# Propolis versus Daktarin® in mucosal wound healing

[1]Zoba H. Ali and [2] Heba Mahmoud Dahmoush

Departments of [1]Oral Biology and [2]Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt. rawya_h2a@yhaoo.com

**Abstract**: The aim of this study was to compare the effect of propolis versus daktarin on mucosal wound healing. Fifty two albino rats were randomly divided into three groups; G 1 (propolis), G 2 (daktarin) and control group. Following the induction of a surgical mucosal wound in the labial mucosa by means of a 1-mm punch-biopsy instrument, biopsy specimens were taken on days 1, 3, 7 and 14 from groups of sacrificed rats and stained with haematoxylin-eosin stains, Mallory's trichrome stain as well as CD68 immunohistochemical stain. Data were analyzed statistically. Histological evaluation of each specimen was done and scoring criteria were used to compare the healing status of wounds. There was no statistical significance between different groups, on day 1. However there was statistically significant difference between G1 and both G2 and control group on day 3. On day 7, statistically significant difference was found between G1 and control group, but there was no statistically significant difference between G1 and G2. Conclusion: Propolis has an enhancing effect on the healing of oral mucosal wounds compared to daktarin.

[Zoba H. Ali and Heba Mahmoud Dahmoush. **Propolis versus Daktarin® in mucosal wound healing.** Life Sci J 2012;9(2):624-636]. (ISSN:1097-8135). http://www.lifesciencesite.com. 95

**Key words:** Propolis; Daktarin®; Labial mucosa; CD68 Immunohistochemical stain.

## 1. Introduction:

Oral mucosal wound or mouth ulcers are sores or open lesions in the mouth which are caused by various disorders [(1)]. Wounds are not just a physical hindrance due to blood loss or tissue damage, but they may threaten the individual survival by development of infection and sepsis due to invasion of micro-organisms or contaminants. Mucosal wounds occur frequently, and the healing of the mucosa is important in most surgical outcomes. And although wound healing in the oral mucosa is improved by sound surgical principles, yet it is also mediated by biologic processes beyond the surgeon's control [(2)]. It should also be noted that ulcers and/or erosions can be the final common manifestation, often clinically indistinguishable, of a wide and complex spectrum of conditions including traumatic lesions, infectious, vesiculo-bullous, neoplastic and gastrointestinal diseases [(3)].

The immediate imperative of the body is to close the wound and prevent establishment of infection [(4)]. Clinically mucosal wound healing in oral cavity occurs by 5 to 7 days [(5, 6)]. It achieves this objective by the means of a rapid and robust inflammatory response, with recruitment of neutrophils, macrophages and lymphocytes to the wound site. This is followed by fibroplasia, ECM synthesis and reorganization [(4)]. This is especially important in the oral cavity which is colonized and contaminated with numerous micro-organisms [(4)].

Thus it is crucial for the oral mucosa to heal healthily and quickly.

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. Propolis has a protecting role for the bee colony. Beehives have been found to be more sterile than most modern day hospitals [(7)].

Hundreds of publications have appeared in the last 40 years describing the biological and health enhancing properties of propolis[(8)]. Propolis has been documented to have many positive medical effects in many fields including an antibacterial, antiviral and antifungal effect [(9-11)]. Also propolis was found to have an effect against parasites [( 12,13)] as an antiulcer (stomach, skin, buccal) [(14,15)]as well as an antioxidant[(10)] . Researches have segregated and tested single substances in propolis; however, it is likely that the presence of a large number of products in propolis may produce a synergistic effect greater than the sum of the effects of individual components [(16)]. Studies evaluating the efficacy of isolated constituents have demonstrated minimal effectiveness compared to the natural compound[(17)]. Similarly **Ahn *et al.*,** [(8)] stated that the health

enhancing effects are found in the ethanol extractable part of propolis called balsam.

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 [18].

Moreover, in vitro antiviral activity of propolis has been attributed to a synergistic action of both flavonoid and flavanol components in propolis[19]. Daktarin® oral gel contains the active ingredient miconazole. Miconazole is an antifungal medicine used to treat infections with fungi and yeasts [20]. Miconazole also has some antibacterial action and kills certain bacteria that may also be present in the infection [21, 22].

However many side effects had been associated with its use in some patients, and the most commonly reported side effects include: nausea, vomiting, diarrhea, allergic reactions and hepatitis [23]. Since daktarin® is widely used in the oral cavity as compared to propolis, it has been the aim of the present study to evaluate the efficiency of propolis in healing oral mucosal ulcers in comparison with daktarin®.

## 2. Materials and Methods

This study was undertaken in the department of Oral Histology and the department of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, Egypt, based on an ethical approved protocol.

Fifty two 3-months old male rats (albino rats) were selected, with an initial weight ranging between 220 and 240 grams. The rats were kept in housing cages (polyethylene, 16×40×30 cm), six animals per cage, with standardized food and water, under a light/dark cycle of 12 h. The cages were kept in a room which had a constant temperature of 25±1°C. In order to prevent the animals from coming in contact with their feces and/or urine, a permeable metal floor was installed in the cages, separating the rats from the lower part of the cage.

All surgical procedures were performed under general anesthesia, by intramuscular administration of 0.1 ml of ketamine hydrochloride (SIGMATEC Company) combined with 0.05 ml of xylazine hydrochloride (ADWIA Company), per 100 g body weight of the animal. After anesthesia, the labial mucosa was antiseptically cleaned with 2% chlorhexidine then a surgical mucosal wound was made in the labial mucosa of all animals by means of a 1-mm punch-biopsy instrument (Acu-Punch, Acuderm Inc., Ft. Lauderdale, FL, USA). The wounds were done so that their depths would include the submucosa.

The same investigator performed all the surgical procedures.

The animals were randomly divided into three groups as follows; group1 (G1; n=20) was treated with propolis applied to the wound site on the labial mucosa three times daily, group2 (G2; n=20) was treated with daktarin applied to the wound site on the labial mucosa three times daily, and group 3 (G3; n=12) was left to heal spontaneously and served as the control group.

Within each group, the rats were subdivided such as 5 rats of G1, 5 rats of G2 and 3 rats of G3 were consecutively sacrificed on days 1, 3, 7 and 14.

The type of propolis used in this study was Bee Propolis extract (Honey paste) (Y.S. Organic Bee Farms 2774N. 4351 Rd. Sheridan, IL 60551 USA). While the Daktarin® used was miconazole nitrate (Janssen Cilag. Pharm. N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium)

Biopsy samples were fixed with 10% formalin and embedded in paraffin for histological examination. Specimens were cut into 5-µm sections and stained with:

1. Haematoxylin-eosin (HE) stains.
2. Mallory's trichrome stain used for collagen fibers identification.

**For immunohistochemical procedure**: Sections of 4-µm thickness were mounted over optiplus slides. These slides were electrically charged. The sections were de-paraffinized and rehydrated, rinsed in PBS and incubated in PBS containing $H_2O_2$ for ten minutes. The primary antibody used was the monoclonal mouse anti-human CD68, clone KP1 (code N1577 Dako). The tissue sections were incubated with the primary antibody over night in moist chamber at 4˚C then rinsed with PBS, 3 times 2 minutes each. The sections were labeled with a streptavidin- biotin method using Dako- LAB vision Catalog CA94539). The sections were visualized with freshly prepared solution containing 3, 3 diaminobenzidin DAB the sections were finally counterstained with methylgreen and viewed by light microscope.

