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RESEARCH ARTICLE

# The Epidermal Growth Factor Receptor Expression in Leptin-Treated Traumatic Oral Ulcer in Rats

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## Abstract

Back ground: The injured tissues need to activate their cellular responses in order to restore their normal histological architecture. Leptin has a significant effect on wound healing by enhancing the expression of many biological molecules and cytokines. The present study aimed to evaluate the effect of topical application of recombinant leptin on induced traumatic oral ulcer healing by mean of immunohistochemical localization of EGF-R. Materials and methods: Forty eight male Albino rats with body weight between (200 -270 gm) and age between 2-3 months, were subjected to traumatic ulcer with 8 mm diameter by surgical blade no.15 on the right side of the buccal mucosa. The animals divided into two groups; control group: the ulcer treated daily with sterilized distilled water, the experimental group: the ulcer treated daily with 10µl of 1 µg/ml recombinant leptin. The rats were sacrificed at 3,7,10 days. The specimens were taken for immunohistochemical study to detect the expression of EGF-R in both control and study groups. Results: The present study showed that the recombinant leptin increased the expression of EGF-R in the ulcer area only at 3rd day of ulceration by both epithelial and mesenchymal cells with highly significant differences than control group. Conclusion: leptin accelerated the healing process in oral mucosal ulcer by increase the expression of EGF-R in early healing period than control.

**Keywords**: *leptin, Oral mucosa, Ulcer, EGF-R.*

## Introduction

The healing process is regeneration and restoration of the lost tissue structure occurred due to trauma or diseases. This process is a collection of multi cellular events include; migration, adhesion, proliferation, differentiation, and extracellular matrix (ECM) formation [1, 2].

The healing process activated when mediators bind to their cell- surface receptors such as growth factors, cytokines and enzymes. There are different types of receptors found on the cell surface waiting to bind with their specific ligand to transduce its signal for specific cell function [3].

One of these receptors is the Epidermal growth factor receptor (EGF-R), which is a transmembrane glycoprotein belongs to the ErbB receptors family or human EGF receptor (HER) family of tyrosine kinases that transports the signals from the ECM to the cells by binding specific ligands [4]. This receptor plays a role in organ development, differentiation, migration, apoptosis inhibitor, and wound healing [5, 6]. The polypeptide hormone leptin, a product of the obese (ob) gene, with molecular weight of 16 kDa, secreted predominantly by adipocytes into the blood stream as a free , or as a protein. In addition to its influences on the body weight homeostasis [7].

It also has a role in a different physiological action such as hematopoiesis [8], bone formation [9], angiogenesis [10], and wound healing [11]. The multi-functionality of leptin made it not only as a systemic hormone but also as a local growth factor [12].

## Materials and Methods

In the present study 48 albino male rats weighting (200 -270 gm), aged (2-3) months were used in this study. The animals were randomly divided into two groups (control,

and study), each group was consisted of 24 rats. They maintained under control conditions of temperature, drinking and food consumption.

All experimental procedures were carried out in accordance with the animal experimentation ethical principles of the Biotechnical Research Center at Al-Nahrain University. A dose of ketamine (50 mg/kg) and xylazine (5 mg/kg) were injected intraperitoneally as anesthetic [13]. The mucosal ulceration with 8 mm diameter was performed on the right side of the buccal mucosa by abrasion with a surgical scalpel blade no.15 [14].

The control group (24 rats): the ulcers treated daily with 10µl of sterilized distalled water. While the experimental group (24 rats): the ulcers treated daily with 10µL of 1µg/ml recombinant leptin protein from Abcam Company UK (ab646). Then the animals were sacrificed according to 3 healing intervals into 3, 7, and 10 days (16 rats from both groups in each period).

Then the specimens from each rats were taken and prepared for histological (H&E stain) and for immunohistochemical localization of EGF-R by using of Anti- EGF-R antibody, Rabbit monoclonal [EP38Y], (ab52894) , and detection kit (ab80436) from Abcam company UK. Under light microscope at x40, a five fields were chosen from epithelium area, and another five fields from lamina properia from each tissue section, captured by digital camera (5 pixel), and the images evaluated imported to computer.

