



ORIGINAL RESEARCH PAPER

Dental Science

Evaluation of role of propolis in healing of traumatic oral ulcers. An animal experimental study.

KEY WORDS: Traumatic oral ulcers, propolis, Histopathologic healing, experimental rabbits.

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ABSTRACT

Traumatic ulcer is the most prevalent ulcers found in the oral cavity. Most of the currently used medications for treatment of oral ulcers focus on providing symptomatic relief and preventing secondary infections. Propolis is a bee product used in some cultures as treatment for mouth ulcers, and it is known for its anti-inflammatory, anti-microbial and immune system regulatory properties. The aim of the study was to test the efficacy of propolis in healing of oral ulcers on experimental rabbits.

Methodology: Traumatic oral ulcers were inflicted in 12 experimental rabbits with the help of punch biopsy instrument. The animals were then divided in two groups, Group I was untreated control group and Group II was topically treated with non alcohol extract of propolis. Histological healing of the ulcers was evaluated on day 7 and day 14.

Statistical analysis was done by using descriptive and inferential statistic methods using student's unpaired t test.

Results: Group treated with propolis showed slightly improved healing than untreated control group but the difference was not statistically significant for both day 7 and day 14.

Introduction:

Ulcers can be defined as breach in epithelium or mucosa. Oral mucosal ulcers are caused by various disorders¹. The estimated point prevalence of oral ulcers worldwide is 4%, with aphthous ulcers being the most common, affecting as many as 25% of the population worldwide². According to size they can be categorized as Minor and Major ulcers. Minor ulcers (size less than 1cm) affect almost 80% population in some or the other part of their life². The Etiology for oral ulcers include trauma, infections, vesico-bullous diseases, neoplasia, nutritional deficiencies, aphthous stomatitis, gastrointestinal diseases etc¹.

Ulcers resulting from traumatic injuries are one of the most common types of ulcers encountered in clinical practice³. Acute traumatic ulcers are caused by accidental biting during mastication, sharp pointy food etc. Such ulcers generally heal quickly within few days without any complications. However chronic ulcers caused due to chronic trauma from sharp edges of teeth, restorations and appliances particularly ill-fitting dentures etc may take longer time to heal. Prevalence of such traumatic ulcers may vary from 3.5 % to 15.6% in surveys conducted across the world⁴⁻⁸. Clinically mucosal wound healing in oral cavity occurs by 5 to 7 days^{9,10}. This is achieved by the means of acute, robust inflammatory response, with recruitment of neutrophils, macrophages and lymphocytes to the wound site. This is followed by inflow of fibroblasts, ECM synthesis and reorganization¹¹. This is especially important in the oral cavity which is colonized and contaminated by numerous microorganisms¹¹. It is therefore crucial for the oral mucosa to heal healthily and as quickly as possible.

The current approach for treatment of oral ulcers commonly deals with providing symptomatic relief by topical anesthetics, and or preventing secondary bacterial infections through topical antibiotics. Not much emphasis has been given in the past on agents which can actually promote healing of oral ulcers, and data

available regarding the same is very limited.

Medicinal Plants, herbal derivatives and animal products like turmeric, aloe vera, honey, bees wax, etc. have shown immense potential for the management and treatment of wounds. A large number of such products have been used by tribal and folklore in many countries for the treatment of wounds and ulcers. In the recent past these products have gained a lot of popularity in field of research and medicine.¹²

Propolis is a golden-dark brown resinous substance that worker bees gather and pack on their hind legs from the sap of trees, shrubs and flower blossoms, the resinous substance of propolis is then carried back to their colony combined with beeswax then used by the bees as a sealant and sterilant in and around the hive.¹³ Propolis is widely used to prevent and treat colds, wounds and ulcers, rheumatism, sprains, heart disease, diabetes and dental caries due to its diverse biological properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, antiulcer and anti-HIV activities.¹⁴ In spite of the big compositional differences of the different propolis types depending on its botanical origin, it is astonishing that the biological effects of the different propolis types are very similar. Antibacterial activity has been demonstrated against both, gram positive and gram negative bacteria, both aerobic and anaerobic types. Due to its wide biological and pharmacological properties propolis can prove to be effective therapeutic agent for treatment of oral ulcers.

The current study was planned with the aim to evaluate the efficacy of alcohol free extract of propolis in healing of traumatic oral ulcers in experimental rabbits.

Objectives:

1. To evaluate histopathologically the healing of traumatic oral ulcers in untreated experimental rabbits.
2. To evaluate histopathologically the healing of traumatic oral

ulcers in experimental rabbits treated with alcohol free propolis extract.

3. To compare the above parameters with each other.

Sample selection: 24 healthy Male/Female Rabbits were selected for the study, all between 6 months and 3 years of age weighing between 2.5kg to 3.5 kg. Pregnant and diseased rabbits were excluded from the study.

Procedure: Artificially a traumatic ulcer was inflicted on labial mucosa of all the specimens with the help of a 5 mm punch biopsy instrument, under Ketamine injection on Day 0. Then the specimen were then divided into 2 groups (n=12).

