Original Article

# Effect of Curcumin Loaded Poly (lactic-co-glycolic acid) Nanoparticles Versus Native Curcumin on the Healing of the Tongue Ulcer in Albino Rats (Histological, histochemical and Immuno-histochemical Study)

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## ABSTRACT

**Background:** Curcumin is a component of turmeric, a medicinal comestible herb that possesses anti-inflammatory activity having the ability to heal oral ulcers, but its extremely low oral bioavailability hampers its application. Poly (lactic-co-glycolic acid) nanoparticles were used for curcumin delivery to increase its bioavailability.

**Aim of the Study:** Is to compare the effects of curcumin-loaded poly (lactic-co-glycolic acid) nanoparticles versus native curcumin on a chemically induced ulcer at the tip of the inferior surface of the tongue in Albino rats.

**Materials and Methods:** Seventy adult male Albino rats were divided into five groups. The negative control group (group I), the positive control group (group II), poly (lactic-co-glycolic acid) nanoparticles treated group (group III), native Curcumin treated group (group IV) and curcumin loaded poly (lactic-co-glycolic acid) nanoparticles treated group (group V). In all groups except group I, rats were subjected to chemically induced ulcer in the tip of their tongues. Additionally in group III, group IV and group V rats were administrated poly (lactic-co-glycolic acid) nanoparticles vehicle (100mg/kg), native curcumin suspension (100mg/kg) and curcumin loaded poly (lactic-co-glycolic acid) nanoparticles (100mg/kg) respectively by oral gavage, daily till the sacrifice dates. Rats were sacrificed at either six or twelve days. Specimens were processed and stained with Hematoxylin and Eosin stain, Masson's Trichrome stain, and Transforming Growth Factor-β (TGF-β) antibody.

**Results:** Histological examination confirmed that nano-curcumin showed higher re-epithelization and better healing potential compared to other groups. Histochemical and immuno-histochemical examination proved that nano-curcumin displayed an increase in the collagen content and wide immunoexpression in the TGF-β respectively which indicated better healing effect than native curcumin.

**Conclusion:** Administration of nano- curcumin showed better wound healing activity compared to that of native curcumin at the equivalent dose level and at the same time.

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**Key Words:** Curcumin; curcumin nanoparticles; PLGA; tongue ulcer.

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## INTRODUCTION

Oral ulceration is a common mucosal disorder that result from physical or chemical injury, viral, fungal or bacterial infections, allergy, malignancy or as a manifestation of systemic diseases. Oral ulceration causes a breach in oral epithelium exposing nerve endings in the underlying lamina propria, which leads to discomfort, especially when eating peppery or citrus foods[1].

On removing the injurious agent, ulcers normally heal within ten-fourteen days. Infection may prolong healing by stimulating an inflammatory response. Although saliva has antimicrobial properties, the oral environment is considered a challenge to healing because it contains an outsized commensal flora and other oral pathogens which induce inflammation[2].

Oral mucosal wounds revealed accelerated and scarless healing when compared to cutaneous wounds. The oral mucosal healing process includes the same phases as cutaneous wounds including hemostasis, inflammatory, proliferative and remodeling phases; however, the unique outcome in the mucosa supposes that different series of events occur in response to oral mucosal injury[3].

Curcumin is an orange-yellow component of turmeric a spice that is often found in curry powder. Curcumin has been reported to have significant anti-inflammatory, anticoagulant, anti-mutagenic, anti-carcinogenic and anti-oxidant effects. It also has significant wound healing effects as it acts on many stages of the natural cutaneous wound healing process for hastening healing[4].

However, the pharmaceutical significance and therapeutic efficacy of curcumin are limited due to its

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poor water solubility, decreased oral bioavailability, photo-degradation, short-half-life, extensive first-pass metabolism and chemical instability. To overcome these problems, newer strategies have been developed[5].

In recent decades, nanotechnology-based delivery systems have gained widespread uses because of their promising actions and advantages over conventional approaches. Nano-encapsulation of curcumin as poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) showed auspicious potential in enhancing healing of cutaneous excisional wounds[5,6].

## MATERIALS AND METHODS

### *Animals*

Seventy of eight- ten weeks old adult male Albino rats weighing (200-250 gm) were used in this study. The rats were housed in the animal house of the medical research center at Ain Shams University. Rats were kept under good ventilation and an adequate stable diet consisting of fresh vegetables, dried bread, and tap water ad libidum throughout the experimental period. The experiment was carried out according to the regulations and approval of the Research Ethics Committee of the Faculty of Dentistry, Ain Shams University, Egypt FDASU-Rec M061810.

