

Comparative study on the Effect of Platelet Rich Plasma Versus Propolis on Induced Labial Ulcer in Albino Rats

Original
Article

Asmaa Khairy Hamza¹, Raneem F. Obeid³, Rania Ahmed Awwad² and Dina Mohamed Abd Elkhaleq³

Department of Oral Biology, Faculty of Dentistry, ¹Russian University, ²Ain Shams University

³Department of Oral biology, Faculty of Oral and Dental Medicines, Future University in Egypt

ABSTRACT

Introduction: Oral Mucositis is a multi-etiological inflammatory condition affecting patient's quality of life.

Objectives: The current study aims to evaluate the effect of both Platelet rich plasma (PRP) and Propolis on chemically induced labial mucosal ulcer in albino rats.

Material and Methods: 35 adult male albino rats weighing around 200-250 grams were subjected to chemical ulcer induction. Rats were divided into 4 main groups. Group I (negative control): rats did not receive any treatment. Group II in which ulceration was induced in the labial mucosa and left untreated to heal spontaneously. Group III (PRP): in which ulceration was treated by PRP. Group IV (Propolis): in which ulceration was treated by propolis. Each group was further subdivided into 2 equal subgroups A and B in which rats were sacrificed at 5&10 days following ulcer induction respectively. Labial mucosa were dissected and examined histologically and immunohistochemically.

Results: Histologically, group I showed normal findings of keratinized stratified squamous epithelium. Group II showed complete epithelial degeneration at day 5, re-epithelialization at day 10. Group III showed beginning of re-epithelialization at day 5 and continuous epithelial lining at day 10. Group IV showed apparently moderate thickness of epithelium at day 5 and complete regeneration at day 10. Immunohistochemically and statistically, group IV showed highest immunopositive PCNA expression of epithelial cells followed by group III then group I then group II showed the lowest mean.

Conclusions: PRP and Propolis showed vast accelerating effects in healing of chemically-induced ulcers through increased vascularity and enhancing re-epithelialization due to their anti-inflammatory and anti-oxidant potentials. Propolis proved to have superior effects compared to PRP

Received: 07 July 2022, **Accepted:** 15 August 2022

Key Words: Labial mucosa, oral mucositis, platelet-rich plasma, Propolis.

Corresponding Author: Raneem F. Obeid, PhD, Department of Oral biology, Faculty of Oral and Dental Medicines, Future University in Egypt, Egypt, **Tel.:** +20 10 0616 8513, **E-mail:** ranim.farouk@fue.edu.eg - raneemobeid2@gmail.com

ISSN: 1110-0559, Vol. 46, No. 4

INTRODUCTION

Oral Mucositis (OM) is a common disease affecting oral mucosa. It affects around 25% of the general population. Causes of OM can be poor oral hygiene, vitamin deficiency, allergic reactions, adverse drug reactions, emotional or physiological stress and traumatic injuries. It causes distress, severe pain and severely affects patient's quality of life^[1].

Labial mucosa is the most commonly affected site by oral ulceration. In rats it's covered by thin orthokeratinized stratified squamous epithelium while in human it has non-keratinized stratified squamous epithelium^[2].

Oral ulcers are defined as breakdown in the mucous membrane with loss of surface tissue, disintegration and necrosis of epithelial tissue that results from several factors such as radiation in head and neck region, chemotherapy or infection^[3].

During menopause, women pass through hormonal alterations directly affecting oral tissues such as burning

mouth syndrome, in which a burning sensation occurs in normal oral mucosa^[4].

Corticosteroids and antibiotics are commonly used in treatment of chemically induced lesions. However, these treatment modalities may cause oral candidiasis which is a common side effects of their prolonged use^[5].

Wound healing is a complex repair process involving migration of cells to the site of tissue injury through three stages: inflammatory phase, cell proliferative phase and the synthesis of new tissue components (remodelling phase). Each wound healing phase is controlled and regulated by biologically active substances called growth factors (GFs)^[6].

This study was designed to assess the effect of using PRP versus Propolis on chemically induced oral mucosal ulcer in albino rats at different durations.

MATERIALS AND METHODS

The proposal of the present study was reviewed and accepted by the Research Ethics Committee of the Faculty

of Dentistry, Ain Shams University , Egypt. Committee approval number: FDASU-RecIM011922.

Materials

A) Animal Model

Thirty five adult, male albino rats, weighing around 200-250 grams were used in the present study (from 4 to 6 months).

The rats were caged in stainless steel cages (20cm X 40cm) with 4 rats per cage in the Medical Research Center, Faculty of Medicine, Ain Shams University under supervision of a specialized veterinarian during the experimental period. Rats were fed a balanced diet and tap water. Proper ventilation, dark and light cycles and nutrition conditions ensuring good and clean working environment were provided.

B) Chemicals

1. **Propolis:** resin extract, was used in this study in a daily dose of 100mg/kg in 1ml distilled water, administered orally by sterile oral feeding tube^[7].
2. **Formocresol:** Purchased from Prevest DenPro limited from (Bari Brahmana, India) in the form of 15 ml. solution for induction of oral mucositis^[8].
3. **PRP:** A fresh blood sample was obtained from rat's eye vein using a 3 ml disposable syringe. A 3 ml of each animal blood was collected. The blood was centrifuged after being obtained from healthy animals in containers for 13 minutes at a speed of 3,500 rpm at room temperature, then the upper part which is centrifuged blood was discarded, then the remaining blood was centrifuged again for 15 minutes and the samples will then be collected by using auto-pipette and injected around the ulcer margins^[9].

Study Design

The duration of the study was 10 days.

