





# Immunohistochemical Localization of Epidermal Growth Factor Receptor in Rat Buccal Mucosa Treated with Fenugreek Leaf Oil

Sarah A Omran\* 🔎 , Ban A Ghani 🔎

Department of Oral Diagnostic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq

\*Correspondence: sara.alaa2206@codental.uobaghdad.edu.iq Received: 23 April 2024 Revised: 16 June 2024 Accepted: 22 July 2024 Published: 28 December 2024 DOI: https://doi.org/10.30539/9hb0jd31



This article is an open access distributed under the terms and conditions of the Creative Common Attribution License (CC BY 4.0)

Cite: Omran SA, Ghani BA. Immunohistochemical Localization of Epidermal Growth Factor Receptor in Rat Buccal Mucosa Treated with Fenugreek Leaf Oil. Iraqi J. Vet. Med. 2024;48(2):72-80.

## ABSTRACT

The lack of effective treatment for oral mucosal ulcers has motivated clinicians to search for other therapeutic techniques to improve oral ulcer healing. Applying fenugreek to an ulcer triggers the release of its anti-inflammatory components, that might reduce any superfluous inflammatory processes and accelerate wound healing. The aim of this study was to investigate the effect of fenugreek leaf oil on the ulcer associated with the expression of epidermal growth factor receptor (EGFR). A total of 24 adult male rats weighing between 350 and 450 grams and 4-6 months of age were used in this study. The experimental design included: the normal group (no ulcer), the control group (12 ulcers left without treatment to the right), and the study group (12 ulcers on the left side were healed using fenugreek oil). All rats were sacrificed on the 3rd and 7th days of the experimental study, healing durations, and biological samples were prepared for histological and immunohistochemistry analysis of EGFR. The current study exhibited that the EGFR expression increased in ulcer sites treated with fenugreek after 3 days of ulceration with significant differences in comparison with other groups. In conclusion, fenugreek oil was efficient in promoting ulcer healing by enhancing epithelization. Moreover, the positive localization of EGFR increased in the study groups treated with fenugreek oil indicating its potential activity in accelerating the healing process.

which contains many enzymes, acids, antioxidants, and

antibacterial factors (1). In rats, the oral cavity is covered

by variably orthokeratinized squamous epithelium like

buccal, palatine, and labial mucosa. Diet and frequency of

consumption affect the keratin layer's thickness. Stronger

collagen and elastin fibers that adhere to the underlying

**K**<sub>eywords</sub>: fenugreek oil, buccal mucosa ulcer, wound healing, EGFR

**INTRODUCTION** 

The oral mucosa of rats is comparable to that of humans as it consists of overlying epithelium and underlying connective tissues. Depending on the environmental conditions that most animals live in and the way they obtain their diet, which makes them vulnerable to many infections and diseases. Therefore, exposure to injury may be caused by trauma, insect bites, burn, or any sharp instrument, which can result in serious clinical problems. However, all animals have means of defense mechanism against invasion by microorganisms in various parts of their body such as mucus membranes that act as effective physical barriers lining the mouth, nose, and eyelids. Typically, the mucous membranes are coated with secretions that fight microorganisms. For example, the mucous membranes line the oral cavity covered with saliva

akes them vulnerable to efore, exposure to injury ites, burn, or any sharp rious clinical problems. of defense mechanism ms in various parts of nes that act as effective th, nose, and eyelids. nes are coated with sms. For example, the vity covered with saliva

Iraqi J. Vet. Med. 2024, Vol. 48(2):72-80

excite cell proliferation through their activation, consequently, they have a role in angiogenesis, mitogenesis, and accelerating the healing process (5). The healing process starts with the inflammatory phase involves homeostasis and inflammation. When mucosa is injured, the first cells that respond and arrive at the injury site within 12 hours are the neutrophils, which could constitute about 50% of all cells within the injury site after a few hours of wounding (6–8). After 24 hours the neutrophils reach their maximum concentration, and their infiltration will reduce (9).

The formation of new epithelium, blood vessels, granulation tissues, and collagen fibers, is the essential process of the proliferative phase. Within 24-48 h, the keratinocytes move from the edges into the wound, due to the loss of hemi-desmosomal assembly of the epithelial cells and move unto they could find their matching cells. Up to this end, it could be said that the re-epithelialization begins from the injury edge (10). Thereafter, the contraction phase takes place, which is the final phase of wound healing process, where the wound edges are connected again and the distance between these edges is closed. After the wound edges are closed, the wound surface is reduced, and the wound closure is fastened. As the collagen fibers and fibroblasts being regulated and deposited, the unarranged collagen matrix transforms into a highly organized collagen matrix, where their structure becomes almost similar to that of native tissues. At a further stage of the remodeling phase, the macrophages and fibroblasts numbers decrease due to the ends of apoptosis and angiogenesis (11). Medicinal plants can be utilized as an initial therapy for injuries, wounds, irritation, and wounds from surgical procedure due to their abundant mineral and vitamin content. For instance, fenugreek leaf oil is rich in phosphorus, iron, carotene, calcium, riboflavin, thiamine, and arachidonic acid. The constituents present in medicinal plants can facilitate expedited recovery and enhance the regeneration of tissues at the site of injury. (12). Fenugreek leaves contain linoleic acid, which serves as a precursor to arachidonic acid and plays a crucial role in the sequence of the inflammatory process. These substances accelerate the inflammatory process by functioning as mediators of inflammation (13).

