (Zila Pharmaceuticals, Phoenix, AZ) is all that is necessary. Pain relief of minor lesions can be obtained with use of a topical anesthetic agent or topical diclofenac (5). In mild cases, topical tetracycline suspension, rinsed round the mouth several times a day relieves soreness and may hasten healing by controlling secondary infection (14). Mitsuhashi et al. (19) applied an animal oral mucositis model which was induced in hamsters through a combination treatment of 5-fluorouracil and mild abrasion of the cheek pouch. Each drug was administered topically to the oral mucosa of hamsters, and the process of healing of damaged oral mucositis was examined by measuring the size of the mucositis. Azulene ointment did not reduce the size of the mucositis compared with the vaseline-treated control group. Polaprezinc-sodium alginate suspension significantly improved the recovery from 5-fluorouracil-induced damage. In contrast, local treatment with dexamethasone exacerbated the mucositis markedly. These results suggested the healing effect of polaprezinc-sodium alginate suspension and the risk of steroids to severe oral mucositis induced by chemotherapy. Lee et al. (20) tested the efficacy of oral recombinant human epidermal growth factor (rhEGF) against radiation-induced oral mucositis in a rat model. They suggested that orally administered rhEGF decreased radiation-induced oral mucositis in rats. Consequently; there are many kinds of therapeutic agents which can be used locally in oral mucosal ul-

cers. TGO is one of them. TGO can be applied easily and can prevent the side effects caused by conventional therapies and drugs. TGO has not got any side effects (21). Prosthetic stomatitis may heal with TGO. Elbek et al suggested that TGO was found effective in shortening of the duration of mucositis and relieving of pain. Moreover, in another study (21) TGO was found effective in lengthening of the duration of recurrence time and relief from pain. In the literature, there was no study on TGO and its wound healing effect. But in our study, we found that oral mucosal ulcers can heal with TGO and that TGO was non-irritant. This study stresses the importance of wound-healing agents and their selection. Our results are completely original.

Cavanagh et al. (22) described 12 cases of wound breakdown after radical vulvectomy being dressed with honey. According to the author, honey was found to be non-irritant and much more effective than topical antibiotics. Also, the present in vitro study revealed the same result for honey. A recent review on the successful utilization of honey as a dressing on infected wounds shows that many authors support the use of honey in infected wounds and some suggest the prophylactic use of honey on the wounds of patients susceptible to methicillin-resistant staphylococcus aureus and other antibiotic-resistant bacteria (23, 24, 25, 26, 27). The antibacterial properties of honey prevent microbial growth in the moist wound environment created, and unlike topical antiseptics, honey causes no tissue damage. In recent years there have been a number of reports in the medical literature regarding the use of honey as a therapeutic agent, in clinical observations (28). The mechanism of antimicrobial action of honey is most likely a combination of a number of different factors. The low water activity of honey is inhibitory to the growth of the majority of bacteria. Honey is mildly acidic, with a pH between 3,2 and 4,5. Low pH alone is inhibitory to many pathogenic bacteria. Gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid. Hydrogen peroxide is produced within honey by the action of the bee-derived enzyme glucose oxidase and has known antimicrobial activity (2, 29, 30). Furthermore, Weston et al emphasized that benzoic acid, sinamic acid and flavonoid played a role in the antimicrobial effect of honey (31). Molan (32) declared that the remarkably rapid effect of honey in cleaning up wounds is due to a combina-

tion of the osmotic outflow and bioactive effect of honey. It contains the enzyme glucose oxidase which becomes active when honey is diluted and produced hydrogen peroxide. In the present study we concluded that honey dilutes when used in the mouth due to saliva. Increasing the effectiveness of the honey depends on increasing the application time. So it can be recommended for wound healing more than 2x1. Osmolarity, acidity, the generation of hydrogen peroxide on dilution and the presence of unidentified phytochemicals have been suggested to contribute to the antimicrobial potency of honey, but geographical location, floral origin, and postharvesting treatment condition may also be important (33). Studies with animal models have provided evidence of the stimulation of healing by honey (26, 33, 34). Moreover, we observed that mucosal healing was stimulated in rats by honey application. The high sugar content of honey inhibits bacterial growth. Slow and low level production of hydrogen peroxide within wounds kills bacteria without causing tissue damage and also aids in debridement of wounds. Therapeutic honeys also produce a moist wound environment (8).

Dunford et al. (35) found that Manuka honey deodorized a wide range of acute and chronic wounds, such as abscesses, diabetic foot ulcers, and leg ulcers, and believed it to be a result of the antibacterial action of the honey against the infection causing the malodor. In some recent cases of fungating wounds, honey was the only effective agent in controlling the malodor. Alcaraz (36) and Kelly (37) noted that honey was effective in the treatment of infectious cutaneous diseases. Our results indicate that honey should be used for a long period in oral mucosal wounds. The physical properties of honey make it effective as a wound dressing. Because of its viscosity, it provides a protective barrier. Honey is non-irritant, cheap and is easily used. It can be found in all supermarkets. Hydroxyproline is located in collagen. Its content in various tissues, plasma and urine is thought to be a reliable indicator of collagen catabolism (10, 11, 12). In the present study, the level of hydroxy-proline (Hyp) in surgical wound was tested since the level of wound healing was obtained.

In the present study, we observed that TGO agent was beneficial as an oral ulcer healing agent. Honey was found effective in shortening the duration of wound healing. Thus, the mechanisms underlying the wound healing controlled by honey requires further investigation.

CONCLUSION

Honey is an effective agent in oral mucosal ulcers. Therapeutic value of honey is more effective in comparison with Glyceroxytriester (TGO).

We believe that it can replace the conventional dressing materials in the near future. Thus, further in vivo investigations are required to support these data.

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