A) Animal Grouping

Animals were divided into four main equal groups as following:

Group I: (control group) in which rats were kept at a healthy state throughout the whole experiment without intervention.

Group II: in which ulceration was induced in the lining mucosa of the lip and rats kept untreated. This group was divided into two equal sub -groups as follows:

- Sub-group IIA: rats were sacrificed after 5 days of ulcer induction.
- Sub-group IIB: rats were sacrificed after 10 days of ulcer induction.

Group III (PRP group): in which ulceration was induced in the mucosa of the rat's lip and rats were treated

by PRP. This group also was divided into 2 equal sub-groups:

- Sub-group IIIA: rats were sacrificed after 5 days of ulcer induction.
- Sub-group IIIB: rats were sacrificed after 10 days of ulcer induction.

Group IV (Propolis group): in which ulcer was induced in the lining mucosa of the lip and rats were treated by propolis application. This group was divided into 2 equal sub-groups:

- Sub-group IVA: in which rats were sacrificed after 5 days of ulcer induction.
- Subgroup IVB: in which rats were sacrificed after 10 days of ulcer induction.

B) Ulcer Induction

After anesthesia, animals were restrained and their mouths were kept open for topical application, using clamp forceps, then a cotton swap soaked in formocresol was applied to the labial mucosa for 60 seconds to create a circular ulcer after 48hr^[10].

C) Ulcer Treatment

After 48hrs of formocresol application, rats were anesthetized by (21mg/kg body weight) intramuscular injection of ketamine. Then 0.5ml of PRP was injected around the ulcer margins in PRP group. The propolis group was treated with propolis application directly on the lesion. At the end of the experiment rats were sacrificed by overdose of ketamine and unwanted body parts were properly disposed in the incinerator of Ain-Shams hospitals. Sample Preparations:

The labial mucosa of all rats was excised free and instantly fixed in 10% phosphate buffered formaldehyde (PBF) solution for 48 hours. Specimens were then appropriately dehydrated by transmitting them through rising concentrations of alcohol. The specimens were transmitted to xylene to be cleared from alcohol and then impregnated in paraffin and embedded in the paraffin wax blocks. Four µm-thick sections were then produced and put in xylene and transmitted in reducing concentrations of alcohol then distilled water for removal of paraffin wax. The sections were finally stained by Hematoxylin & Eosin (H&E), and Anti-Proliferating Cell Nuclear Antigen (Anti-PCNA) which is a marker for cell regeneration^[11].

Immuno-histochemical Staining

Following deparaffinization and rehydration of paraffin embedded sections, the sections were stained using mouse anti-proliferating cell nuclear antigen (PCNA). Sections were first dewaxed in xylene. Excess xylene was removed by washing the slides in ethanol. After treating the tissue with pepsin for 20 min at 37°C, the slides were washed with PBS. Sections were then incubated in 0.3% hydrogen peroxide (H₂O₂) at room temperature for 30 minutes to

inhibit the activity of endogenous peroxidase. Then, slides were submerged in protein blocking solution of 5% horse serum with 1% goat serum. The primary antibodies were then added for 18 h at 4°C. The slides were then washed with PBS, blocked again with protein-blocking solution for 1 h, and incubated with horseradish peroxidase-conjugated anti-rabbit antibody at 1:200 dilutions for 1 h at room temperature. Slides were washed again in PBS thrice and then incubated with 3, 3'-diaminobenzidine for 10 min. After the excess 3, 3'-diaminobenzidine was washed off, counterstaining was done with Gill's #3 hematoxylin. Specimens were mounted in DPX and examined under light microscope. Positive reaction was detected as brown nuclear discoloration^[12].

Histomorphometric Analysis

Image J software (Version 1.41a, NIH, USA) was used for histomorphometric analysis, in the Precision Measurements Unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. Capturing of all images was done using digital cameras (EOS 650D, Cannon, Japan) that was mounted on a light microscope (BX60, Olympus, Japan). Images were transferred to the computer system for analysis. Images of PCNA immune-stained sections were analyzed by the software to count the number of immune-positive cells per unit area in the epithelium and connective tissue of the studied specimens. Also, the same software was used to count the number of inflammatory cells, vacuolated epithelial cells and area percentage for collagen fibers for H&E-stained sections. Three representative fields from each rat were assessed to display the whole epithelium as well as proper anti-PCNA reaction^[13].

Statistical analysis

Numerical data of mean number of inflammatory cells, number of vacuolated epithelial cells, and mean of collagen fibers area percentage as well as mean of PCNA positive cells were examined for normality among tested groups by monitoring the distribution of data. One-way ANOVA test was used to compare between groups. When ANOVA test was significant, Bonferroni's post-hack test was done for pair-wise comparisons between each two groups. The significance level was performed using IBM SPSS Statistics Version 21 for Windows.

LIGHT MICROSCOPIC RESULTS

Hematoxylin & Eosin (H&E)

Group I (control group): Examination of the H&E-stained sections of the labial mucosa of group I showed normal appearance of keratinized stratified squamous epithelium of apparently healthy thickness. Lamina propria showed well-organized collagen fibers intermingled with spindle shaped fibroblast. Blood vessels of variable diameters were seen and few inflammatory cells were detected close to blood vessels (Figure 1a).

Group II (untreated ulcerated labial mucosa):
Subgroup IIA (at 5 days): In this group, complete loss of the epithelial layer over the ulcerated area with discontinuous keratin layer were observed. Presence of intracellular vacuolations in the prickle cell layer in adjacent areas was noticed. Extracellular edema and inflammatory cells infiltration were evident in most areas in the underlying lamina propria (Figure 1b).