However, the epidermal growth factor receptor "transmembrane tyrosine kinase receptor" is one of the crucial growth factors that have a significant role in wound healing (14). The binding of ligands, such as EGF to receptor activates and stimulates intracellular signaling cascades that control various cellular responses, such as DNA synthesis and cell proliferation, differentiation, migration, adhesion, angiogenesis, inhibition of apoptosis, and survival (15–17). Epidermal growth factor receptors have demonstrated expression on vascular endothelial cells, keratinocytes, and normal skin fibroblasts (18). The staining of EGFR can be observed in the cell membrane and cytoplasm of keratinocytes as well (19). In addition, the EGFR motivates the proliferation of epithelium after injury and accelerates wound closure (20–22). Extensive research has focused on wound care, including exploring novel therapy approaches and advancements in the treatment of both acute and chronic wounds. These advancements include the utilization of herbal remedies to enhance healing and minimize the adverse effects of medications. Fenugreek has many potential benefits that can be utilized in the wound healing process; however, this field needs to be more investigated and applied to various wound sites and different possible tissues. The aim of this study was to investigate the effect of fenugreek leaf oil on ulcer healing associated with the expression of epidermal growth factor receptor (EGFR).

## MATERIALS AND METHODS

## **Ethical Statement**

All procedures used in this study were approved by the local Scientific Research Committee of the College of Dentistry, University of Baghdad for ethical principles of animal experimentation guidelines on the care and use of animals in research of animal welfare (Approval Number: 879, dated 3-12-2023).

## Animals

From October 2023 to January 2024, twenty-four Wister albino male rats of 350-450 g weight, and 2-3 months of age. The animals were housed in the Veterinary clinic in Al-Najaf City and cared out in accordance with Baghdad University for ethical standards of animal investigation. The animals were kept in polycarbonate cages on a layer of wood shavings, in groups of four per cage, under standard laboratory control conditions at room temperature, fed with a standard rat chow pellet diet, and free access to tap water.

#### **Experimental Design**

The animals were randomly divided into three groups: the normal group, the control group, and the study group. All animals were subjected to traumatic ulcers of 5 mm diameter, and 1 mm depth by using a round diamond bur size 1mm on the right and left buccal mucosa for each rat. The experimental design was partitioned in the following manner: normal group (24 specimens of normal tissue) in which absence of ulcer initiation or treatment, control group (24 ulcers, on the right side): ulcers were irrigated with normal saline and left without treatment for spontaneous healing, and study group (24 ulcers, on the left side): was treated once daily with fenugreek oil.

#### **The Surgical Procedure**

Every animal was weighed in order to determine the precise amount of general anesthetic that would be administered. In a well-sterile environment, general anesthesia was administered by injecting a combination of 50 mg/kg ketamine with 5 mg/kg xylazine into the peritoneal cavity in the bottom abdomen region, after being sterilized using a 70% alcohol solution (23). Mucosal ulcer induction was done by utilizing a round diamond bur (24) of 5mm diameter, and 1mm in depth (25) to the right and

left buccal mucosa of each rat, (Figure 1 A). Daily local application of  $10 \ \mu$ L of fenugreek oil by micropipette on the study group and the control groups were rinsed with distilled water (6) (Figure 1 B).

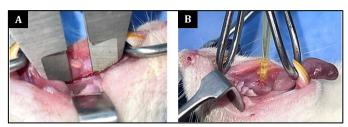


Figure 1. (A) Ulcer induction, (B)Oil application

Animals were sacrificed according to the healing durations of 3 and 7 days, with a total of twelve animals for each duration. The rats were euthanized by administering an excessive amount of an anesthetic solution. (26). Thereafter. the wound region dissected was perpendicularly by utilizing a surgical scalpel (no. 15) to remove a 5 mm slice outside borders of the wound (25). The dissected samples were then inserted in 10% freshly formalin (27), for histological prepared and immunohistochemical localization of epidermal growth factor receptor expression.

## Immunohistochemical Staining for EGFR Detection

The staining procedure was decided depending on the manufacturer's data sheets. Tissue sections (4 um thick) were cut, stretched in hot water (45°C), and mounted on positive charge slides. De-paraffinization was performed by heating at 65°C for 30 min and immersing in xylene for 5 min twice. Slides were rehydrated sequentially in absolute alcohol, 90%, 80%, and 70%, followed by distilled water (5 min each). Antigen retrieval involved immersing slides in Tris-EDTA (pH 9) at 99°C for 30 min, then cooling for 15 min. Slides were washed with phosphate-buffered saline (PBS, pH 7.4) and treated with hydrogen peroxide to block peroxidase activity, followed by incubation with primary antibody (EGFR, 1:50) overnight at 37°C in a humid chamber. After washing, a mouse linker was applied for 10 minutes, followed by a secondary antibody (horseradish peroxidase) for 30 min at 37°C. Diaminobenzidine (DAB) was added as a chromogen for 5 minutes, followed by rinsing. Counterstaining with Myer's hematoxylin was performed for 1-2 min, and slides were rinsed and dehydrated in graded alcohols, cleared in xylene, and mounted with DPX. Slides were allowed to dry for 30 min before analysis.

#### **Immunohistochemical Analysis**

Each tissue section contained two major areas: the layer of epithelium and the lamina propria. At power  $40 \times$  by using a light microscope, 5 areas from the epithelium region and 5 areas from connective tissue were selected for each tissue section, these areas were recorded via a digital camera that is linked to the microscope lens. Thereafter, the images were stored on the computer for analysis. The

calculations of staining results were accomplished by performing. The Aperio positive pixel count algorithms application is part of the Aperio Image Scope software v12.4.6.5003, developed by Aperio Knowledges Inc, USA. Weak positive numbers were neglected because it was represented as a reflection at the back of the image (28-32). The expression of EGFR protein was summarized into the four-range index: 0; no staining, 1 +; less than 25% positive cells for weak staining, 2 +; 26-50% positive cells for moderate staining, 3+; higher than 50% positive cells for strong staining (33). The intensity of EGFR expression in cell membranes was assorted on a four-scale index as follows: 0 (no staining or staining < 10% of epithelial cells), 1 (weak staining,  $\leq 10\%$ ), 2 (moderate staining, >10%), and 3 (intense staining, >10%). For statistics investigation, these types were separated into two groups, 0 (negative: absent or weak staining 0, 1) and 1 (positive; moderate and intense 2, 3) therefore intensity of EGFR can be categorized into, score 0, <10% of epithelial cells and score 1, >10% of epithelial cells (34,35).