The evaluation of staining results was achieved by applying (Aperio positive pixel count algorithms program (from Aperio Image Scope software v11.1.2.760 (Aperio Technologies Inc, USA)), we neglected the weak positive reading in yellow color. The average of mean positive percentages for each five area from epithelium and lamina poroperia were obtained and considered as the value of expression of EGF-R per slide.

## Results

At 3rd day in the control group the weak positive expression for EGF-R was detected in the epithelium margin of the ulcer mainly in the basal and spinosum layers. In lamina properia the positive reactivity was detected in the ECM, and endothelial cells of blood vessels (Fig. 1. A&B).The study group showed strong positive immune staining for EGF-R in the new regenerated epithelium around the ulcer margin .In lamina properia the positive reaction was in the ECM, and endothelial cells (Fig. 1. C&D).At7th day in control group the strong positive reactivity was seen in the basal layer of epithelium.

The ECM of the lamina properia, fibroblast cells, and endothelial cells showed positive reaction (Fig 2.A&B).While the study group moderate immune reactivity were seen in the spinosum and granulosum layers of new epithelium. The EGF-R expression level was weak at the center of the ulcer and became negative in peripheral area of lamina properia (Fig. 2. C&D).At 10th day in the control group there was moderate positive staining reactivity in the epithelium cells was seen mainly in the granulosum layer and in some area of basal layer. In lamina properia, new collagen fibers, fibroblast cells, and endothelial cells showed weak positive reaction to the EGFR (Fig. 3.A&B).

While in the study group the epithelium tissue showed weak positive reactivity to the EGFR and the lamina properia showed negative expression to the EGF-R (Fig. 3.C&D).The mean percentage differences between control and study groups in positive immuno reactivity to EGFR was illustrated in (Fig.4).It showed that the highest mean percentage value for expression for EGFR were seen in both epithelium and lamina properia of study group at 3rd day.

Table-1, illustrated that the highly significant differences between the study and control group in EGF-R expression in epithelium were seen at 3rd day and 7th day and significant at 10th days. Regarding lamina properia, the result showed significant differences between the study and control groups in EGF-R expression at 3rd and highly 7th days, and non-significant differences at 10th days.

Table-2 showed that there was significant difference between epithelium and connective tissue for expression of EGF-R in control group except at 10th day it was highly significant difference. For the study group there was significant difference between epithelium and lamina properia in the expression of EGF-R at 3rd and 10th day and highly significant at 7th day. Regarding to the duration differences in each control and

 

study group, the ANOVA test was used as shown in Table-3. For both control and study groups, the epithelium and lamina properia showed highly significant differences in all duration for positive expression for EGF-R.