Subgroup IIB (at 10 days): The ulcerated area showed newly formed thin epithelial lining with short rete pegs. Intra-cellular vacuolations within the prickle cell layer were fewer than the previous group. Also, the underlying lamina propria showed irregularly arranged collagen fibers as well as areas of hyalinized collagen (Figure 1c).

Group III (ulcer treated with PRP):

Subgroup IIIA (PRP at 5 days): Newly formed epithelial lining with detached of the keratin layer were seen. Intracytoplasmic cell vacuolations in basal and para-basal cell layers were detected. Lamina propria showed few inflammatory cells and localized areas of extracellular edema (Figure 1d).

Subgroup IIIB (PRP at 10days): This group showed normal appearance of the keratinized stratified squamous epithelium with short rete pegs except in some areas in which the epithelium and its keratin coverage were reduced in thickness with evidence of fewer intracytoplasmic epithelial cell vacuolations. The underlying lamina propria showed well-developed collagen fibers with no extracellular edema compared to subgroup IIIA (Figure 1e).

Group IV (ulcer treated with propolis):

Subgroup IVA (propolis at 5 days): The epithelial layer of this group showed well developed ortho-keratinized stratified squamous epithelium with well-formed keratin layer. Well-developed collagen fiber bundles and blood vessels of variable diameters were also detected in the underlying lamina propria (Figure 1f).

Subgroup IVB (propolis at 10days): This subgroup showed complete epithelial regeneration with well-developed thick and numerous rete pegs. The epithelium appeared well-developed with thick keratin layer of even thickness. Keratohyalin granules appeared in the granular cell layer. The underlying lamina propria showed few inflammatory cells, well-formed collagen fiber bundles and blood vessels of variable diameters (Figure 1g).

Immuno-histochemical results (PCNA antibodies)

Group I (negative control): The epithelium of labial mucosa of rats in this group displayed areas with positive PCNA reactions in the basal and para-basal cell layers alternating with areas of negative PCNA reactions (Figure 2a).

Groups II, III, IV

Subgroups A: Ulcerated labial mucosa after 5 days: In subgroups (IIA) the epithelium showed few positive PCNA

reactions (Figure 2b), (group IIIA) showed moderate positive reaction (Figure 2c) while in group (IVA) the epithelium showed strong positive reaction in basal, para-basal cell layers and in the lamina propria (Figure 2d).

Subgroups B: Ulcerated labial mucosa after 10 days: The regenerated epithelium in subgroup (IIB positive control) showed few positive PCNA reaction (Figure 2e), while in subgroups (IIIB & IVB) showed strong positive PCNA reaction in basal, para-basal cell layers and in lamina propria (Figures 2 f,g).

Statistical results

Statistical results of the present study are demonstrated in the following tables.

A) Comparison Between 5 and 10 Days According to the number of vacuolated epithelial cells within each group:

The number of vacuolated epithelial cells is presented in (Table A). This table shows no statistically significant difference between follow-up periods according to the number of vacuolated cells for all tested groups ($p>0.05$). For group II, 5 days (78.00 ± 21.97) showed an insignificant difference with 10 days (56.80 ± 11.71) at $p=0.093$. For PRP group, 5 days (30.00 ± 8.75) showed an insignificant difference with 10 days (21.80 ± 4.32) at $p=0.097$. For

propolis group, 5 days (16.40 ± 6.19) showed an insignificant difference with 10 days (10.60 ± 4.93) at $p=0.140$.

B) Comparison Between 5 and 10 days according to number of inflammatory cells within each group:

The number of inflammatory cells is presented in (Table B). Which showed no statistically significant difference between the groups according to the number of inflammatory cells in each group.

C) Comparison between groups according to area percentage (%) of hyalinized collagen at 5 & 10 days:

(Table C) shows statistically significant difference between groups according to % of hyalinized collagen in positive control group.

D) Comparison between PCNA positive epithelial cells:

High statistically significant difference between subgroups (IIA, IIIA & IVA) was found, where subgroup IVA showed the highest mean value (76.40 ± 10.43) while subgroup IIA showed the lowest mean value (19.40 ± 14.96). The data represented in (Table D).

There was high statistical significance difference between subgroups (IIB, IIIB & IVB) in which the highest value was shown in subgroup IVB (93.80 ± 26.61) and the lowest mean value was shown in subgroup IIB (16.40 ± 3.58).