### *Materials*

- Acetic acid (50%).
- Carboxy-methylcellulose (CMC) (0.5%).
- PLGA NPs.
- Native Curcumin (NCr).
- Cr loaded PLGA NPs (Cr PLGA NPs).
- These materials were purchased from Nano-Gate Company, Nasr City, Cairo, Egypt.

### *Preparation method of curcumin loaded PLGA nanoparticles*

In this study, Cr PLGA NPs were prepared in Nano-Gate Company, they were composed of a hydrophilic surface and a hydrophobic core providing a drug-carrying reservoir and also enabling them to dissolve in aqueous solutions to overcome the curcumin's limitation. Curcumin loaded nanoparticles were prepared according to the solid-in-oil-in-water (s/o/w) emulsion technique. PLGA (45 mg) was dissolved in dichloromethane for six hours to obtain a uniform PLGA solution. Five milligrams of curcumin were added to the PLGA solution and sonicated at 55 W for 1 min to produce the solid-in-oil primary emulsion. This emulsion was added to 20 ml of polyvinyl alcohol solution (1% w/v) and again sonicated at 55 W for two minutes to get the final solid-in-oil-in-water emulsion. Empty PLGA NPs were prepared without the addition of curcumin using the same procedure. The resulted nano-sized particles were stirred in the emulsion for three hours for solvent evaporation. The final emulsion was centrifuged at 15,000 g for fifteen minutes to remove the residual solvent. The NPs obtained were washed thrice with deionized distilled water and finally re-suspended in deionized water and dried on a lyophilizer. The NPs were stored at 4 °C until further use in the form of suspension[7,8]. By the transmitted electron microscope, the particle shape was spherical with an average size of 160 ±20 nm (Figures 1A, 1B).

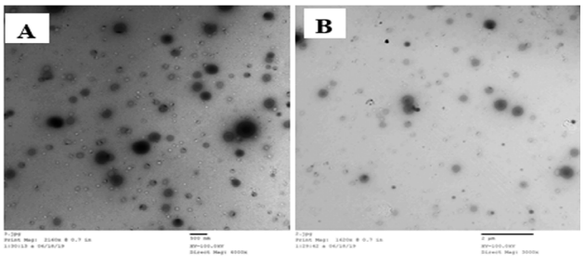


**Fig. 1A, 1B:** Transmitted electron micrograph showing the PLGA nanoparticles characters (x4000, x3000).

### *Experimental Design*

The animals were randomly divided into five equal groups, each group consisting of fourteen rats which was furtherly sub-divided into two equal subgroups, seven rats each:

- Subgroup A: where rats were sacrificed at six days of ulcer induction.
- Subgroup B: where rats were sacrificed at twelve days of ulcer induction.

**1- Negative control group (group I):** Rats were not subjected to ulcer induction and didn't receive any treatment.

**2- Positive control group (group II):** Rats were subjected to ulcer induction and didn't receive any further treatment.

**3- PLGA NPs treated group (group III):** Rats were subjected to ulcer induction followed by administration of 100mg/kg of PLGA NPs vehicle by gastric tube, once daily starting from the day of ulcer induction till the sacrifice dates[8].

**4- Native curcumin-treated group (group IV):** Rats were subjected to ulcer induction followed by administration of 100mg/kg of NCr suspended in 0.5 % CMC delivered by gastric tube, once daily starting from the day of the ulcer induction till the sacrifice dates[8].

**5- Cr PLGA NPs treated group (group V):** Rats were subjected to ulcer induction followed by administration of 100 mg/kg of Cr PLGA NPs by gastric tube, once daily starting from the day of ulcer induction till the sacrifice dates[8].

### *Ulcer induction*

Before the ulcer induction, all rats were anesthetized by intraperitoneal injection of Ketamine Hydrochloride (50mg/kg). Round filter paper of 5.5 mm diameter was socked in 15 ml of 50% acetic acid. To create a round ulcer,

an acid socked filter paper was pressed onto the tip of the inferior surface of the tongue for 60 seconds[9].