The cellular staining pattern of anti-CD68, is granular cytoplasmic of variable intensity. The expression of macrophages was determined by counting the CD68-positive stained cells in 5 fields of magnification 400. The means and standard deviations were recorded for each group, and one-way ANOVA was used to compare the differences of

the means of the groups and determine significant differences as well as Paired Student's t-Test to compare between each two groups at every time point.

The number of CD68-positive cells was expressed as [(number of +ve cells)/per 400 x visual field]

Slides were coded and microscopically examined to be evaluated for the histological parameters of wound healing [24, 25] including the amount of granulation tissue, inflammatory infiltrate collagen fiber deposition as well as endothelial cells in each wound.

**Histological evaluation:**

Scoring criteria were adopted after **Sultana *et al.,*** [26] to compare the healing status of wounds in an ascending order for specific points as follows: Amount of granulation tissue (Profound - 1, Moderate - 2, scanty - 3, absent - 4). Inflammatory infiltrate (Profound - 1, Moderate - 2, few - 3), Collagen fiber orientation (Vertical - 1, Mixed- 2, Horizontal - 3), Amount of early collagen (Profound - 1, Moderate - 2, Minimal - 3, Absent - 4). Amount of mature collagen was (Profound - 1, Moderate - 2, Minimal - 3). In addition, dilated blood capillaries and endothelial cells proliferation was added as a scoring criterion as follows (Profound proliferation - 1, Moderate proliferation- 2, capillary dilatation only - 3).

Total healing score of each case was calculated by adding the score of individual criteria. Lower scores indicated poorer wound healing. While higher scores pointed to a better healing process.

Healing status was graded as follows:

Good (16 - 19), fair (12 - 15) and poor (08 - 11) [26].

**3.Results**

**Histological examination of the groups sacrificed on day one:**

The control group showed moderate inflammatory cell infiltration (neutrophils and macrophages) in the control cases. The cells with positive expression of CD68 (CD68+ macrophages) in control group were [(9.80 ± 3.70)/per 400 x visual field].

Endothelial cells were noticeably dilated.

No excessive granulation tissue was seen as well as no collagen fiber deposition as seen both histologically and by Mallory's trichrome stain. (Fig.1)

Group 1 treated with propolis showed few inflammatory cell infiltrations (neutrophils and macrophages) in all cases. (Fig.2) CD68+ macrophages were [(6.00 ± 2.24)/per 400 x visual field]

Endothelial cells showed normal number and architecture with the exception of one case which revealed dilated blood vessels.

No excessive granulation tissue was seen as well as no collagen fiber deposition.

Group 2 treated with daktarin showed moderate inflammatory cell infiltration (neutrophils and macrophages) in 80% of cases and mild inflammatory cell infiltration in 20%. CD68+ macrophages in group 2 were [(8.40 ± 3.78)/per 400 x visual field]

Using ANOVA test and Paired Student's t-Test the difference in mean macrophage count (CD68+ macrophages) did not show any statistical significance between different groups, $P > 0.05$ (Tables I-IV and histogram I)

All cases revealed dilated blood vessels and 40% of them their dilatation was extreme.

No excessive granulation tissue was seen as well as no collagen fiber deposition

**Histological examination of the groups sacrificed on day three:**

The control group showed profound inflammatory cell infiltration (neutrophils and macrophages) in control cases. (Fig. 3) The cells with positive expression of CD68 (CD68+ macrophages) in control group were [(22.0 ± 6.82)/per 400 x visual field] .

Endothelial cells showed proliferation and attempts of excessive blood vessel formation.

Excessive granulation tissue was seen as well as collagen fiber deposition in a mesh like pattern.

Interrupted epithelization covered the granulation tissue.

Group 1 treated with propolis showed a few inflammatory cell infiltrations (neutrophils and macrophages) in 4 cases (Fig. 4) while 1 case still showed moderate inflammatory cell infiltration. CD68+ macrophages in group 1 were [(10.8± 4.09)/per 400 x visual field]

Endothelial cells showed normal number and architecture.

No excessive granulation tissue was seen. A small amount of well organized horizontally oriented collagen fibrils were seen.

Evidence of early epithelization was seen in all cases of group 1

Group 2 treated with daktarin® showed profound inflammatory cell infiltration (neutrophils and macrophages) in one of cases, moderate inflammatory cell infiltration in 3 of the cases and few inflammatory cell infiltration in one case. The cells with positive expression of CD68 (CD68+

macrophages) in group 2 were [(24.8± 5.50)/per 400 x visual field]

Using ANOVA test, there was a highly significant difference in mean macrophage count (CD 68+) between different groups, (*P*=0.004). (Table V and histogram II). Using Paired Student's t-Test CD68+ macrophages in group 1 were significantly lower than those in both control group and group 2, *P* <0. 01. However, no statistical significance was found between G2 and control group *P*>0.05 (Tables VI-VIII)

All cases revealed dilated blood vessels but no endothelial proliferation. A moderate amount of granulation tissue was seen as well as collagen fibrils deposition. Some of the collagen fibrils showed disorganization while some were well organized.

Evidence of early epithelization was seen in all cases of group 2 except the one showing profound inflammatory cell infiltration. (Fig. 5)

**Histological examination of the groups sacrificed on day seven:**

The control group showed a marked decrease in inflammatory cell infiltration with only sporadic chronic inflammatory cells seen in the examined fields. The cells with positive expression of CD68 were [(15.2± 5.31)/per 400 x visual field].

A slight increase in the number of blood vessels than normal was seen, as well as more than group 1 and group 2.

Granulation tissue still persisted under an incompletely epithelized wound surface which showed re-epithelization delay compared to the other two groups. Collagen bundles showed some organization, yet with delayed collagen build up and delay in scar maturation compared to the other groups. Collagen showed mainly a wavy or longitudinal organization of the fibers (Figs. 8 & 9) .

Group 1 treated with propolis showed almost no inflammatory cells infiltration with normal number and architecture of the blood vessels. CD68+ macrophages were [(5.40 ± 2.07)/per 400 x visual field] (Fig. 6).

No excessive granulation tissue was seen. And the collagen bundles showed complete organization. Complete epithelization was seen (Fig. 10) .

Group 2 treated with daktarin showed very few inflammatory cells in 2 cases and few inflammatory cells in 3 cases. The cells with positive expression of CD68 were [(8.60 ± 1.67)/per 400 x visual field] (Fig. **7**).

Using ANOVA test, there was a highly significant difference in mean macrophage count (CD 68+) between different groups, (*P*=0.004). (Table IX and histogram III). Using Paired Student's t-Test CD68+ macrophages in group 1 were significantly lower than those in both control group and group 2, *P* <0. 01. Moreover, there was a statistical significance between G2 and control group ($p<0.05$and $>0.01$) (Tables X-XII)

All cases revealed normal vascularity. Granulation tissue was scanty and showed complete epithelization. Collagen fibers showed good organization, with few areas showing a mixed horizontally oriented and mesh like pattern of collagen organization (Fig.11)

Histological examination o f the groups sacrificed on day fourteen:

All three groups including the control group, group 1 and group 2 showed clearance of all inflammatory cells and normal vascularity. No granulation tissue was seen. And the collagen bundles showed complete organization. Complete epithelization was seen. No histopathological differences were seen between the different groups.