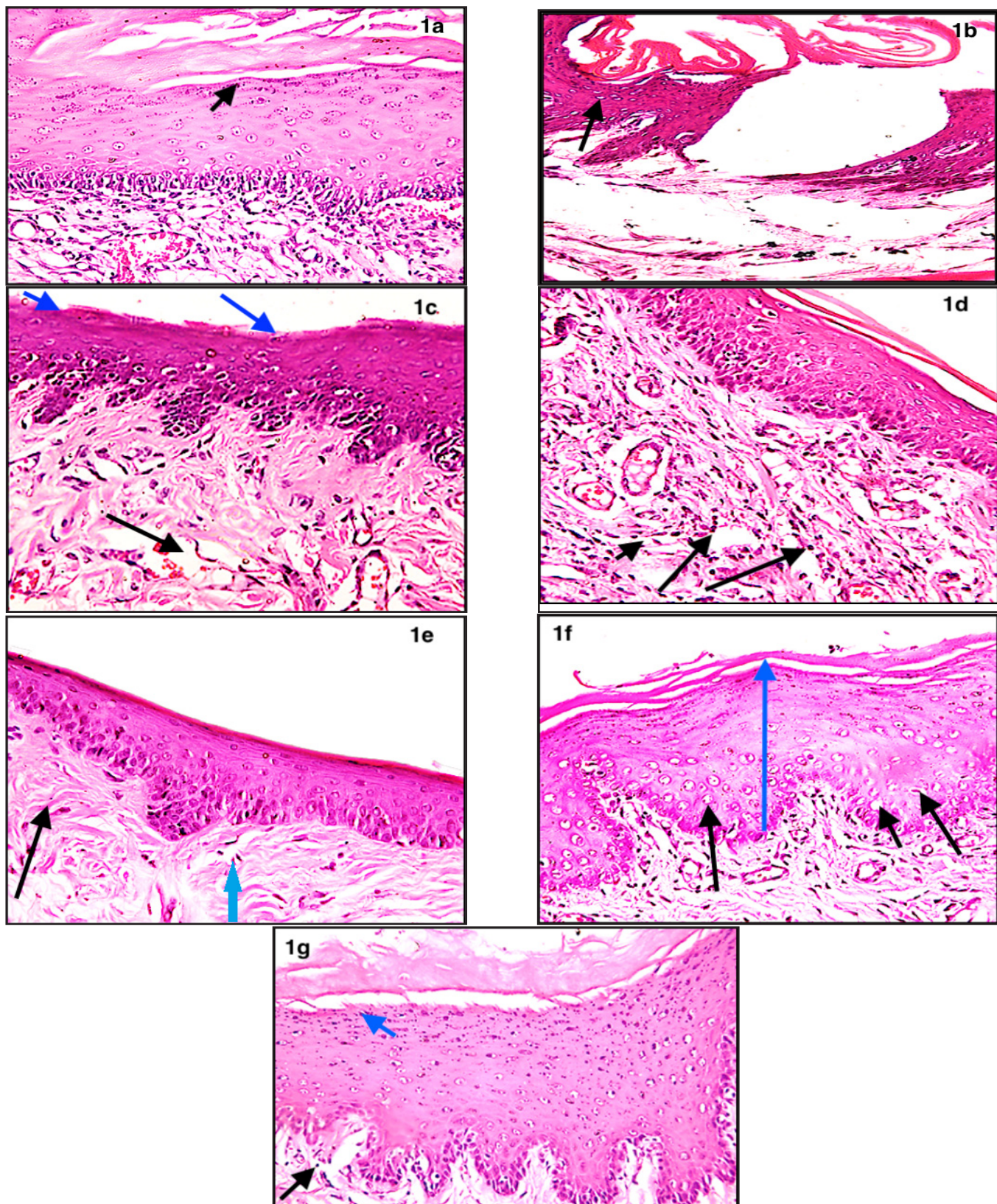


Fig. 1: (a): A photomicrograph of group I (control group) showing keratohyalin granules within the granular cell layer (black arrow); (b) group II , subgroup (IIA) showing numerous intracellular vacuolations within the prickle cell layer (black arrow); (c) subgroup (IIB) revealed keratin detachment in some regions (blue arrows) and some dilated blood vessels (black arrow); (d) Group III, subgroup (IIIA PRP): showing lamina propria with few areas of extracellular edema and degeneration with some inflammatory cells (black arrows); (e) subgroup (IIIB PRP): well developed collagen fibers (black arrow) and mild inflammatory cells infiltrate (blue arrow); (f) subgroup (IVA propolis): showing apparently increased thickness of the keratinized stratified squamous epithelium (blue arrow) as well as presence of some intracytoplasmic cell vacuolations (black arrows) within the prickle cell layer; (g): subgroup (IVB) showing Apparent keratohyalin granules within the granular cell layer (blue arrow), Few inflammatory cells, well developed collagen fibers and lack of extracellular edema were observed in the underlying lamina propria (black arrow) (H&E.org.mag x400).