- The pain was controlled by the administration of Diclofenac sodium at a dose of 5mg/kg once daily[10].
- At the end of the experiment at either day six or twelve, rats were sacrificed by an overdose of anesthesia. The rat's tongues were immediately dissected followed by getting rid of the sacrificed rat bodies in the incinerator of Ain Shams University hospital. After the tongues were excised, the areas of interest were fixed immediately in 10% buffered formalin solution. The specimens were then processed and embedded in the paraffin wax blocks to be sectioned by microtome to a thickness of four to six microns to be assigned for histological, histochemical and immuno-histochemical staining.

### *Histological Examination*

### *Routine H&E staining*

Sections were stained with H&E stain for routine histological examination using light microscope by the conventional method[11].

### *Masson's Trichrome stain*

This histochemical special stain was used for the detection of collagen and to discriminate between mature and immature collagen fibers in tissues during the course of wound healing. The immature collagen fibers stain blue while the mature ones display varying hues of red[12].

### *Immuno-histochemical Examination*

Using rabbit anti-human polyclonal TGF-B antibody to evaluate the expression level of TGF-β protein for cell proliferation and efficient and rapid healing, (code number: 142588. USBiological Company, United States). The antibody is characterized with species reactivity to human and rats. Blockage of the endogenous peroxidase activity was done by the immersion of the sections in methanol with 0.3% hydrogen peroxide then washed in phosphate buffered saline (PBS). The sections were incubated with primary antibody in a dilution 1:10-1:100 according to the manufacturer's recommended protocol. Specimens were incubated with primary antibody enhancer for ten minutes at room temperature. high pressure ramp polymer) by (Horseradish peroxidasewas added and incubated for fifteen minutes at room temperature. After each of the previous steps specimens were washed four times in PBS, three minutes each. The sections were covered by substrate chromogen solution then rinsed with PBS. Finally, counterstained with Harris Hematoxylin for one-four minutes and then were washed in water bath for seven-eight minutes, PBS for one minute, tap water for three minutes and then covered. Specimens were mounted using a permanent mounting media (DPX)[13,14]. Positive immunological reaction was detected as brown discoloration in epithelial and underlying connective tissue cells, collagen fibers and endothelial cells of the blood vessels.

### *Histochemical and Immuno-histochemical assessment*

For each positive Masson's trichrome section and for each positive anti TGF-β section, five microscopic fields were captured at original magnification of X400. This was performed using a digital camera (EOS 650D, Cannon, Japan) which was mounted on a light microscope (BX40F4, Olympus, Japan). Images of X400 magnification were then transferred to the computer system for analysis. All the steps of histochemical and immunohistochemical assessment were carried out using image J, 1.41 a (NH, USA) image analysis software and were performed in the precision measurements unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. For evaluation of Masson's Trichrome stain, the area percentage of newly formed blue stained collagen fibers was measured automatically. The mean area percentage for each case was calculated. For evaluation of TGF-β immuno-reactivity, the area fraction of immuno-positively stained area was measured automatically. The area fraction represented the percentage of immuno-positively area to the total area of the microscopic field. The mean area fraction for each case was calculated. The collected data were tabulated in Microsoft excel sheet.

### *Statistical analysis*

Data were tabulated, coded then analyzed using the computer program Statistical Package for Social Science (SPSS) version 26.0 to obtain descriptive data. They were calculated in the form of Mean ± Standard deviation SD and analytical statistics in the statistical comparison between the different groups, the significance of difference was tested using One way ANOVA (analysis of variance) which was used to compare between more than two groups of numerical (parametric) data. Then followed by post-hoc tukey test and Student's t-test (Unpaired) that were used to compare between mean of two different groups of numerical (parametric) data. *P value* less than 0.05 was considered statistically significant.

## RESULTS

### *H&E staining*

### *1) Negative control group (group I)*

Subgroup 1A and 1B: At six and twelve days, the H&E stained sections of the inferior surface of the tongue showed normal structure of keratinized epithelium which covered normally appeared lamina propria (Figures 2A, 2C). Higher magnification of the epithelium showed normal architecture and layering of keratinized stratified squamous epithelium ; basal, prickle, granular and keratin layers and mitotic activity at basal and parabasal layer. Lamina propria showed collagen fibers and many fibroblasts (Figures 2B, 2D).

### *2) Positive control group (group II)*

Subgroup 2A: At the six days post ulceration, the H&E stained sections of the inferior surface of the tongue showed loss of the covering epithelium. The ulcer area was filled with granulation tissue showing dense inflammatory cell infiltration and many dilated blood vessels (Figure 3A). Higher magnification of thin epithelial edge showed mitotic figures, apoptotic activity and dilated blood vessels in the lamina propria (Figure 3B).