The progression of healing was assessed histologically on the basis of individual scoring criteria adopted after **Sultana *et al.,*** [26] used on days 1, 3, 7 and 14. The results showed that wounds healed progressively with time.

On day 1 healing status was poor for all examined cases. On day 3 healing status was poor for all control group, as well as 40% of G1 and 80% of G2. However, 60% of G1 and 20% of G2 showed fair healing. On day 7; healing status was fair for all control group, as well as 40% of G1 and 80% of G2, while 60% of G1 and 20% of G2 showed good healing. On day 14, healing status was fair for 50% of control group, as well as 40% of G2. But, healing was good for 100% of G1 and 60% of G2 as well as 50% of control group.

From the above findings it can be summarized that the peak of inflammatory cells infiltration was seen at the control group not treated by either propolis or daktarin, with declining cell numbers in all groups after one week. The group treated with propolis showed a noticeable low inflammatory cell infiltrate at all time points along the study. The cells with positive expression of CD68 (CD68+ macrophages) were significantly lower than both control group and group 2 at day 3 (*P* <0. 01), but was significantly lower compared to control group only on day 7. Inflammatory cells completely cleared by day 14 for all groups.

All cases showed dilated blood vessels with variable intensities on day 1, however only the control group showed endothelial cell proliferation on day 3 which persisted till day 7 but with a fewer number.

Granulation tissue was noticeably found in the early stages of wound healing in both the control

group and group 2; more prominently in the control group. Granulation tissue decreased by day 7, but remained more in the control group compared to group 1 and group 2. No granulation tissue was found in any of the groups by day 14.

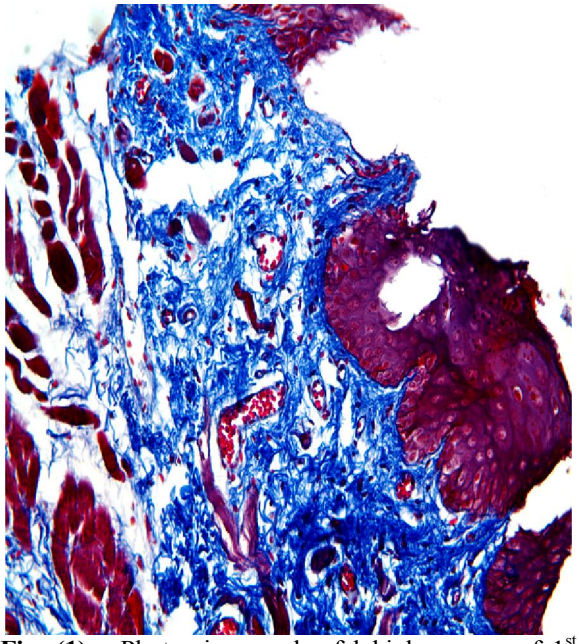


**Fig. (1):** Photomicrograph of labial mucosa of 1st postoperative day in control group (G 3) showing wound site and underlying disorganized collagen (Mallory trichrome x 100).

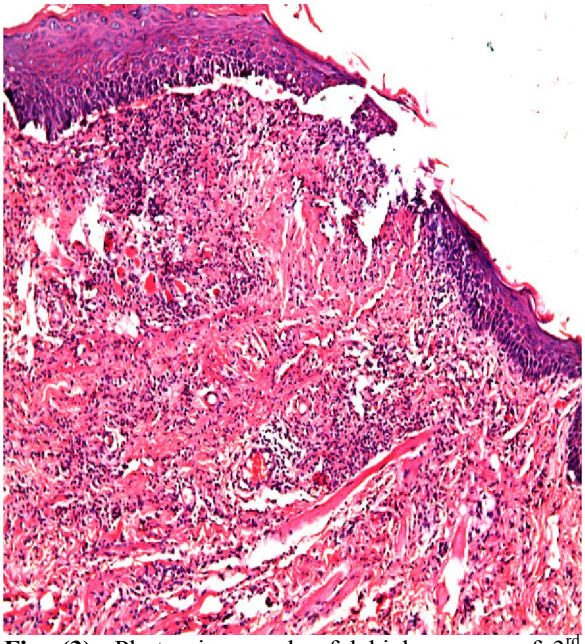


**Fig. (3):** Photomicrograph of labial mucosa of 3rd postoperative day in control group showing severe inflammatory cell infiltrate. (H & E stain x 100).

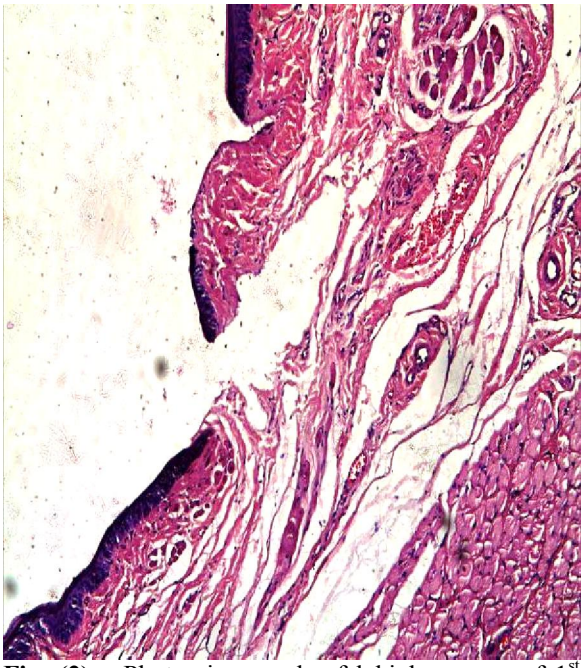


**Fig. (2):** Photomicrograph of labial mucosa of 1st postoperative day in propolis group showing wound site with few inflammatory cell infiltration. (H&E stain x 100).

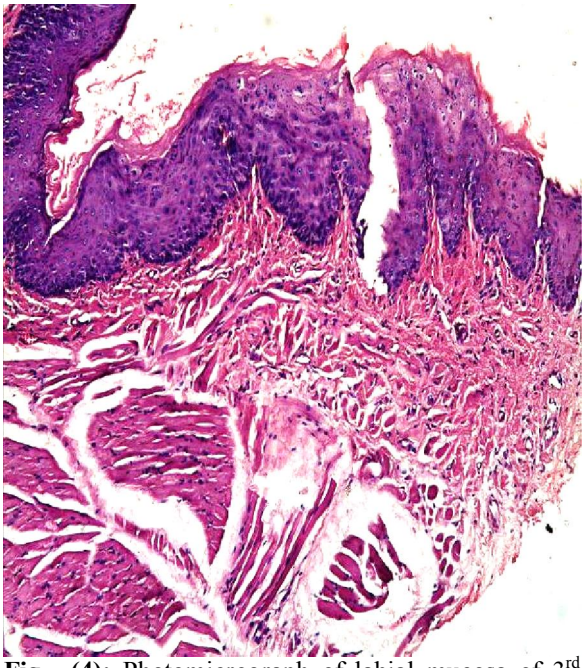


**Fig. (4):** Photomicrograph of labial mucosa of 3rd postoperative day showing mild inflammatory cell infiltrate in G1. (H & E stain x 100).