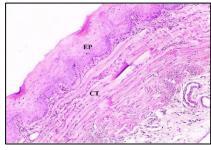
#### **Statistical Analysis**

After collecting the staining results from the immunohistochemical sections and applying the scoring index. The experimental data were analyzed using the Statistical Package for Social Sciences (SPSS) software v26 and MS Office Excel 2019. The details of the participants were only associated with their respective serial numbers, and the acquired data was managed on a regular basis. The data was quantified using statistical measures such as the mean, standard deviation, standard error, and minimum and maximum values. Independent t-test (for normally distributed data) and Mann-Whitney U test (for nonnormally distributed data) were used alternatively to assess the difference between 2 independent continuous variables. Paired t-test (for normally distributed data) and Wilcoxon signed ranks test (for non-normally distributed data) test were used alternatively used to assess the difference between 2 related continuous variables. A significant level of 0.05 or lower, with a confidence level of 95%, proved significant.

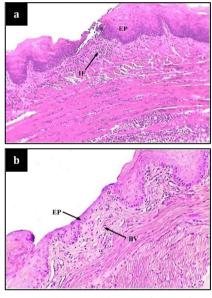
#### **RESULTS**

## **Histological Results**

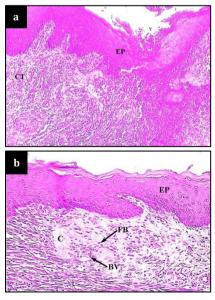
The normal tissue segment was observed by a microphotograph that revealed keratinized epithelium, fibroblasts, and collagen fibers in lamina propria, (Figure 2). On the 3rd day of the control group, the wound area displays migrating epithelial cells at wound edges, and inflammatory cell infiltration (Figure 3 a). The ulcer site of the study group shows the epithelial bridge at the surface, lamina propria shows blood vessels (BV) (Figure 3 b). A view of the 7th day of the control group showed newly formed epithelium sealing the wound surface (Figure 4 a). A view of the ulcer site of the study group showed thickened newly formed epithelium. Lamina propria, shows remodeling collagen fibers, fibroblasts, and numerous blood vessels, (Figure 4 b).



**Figure 2.** View shows keratinized stratified squamous epithelium (EP) and fibrous connective tissue (CT). H&E in 20×



**Figure 3**. (a) View of the control group shows epithelium (EP), and inflammatory cells (IF). H&E in x20. (b) A view of the study group shows the epithelium (EP), and blood vessels (BV), on the third day. H&E in 20×



**Figure 4. (a)** View of the control group shows new epithelium (EP), and connective tissue (CT). H&E in x20. **(B)** View of the study group shows epithelium (EP), fibroblasts (FB), collagen fibers (C), and blood vassels (BV), on the seventh day. H&E in 20×

#### Immunohistochemical results of EGFR

Normal group: weak positive expression of EGFR was noticed at the basal layer in a few epithelial cells, and fibroblast cells of the lamina propria, (Figure 5). The statistical results showed that there are significant differences between normal and experimental groups (control and study groups) in which, the normal group exhibits the most minimal average values in terms of both percentage and intensity of the epithelium and lamina propria of the healing periods (Tables 1-3 and Figure 8).

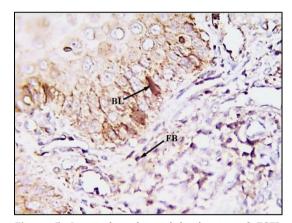


Figure 5. Immunohistochemical localization of EGFR expression of EGFR at fibroblasts (FB), basal layer (BL) in normal group at  $40 \times$ 

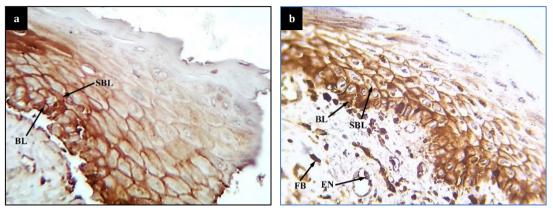
The duration comparison between groups illustrated in (Table 3) showed significant differences between the normal and control groups in epithelium with a P value (0.001) on the 3rd day and a P value (0.000) on the 7th day. Also, there was significant differences between normal and study groups with P value (0.000) in the 3rd day and P value (0.050) in 7th day. Based on these results, the normal group has the lowest mean value of positivity in the epithelium. In the lamina propria, there were significant differences between normal and control groups with a P value (0.000) on the 3rd and 7th day. Also, there were significant differences with the P value (0.000) on the 3rd day and the P value (0.021) on the 7th day between normal and study groups, consequently, the normal group exhibited the lowest average positive reaction in the lamina propria.

On the 3-day period, the control group presented moderate positive EGFR expression in epithelium at suprabasal and basal cell layers, (Figure 6-a). The microphotograph of the study group displayed strong positive expression in epithelium specifically at the basal and suprabasal cell layers, while in lamina propria, positive expression was seen by fibroblasts and endothelial cells, (Figure 6-b). The mean difference of normal and experimental groups showed the maximum mean values of EGFR on the third day of the study group in epithelium and lamina propria respectively. (Tables 1, 2) and (Figure 8). This means there were significant variances on the third day between the control and study groups with P values (0.001 and 0.002) in percentage and intensity respectively (Table 4).