Subgroup 2B: At the twelve days post ulceration, the ulcer area was covered by apparently moderate thickness of keratinized epithelium compared to the unwounded epithelium and showed shallow rete pegs (Figure 3C). Higher magnification showed the epithelium with mitotic and many apoptotic cells. Lamina propria showed some inflammatory cells, collagen fibers, fibroblasts and numerous blood vessels engorged with RBCs (Figure 3D).

### *3) PLGA NPs treated group (group III)*

Subgroup 3A: At six days post ulceration, loss of the epithelial coverage was seen. Granulation tissue at the ulcer bed was filled with inflammatory cell infiltration (Figure 4A). Higher magnification, showed apparently thin epithelial edge. The granulation tissue was filled with chronic inflammatory cells, fibroblasts and collagen fibrils (Figure 4B).

Subgroup 3B: At twelve days post ulceration, the inferior surface of the tongue of rats treated with PLGA NPs showed healed ulcer with apparently thin keratinized epithelial layer (Figure 4C). Higher magnification showed nearly flat rete pegs of the epithelium, few mitotic figures and apoptotic cells. Lamina propria showed few inflammatory cells and some fibroblasts (Figure 4D).

### *4) Native curcumin-treated group (group IV)*

Subgroup 4A: At six days post ulceration, loss of the epithelial coverage was observed. The ulcer bed was filled with granulation tissue infiltrated by inflammatory cells (Figure 5A). Higher magnification showed apparently thin epithelium at the edge with apoptotic cells and mitotic figures. Granulation tissue with inflammatory cells. Blood vessels and condensed collagen fibers with fibroblasts were seen in the lamina propria (Figure 5B).

Subgroup 4B: At twelve days post ulceration, healed ulcer showed keratinized epithelium coverage. Connective tissue showed dilated blood vessels, some were engorged with RBCs and collagen fibers were seen (Figure 5C). Higher magnification showed moderately thick keratinized stratified epithelium developing apoptotic cells and moderately long rete pegs. Connective tissue with some fibroblasts, collagen fibers and scarce inflammatory cells (Figure 5D).

### *5) Cr PLGA NPs treated group (group V)*

Subgroup 5A: At six days post ulceration, creeping of the two epithelial edges over the ulcer bed was seen (Figure 6A). Higher magnification showed epithelial edge with apoptotic cells and mitotic figures. Connective tissue showed few inflammatory cells, many fibroblasts and collagen fibers (Figure 6B).

Subgroup 5B: At twelve days post ulceration, re-epithelization of keratinized epithelium with almost normal epithelial thickness and architecture over the lamina propria was seen (Figure 6C). Higher magnification showed well-developed rete pegs of the epithelium. The underlying connective tissue showed many fibroblasts and organized collagen fibers. Few blood vessels showed dilatation while others appeared normal (Figure 6D).

### *Masson's Trichrome stain results*

### *1) Negative control group (group I)*

Subgroup 1A and 1B: At six and twelve days, light microscopic examination of specimens stained with Masson's Trichrome special stain in the lamina propria under the epithelium showed apparently almost equal amounts of mature red stained collagen fibers and immature blue stained collagen fibers (Figures 7A, 7B).

### *2) Positive control group (group II)*

Subgroup 2A: At six days post ulceration, light microscopic examination of specimens stained with Masson's Trichrome special stain in the lamina propria under the ulcerated area showed dominance of mature collagen fibers stained in red and focal distribution of immature fine blue stained collagen fibers (Figure 8A).

Subgroup 2B: At twelve days post ulceration, the lamina propria under the healed ulcer showed dominance of immature fine collagen fibers (Figure 8B).

### *3) PLGA NPs-treated group (group III)*

Subgroup 3A: At six days post ulceration, the lamina propria under the ulcerated area showed horizontally arranged dense and thick immature collagen fibers (Figure 9A).

Subgroup 3B: At twelve days post ulceration, the lamina propria under the healed ulcer showed dense and thick randomly arranged immature collagen fibers (Figure 9B).

### *4) Native curcumin-treated group (group IV)*

Subgroup 4A: At six days post ulceration, the lamina propria under the ulcer edge showed thick scattered immature blue stained collagen fibers (Figure 10A).

Subgroup 4B: At twelve days post ulceration, the lamina propria under the healed ulcer showed coarse, radially organized bundles of immature collagen fibers (Figure 10B).