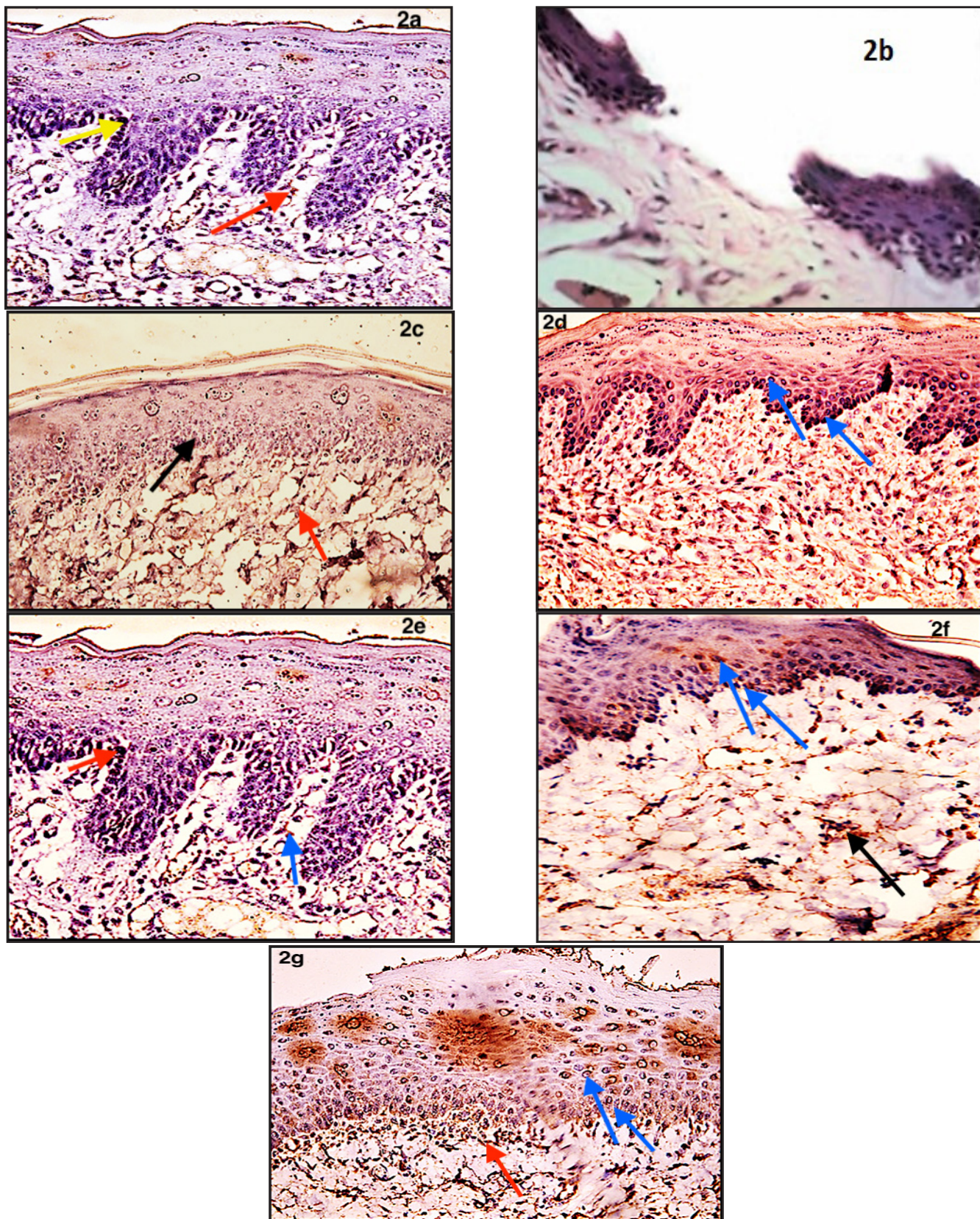


Fig. 2: (a): A photomicrograph of group I showing positive CNA reactions in the basal cell layer (yellow arrow) and the underlying lamina propria (red arrow); (b) group II, subgroup (IIA) revealed the ulcerated region with few positive PCNA reaction in the basal cell layer (green arrow); (c) subgroup (IIB): revealed mild positive PCNA reaction in the basal cell layer (black arrow) and lamina propria (red arrow); (d) subgroup (IIIA PRP): showing marked immuno positive PCNA reactions in the basal and para-basal cell layers (blue arrows); (e) subgroup (IIIB PRP): showing immuno positive PCNA reaction in the basal cell layer (red arrows) and the lamina propria (blue arrow); (f) subgroup (IVA): showing mild positive reaction in the basal and Para -basal cell layers (blue arrow) as well as in the lamina propria (black arrow); (g) subgroup (IVB): showing marked positive PCNA reaction in the basal, para-basal cell layers (blue arrow) and the lamina propria (red arrows) (Anti-PCNA org. mag.x400).

Table A: Shows the number of vacuolated epithelial cells in different groups. This table shows no statistically significant difference between follow-up periods according to No. of vacuolated cells for all tested groups ($p>0.05$).

Groups	No. of Vacuolated Cells			ANOVA		
	Range	Mean±SD	Change%	Bonferroni	F	p-value
Control Group	9–23	15.80 ^B ±5.26				
Group II	45–71	56.80 ^A ±11.71	259.5%	<0.001**	41.919	<0.001**
PRP Group	17–28	21.80 ^B ±4.32	38.0%	0.567		
Propolis Group	6–18	10.60 ^B ±4.93	-32.9%	0.671		

Table B: Shows the number of inflammatory cells which shows no statistically significant difference between groups according to no. of inflammatory cells in each group.

Groups	Inflammatory Cells			ANOVA		
	Range	Mean±SD	Change%	Bonferroni	F	p-value
Control Group	4–19	10.20 ^B ±6.06				
Group II	10–34	22.80 ^A ±9.55	123.5%	0.033*	6.147	0.006*
PRP Group	4–16	9.60 ^B ±5.03	-5.9%	0.999		
Propolis Group	3–12	6.60 ^B ±3.78	-35.3%	0.815		

Table C: Shows statistically significant difference between groups according to % of hyalinized collagen in group II.

Groups	% Hyalinized collagen			ANOVA		
	Range	Mean±SD	Change%	Bonferroni	F	p-value
Control Group	0.078–2.82	0.91 ^B ±1.13				
Group II	10.33–26.49	17.9 ^A ±6.25	1867.0%	<0.001*	37.596	<0.001**
PRP Group	0.05–0.47	0.22 ^B ±0.17	-75.8%	0.737		
Propolis Group	0.00–0.61	0.27 ^B ±0.31	-70.3%	0.752		

Table D: Showing high statistical significance difference between subgroups regarding the number of immunopositive cells.

Groups	No of PCNA positive cells			ANOVA		
	Range	Mean±SD	Change%	Bonferroni	F	p-value
Control Group	27–93	70.20 ^A ±27.71				
Group II	12–20	16.40 ^C ±3.58	-76.6%	0.003*	13.272	<0.001**
PRP Group	48–70	57.00 ^B ±9.80	-18.8%	0.724		
Propolis Group	64–137	93.80 ^A ±26.61	33.6%	0.277		

DISCUSSION

Oral ulcers are common conditions which are caused by various disorders and may severely affect life quality^[13].