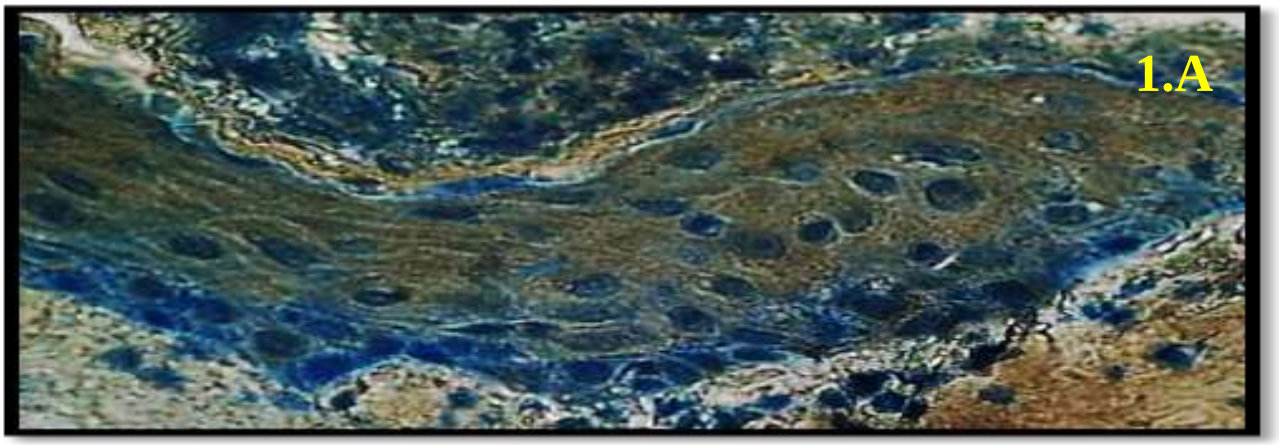


**Figure 1: A: ECF-R expression in control group at 3rd day in new regenerating epithelium x 40**

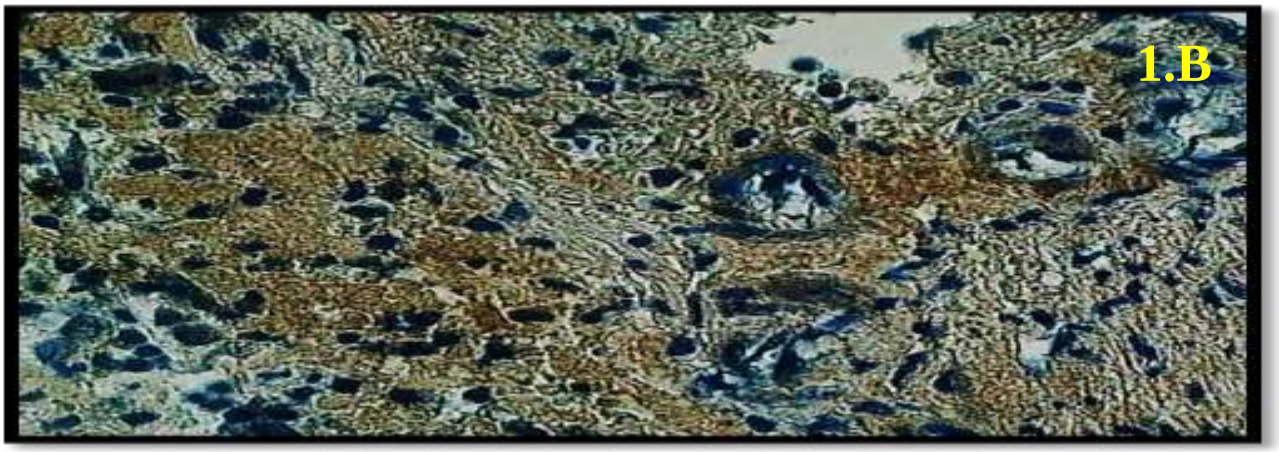


**Figure 1.B: EGFR expression at 3d day in control group, in lamina properia, x 40**

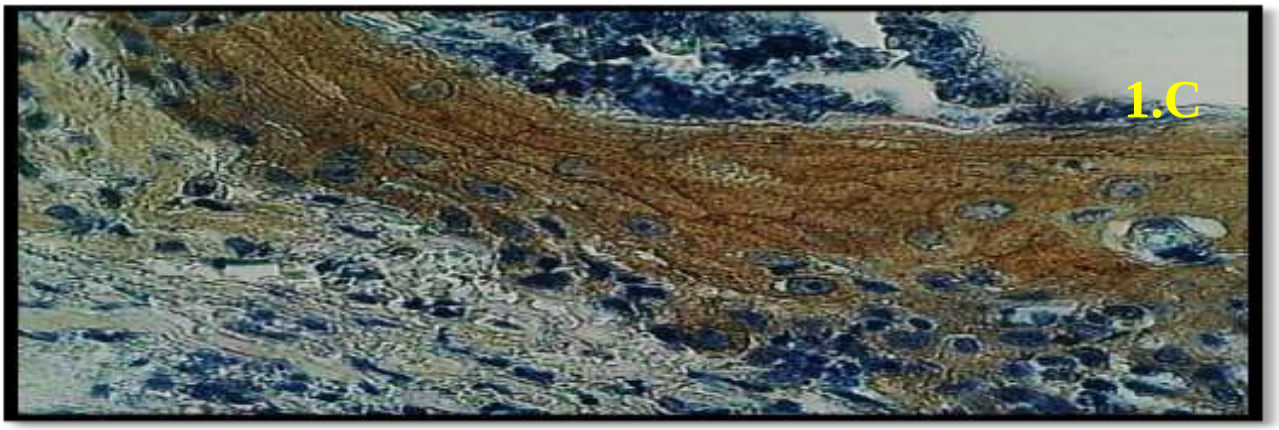


**Figure 1.C: EGF-R expression at 3d day in study group, in new regenerating epithelium x40**


**Figure 1.D: EGFR expression at 3d day in study group in lamina prperia, x40**

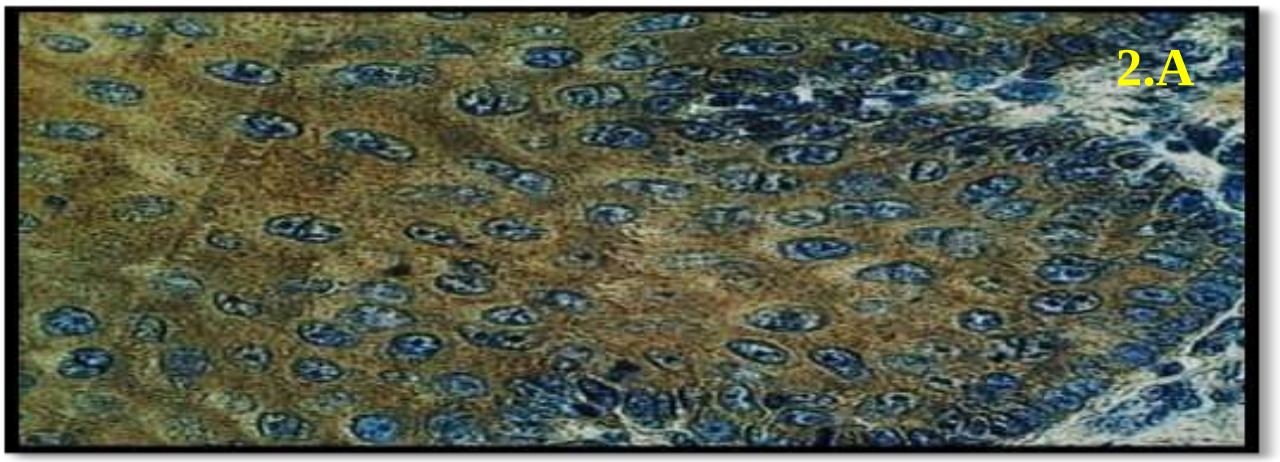


**Figure 2.A: EGFR expression at 7th day in control group in epithelium x40**


**Figure 2.B: EGFR expression at 7th day in control group in lamina properia. x40**

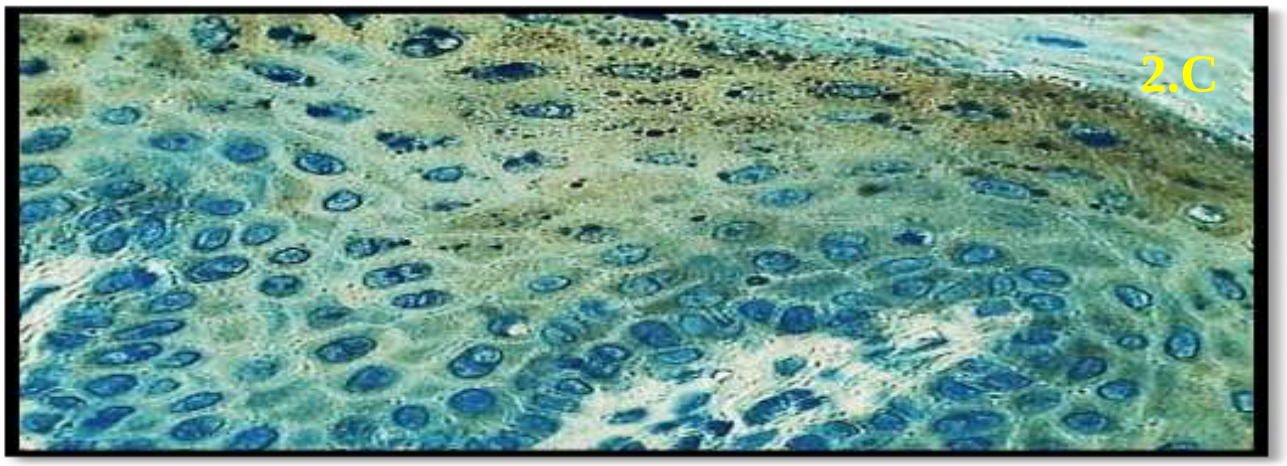