### 5) Cr PLGA NPs-treated group (group V)

Sub group 5A: At six days post ulceration, the lamina propria under the ulcer edge showed dominance of wavy

haphazardly arranged immature collagen bundles with areas of condensation (Figure 11A).

Subgroup 5B: At twelve days post ulceration, lamina propria under the healed ulcer showed coarse and organized dense bundles of immature collagen fibers (Figure 11B).

### *Immuno-histochemical results*

#### *1) Negative control group (group I)*

Subgroup 1A and 1B: At six and twelve days, normal sections of the inferior surface of rat tongue showed negative immuno-staining of epithelium and the lamina propria (Figures 12A, 12B).

#### *2) Positive control group (group II)*

Subgroup 2A: At the six days post ulceration, the granulation tissue showed immuno-expression of TGF-β stain while few immuno-labelled cells were seen in the epithelial edges of the ulcer and in the lamina propria (Figure 13A).

Subgroup 2B: At the twelve days post ulceration, the healed epithelium showed some basal immuno-positively brown stained cells while the lamina propria showed generalized immuno-positivity for TGF-β stain (Figure 13B).

#### *3) PLGA NPs-treated group (group III)*

Subgroup 3A: At six days post ulceration, the basal and supra-basal layers of the epithelial edges, the granulation tissue and the lamina propria showed presence of immuno-positively stained cells for TGF-β stain (Figure 14A).

Subgroup 3B: At the twelve days post ulceration, the healed ulcer showed some immuno-positively stained basal cells for TGF-β stain in the epithelium and generalized immuno-positivity for TGF-β stain in the lamina propria (Figure 14B).

#### *4) Native curcumin-treated group (group IV)*

Subgroup 4A: At the six days post ulceration, epithelial edges showed few immuno-positively stained cells for TGF-β stain while the granulation tissue and the lamina propria showed wide distribution of immuno-positivity of TGF-β stain (Figure 15A).

Subgroup 4B: At the twelve days post ulceration, the basal layer of the epithelium, the lamina propria and the endothelial lining of blood vessels showed scarce immuno-positively stained cells for TGF-β stain (Figure 15B).

#### *5) Cr PLGA NPs-treated group (group V)*

Subgroup 5A: At six days post ulceration, epithelium edges showed basal immuno-positively stained cells for TGF-β stain. The lamina propria showed wide distribution of immuno-positivity stained cells for TGF-β stain (Figure 16A).

Subgroup 5B: At twelve days post ulceration, the healed ulcer showed negative immuno-staining for TGF-β stain of epithelium and the lamina propria (Figure 16B).

### *Statistical results*

#### *1- Masson's Trichrome stain statistical results*

#### *(A) Using one way analysis ANOVA followed by post-hoc tukey test*

**At six days post ulceration**

The positive control group showed the statistically significant lowest mean area percentage of newly formed immature collagen fibers of all groups. It revealed significant difference compared to negative control group.

Concerning the PLGA NPs group, it revealed no significant difference compared to the negative control group and positive control group. Meanwhile it revealed a significant decrease compared to NCr group and Cr PLGA NPs group.

NCr group revealed a significant increase compared to negative, positive control and PLGA NPs groups, while it showed a significant decrease compared to Cr PLGA NPs group.

The Cr PLGA NPs group revealed the statistically significant highest mean area percentage of newly formed immature collagen fibers of all groups (Table 1, Figure 17).

**At twelve days post ulceration**

The positive control group showed the statistically significant lowest mean area percentage of newly formed immature collagen fibers of all groups. It revealed significant difference compared to negative control group.

Concerning the PLGA NPs group, it revealed no significant difference compared to the negative control group and positive control group. Meanwhile it revealed a significant decrease compared to NCr group and Cr PLGA NPs group.

The NCr group revealed a significant increase compared to negative, positive control and PLGA NPs groups, while it showed a significant decrease compared to Cr PLGA NPs group.

The Cr PLGA NPs group revealed the statistically significant highest mean area percentage of newly formed immature collagen fibers of all groups (Table 1, Figure 17).

#### *(B) Using student's t-test (unpaired)*

In all groups, the mean area percentage of newly formed immature collagen at six days post ulceration was significantly lower than that at twelve days post ulceration (Table 2, Figure 18).