A rat's labial mucosa was the tissue of choice because this is the most common site affected by oral ulceration^[14].

PRP application is one of the modalities that has been used to verify its effect in treating OM. PRP provides a variety of cytokines and GFs that stimulates and initiate the healing process. These GFs includes TGF-B, PDGF, ECGF, ILGF, VEGF and fibronectin. They promote tissue healing via induction of extracellular matrix synthesis by existing fibroblasts as well as stimulation of proliferation of mesenchymal cells. Moreover, PRP has an important role for decreasing pain and eating discomfort associated with OM^[15].

Propolis was chosen in this study because it possesses biological and pharmacological properties

such as anti-inflammatory, anti-oxidant, anti-microbial, immunostimulant and wound-healing activity^[13].

The current study investigated the effect of PRP and Propolis by H&E stains as a routine histologic examination to detect structural changes in both epithelium and CT in all groups.

Examination of the H&E-stained sections of Group II revealed apparent thinning, discontinuity of the epithelium and numerous intracellular vacuolations of epithelial cells which was statistically significant when compared to control group (Group I). This could be attributed to apoptotic changes within epithelial cells, matching the results of (Awwad, 2020)^[16] who also stated that the apoptotic changes seen in the epithelium could be attributed to increased reactive oxygen species (ROS) production due to the underlying inflammatory process. The lamina propria showed areas of extracellular edema due to degeneration of extracellular elements and accumulation of inflammatory exudates. Also, presence of hyalinized

collagen fibers as well as inflammatory cell infiltration due to oxidative stress secondary to free oxygen radicals accumulated in the tissues as a result of the induced inflammation. These oxygen free radicals stimulate matrix metalloproteases (MMPs) in the tissue which in turn destroys the collagen fibers, which are in agreement with (Ishiyama et al., 2002)^[17] who found increased number of inflammatory cells and destroyed collagen fibers in the connective tissue of buccal mucosa of albino rats after ulcer induction.

Light Microscope examination of the H&E-stained sections of PRP (subgroup IIIA), showed thin layer of stratified squamous epithelium with discontinuous keratin layer in comparison to (subgroup IIIB which showed well-formed epithelial covering and well-developed keratin layer. This was in addition with Rashed et al., 2019^[15] who reported increased density of collagen fibers by day 10 of ulcer healing using PRP due to increased levels of FGF, PDGF, EGF which promote wound closure due to proliferation of fibroblast, thus enhancing collagen deposition, vascularization, granulation-tissue-formation and myo-fibroblast formation which led to wound reduction and eventually its closure.

In the present study, subgroup IVA revealed well-developed epithelial layer while subgroup IVB showed great improvement in the epithelium with significant reduction in the epithelial vacuolated cells. The same subgroup showed reduction in the inflammatory cells in the underlying lamina propria. These findings were matching to those of (Mahattandul et al., 2018)^[18] who found reduction in inflammatory cells count on 3rd – 5th days following ulcer induction in hard palate of albino rats, they attributed that to the anti-inflammatory and bacteriostatic effect of propolis. Propolis is considered a significant antimicrobial against both gram +ve, gram -ve, aerobic and anaerobic bacteria. These properties are due to presence of Artepillin C in its chemical composition that causes activation of natural defense of the micro-organism by altering its cell membrane causing its disruption thus leads to reduction of bacterial activity. Moreover, Artepillin C shows anti-inflammatory activity mediated by modulation of NF-Kappa B and inhibition of prostaglandin E2 and nitric oxide^[19].

PCNA antibody has been used to detect proliferating cells as it's considered a valuable tool for assessing cell proliferation throughout wound healing^[20].

Immunohistochemical examination of group IIA specimens revealed few reactions in both basal cell layer and the underlying lamina propria. These results were in agreement with (Mohsen R, et.al.)^[21] who pointed out that after buccal ulceration, fewer positive cells were present in basal and para-basal cell layers of epithelium. This can be explained by the markedly reduced proliferation potential of epithelium of this group. Moreover, the presence of inflammatory end-products which might affected the dividing ability of basal epithelial cells rendering them incapable to function properly.

In this study, group IIB showed reduced PCNA expression in both basal cell layer and the underlying lamina propria compared to the treated groups. These results were parallel with those of (Lee et.al, 2016)^[22] who reported the reduction of PCNA immuno-positive cells after administration of formocresol. They stated that formocresol has severe cytotoxic effect due to presence of formaldehydes which cause severe necrosis to gingiva, lips, tongue and also has role in osteomyelitis. Also, formocresol has the ability to inhibit angiogenic factor at ulcer base and margins.

In the current study, the number of PCNA positive nuclei in epithelial tissue of group IIIa was significantly increased in comparison to that of group II at same day. The expression of PCNA positive cells in the underlying lamina propria was significantly high in group IIIB than in group IIIA. These results are in parallel with (Rashed et. al., 2019)^[15] who found the same results using a different approach by inducing the ulcer on buccal mucosa by formocresol. The author stated that PRP has a wide pool of growth factors and signals that help in wound healing by replacing senescent resident cells to allow normal healing process. Moreover, PRP achieve regenerative process rather than repair.

In the current work, examination of the PCNA stained specimens of group IVA (propolis 5days) showed significantly more positive cells in basal, para-basal cell layers of epithelium as well as in the underlying lamina propria when compared statistically to group II. These findings confirm the potential of propolis to act as anti-inflammatory agent. These were matching the results of (Elfiki et.al, 2017)^[19] who found out that propolis has huge effects on the inflammatory changes associated with oral mucositis and the proliferating capacity of the epithelium as it reduces pain and size of ulcer among cancer patients.