**Figure 2.C: EGFR expression at 7th day in study group in epithelium x40**


**Figure 2.D: EGFR expression at 7th day in study group in lamina prperia x40**

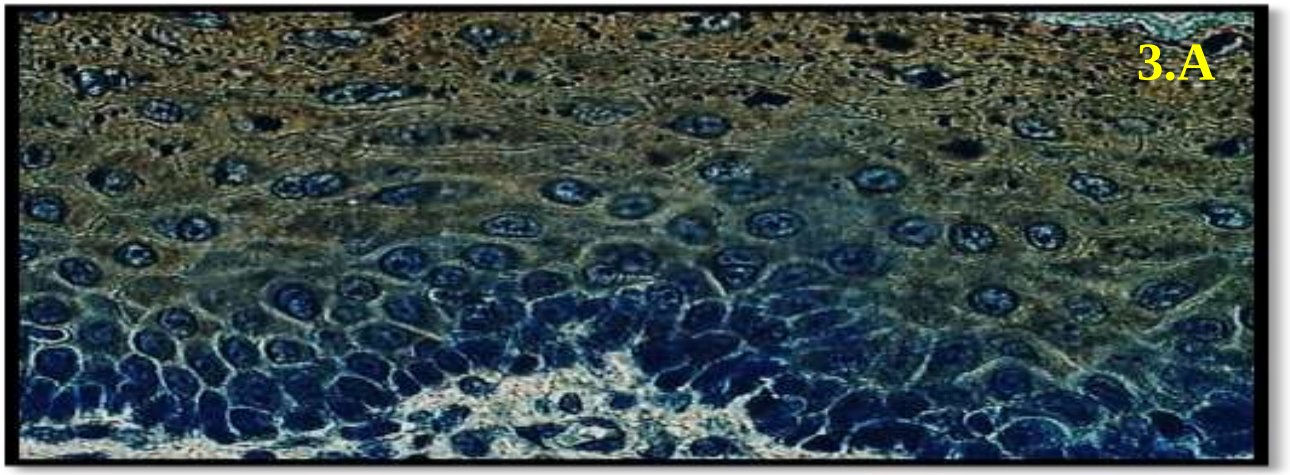


**Figure 3.A: EGFR expression at 10th day in control group in epithelium x40**

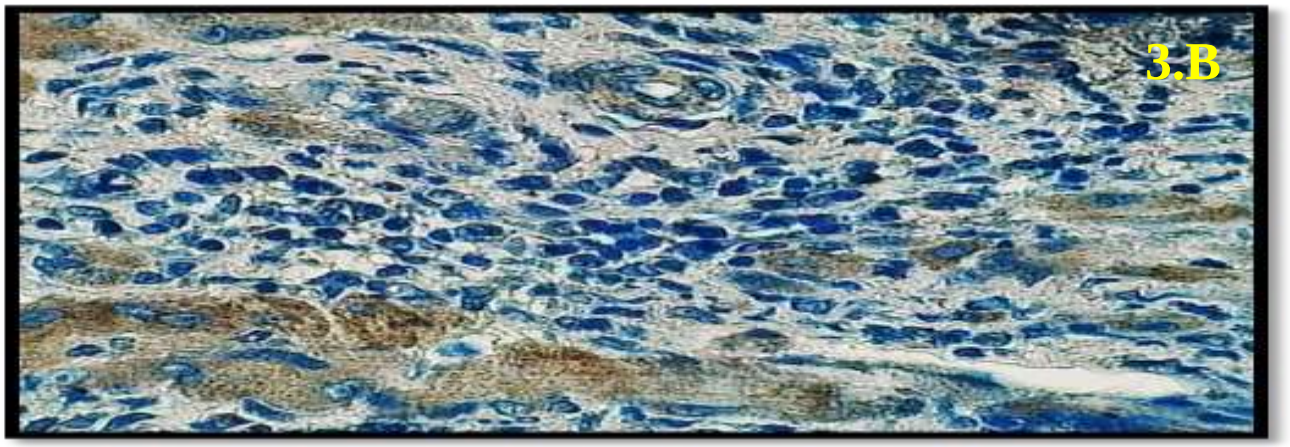


**Figure 3.B: EGFR expression at 10th day in control group in lamina properia , x40**


**Figure 3.C: EGFR expression at 10th day in study group in epithelium x40**

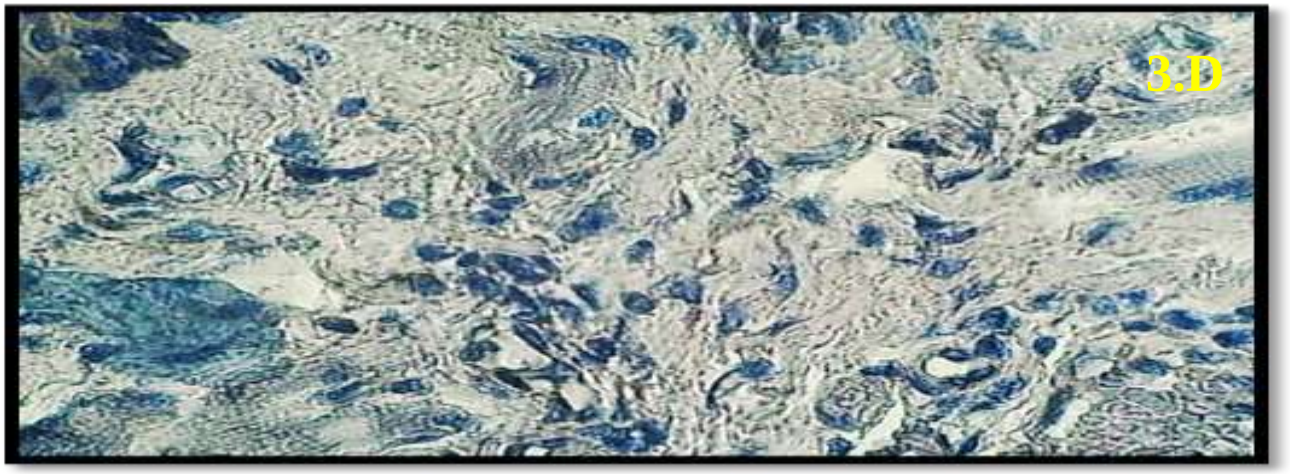


**Figure 3.D: EGFR expression at 10th day in study group in lamina prperiax 40**

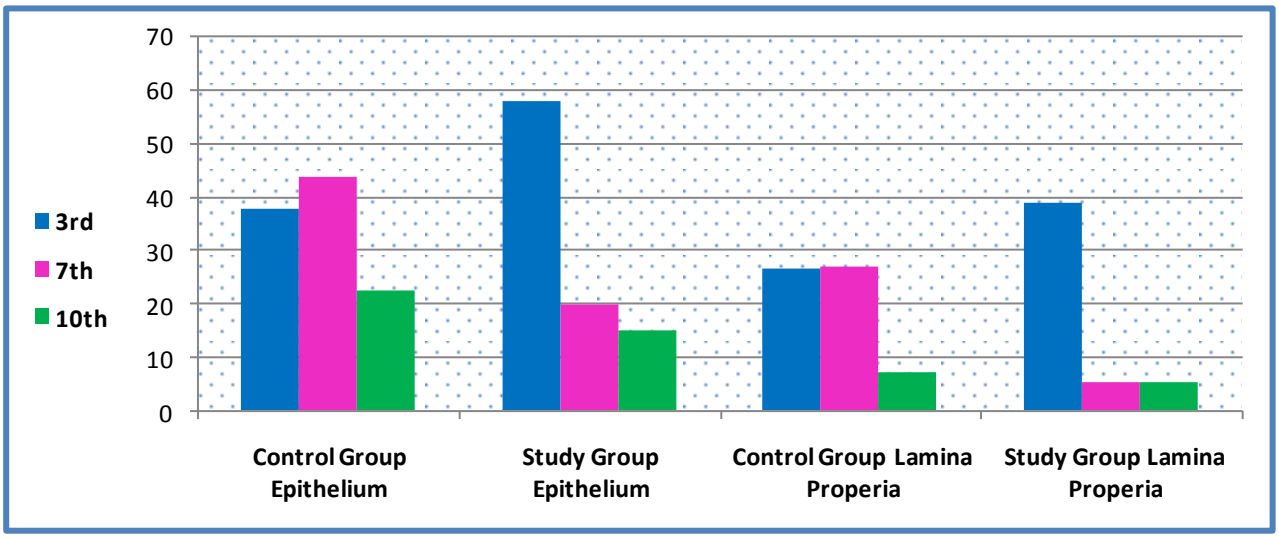


**Figure 4: The mean positive percentages of EGF-R in epithelium and lamina properia in control and study groups for different periods**

**Table 1: Groups' comparison for Positive cells expressed EGF-R in both epithelium and lamina properia at each