#### *2- TGF-β stain statistical results*

#### *(A) Using one way analysis ANOVA followed by post-hoc tukey test*

**At six days post ulceration**

The negative control group showed the statistically significant lowest mean area percentage of TGF-β immuno-positivity of all groups.

Concerning the positive control group, it revealed a significant increase of the mean area percentage of TGF-β immuno-positivity compared to the negative control group.

The PLGA NPs group revealed a significant increase of the mean area percentage of TGF-β immuno-positivity compared to the negative control group while it showed no significant difference compared to positive control group.

The NCr group revealed a significant increase to the mean area percentage of TGF-β immuno-positivity compared to PLGA NPs, negative and positive control groups, while it showed a significant decrease compared to Cr PLGA NPs group.

In the Cr PLGA NPs group, it revealed the statistically significant highest mean area percentage of TGF-β immuno-positivity compared to all groups (Table 3, Figure 19).

**At twelve days post ulceration**

The positive control group showed the statistically significant highest mean area percentage of TGF-β immuno-positivity of all groups.

Concerning the PLGA NPs group, it revealed a significant decrease compared to the positive control group. Meanwhile it revealed a significant increase compared to negative control group, NCr group and Cr PLGA NPs group.

The NCr group revealed a significant decrease to the mean area percentage of TGF-β immuno-positivity compared to the positive control group and PLGA NPs group, while it showed a significant increase compared to negative control group and Cr PLGA NPs group.

The Cr PLGA NPs group revealed no significant difference to the mean area percentage of TGF-β immuno-positivity compared to the negative control group, while it showed significant decrease to the mean area percentage of TGF-β immuno-positivity compared to the positive control, PLGA NPs and NCr groups where the Cr PLGA NPs group showed negative immuno-histochemical reaction (Table 3, Figure 19).

***(B) Using student's t-test (unpaired)***

At six days in the negative control group, there was no significant difference to the mean area percentage of TGF-β immuno-positivity compared to at twelve days of the same group.

At six days post ulceration in the positive control group and PLGA NPs group, the mean area percentage of the TGF-β immuno-positivity was significantly lower than that at twelve days post ulceration of the same group.

At six days post ulceration in the NCr group, the mean area percentage of the TGF-β immuno-positivity was significantly higher than that at twelve days post ulceration of the same group.

At six days post ulceration in the Cr PLGA NPs group the mean area percentage of the TGF-β immuno-positivity was significantly higher than that at twelve days post ulceration of the same group which showed the least mean area percentage (Table 4, Figure 20).