Our immune-histochemical investigations of labial mucosa receiving propolis of group IVB (propolis 10 days) showed strong PCNA reaction in basal and suprabasal cell layers of epithelium and the lamina propria. This indicated the importance of propolis as a potent anti-oxidant and anti-inflammatory agent that allows the proliferation of cells in different phases of cell cycle. This was in agreement with (Yossef et.al, 2017)^[23] who found in their study that the effect of propolis in gingival ulcer was correlated with cellular proliferation and differentiation. They also stated that propolis not only inhibit apoptosis but also, improves the metabolic activity of cells.

CONCLUSIONS

The present study concluded the histological, immunohistochemical and statistical results of both PRP and Propolis could ameliorate the effects of induced ulceration on the labial mucosa of albino rats although propolis proved to have superior effects compared to PRP. These effects were correlated to the duration of treatment.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Arafa MG, Ghalwash D, El-Kersh DM, Elmazar MM. Propolis-based niosomes as oromuco-adhesive films: A randomized clinical trial of a therapeutic drug delivery platform for the treatment of oral recurrent aphthous ulcers. *Sci Rep.* 2018;8(1):1–14. DOI: 10.1038/s41598-018-37157-7.
2. Wahba A, Farid M, Adawy H. A Comparative Study between the Combined Effect of Frankincense and Myrrh versus the Effect of Triamcinolone Acetonide on Healing of Induced Labial Mucosal Ulcer in Albino Rats. *Al-Azhar Dent J Girls.* 2017;4(4):339–45. DOI: 10.21608/ADJG.2017.5281 .
3. Baharvand M, Jafari S, Mortazavi H. Herbs in oral mucositis. *J Clin diagnostic Res JCDR.* 2017;11(3):ZE05. DOI: 10.7860/JCDR/2017/21703.9467.
4. Suri V, Suri V. Menopause and oral health. *J Midlife Health.* 2014;5(3):115. DOI: 10.4103/0976-7800.141187.
5. Gilvetti C, Porter SR, Fedele S. Traumatic chemical oral ulceration: a case report and review of the literature. *Br Dent J.* 2010;208(7):297–300. DOI: 10.1038/sj.bdj.2010.295.
6. Echtermeyer F, Streit M, Wilcox-Adelman S, Saoncella S, Denhez F, Detmar M, et al. Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. *J Clin Invest.* 2001;107(2):R9–14. DOI: 10.1172/JCI10559.
7. Alamoudi NM, El Ashiry EA, Farsi NM, El Derwi DA, Atta HM. Treatment of oral ulcers in dogs using adipose tissue-derived mesenchymal stem cells. *J Clin Pediatr Dent.* 2014;38(3):215–22. DOI: 10.17796/jcpd.38.3.193115427jg6vl60.
8. Tseveenjav B, Furuholm J, Mulic A, Valen H, Maisala T, Turunen S, et al. Survival of primary molars with pulpotomy interventions: Public oral health practice-based study in Helsinki. *Acta Odontol Scand.* 2021;79(8):636–41. DOI: 10.1080/00016357.2021.1928747.
9. Messori MR, Nagata MJH, Furlaneto FAC, Dornelles RCM, Bomfim SRM, Deliberador TM, et al. A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. *RSBO Rev Sul-Brasileira Odontol.* 2011;8(3):299–304. DOI: 10.1002/14651858.CD002781.
10. Girish MS, Anandakrishna L, Chandra P, Nandlal B, Srilatha KT. Iatrogenic injury of oral mucosa due to chemicals: A case report of formocresol injury and review. *IOSR J Dent Med Sci.* 2015;14:1–5. DOI: 10.9790/0853-1901020104.
11. Öztekin A, Öztekin C. Vitamin D levels in patients with recurrent aphthous stomatitis. *BMC Oral Health.* 2018;18(1):186. DOI:10.1186/s12903-018-0653-9.
12. EL-Komy MHM, Hassan AS, Raheem HMA, Doss SS, EL-Kaliouby M, Saleh NA, et al. Platelet-rich plasma for resistant oral erosions of pemphigus vulgaris: A pilot study. *Wound Repair Regen.* 2015;23(6):953–5. DOI: 10.1111/wrr.12363.
13. Agnihotri A, Kaur A, Arora R. Oral Ulceration and Indian Herbs: A Scoping Review. *Dent J Adv Stud.* 2020;8(03):71–9. DOI:10.1055/s-0040-1716316.
14. Onuma H, Mastui C, Morohashi M. Quantitative analysis of the proliferation of epidermal cells using a human skin organ culture system and the effect of DbcAMP using markers of proliferation (BrdU, Ki-67, PCNA). *Arch Dermatol Res.* 2001;293(3):133–8. DOI: 10.1007/s004030000195.
15. Rashed FM, GabAllah OM, AbuAli SY, Shredah MT. The effect of using bone marrow mesenchymal stem cells versus platelet rich plasma on the healing of induced oral ulcer in albino rats. *Int J stem cells.* 2019;12(1):95–106. DOI: 10.15283/ijsc18074.
16. Awwad RA. Histological and Immunohistochemical Evaluation of the Ameliorating Role of Propolis and/or Intermittent Fasting on Induced Oral Mucositis in Albino Rats. *Egypt Dent J.* 2020;66(4-October (Oral Medicine, X-Ray, Oral Biology & Oral Pathology)):2323–37. DOI: 10.21608/EDJ.2020.40454.1223.
17. Ishiyama H, Kawai K, Azuma A, Nagano C. Therapeutic effect of rebamipide in a modified acetic acid-induced buccal mucosal ulcer model. *Inflammopharmacology.* 2002;10(4):391–9. DOI:10.1163/156856002321544864.
18. Mahattanadul S, Mustafa MW, Kuadkaew S, Pattharachayakul S, Ungphaiboon S, Sawanyawisuth K. Oral ulcer healing and anti-candida efficacy of an alcohol-free chitosan-curcumin mouthwash. *Eur Rev Med Pharmacol Sci.* 2018;22(20):7020–3. DOI: 10.26355/eurrev_201810_16173.
19. Al Jaouni SK, Al Muhayawi MS, Hussein A, Elfiki I, Al-Raddadi R, Al Muhayawi SM, et al. Effects of honey on oral mucositis among pediatric cancer patients undergoing chemo/radiotherapy treatment at King Abdulaziz University Hospital in Jeddah, Kingdom of Saudi Arabia. *Evidence-Based Complement Altern Med.* 2017;2017. DOI: 10.1155/2017/5861024.
20. Sook LIM, Seungwon S. Effects on Cell Wall Compositions of *Candida albicans* by Thymol Isolated from *Thymus vulgaris*. *춘계총회 및 학술대회.* 2007;310.
21. Mohsen ROM, Halawa AM, Hassan R. Role of bone marrow-derived stem cells versus insulin on filiform and fungiform papillae of diabetic albino rats (light, fluorescent and scanning electron microscopic study). *Acta Histochem.* 2019;121(7):812–22. DOI: 10.1016/j.acthis.2019.07.007.