duration**

| Day | Site | Control | | Study | | T-test | P-value |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | | |
| 3rd | Epithelium | 37.98 | 4.264 | 58.08 | 9.848 | 5.837 | 0.001 HS |
| | Lamina properia | 26.65 | 11.226 | 39.08 | 7.937 | 3.304 | 0.013 S |
| 7th | Epithelium | 43.63 | 10.809 | 19.69 | 3.943 | 5.588 | 0.001 HS |
| | Lamina properia | 27.08 | 3.993 | 5.222 | 0.770 | 16.195 | 0.000 HS |
| 10th | Epithelium | 22.46 | 4.507 | 14.922 | 8.990 | 2.719 | 0.030 S |
| | Lamina properia | 6.991 | 2.904 | 5.083 | 0.941 | 1.909 | 0.099 NS |

**Table 2: Sites' comparisons for Positive cells expressed EGF-R in both groups at each duration**

| Day | Group | Mean different | SE | T-test | P-value |
|---|---|---|---|---|---|
| 3rd | Control | 11.325 | 3.773 | 3.004 | 0.020 S |
| | Study | 18.995 | 4.672 | 4.065 | 0.005 S |
| 7th | Control | 16.542 | 3.944 | 4.195 | 0.004 S |
| | Study | 14.435 | 1.361 | 10.600 | 0.000 HS |
| 10th | Control | 15.475 | 1.287 | 12.016 | 0.000 HS |
| | Study | 9.838 | 3.283 | 2.996 | 0.020 S |

**Table 3.18: ANOVA test for duration differences in epithelium and lamina properia in both groups**

| Marker | Groups | | F-test | P-value |
|---|---|---|---|---|
| E.G.R | Control | Epithelium | 18.557 | P<0.001 HS |
| | | Lamina properia | 21.038 | P<0.001 HS |
| | Study | Epithelium | 69.528 | P<0.001 HS |
| | | Lamina properia | 142.837 | P<0.001 HS |

## Discussion

The healing process depends on many different cellular activities. During this process every cell must be respond to local and distant signals for integrated different cellular actions.

The transmembrane receptor such as; epidermal growth factor receptor (EGF-R), which is one of many receptors enabling cells to receive information from the surrounding environment; and transmit intracellular responses.

Many studies mentioned to the roles of EG-R activation signaling pathway after binding with it's ligands, in a number of biological responses like; inflammatory regulation, migration, proliferation and cellular differentiation [15, 17]. In the present study the EGF-R expression showed highest mean positivity percentages in the keratinocytes in all epithelial cell layers of leptin-treated group only at 3rd day with highly significant differences than control group. Later on the expression decreased to the lowest mean value at 7th and 10th day, and it was restricted to keratinocytes in the basal and granulosum layers of epithelial cells.