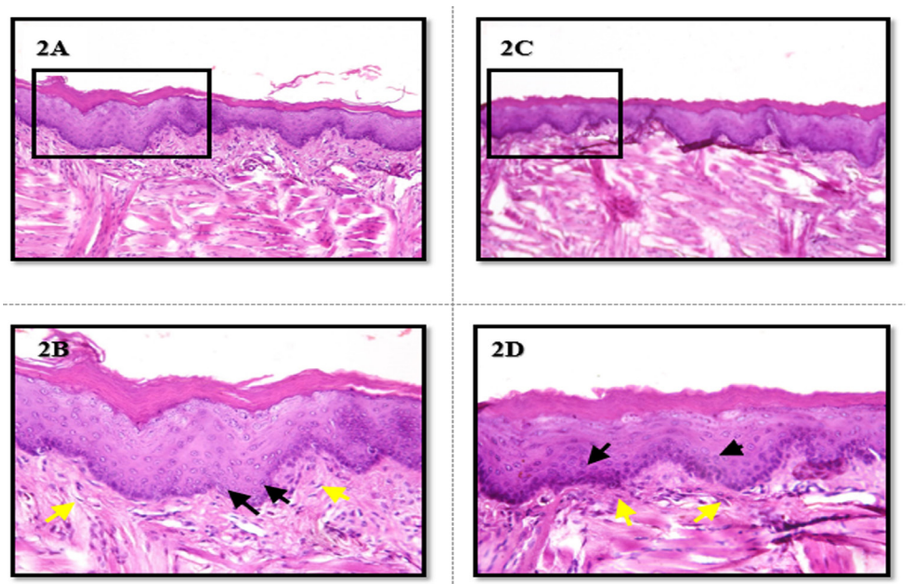


Fig. 2A: Photomicrograph of the normal section of inferior surface of the tongue of subgroup 1A showing normal structure of keratinized epithelium covering normal lamina propria (H&E stain, x100). Fig. 2B: Higher magnification of the black inset of fig. 2A showing normal architecture and layering of keratinized stratified squamous epithelium; basal, prickle, granular and keratin layers with mitotic activity at basal and parabasal layers (black arrows). Lamina propria showing collagen fibers and many fibroblasts (yellow arrows) (H&E stain, x400). Fig. 2C: Photomicrograph of the normal section of inferior surface of the tongue of subgroup 1B showing normal structure of keratinized epithelium covering normally appearing lamina propria (H&E stain, x100). Fig. 2D: Higher magnification of the black inset of fig. 2C showing normal architecture and layering of keratinized stratified squamous epithelium ; basal, prickle, granular and keratin layers and mitotic activity at basal and parabasal layer ( black arrows). Lamina propria showing collagen fibers with many fibroblast (yellow arrows) (H&E stain, x400).