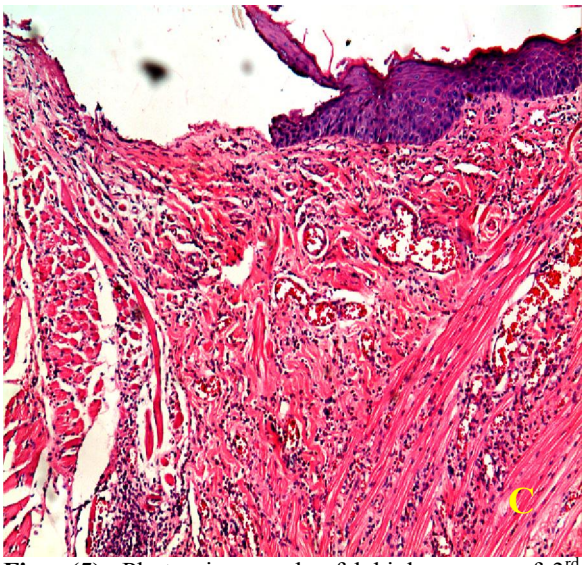



**Fig. (5):** Photomicrograph of labial mucosa of 3rd postoperative day showing profound inflammatory cell infiltrate and dilated blood vessels in one of the lesions in G2. Note the lack of epithelization with severe inflammatory response. (H & E stain x 100).

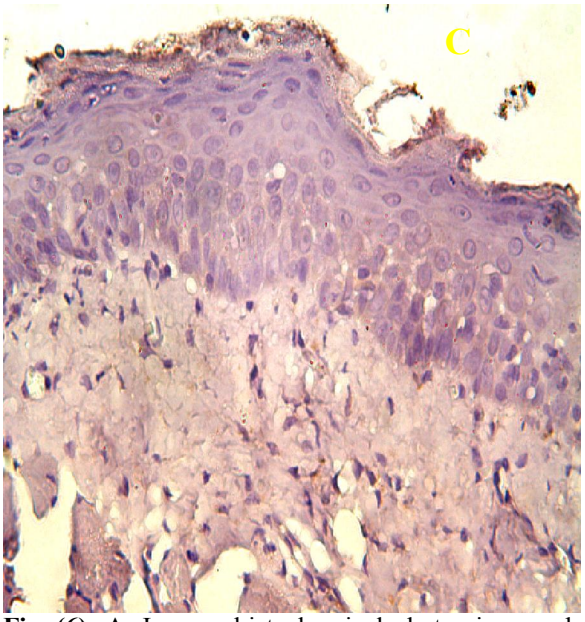



**Fig. (6): A:** Immunohistochemical photomicrograph of labial mucosa of 7th postoperative day in G1. (CD68 x 400).

Collagen fiber deposition was present in all groups starting in the early stage of wound healing (3-day). Collagen appeared bluish when examined by Mallory's trichrome stain. There was a quantitative increase in collagen synthesis which increased with time in subsequent groups. Collagen fibers orientation ranged from a mesh like organization in the control group to horizontally oriented fibrils in group 1. Mixed orientation was seen in group 2. Collagen fibrils showed organization and maturation on day 7 in all cases, although collagen in the control group showed some delay in scar maturation compared to the other groups. Collagen fibers showed maturation and horizontal organization in all cases on day 14.

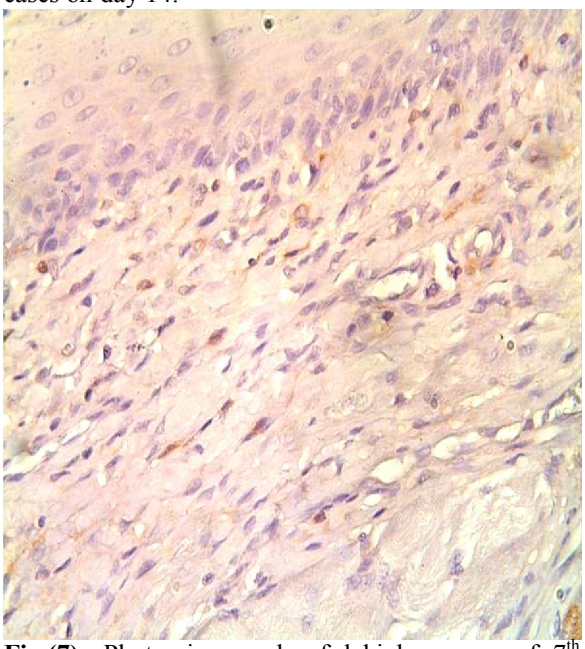


**Fig.(7):** Photomicrograph of labial mucosa of 7th postoperative day showing mild inflammatory cell infiltrate in G2. (CD68 x 400)

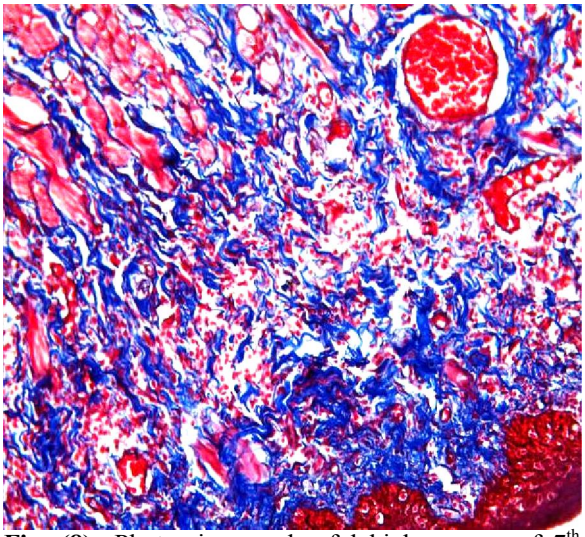


**Fig. (8):** Photomicrograph of labial mucosa of 7th postoperative day showing short wavy, longitudinally arranged collagen bundles in control group. (Mallory's Trichrome stain x 200)

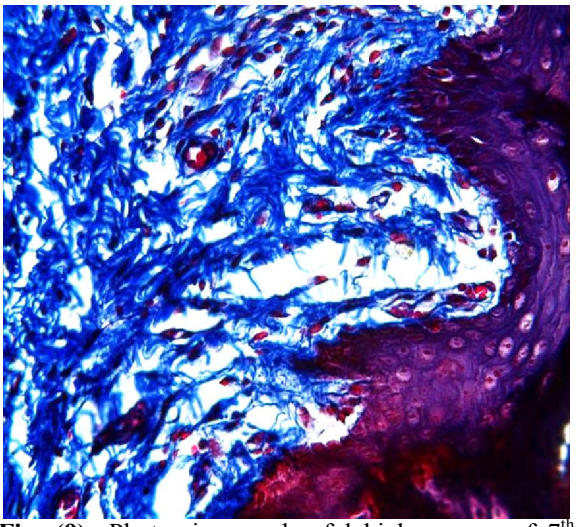


**Fig. (9):** Photomicrograph of labial mucosa of 7th postoperative day showing longitudinally arranged collagen bundles in control group. (Mallory's Trichrome stain x 400)