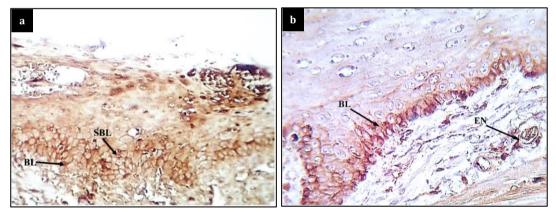
During the 7-day period, the positive immunohistochemical localization of EGFR in the control

group was noticed in basal and suprabasal epithelial layers, (Figure 7-a). Tissue section of study group displayed moderate reaction in basal layer, and endothelial cells of lamina propria, (Figure 7-b). The statistical results showed significant variances among the experimental groups on the seventh day duration, with a P value (0.000) in which the control group had a higher mean value of EGFR than the study group in percentage and intensity of epithelium and the lamina propria. (Tables 1, 2, 4) and (Figure 8). The statistical results in (Table 3) showed significant

differences between the 3rd and 7th day in the control and the study groups with a P value of (0.019) and (0.002), respectively, in percentage and intensity in the epithelium. Significant differences were recorded between the 3rd and 7th day in study groups with a P value of (0.000) in lamina propria. There were non-significant differences between the 3rd and 7th day in the control groups, where the P values were (0.158) and (0.854) in NP% and IP%, respectively, in lamina propria.



**Figure 6.** Immunohistochemical localization of of EGFR at day 3 in suprabasal layer (SBL), basal layer (BL), fibroblast (FB), and endothelial cells (EN) (a-control group: positive expression, and b-study group: strong reactivity) at 40×



**Figure 7.** Immunohistochemical localization of EGFR positive expression at day 7 in suprabasal layer (SBL), basal (BL), and endothelial cells (EN), (a-control group, and b-study group) at 40×

| Day   | Group   | Sub-group | NO. | Mean  | S.D.  | S.E. | Minimum | Maximum |
|-------|---------|-----------|-----|-------|-------|------|---------|---------|
| Day 0 | Normal  | NP%       | 12  | 25.42 | 7.48  | 2.16 | 15.67   | 35.32   |
|       |         | IP%       | 12  | 17.91 | 4.88  | 1.41 | 11.55   | 25.49   |
| Day 3 | Control | NP%       | 12  | 42.43 | 11.32 | 3.27 | 27.78   | 60.97   |
|       |         | IP%       | 12  | 34.29 | 12.65 | 3.65 | 12.64   | 56.94   |
|       | Study   | NP%       | 12  | 60.58 | 7.45  | 2.15 | 53.4    | 80.39   |
|       |         | IP%       | 12  | 49.68 | 8.17  | 2.36 | 41.95   | 71.99   |
| Day 7 | Control | NP%       | 12  | 56.01 | 4.78  | 1.38 | 47.36   | 63.98   |
|       |         | IP%       | 12  | 48.18 | 5.94  | 1.71 | 39.03   | 60.57   |
|       | Study   | NP%       | 12  | 31.67 | 5.96  | 1.72 | 23.24   | 42.15   |
|       |         | IP%       | 12  | 24.77 | 4.03  | 1.16 | 17.97   | 31.85   |

Table 1. Statistical mean values of EGFR regarding epithelium

Percentage NP%, Intensity IP%

Day S.D. S.E. Minimum Maximum Sub-group NO. Mean Group Dav 0 Normal NP% 12 17.34 2.01 0.58 13.32 20.97 IP% 12 10.86 2.44 0.7 7.23 16.96 Dav 3 Control NP% 12 34.74 4.44 1.28 27.76 42.36 IP% 12 28.1 5.96 1.72 17.14 36.84 Study NP% 53.04 14.21 32.18 75.31 12 4.1 IP% 12 14.35 4.14 26.78 70.7 46.68 Day 7 NP% 48.49 Control 12 38.07 6 1.73 29.93 IP% 12 27.6 5.62 38.66 1.62 1.69 Study NP% 1.91 11.33 36.52 12 22.6 6.63 IP% 1.59 12 15.35 5.51 9.11 28.1

Table 2. Statistical mean values of EGFR regarding lamina propria

Percentage NP%, Intensity IP%

#### Table 3. Duration comparison regarding EGFR expression

|            |                |                                         |          | Comp    | arison             |
|------------|----------------|-----------------------------------------|----------|---------|--------------------|
| EGFR       | Group          | Duration                                | Subgroup | T-test  | P-value            |
| Epithelium | Normal/Control | Between 0 and 3 days                    | NP%      | -3.175  | $0.001^{*1}$       |
|            |                |                                         | IP%      | -4.187  | 0.001*2            |
|            | Normal/Control | Between 0 and 7 days                    | NP%      | -4.157  | $0.000^{*1}$       |
|            |                |                                         | IP%      | -13.651 | 0.000*2            |
|            | Normal/Study   | Between 0 and 3 days                    | NP%      | -4.157  | 0.000*1            |
|            |                |                                         | IP%      | -4.157  | 0.000*1            |
|            | Normal/Study   | Between 0 and 7 days                    | NP%      | -1.963  | 0.050*1            |
|            |                |                                         | IP%      | -3.759  | 0.001*2            |
|            | Control        | Between 3 and 7 days                    | NP%      | -2.353  | 0.019*4            |
|            |                |                                         | IP%      | -3.023  | 0.012*3            |
|            | Study          | Between 3 and 7 days                    | NP%      | 3.059   | 0.002*4            |
|            |                |                                         | IP%      | 3.059   | 0.002*4            |
| Lamina     | Normal/Control | Between 0 and 3 days                    | NP%      | -4.157  | 0.000*1            |
|            |                |                                         | IP%      | -9.272  | 0.000*2            |
|            | Normal/Control | Between 0 and 7 days                    | NP%      | -11.355 | 0.000*2            |
|            |                |                                         | IP%      | -9.464  | 0.000*2            |
|            | Normal/Study   | Between 0 and 3 days                    | NP%      | -8.62   | 0.000*2            |
|            | , .            | -                                       | IP%      | -8.525  | 0.000*2            |
|            | Normal/Study   | Between 0 and 7 days                    | NP%      | -2.63   | 0.021*2            |
|            | , .            | -                                       | IP%      | -2.578  | 0.021*2            |
|            | Control        | Between 3 and 7 days                    | NP%      | -1.412  | $0.158^{4}$        |
|            |                | , i i i i i i i i i i i i i i i i i i i | IP%      | 0.188   | 0.854 <sup>3</sup> |
|            | Study          | Between 3 and 7 days                    | NP%      | 6.234   | 0.000*3            |
|            | -              | , i i i i i i i i i i i i i i i i i i i | IP%      | 6.765   | 0.000*3            |