22. Lee C, Choi Y, Park S. Mandibular bone necrosis after use of paraformaldehyde-containing paste. *Restor Dent Endod.* 2016;41(4):332–7. DOI: 10.5395/rde.2016.41.4.332.
23. Yossef MM, Bayoumi DA. Histological study of the effect of propolis on gingival tissue of albino rat. *Egypt Dent J.* 2017;63(1-January (Oral Medicine, X-Ray, Oral Biology & Oral Pathology)):477–90. DOI: 10.21608/EDJ.2017.74785.

المخلص العربي

دراسة مقارنة لتأثير البلازما الغنية بالصفائح الدموية مقابل صمغ العسل علي القرحة
الشفية المستحثة في الجرذان البيضاء
"دراسة هستولوجية و هستوكيميائية مناعية"

اسماء خيرى حمزه^١، رنيم فاروق عبيد^٢، رانيا احمد عواد^٢، دينا قشقوش^٣

قسم بيولوجيا الفم، كلية طب اسنان، الجامعة الروسية،^٢ جامعه عين شمس

^٣قسم بيولوجيا الفم، كلية طب الفم والاسنان، جامعه المستقبل بالقاهرة

المقدمه: التهاب الغشاء المخاطي للفم هو حالة النهائية متعددة المسببات تؤثر على نوعية حياة المريض.

الهدف من البحث: تهدف الدراسة الحالية إلى تقييم تأثير كل من البلازما الغنية بالصفائح الدموية وقدره صمغ العسل

المواد و طرق البحث: تعرض ٣٥ من ذكور الجرذان البيضاء البالغة التي تزن حوالي ٢٠٠-٢٥٠ جراما لتحريض القرحة الكيميائية. تم تقسيم الجرذان إلى ٤ مجموعات رئيسية. المجموعة الأولى: لم تتلق الجرذان أي علاج. المجموعة الثانية التي تم فيها تحفيز التقرح في الغشاء المخاطي للشفاة وترك الشفاة دون علاج. المجموعة الثالثة: حيث تم علاج التقرح بواسطة البلازما الغنييه بالصفائح الدموية. المجموعة الرابعة: حيث تم علاج التقرح بواسطة صمغ العسل. تم تقسيم كل مجموعة إلى مجموعتين فرعيتين متساويتين A و B حيث تم التضحية بالجرذان في ٥ و ١٠ أيام بعد تحريض القرحة على التوالي. تم تشريح الغشاء المخاطي للشفة وفحصه من الناحية النسيجية والمناعية الكيميائية.

النتائج: من الناحية النسيجية، أظهرت المجموعة الأولى نتائج طبيعية للنسيج الطلائي الحرشفي الطبقي الكيراتيني. أظهرت المجموعة الثانية أظهرت فقد كامل للنسيج الطلائي فوق المنطقه المتقرحه في اليوم ٥، وإعادة بنائه في اليوم ١٠. أظهرت المجموعة الثالثة إعادة بناء النسيج الطلائي غير مكتمل في اليوم ٥ والبطانة النسيجية مكتمله في اليوم ١٠. أظهرت المجموعة الرابعة على ما يبدو سمكا معتدلا من النسيج الطلائي في اليوم ٥ وتجديدا كاملا في اليوم ١٠. من الناحية المناعية والكيميائية والإحصائية، أظهرت المجموعة الرابعة أعلى تعبير PCNA إيجابي للخلايا الطلائية تليها المجموعة الثالثة ثم المجموعة الأولى ثم المجموعة الثانية أظهرت أدنى متوسط.

الاستنتاجات: أظهر العلاج بواسطه البلازما الغنييه بالصفائح الدموية و صمغ العسل تأثيرات متسارعة واسعة في شفاء القرحة المستحثة كيميائيا من خلال زيادة الأوعية الدموية وتعزيز إعادة بناء الخلايا الطلائية بسبب إمكاناتها المضادة للالتهابات والمضادة للأكسدة. أثبت صمغ العسل أن له تأثيرات متفوقة مقارنة ب البلازما الغنييه بالصفائح الدموية