This result agree with immunohistochemical investigation done by leydon *et al.*, 2014[18] they found that the positive expression of EGF-R was observed at the acute phase of wound healing in vocal fold injury, and the expression became more abundant in all epithelial cell layers at day 3, then it was restricted primarily to cells in the basal and most superficial layers of epithelial cells at day 5, make a suggestion that receptor playing a key role in cell growth and wound repair. EGF-R has effects on the early re-epithelialization; they noticed that the EGF-R null mice exhibits delayed wound healing, because the early proliferation and migration of keratinocytes are greatly impaired at the first 3 days [19].

The migration of epithelial cells at the wounded edge displayed the strongest EGF-R signaling activation, which suggested that the wound closure was dependent on EGF-R activation [20]. The EGF-R can enhance mitogenic activity and stimulating motility of epithelial cell during re-epithelialization [21, 22]. Corneal wound healing delayed when they used EGF-R inhibitor systemically; the epithelial cells proliferation, stratification, and thickness were decreased [23].

Wound- induced shedding of EGF as autocrin-paracrin ligand for EGF-R; this will result in transactivation of EGF-R and enhanced keratinocyte proliferation and migration in corneal wound healing [24]. In the lamina properia the expression of EGF-R detected at 3rd day in ECM, cell membrane of the fibroblasts, endothelial cells and inflammatory cells in both control and study groups. The highest mean percentages of positive cells which expressed EGF-R were seen in study group with significant differences than control group only at this period, and then the EGF-r decreased to its lowest mean positive percentage from the 7th day. This result match Ogunwobi and Beales , 2008 [25] who reported that the exogenous leptin can activates the EGF-R to 67 % after 6 hours of administration either directly by potently increased gene expression of both lignds EGF and TGFα; or indirectly by MMP-mediated extracellular shedding of the ligands EGF-R, EGF and TGFα, leading to ligand-mediated transactivation of EGR. The two pathway of EGF-R activation essentially stimulator of multiple cell events such as; re-epithelization, fibroblast migration, proliferation, wound contraction, and angiogenesis [26].

The delayed of wound healing in EGFR-null mice caused by an abnormally intense and diffuse inflammatory cells infiltrate especially the neutrophils which is leads to establishment of an unbalanced microenvironment, dominated by proteolytic rather than protective mechanisms [27]. This result converges with Scholes *et al.*, 2001, who reported that the EGFR and its ligands are present on human macrophages. The leukocytes release EGF-R ligands; TGF-α and EGF. TGF- α and activate EGF-R which upregulates the CXC8/IL-8 a selective neutrophil chemoattractant, that stimulates epithelial proliferation and migration, and endothelial cell proliferation [28].

The EGF-R can enhanced the fibroblasts functions in matrix deposition, remodeling, and wound contraction by increasing the expression of MMP (MMP-1,MMP-2, and MMP-3) on fibroblasts and downregulated in EGF-R deficient cells [29].When the EGF-R bind to its ligand EGF in vitro lead to angiogenic activation [30]. EGF-R express on micro -vascular endothelial cells (MVECs) and when its activated by EGF or TGF-α stimulate the MVECs to tube formation in vitro [31].

EGF-R activated by the EGF; stimulate endothelial cells to secrete several proteases and plasminogen activators and degrade vessel basement membrane which allows the endothelial cells to invade the surrounding matrix [32, 33]. Vascular smooth muscle cells (VSMCs) express all EGF-R family members along with their respective ligands and the activation of EGF-R that expressed by endothelial cells by its ligands such as

 

EGF,TGF-α lead to stimulates receptor phosphorylation of SMCs leading to migration of SMCs and vessel maturation [34, 35].

## Conclusion

According to our knowledge the present study is the first one that evaluated the effect of topical application of leptin on abrasion mucosal ulcer healing, and assessment of the expression of EGF-R in traumatic ulcer healing.

In our study we noticed that leptin accelerated the healing process in oral mucosal ulcer by increase the expression of EGF-R in early healing period than control.

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