\*Significant result. <sup>1</sup>Mann-Whitney U test. <sup>2</sup>Independent t-test. <sup>3</sup> Wilcoxon signed ranks test. <sup>4</sup>Paired t test. Percentage NP%, Intensity IP%.

| Table 4. Group comparison regarding EGFR expression | 1 |
|-----------------------------------------------------|---|
|-----------------------------------------------------|---|

|            | Duration | Subgroup | Group comparison |                 |  |
|------------|----------|----------|------------------|-----------------|--|
| EGFR       |          |          | T-test           | <i>P</i> -value |  |
| Epithelium | 3 days   | NP%      | -3.406           | 0.001*1         |  |
| -          |          | IP%      | -3.06            | 0.002*1         |  |
|            | 7 days   | NP%      | 4.157            | 0.000*1         |  |
|            |          | IP%      | 11.308           | 0.000*2         |  |
| Lamina     | 3 days   | NP%      | -3.032           | 0.002*1         |  |
|            |          | IP%      | -4.142           | 0.001*2         |  |
|            | 7 days   | NP%      | 5.996            | 0.000*2         |  |
|            | 5        | IP%      | 5.394            | 0.000*2         |  |

Significant result. <sup>1</sup>Mann-Whitney U test. <sup>2</sup>Independent t-test. <sup>3</sup> Wilcoxon signed ranks test. <sup>4</sup>Paired t test. Percentage NP%, Intensity IP%.

## DISCUSSION

The histological observation allowed both the quantitative and the qualitative evaluation of the structures of the tissue which is considered a powerful tool for the assessment of the process of healing (36). The microscopical examination of tissue sections at ulcer sites of the current study revealed newly formed epithelium sealing the wound surface in the group treated with

fenugreek oil on the 3rd day, which was attributed to the anti-bacterial anti-oxidant and anti-inflammatory effects that decreased the time required for the healing process by avoiding infection and accelerating the inflammatory process as reported by previous studies (6, 37,38), also full re-epithelialization as complete epidermal regeneration, presumably achieved through antioxidant activities of the plant (39). Results of a study conducted by Cavalcante et al., (40) showed remodeled connective tissue sometimes with a discreet inflammatory infiltrate and sometimes with the absence of inflammation almost compatible with the results of this investigation.

Growth factors are dynamic regulators for numerous cellular mechanisms. They act as signaling molecules among the cells. Moreover, they bind to specific receptors on the surface of the targeted cell (41). The EGF family of growth factors are the best-considered growth factors in wound repair. The essential members associated with wound healing include EGF, TGF- $\alpha$ , and EGF-HB (42). Epidermal growth factor (EGF) receptors participate in mucosal wound repairing through activation, proliferation, and migration of keratinocytes, endothelial cells, and fibroblasts and simplifies epithelial regeneration (43). Activation of the EGFR has an essential role due to the increasing epithelial cell proliferation and migration, thus, promoting re-epithelialization of acute wounds (44-46). The rats were chosen as animal models for the experimental procedures. Because the morphology and physiology of their oral apparatus and their similarity to the oral mucosa of humans besides, due to their low cost and small body size, which make them easy to handle and care, and they can also be investigated in collective numbers (47).

In the present study, the lowest mean value of positive expression was recorded in the normal group in the basal layer, which showed weak positive expression at the cell membrane and cytoplasm of keratinocytes of normal tissues, this could be due to that those cells are in their arresting phase and undergo minor proliferation in line with results matched with previous studies (19,48) in which, normal epithelium showed mainly negative expression of EGFR in epithelial layers with weak positive reaction at spinosum layer in some regions. In the current study, the lamina propria showed a few of positive cells expressed with weak intensity in the extracellular matrix. Thus, having lower mean value, this result agrees with Le and Gerber (18) who demonstrated the positive expression noticed by vascular endothelial cells, keratinocytes, and fibroblasts.

Regarding experimental groups, after 3 days duration, the EGFR expression presented highest mean value of percentage and intensity in basal and parabasal layers of epithelium with significant difference between study and control groups of the same duration. However, the mean values decreased after 7 days of treatment and it was limited to basal layer cells. Based on previous studies (48,49), the positive expression of EGFR was detected at the early stage of wound repair and became considerable in all epithelial cell layers. However positive expression was constrained mostly to cells in basal and most superficial layers of epithelial cells from day 5 and forward, which makes a proposal that the receptor has an important effect in cell growth and wound healing, which complies with current study. Epithelialization is a critical stage of wound healing, during which keratinocytes undergo rapid cell division and movement to generate a fresh protective layer that covers the wound site (50). Moreover, the investigational groups at 3rd display the presence of an

abundance of fresh collagen fibers accompanied by a significant number of blood vessels and actively growing granulation tissue, as stated by previous studies (51,52).