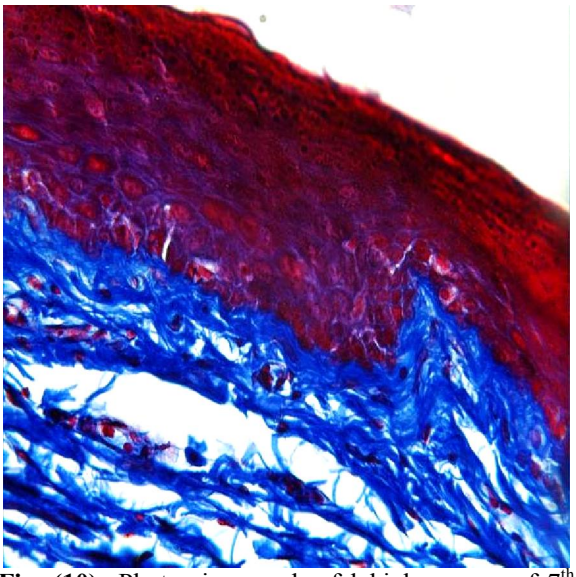


**Fig. (10):** Photomicrograph of labial mucosa of 7th postoperative day showing well organized horizontally oriented collagen deposition in G1. An indication of good healing (Mallory's trichrome stain x 400)

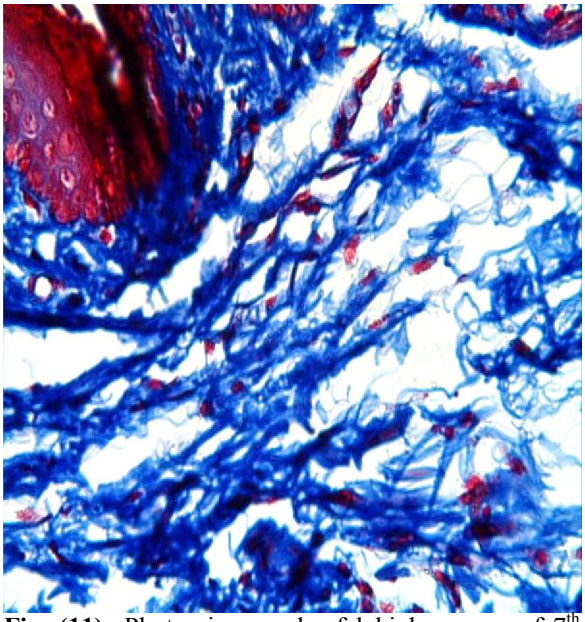


**Fig. (11):** Photomicrograph of labial mucosa of 7th postoperative day showing a mixed horizontally oriented and mesh like pattern of collagen organization in G2 (Mallory's Trichrome stain x 400)

Histologically assessed scoring criteria showed that healing progressed with time in all groups. G1 treated with propolis showed advanced healing when compared with the other two groups at all time points. Early epithelization was seen starting day 3 in all groups, however, cases showing profound inflammatory cells infiltration did not show epithelization in this early stage. On day 7, all examined cases showed epithelization.

**Table I: difference in mean macrophage count (CD 68) between different groups after 1day using ANOVA statistical test:**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | F-Value | *p*-Value |
| Control | 9.80 ± 3.70 | 1.679 | 0.228 |
| Propolis | 6.00 ± 2.24 | | |
| Daktarin® | 8.40 ± 3.78 | | |

**No significant difference, ($p$>0.05).**

**Table II: Difference in mean macrophage count (CD 68) between Control and Propolis groups after 1day using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 9.80 ± 3.70 | 1.9649 | 0.0850 |
| Propolis | 6.00 ± 2.24 | | |

Not significant difference, ($p$>0.05).

**Table III: Difference in mean macrophage count (CD 68) between Control and Daktarin® groups after 1day using Paired Student's t-Test**

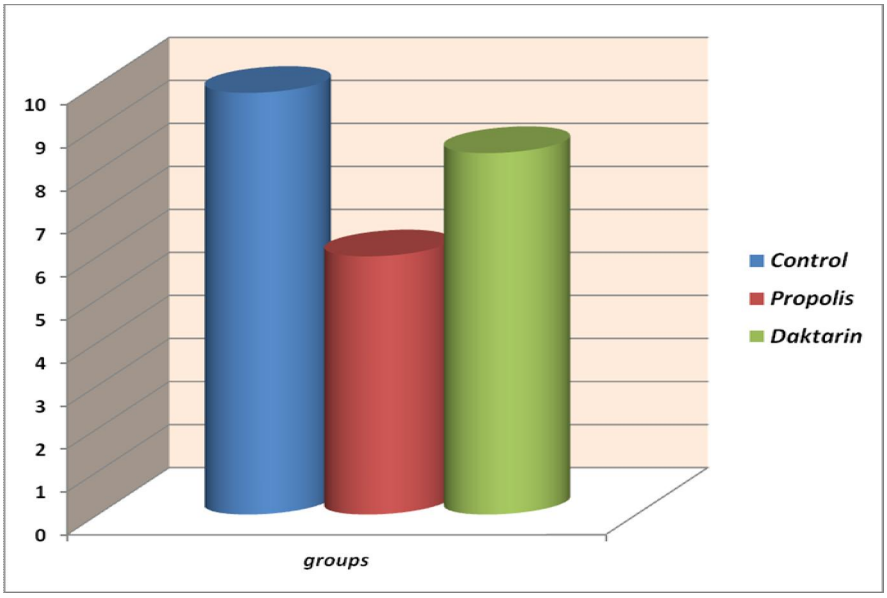
| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 9.80 ± 3.70 | 0.5916 | 0.5704 |
| Daktarin® | 8.40 ± 3.78 | | |

Not significant difference, ($p$>0.05).

**Table IV: Difference in mean macrophage count (CD 68) between Propolis and Daktarin® groups after 1day using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Propolis | 6.00 ± 2.24 | 1.2216 | 0.2566 |
| Daktarin® | 8.40 ± 3.78 | | |

Not significant difference, ($p$>0.05).