Group I: No drug therapy was provided for the specimen of this group (Untreated Control Group).

Group II: Specimen of this group were topically administered topically alcohol free extract of propolis from day 1 to day 14 daily, three times a day at 8 hourly interval.

Visual monitoring of healing of the ulcers was done on day 1,7,14. Biopsy specimens of the edge of the healing ulcer was obtained on day 7 and day 14 from both groups and was stained with Picrosirius stain for collagen and Heamatoxyllin and Eosin stain for tissue inflammation. Histological examination of each specimen was done and scoring criteria of Sultana et al in 200915 was used to compare the quality of healing of wounds (Table 1).

Calculation of Healing Score:

Total healing score of each case was calculated by adding the score of individual criteria. Lower scores indicated poorer wound healing, while higher scores indicated better healing process. Healing status was graded as follows: Good (16 -19), fair (12 - 15) and poor (08 -11).

Table 1: Scoring criteria to compare the healing status of wounds in an ascending order (Sultana et al).¹⁰

Sr no	Histopathological findings	Score
1	Amount of granulation tissue	Profound -1, Moderate - 2, Scanty - 3, Absent - 4.
2	Inflammatory infiltrate	Profound -1, Moderate - 2, Few - 3
3	Collagen fiber orientation	Vertical -1, Mixed- 2, Horizontal - 3
4	Amount of early collagen	Profound -1, Moderate - 2, Minimal - 3, Absent - 4
5	Amount of mature collagen	Profound -1, Moderate - 2, Minimal - 3
6	Dilated blood capillaries and endothelial cells proliferation	Profound proliferation -1, Moderate proliferation- 2, capillary dilatation only - 3

Statistical analysis: Data was statistically analyzed was done by using descriptive and inferential statistic methods using unpaired t test and software used in the analysis was SPSS 17.0 version and p < 0.05 was considered as significant.

Results: Mean Healing scores based on the histologic criteria of sultana et al of both groups were calculated and compared on day 7 and day 14. The group I (untreated control) showed slightly poorer healing on day 7 in comparison with Group II which was treated with Propolis (Table no 2). Mean Healing scores on 14 showed fair healing with both groups, however Group II showed slightly better healing than Group I (Table no 2). However the difference in healing was not found to be statistically significant between both groups for day 7 and day 14.

Table 2: Comparison of mean scores in both the groups at 7th and 14th day by unpaired t test

Time	Group	N	Mean	Std. Deviation	p-value
7th day	UC	12	8.58	0.79	0.278
	Propolis	12	8.92	0.67	

14th day	UC	12	14.33	0.98	0.137
	Propolis	12	15.00	1.13	

Table 1 shows that at 7th day there was no statistically significant difference (p>0.05) existed in mean score of Uc group (8.58 ± 0.79) as compared to propolis group (8.92 ± 0.67). Similarly at 14th day in Uc group the mean score was 14.33 ± 0.98 and in propolis group was 15.00 ± 1.13, which was again statistically not significant (p>0.05).

Discussion:

In this study wound healing by second intention was chosen because it is a clinical condition that is frequently encountered in traumatic oral ulcers and by the oral surgeons. An experimental time period of 14 days was chosen because most wounds even if infected would show complete or nearly complete healing by the end of this time period.

Rabbits were selected for the study as the studies conducted on animals are of low cost and provide useful information that could be difficult to obtain in humans. In studies conducted on humans, it is difficult to eliminate biases in relation to their behavioral variables, and standardize and maintain the same living conditions during the entire experiment. Also standardization of variables like size and depth of ulcer would not have been possible with humans as it was with rabbits. Thus the use of rabbits in this work produced simple information but still capable of encouraging further researches in this area of knowledge.

The surgical mucosal wounds were made in the labial mucosa of all animals by means of a 5-mm punch-biopsy instrument before being removed with a scalpel from the rabbit's labial mucosa. This method is very effective for creating uniform ulcer diameters.

Calculation of Healing score on basis of Histopathological evaluation: Healing score was calculated on the basis of histopathological examination by using the method of Sultana et al¹⁵. On day 7 the Group II (treated with propolis) showed slightly superior healing than Group I, the healing status further improved for both groups on day 14. The difference in healing score between both the groups was not found to be statistically significant for day 14, but Group II showed slightly higher healing scores than those of Group I (Table).

The slightly improved healing scores with Group II on day 7 and day 14 can be attributed to the wide biological and pharmacological properties of propolis such as tissue regenerative properties, anti microbial properties, antioxidant activity and antiulcer activity. Similar results were reported by Zoba H. Ali and Heba Mahmoud Dahmouh (2012)¹⁶ and M. Abdulrhman et al (2012)¹⁷.

Conclusion: Propolis a bees product used in the current study is found to have an enhancing effect on the healing of traumatic oral mucosal wounds in experimental rabbits as compared to the untreated control group although the difference was not found to be significantly higher. It can be linked to the antimicrobial, antioxidant, anti-inflammatory and cytoprotective properties of its components. Further in vivo studies will be required for confirming its therapeutic effects of on oral lesions in human subjects.

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