However, in lamina propria, the EGFR expression was observed at day 3 in fibroblast cells in both control and study groups. The EGFR positive expression of study groups showed the maximum average values of percentage compared to the control group. Afterward, the expression of EGFR was restricted. The lower mean value of positive expression of EGFR was noted in study group with a significant variance compared to control group on the seventh day duration. The result matched with Abed and Al-Ghaban (53). The matrix deposition by fibroblast cells improved by EGFR presence and wound closure due to increasing the expression of matrix metalloproteinases on fibroblasts and enhancing their activity (54,55).

According to the results of the current study, it is proposed that the epithelial cells contribute in all phases of regeneration in the process of new epithelium formation through cell signaling, thus the epithelial cells post-injury adapted to divide mainly in basal and suprabasal layers and therefore terminal differentiation hadn't occurred until day 7. The existence of activated EGFR in the epithelium played a significant role in wound healing and cell growth.

It is concluded that fenugreek was effective in accelerating the healing process. The expression of EGFR was increased by mucosal cells specifically in epithelial cells in study groups treated with fenugreek oil referred to its significant role in the healing process, due to oil containing bioactive components that accelerate the process of repair.

#### **ACKNOWLEDGEMENTS**

N/A

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

#### REFERENCES

- 1. Faustich JS, Stepaniuk KS, Robinson NA, Piedra-Mora C. Oral cavity. Vet Cytol. 2020;359–79. <u>10.1002/9781119380559.ch30</u>
- 2. Treuting PM, Dintzis S, Montine KS. Comparative anatomy and histology: a mouse, rat, and human atlas. Academic Press; 2017.
- 3. Kumar V, Abbas A, Aster J, Turner J. Robbins & Cotran Pathologic Basis of Disease Tenth Edition. Elsevier. 2021.
- Salleh A, Fauzi MB. The in vivo, in vitro and in ovo evaluation of quantum dots in wound healing: A review. Polymers (Basel). 2021;13(2):191. <u>10.3390/polym13020191</u>
- Masi ECDJ De, Campos ACL, Masi FDJ De, Ratti MAS, Shin Ike I, Masi RDJ De. The influence of growth factors on skin wound healing in rats. Braz J Otorhinolaryngol. 2016;82:512–21. 10.1016/i.bjorl.2015.09.011
- Kamil NB, Al-Ghaban NM. Evaluation of effect of local exogenous application of Myrrh oil on healing of wound incisions of facial skin (Histochemical, Histological and Histomorphometrical study in rabbits). J Baghdad Coll Dent. 2019;31(4):71–8. 10.26477/jbcd.v31i4.2724
- Sinno H, Malhotra M, Lutfy J, Jardin B, Winocour S, Brimo F, et al. Accelerated wound healing with topical application of complement C5. Plast Reconstr Surg. 2012;130(3):523–9. 10.1097/PRS.0b013e31825dc02d
- 8. Wu MCL, Brennan FH, Lynch JPL, Mantovani S, Phipps S, Wetsel RA, et al. The receptor for complement component C3a mediates

protection from intestinal ischemia-reperfusion injuries by inhibiting neutrophil mobilization. Proc Natl Acad Sci. 2013;110(23):9439–44. 10.1073/pnas.1218815110

- Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014;14(3):141–53. <u>10.1038/nri3608</u>
- Bykov VL, Leontiyeva I V, Kulayeva V V, Iseyeva YA. TISSUE, CELLULAR AND MOLECULAR BIOLOGY OF HEMOSTASIS AND REEPITHELIZATION IN THE HEALING WOUNDS OF THE ORAL MUCOSA. Morphology. 2019;156(6):64–76.
- 11. Narayan R. Encyclopedia of biomedical engineering. Elsevier; 2018.
- Arya P, Kumar P. Bioactive Compounds in Fenugreek. Spice Bioact Compd Prop Appl Heal Benefits. 2022; <u>10.1201/9781003215387-13</u>
- Fetterman Jr JW, Zdanowicz MM. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. Am J Heal Pharm. 2009;66(13):1169–79. <u>10.2146/ajhp080411</u>
- 14. Herbst RS. Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. 2004;59(2):S21–6. 10.1016/j.ijrobp.2003.11.041
- Berasain C, Avila MA. The EGFR signalling system in the liver: from hepatoprotection to hepatocarcinogenesis. J Gastroenterol. 2014;49:9–23. <u>10.1007/s00535-013-0907-x</u>
- Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J. 2011;278(1):16–27.10.1111/j.1742-4658.2010.07919.x
- 17. Santra M, Santra S, Zhang J, Chopp M. Ectopic decorin expression upregulates VEGF expression in mouse cerebral endothelial cells via activation of the transcription factors Sp1, HIF1 $\alpha$ , and Stat3. J Neurochem.2008;105(2):324–37.10.1111/j.1471-4159.2007.05134.x
- Le T, Gerber DE. Newer-generation EGFR inhibitors in lung cancer: how are they best used? Cancers (Basel). 2019;11(3):366. <u>10.3390/cancers11030366</u>
- Chen J, Zeng F, Forrester SJ, Eguchi S, Zhang MZ, Harris RC. Expression and function of the epidermal growth factor receptor in physiology and disease. Physiol Rev. 2016;96(3):1025–69. 10.1152/physrev.00030.2015
- Cuylen S, Blaukopf C, Politi AZ, Müller-Reichert T, Neumann B, Poser I, et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes.Nature.2016;535(7611):308–12. <u>10.1038/nature18610</u>
- 21. Venur VA, Cohen J V, Brastianos PK. Targeting molecular pathways in intracranial metastatic disease. Front Oncol. 2019;9:99. 10.3389/fonc.2019.00099
- Wang S, Zhang Z, Peng H, Zeng K. Recent advances on the roles of epidermal growth factor receptor in psoriasis. Am J Transl Res. 2019;11(2):520.
- Tsukamoto A, Niino N, Sakamoto M, Ohtani R, Inomata T. The validity of anesthetic protocols for the surgical procedure of castration in rats. Exp Anim. 2018;67(3):329–36. <u>10.1538/expanim.18-0003</u>
- 24. Choung HW, Lee SH, Ham AR, Lee NR, Kim B, Pang KM, et al. Effectiveness of low-level laser therapy with a 915 Nm wavelength diode laser on the healing of intraoral mucosal wound: an animal study and a double-blind randomized clinical trial. Medicina (B Aires). 2019;55(8):405. <u>10.3390/medicina55080405</u>
- Wang X, Yan L, Tang Y, He X, Zhao X, Liu W, et al. Anti-inflammatory effect of HGF responses to oral traumatic ulcers using an HGF-Tg mouse model. Exp Anim. 2022;71(2):204–13. <u>10.1538/expanim.21-0141</u>
- Mohamed IF, Ghani BA, Fatalla AA. Histological evaluation of the effect of local application of Punica granatum seed oil on bone healing. Int J Biomater. 2022;2022. <u>10.1155/2022/4266589</u>
- Dantas TS, de Barros Silva PG, Verde MEQL, Júnior A de LR, Cunha M do PSS, Mota MRL, et al. Role of inflammatory markers in prognosis of oral squamous cell carcinoma. Asian Pacific J Cancer Prev APJCP. 2019;20(12):3635. <u>10.31557/APJCP.2019.20.12.3635</u>
- 28. Nassar A, Cohen C, Agersborg SS, Zhou W, Lynch KA, Barker EA, et al. A multisite performance study comparing the reading of