**Histogram I: Showing difference in macrophage count (CD 68) between different groups after 1day.**

**Table V: Difference in mean macrophage count (CD 68) between different groups after 3days using ANOVA statistical test:**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | F-Value | *p*-Value |
| Control | 22.0 ± 6.82 | 8.814 | 0.004** |
| Propolis | 10.8± 4.09 | | |
| Daktarin® | 24.8± 5.50 | | |

** High significant difference, ($p$<0.01).

**Table VI: Difference in mean macrophage count (CD 68) between Control and Propolis groups after 3days using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 22.0 ± 6.82 | 3.6003 | 0.0070** |
| Propolis | 10.8± 4.09 | | |

** High significant difference, ($p$<0.01).

**Table VII: Difference in mean macrophage count (CD 68) between Control and Daktarin® groups after 3days using Paired Student's t-Test**

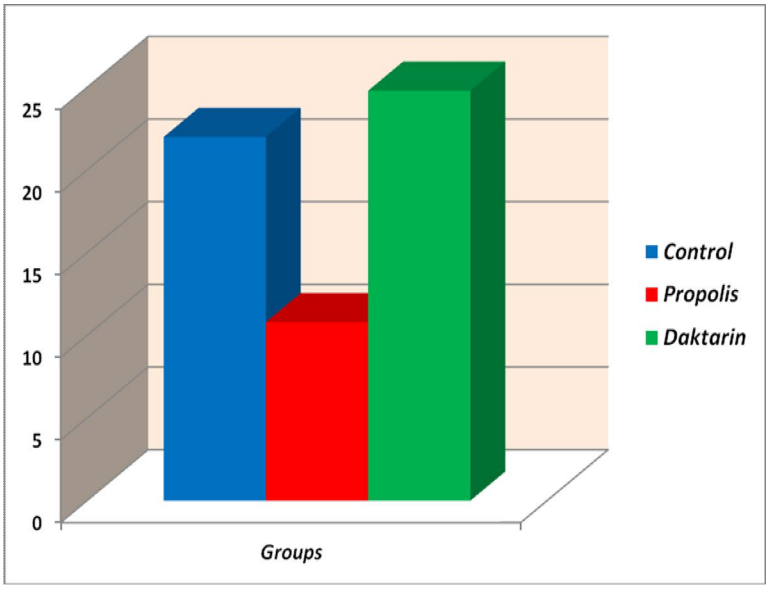
| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 22.0 ± 6.82 | 0.7149 | 0.4950 |
| Daktarin® | 24.8± 5.50 | | |

Not significant difference, ($p$>0.05).

**Table VIII: Difference in mean macrophage count (CD 68) between Propolis and Daktarin® groups after 3days using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Propolis | 10.8± 4.09 | 5.0936 | 0.0009** |
| Daktarin® | 24.8± 5.50 | | |

** High significant difference, ($p$<0.01).




**Histogram II: Showing difference in macrophage count (CD 68) between different groups after 3days.**

**Table IX: Difference in mean macrophage count (CD 68) between different groups after 7days using ANOVA statistical test:**

| group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | F-Value | *p*-Value |
| Control | 15.2± 5.31 | 10.61 | 0.002** |
| Propolis | 5.40 ± 2.07 | | |
| Daktarin® | 8.60 ± 1.67 | | |

** High significant difference, ($p$<0.01).

**Table X: Difference in mean macrophage count (CD 68) between Control and Propolis groups after 7days using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 15.2± 5.31 | 4.7012 | 0.0015** |
| Propolis | 5.40 ± 2.07 | | |

** High significant difference, ($p$<0.01).

**Table XI: Difference in mean macrophage count (CD 68) between Control and Daktarin® groups after 7days using Paired Student's t-Test**

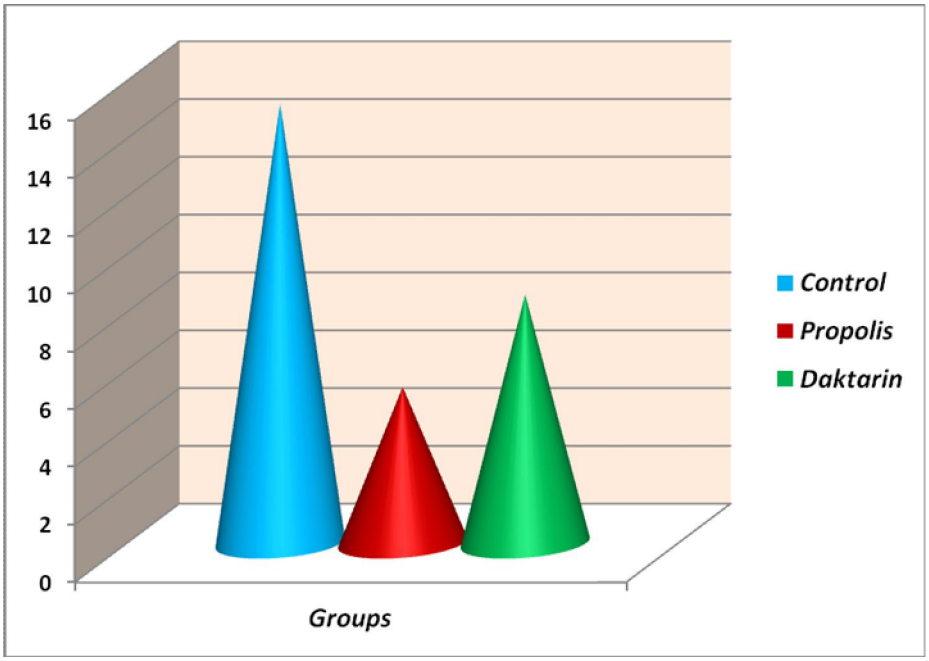
| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Control | 15.2± 5.31 | 3.5109 | 0.0080** |
| Daktarin® | 8.60 ± 1.67 | | |

** High significant difference, ($p$<0.01).

**Table XII: Difference in mean macrophage count (CD 68) between Propolis and Daktarin® groups after 7days using Paired Student's t-Test**

| Group | Mean macrophage count (CD 68) | | |
|---|---|---|---|
| | M±SD | t-Value | *p*-Value |
| Propolis | 5.40 ± 2.07 | 2.6854 | 0.0277* |
| Daktarin® | 8.60 ± 1.67 | | |

Significant difference, ($p$<0.05and >0.01).




**Histogram III: Showing difference in macrophage count (CD 68) between different groups after 7days.**

**4. Discussion:**

We chose to study wound healing by second intention 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 healing by the end of this time period.

A chief strength of this study was that all of the wounds were made under the same experimental conditions and were standardized for size, depth and site. The choice of male rats also cancelled the effect of sex hormones on wound healing. Sex hormones likely modulate oral mucosal wound healing.

Studies in rats are of low cost and provide useful information that could be difficult to obtain in

humans. In studies with 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. Thus, the use of rats in this work produced simple information but still capable of encouraging further researches in this area of knowledge.

In the present study, surgical mucosal wounds were made in the labial mucosa of all animals by means of a 1-mm punch-biopsy instrument before being removed with a scalpel from the rat's labial mucosa. This method is very useful for creating uniform ulcer diameters.

Since reduced wound inflammation is associated with improved tissue repair [27, 28], we examined inflammatory cell infiltration in the oral mucosal wounds of different groups. Whereas macrophages infiltration was found in all the studied specimens peaking in day 3 for all groups, the cells with positive expression of CD68 (CD68+ macrophages) were significantly lower than both control group and the group treated with daktarin at day 3 ($P$ <0. 01), but was significantly lower only in comparison to control group on day 7

Neutrophils and macrophage infiltration is the most prominent feature of the innate response, but they are also a double edged sword. They are aggressive against microbes, but they cause major collateral damage by releasing a corrosive cocktail of protease enzymes and active oxygen species [4].

Even though crucial for antibacterial defense, neutrophils and macrophages can become an unwelcome, damaging presence in a wound, if they stay in residence for too long [4].