immunohistochemical slides on a computer monitor with conventional manual microscopy for estrogen and progesterone receptor analysis. Am J Clin Pathol. 2011;135(3):461-7. 10.1309/AICP4VFKA5FCMZNA

- Krajewska M, Smith LH, Rong J, Huang X, Hyer ML, Zeps N, et al. Image analysis algorithms for immunohistochemical assessment of cell death events and fibrosis in tissue sections. J Histochem Cytochem. 2009;57(7):649–63. <u>10.1369/jhc.2009.952812</u>
- Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz APN, et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol. 2012;7(1):1–10. <u>10.1186/1746-1596-7-42</u>
- Jarullah HA, Ja'afar HAR, Alhabib MF. The Effects of Estradiol and Progesterone Therapy on Markers Expression of Vimentin and Desmin in Rat Uterine Tube. Immunohistochemical Study. Med J Babylon. 2014;11(2).
- Tsujikawa T, Kumar S, Borkar RN, Azimi V, Thibault G, Chang YH, et al. Quantitative Multiplex Immunohistochemistry Reveals Myeloid-Inflamed Tumor-Immune Complexity Associated with Poor Prognosis. Cell Rep. 2017;19(1):203–17. 10.1016/j.celrep.2017.03.037
- Saeed AK, Salmo N. Epidermal growth factor receptor expression in mice skin upon ultraviolet B exposure-Seborrheic Keratosis as a coincidental and unique finding. Adv Biomed Res. 2012;1. 10.4103/2277-9175.100178
- Taoudi Benchekroun M, Saintigny P, Thomas SM, El-Naggar AK, Papadimitrakopoulou V, Ren H, et al. Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. Cancer Prev Res. 2010;3(7):800–9. <u>10.1158/1940-6207.CAPR-09-0163</u>
- Diniz-Freitas M, García-Caballero T, Antúnez-López J, Gándara-Rey JM, García-García A. Pharmacodiagnostic evaluation of EGFR expression in oral squamous cell carcinoma. Oral Dis. 2007;13(3):285–90. <u>10.1111/j.1601-0825.2006.01280.x</u>
- Masson-Meyers DS, Andrade TAM, Caetano GF, Guimaraes FR, Leite MN, Leite SN, et al. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020;101(1–2):21–37. 10.1111/iep.12346
- 37. Abed SS, Al-Ghaban NM, Ibrahim NA. The Biological Effects of Leptin as Topical Treatment for Traumatic Oral Ulcer in Rats.
- Hameed Alwan M, A Ghani B. Histological Evaluation of Local Application of Flavonoid Extract of Capparis Spinosa on Wound Healing in Alloxan-Induced Diabetic Rats. Diyala J Med. 2020;18(2):1–13. 10.26505/DJM.18014700609
- Ktari N, Trabelsi I, Bardaa S, Triki M, Bkhairia I, Salem RBSB, et al. Antioxidant and hemolytic activities, and effects in rat cutaneous wound healing of a novel polysaccharide from fenugreek (Trigonella foenum-graecum) seeds. Int J Biol Macromol. 2017;95:625–34. <u>10.1016/j.ijbiomac.2016.11.091</u>
- Cavalcante GM, Paula RJS de, Souza LP de, Sousa FB, Mota MRL, Alves APNN. Experimental model of traumatic ulcer in the cheek mucosa of rats. Acta Cir Bras. 2011;26(3):227–34. <u>10.1590/S0102-86502011000300012</u>
- Kręcicki T, Jeleń M, Zalesska-Kręcicka M, Rak J, Szkudlarek T, Jeleń-Krzeszewska J. Epidermal growth factor receptor (EGFR), proliferating cell nuclear antigen (PCNA) and Ki-67 antigen in laryngeal epithelial lesions. Oral Oncol. 1999;35(2):180–6. 10.1016/S1368-8375(98)00100-6
- Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol. 2005;1(1):10–2005. <u>10.1038/msb4100014</u>
- 43. Bodnar RJ. Epidermal growth factor and epidermal growth factor receptor: the Yin and Yang in the treatment of cutaneous wounds and cancer.Adv wound care. 2013;2(1):24–9. <u>10.1089/wound.2011.0326</u>
- Rheinwald JG, Green H. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature. 1977;265(5593):421–4. <u>10.1038/265421a0</u>
- 45. Hudson LG, McCawley LJ. Contributions of the epidermal growth factor receptor to keratinocyte motility. Microsc Res Tech.