Hence, lower inflammatory responses have been associated with faster wound healing [29, 30].

There was a decrease in CD68 positive cells in the group treated with propolis at all time points along the study. This was of a statistical significance when compared to both control group and group 2 on day 3, ($P$ <0. 01)

However, on day 7, the group treated with propolis showed significantly lower macrophage count compared to the control group, $P$<0. 01, but did not show statistical significance compared to group 2.

The finding of reduced inflammatory cell infiltration in oral wounds treated by propolis is in keeping with the accelerated repair. The anti-inflammatory activity of propolis has been reviewed by **Almeida and Menezes,** [31]. Propolis has inhibitory effects on mieloperoxidase activity, NADPH-oxidase ornithine decarboxilase, tirosine-protein-kinase, and hyaluronidase from guinea pig mast cells. This anti-inflammatory activity can be explained by the presence of active flavonoids and cinnamic acid derivatives. The former includes acacetin, quercetin, and naringenin the latter includes caffeic acid phenyl ester (CAPE) and caffeic acid (CA)[31]. On the other hand, **Santos *et al.,*** [32] observed that propolis propolis gel and Daktarin showed complete clinical remission of palatal edema and erythema and concluded that the efficacy of propolis was comparable to Daktarin.

Nevertheless, in the oral cavity, propolis had been found to inhibit different pathogenic microbes such as bacteria, fungi and viruses[33-35] and can be successfully applied against the different stomatological pathologic conditions: stomatitis, paradontosis, gingivitis and caries[34,36,37].

In the present study, granulation tissue was noticeably found in the early stages of wound healing in both the control group and group 2; more prominently in the control group. This is in line with **Stephens *et al.,*** [38,40] who stated that oral mucosal fibroblasts produce HGF as well as keratinocyte growth factor, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF-2). Following injury, fibroblasts migrate into the wound, proliferate and produce the matrix proteins (fibronectin, hyaluronic acid, collagen and proteoglycans) and, in doing so, form granulation tissue [41]. They also interact with keratinocytes, releasing growth factors and cytokines that play a further role in modulating wound repair [42]. The composition of the ECM (and thus the final wound healing outcome) can be altered by the balance between the MMPs and TIMPs enzymes produced by fibroblasts.

In this study, granulation tissue decreased by day 7, but remained more in the control group compared to group 1 and group 2. No granulation tissue was found in any of the groups by day 14.

The orderly collagen formations at different stages of wound healing at different days have been recognized as histologic characteristics of healing. These include increased diameter, increased inter-fibril binding, and rearrangement of fibrils with time to become more organized in a manner that maximizes strength[43]. Based on these histological parameters it was noticed in our study that collagen fiber deposition was present in all groups starting in the early stage of wound healing (3-days). Increasing with time until it reached a maximum mature arrangement by day 14.

This is in accordance with **Cotran *et al.,*** [44] and **Barbul,** [45].

Collagen fibers orientation ranged from a mesh like organization in the control group to horizontally oriented fibrils in group 1. Mixed orientation was seen in group 2. Collagen fibrils showed organization and maturation on day 7 in all cases. Horizontal collagen orientation during wound healing had been reported by **Mustafa,** [24] and **Barbul,** [45]. Although collagen in the control group showed some delay in scar maturation compared to the other groups, collagen fibers showed maturation and horizontal organization in all cases on day 14, hence indicating complete healing.

In this current study, all cases showed complete epithelization on day 7, this is in agreement with Yilmaz et al', who when evaluated the therapeutic effectiveness of honey on oral mucosal ulcers stated that the wounds of all their studied groups were covered by new mucosa epithelium and were similar to the normal one on day 7 and 14. [46]

**Conclusion:**

Propolis has an enhancing effect on the healing of oral mucosal wounds. It was linked to decreased inflammatory reaction. Therapeutic value of propolis in oral mucosal wound healing is more effective compared to daktarin. Further investigations about the therapeutic effects of propolis on oral lesions might substitute or aid the conventional treatment methods. Thus, further in vivo investigations are required to support this assumption.

**Corresponding author:**
Zoba H. Ali, Oral Biology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt. rawya_h2a@yahoo.com

**References:**

1. Gonsalves W.C, Chi A.C, Neville B.W. (2007): Common oral lesions: Part I. Superficial mucosal lesions. Am. Fam. Physician;75(4):501-507.
2. Markiewicz M.R, Margarone III J.E, Barbagli G, Scannapieco F.A. (2007): Oral Mucosa Harvest: An Overview of Anatomic and Biologic Considerations, EAU-EBU Update Series 5,:179-187.
3. Campisi G, Compilato D, Cirillo N, Ciavarella D, Panzarella V, Amato S, Lo Muzio L. (2007): Oral ulcers: three questions on their physiopathology. Minerva Stomatol.; 56 (5):293-302.
4. Enoch S, (2009): Scarless healing: oral mucosa as a scientific model Wounds UK, , 5(1):42-48.
5. Birn H, Winther J. E. (1982): Surgical standard procedures suture. In : Manual of Minor Oral Surgery. London :W.B Saunders ,1982 :22.
6. Gordon R, Seward M.H, David A, Gowan M.C.(1987): Intraoral incisions and suturing. In: Killey and Kay's outline of oral surgery part one. 2nd ed. India: 10p publishing limited, 1987: 42.
7. Marcucci MC, Ferreres F,Garc a-Viguera C, Bankova VS, De Castro SL, Dantas AP, Valente PHM , Paulino N. 2001: Phenolic compounds from Brazilian propolis with pharmacological activities. J Ethnopharmacol 74: 105-112.
8. Ahn M-R, Kunimasa K, Ohta T, Kumazawa S, Kamihira M, Kaji K, Uto Y, Hori H, Nagasawa H, Nakayama T. (2007): Suppression of tumor-induced angiogenesis by Brazilian propolis: Major component artepillin C inhibits in vitro tube formation and endothelial cell proliferation. Cancer Letters; 252 (2): 235-243.
9. Banskota A.H, Tezuka Y, Kadota S. (2001): Recent progress in pharmacological research of propolis. Phytotherapy Res.; 15 (7): 561-571.
10. Farooqui T, Farooqui A. (2010): Molecular Mechanism Underlying the Therapeutic Activities of Propolis: A Critical Review. Curr Nutr Food Sci.; 6: 188-199.
11. Sforcin J.M, Bankova V. (2011): Propolis: Is there a potential for the development of new drugs? J Ethnopharmacol.; 133: 253-260
12. Pontin K, Da Silva Filho A.A, Santos F.F, Silva M.L, Cunha W.R, Nanayakkara N.P, Bastos J.K, de Albuquerque S. (2008): *In vitro* and *in vivo* anti-leishmanial activities of a Brazilian green propolis extract Parasitol Res.; 103(3):487-492.
13. Ozbilge H, Kaya E.G, Songul A.S, Silici S. (2010): Anti- leishmanial activities of ethanolic extract of Kayseri propolis. African J. Microbiology Res.; 4 (7): 556-560.
14. De Barros M.P, Sousa J.B, Bastos J.K, De Andrade S.F. (2007): Effect of Brazilian green propolis on experimental gastric ulcers in rats. J. Ethnopharmacology.;110 (3): 567-571.
15. Hsiao G, Lee J.J, Lin K. H, Shen C.H, Fong T.H, Chou D.S, Sheu J.R. (2007): Characterization of a novel and potent collagen antagonist, caffeic acid phenethyl ester, in human platelets: *In vitro* and *in vivo* studies. Cardiovascular Res.;75 (4): 782-792.
16. Wegner T, Wagner H. The active components and the pharmacological multi-target principle of STW (Iberogast®). Phytomedicine, 2006;13 (Suppl.1),20-35.
17. Amoros M, Simoes C. M, Girre L, Sauvager F, Cormier M. (1992): Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J Nat Prod; 55(12):1732-1740.
18. Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V.S, Christov R, Popov S. (1999): Antibacterial, antifungal and antiviral activity of propolis of different geographic origin, Journal of Ethnopharmacology; 64: 235-240.
19. Cardile V, Panico A, Gentile B, Borrelli F, Russo A. (2003): Effect of propolis on human cartilage and chondrocytes. Life Sci.;73(8):1027-1035.
20. Ansari S, Prasad R. (1993): Effect of miconazole on the structure and function of plasma membrane of

Candida albicans. Original Res. Article FEMS Microbiology Letters; 1 (114):93-98.
21. Van Cutsem J.M, Thienpont D. (1972): Miconazole, a broad-spectrum antimycotic agent with antibacterial activity. Chemotherapy.;17(6):392-404.
22. Khaliq J.A, Raza S. M, Anis I, Khan M. I. (2009): *In vitro* antifungal and antibacterial activities of extracts of Galium tricornutum subsp. longipedunculatum. J Enzyme Inhib Med Chem.;24(1):192-196.
23. Robert G. Irwin, Andreas H. Grill, Thomas J. Walsh. (1998): Antifungal drugs. Side effects of drugs Annual Vol. 21; 282-292.
24. Mustafa S. (1997): Effect of copper salt of Ibuprofen on wound healing in experimental rats. M.phil: Pathology Thesis, University of Dhaka.; 87.
25. Maria jose E, Marta G, Fernando L, Meana Alvaro, Eva G, Jose L Jorcano, Sara G Llames, (2004): An in vivo model of wound healing in genetically modified skin humanized mice, in Wound healing (original Article). J. Investigative Dermatology; 123: 1-19
26. Sultana J, Molla M.R, Kamal M, Shahidullah M, Begum F, Bashar MA. (2009): Histological differences in wound healing in Maxillofacial region in patients with or without risk factors. Bangladesh J. Pathol.; 24 (1) : 8.
27. Wetzler C, Kampfer H, Stallmeyer B, Pfeilschifter J, Frank S. (2000): Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. J Invest Dermatol.; 115:245–253.
28. Wilgus T.A, Vodovotz Y, Vittadini E, Clubbs E.A, Oberyszyn T.M. (2003): Reduction of scar formation in full-thickness wounds with topical celecoxib treatment. Wound Repair Regen;11:25–34.
29. Dovi J.V, He L.K, DiPietro L.A. (2003): Accelerated wound closure in neutrophil-depleted mice. J Leukoc Biol.;73:448-455.
30. Dovi J.V, Szpaderska A.M, DiPietro L.A. (2004): Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost.;92:275-280.
31. Almeida E.C.D, Menezes H. (2002): Anti-inflammatory activity of propolis extracts: a review 2104. J. Venomous Animals and Toxins including Tropical Diseases.; 8 (2): 191-212.
32. Santos V.R, Gomes R.T, de Mesquita R.A, Mariela de Moura D.G, França E.C, de Aguiar E.G, Naves M.D, Abreu J.A.S, Sheila Abreu R.L. (2008): Efficacy of Brazilian propolis gel for the management of denture stomatitis: a pilot study. Phytotherapy Res.;11 (22): 1544–1547.
33. Santos F.A, Bastos E.F, Maia A.A, Uzeda M, Carvalho M.A.R, Farias L.M, Moreira E.S.A. (2003): Brazilian propolis: physicochemical properties, plant origin and antibacterial activity on periodontopathogens. Phytotherapy Res.;17(3):285-289.
34. Hayacibara M.F, Koo H, Rosalen P.L, Duarte S, Franco E.M, Bowen W.H, Ikegaki M, Cury J.A. (2005): *In vitro* and *in vivo* effects of isolated fractions of Brazilian propolis on caries development. J. Ethnopharmacology.;101 (1-3): 110-115.
35. Scazzocchio F, D'auria F.D, Alessandrini D, Pantanella F. (2006): Multifactorial aspects of antimicrobial activity of propolis. Microbiological Res. ;161 (4): 327-333.
36. Amaral R.C, Gomes R.C, Rocha Dos Santos W.M, Abreu S.L.R, Santos V.R. (2006): Periodontitis treatment with brasilian green propolis gel. Pharmacologyonline.; 3: 336-341.
37. Oliveira ACSS, Murgo DOA. (2006): Estidio da aplicacao da propolis ma odontologia (revisao de literatura) Application of propolis in odonotology, a review, X Encontro Latino Americano de Iniciação Científica e VI Encontro Latino Americano de Pós-Graduação – Universidade do Vale do Paraíba:; 737-740.
38. Stephens P, Hiscox S, Cook H, Jiang W.G, Zhiquiang W, Thomas D.W. Phenotypic variation in the production of bioactive hepatocyte growth factor/scatter factor by oral mucosal and skin fibroblasts. Wound Repair Regen. 2001; 9(1): 34–43
39. Okazaki M, Yoshimura K, Uchida G, Harii K. (2002): Elevated expression of hepatocyte and keratinocyte growth factor in cultured buccalmucosa- derived fibroblasts compared with normal-skin-derived fibroblasts. J Dermatol Sci.; 30(2): 108–115.
40. Shannon D.B, McKeown S.T, Lundy F.T, Irwin C.R. (2006): Phenotypic differences between oral and skin fibroblasts in wound contraction and growth factor expression. Wound Repair Regen.; 14(2): 172–178.
41. Enoch S, Moseley R, Stephens P, Thomas D.W. (2008): The oral mucosa: a model of wound healing with reduced scarring. Oral Surgery.; 1(1): 11–21.
42. Slavin J. (1996): The role of cytokines in wound healing. J Pathol.; 178(1): 5–10.
43. Baum C.L, Arpey C.J. (2005): Normal cutaneous wound healing: clinical correlation with cellular and Molecular events. Dermatol Surg.; 31: 674-686.
44. Cotran R.S, Kumar V, Collin's T. (1999): Tissue repair: cellular growth, Fibrosis and wound healing. In: Robins Pathological Basis of Disease. 6th edition. Philadelphia, USA : W.B. Saunders ; 22-118.
45. Barbul A. (2005): Wound healing. In: Scwartz's Principles of surgery. 8th Ed New York : The McGraw - Hill companies, Inc,; 222-223.
46. Yilmaz N, Nisbet Ö, Nisbet C, Ceylan G, Hoşgör F, Dede ÖD, (2009): Biochemical evaluation of the therapeutic effectiveness of honey in oral mucosal ulcers.Bosnian J. Basic Medical Sci.; 9 (4): 294-295.