**1998;43(5):444–55.**<u>10.1002/(SICI)1097-</u> <u>0029(19981201)43:5%3C444::AID-JEMT10%3E3.0.C0;2-C</u>

- 46. Nizioł M, Ościłowska I, Baszanowska W, Pałka J, Besio R, Forlino A, et al. Recombinant prolidase activates EGFR-Dependent cell growth in an experimental model of inflammation in HaCaT keratinocytes. Implication for Wound Healing. Front Mol Biosci. 2022;9:876348. 10.3389/fmolb.2022.876348
- O'loughlin PF, Morr S, Bogunovic L, Kim AD, Park B, Lane JM. Selection and development of preclinical models in fracture-healing research. JBJS. 2008;90(Supplement\_1):79–84. <u>10.2106/IBJS.G.01585</u>
- 48. Abed SS. Immunohistochemical Expression of Decorin , and Epidermal Growth Factor Receptor in Induced Traumatic Oral Mucosal Ulcer Treated with Leptin in Rats (Histological and Histomorphometrical Studies) A thesis. 2018.
- Leydon C, Imaizumi M, Bartlett RS, Wang SF, Thibeault SL. Epithelial cells are active participants in vocal fold wound healing: an in vivo animal model of injury. PLoS One. 2014;9(12):e115389. 10.1371/journal.pone.0115389

- Alwan MH, Ghani BA. Histological Evaluation of the Wound Healing Activity of Allium Ampeloprasum Methanol Extract. Malaysian J Microsc. 2023;19(2):105–18. <u>10.26477/jbcd.v35i4.3508</u>
- Al-Zubaidy EY, Ghani BA, Ibrahim NR. Local application of Opuntia ficus-indica/Punica granatum oils on cutaneous wound healing: a histochemical study. J Baghdad Coll Dent. 2023;35(4):28–34. 10.26477/jbcd.v35i4.3508
- Muhammad NH, Ghani BA. Histological Evaluation of the Effect of Topical Application of Curcumin Powder and Essential Oil on Skin Wound Healing. J Baghdad Coll Dent. 2015;27(3):58–63. 10.12816/0015036
- 53. Abed SS, Al-Ghaban NM. The Epidermal Growth Factor Receptor Expression in Leptin-Treated Traumatic Oral Ulcer in Rats. J Glob Pharma Technol [Internet]. 2019;11(3):138–46. Available from: www.jgpt.co.in
- Vu TH, Werb Z. Matrix metalloproteinases: Effectors of development and normal physiology. Genes Dev. 2000;14(17):2123–33. 10.1101/gad.815400
- Ravanti L, Kähäri VM. Matrix metalloproteinases in wound repair (review). Int J Mol Med. 2000;6(4):391–407. <u>10.3892/ijmm.6.4.391</u>

## التوطين المناعي النسيجي الكيميائي لمستقبل عامل نمو البشرة في الغشاء المخاطي الشدقي للفئران المعالج بزيت أوراق الحلبة

## سارة علاء عمران، بان جميل عبد الغني

#### قسم علوم تشخيص الفم، كلية طب الأسنان، جامعة بغداد، بغداد، العراق

#### الخلاصة

إن عدم وجود علاج فعال لقرحة الغشاء المخاطي للفم قد حفز الأطباء للبحث عن تقنيات علاجية أخرى لتحسين شفاء قرحة الفم. يؤدي تطبيق الحلبة على القرحة إلى إطلاق مكوناتها المضادة للالتهابات، مما قد يقلل من أي عمليات التهابية زائدة ويسرع شفاء الجروح. كان الهدف من هذه الدراسة هو در اسة تأثير زيت أوراق الحلبة على القرحة المرتبطة بالتعبير عن مستقبل عامل نمو البشرة (EGFR). تم في هذه الدراسة استخدام أربعة و عشرين ذكر أ بالغا من فئران ويستار البيضاء يتر اوح وزنها بين ٢٥٠ و ٢٥٠ جراماً وتتر اوح أعمار هم بين ٤-٦ أشهر. شمل التصميم التجريبي: المجموعة الطبيعية (لا توجد قرحة)، والمجموعة (ع توجد)، والمجموعة الضافر (11 قرحة في الخاسن في ٤٢٠ أل الغامن في التومين الثالث والسابع من الدراسة (14 قرحة ترعدة)، ومحموعة الدراسة (11 قرحة في الجانب الأيسر تم شفاؤها باستخدام زيت الحلبة). تم التصحية بجميع الفئران في اليومين الثالث والسابع من الدراسة التجريبية، ومدة الشفاء، وتم حريم العينات البيولوجية لتحليل الكيمياء النسيجية والمناعية لـ EGFR. أظهرت الدراسة الحالية أن تعبير RGFE إذ في مواقع القرحة المعالجة بعد ٢ أيلم من التقرح مع وردة المغاء، وردة المولي التيمامي وردة الحالية التعربي الحلبة أي تعبير علي معالية التوربي معليم وردة العام القرب الحرفي من التقرحة ع وجود اختلافات، معنوية المولي الكيمياء النسيجية والمناعية لـ EGFR. أظهرت الدراسة الحلية أن تعبير الطبيري إلى ذلك، زاد التوطين الإيجابي لـ EGFR في معلية الشفاء، وردة المعاري. عليم القرحة عن طريق تعزيز تكوين النسيج الطهاري. على ذلك، زاد التوطين الإيجاب وجود اختلافات معنوية مقارنة بالمجموعات الأخرى التحاب في القرحة تعزيز تكوين النسيح الطهاري. ولمواقع القرح ألمي أل

الكلمات المفاحية زُيَّت الحابة، قرحة الْعَشَّاء المخاطي الشدقي، التئام الجروح مستقبل عامل نمو البشرة