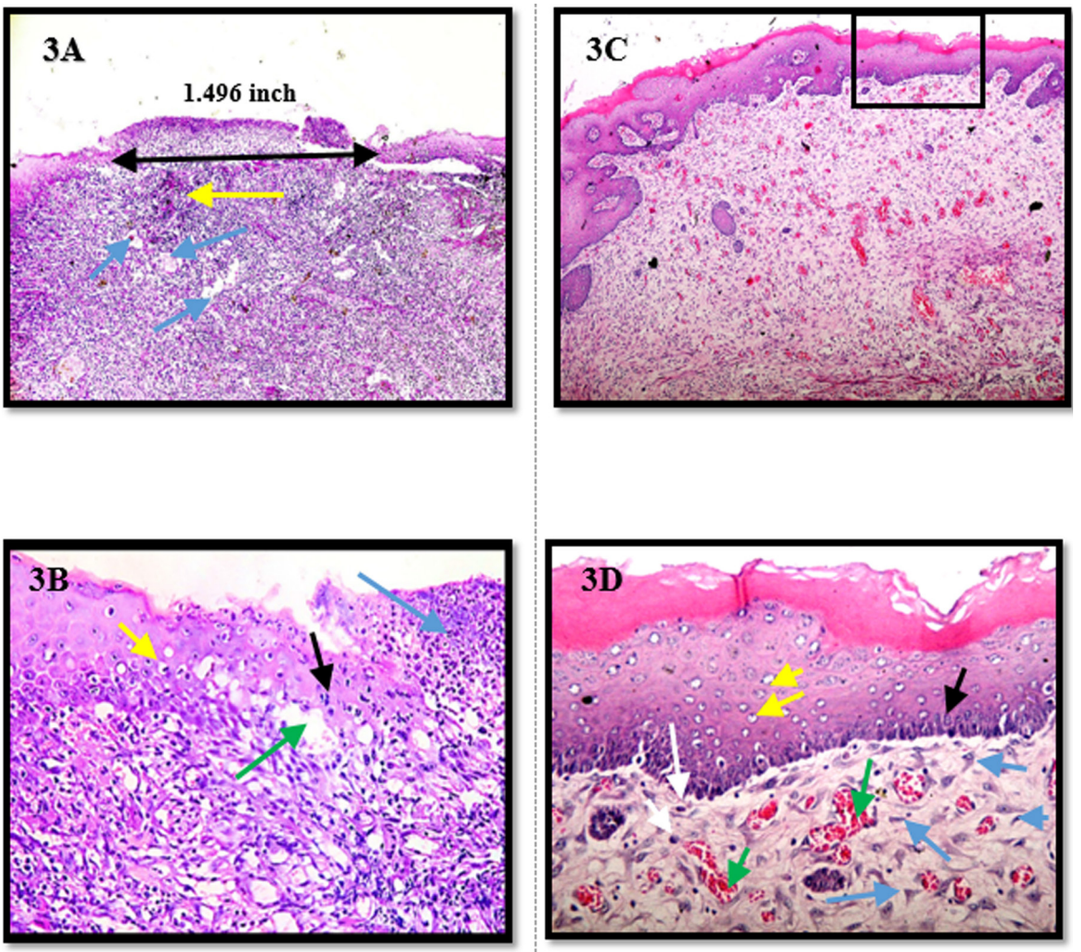


**Fig. 3A:** Photomicrograph of the inferior surface of the tongue of subgroup 2A showing loss of the covering epithelium. The ulcer bed filled with granulation tissue (black arrow), dense inflammatory cell infiltration (yellow arrow) and dilated blood vessels (blue arrows) (H&E stain, x100). **Fig. 3B:** Higher magnification of ulcer edge of subgroup 2A showing granulation tissue with dense inflammatory cells infiltration (blue arrow). The thin epithelial margin show mitotic activity (black arrow), apoptotic activity (yellow arrow) and dilated blood vessels in connective tissue (green arrow) (H&E stain, x400). **Fig. 3C:** Photomicrograph of the inferior surface of the tongue of subgroup 2B showing healed ulcer (black inset) covered by apparently moderate thickness of keratinized epithelium with shallow rete pegs (H&E stain, x100). **Fig.3D:** Higher magnification of the black inset of fig. 3C showing the epithelium with mitotic figures (black arrow) and many apoptotic activities (yellow arrows). Fibroblasts (blue arrows), numerous blood vessels engorged with RBCs (green arrows) and some inflammatory cells (white arrows) are seen in the lamina propria (H&E stain, x400).

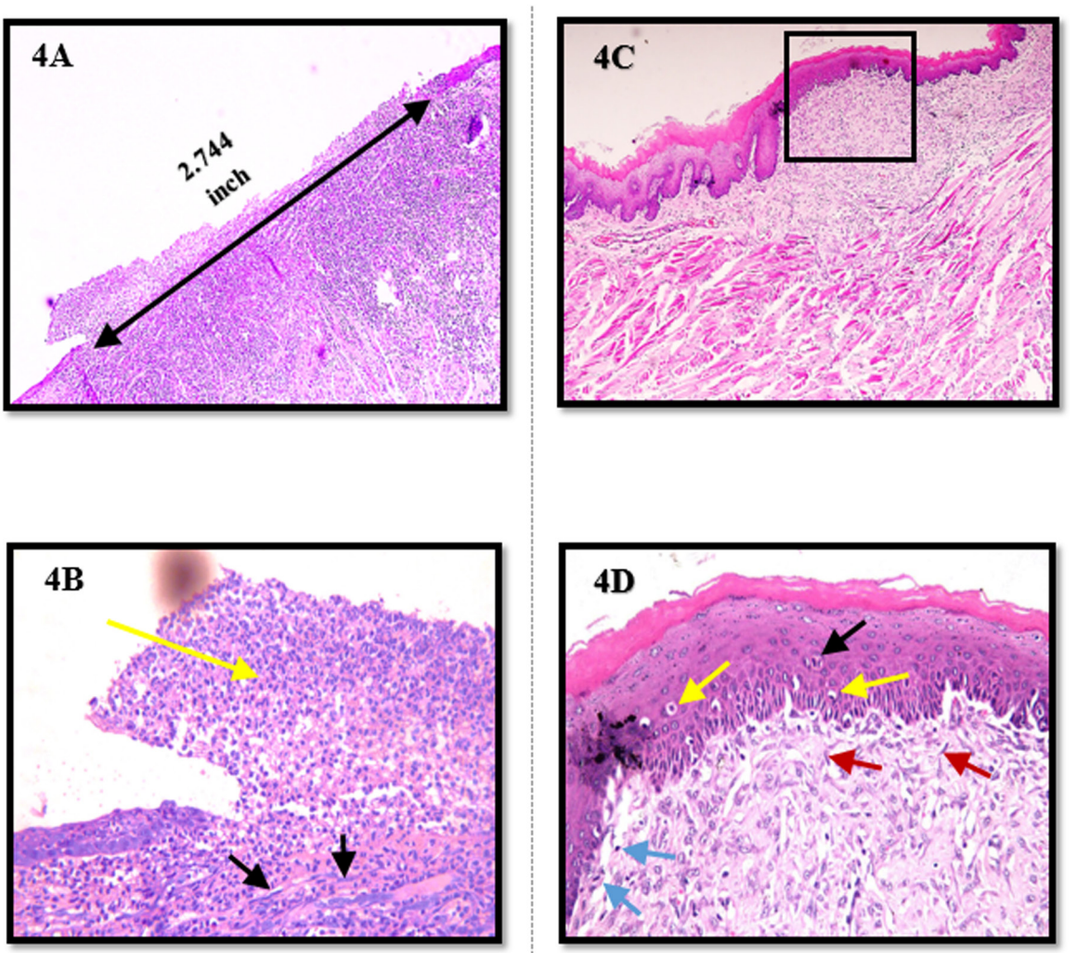


**Fig. 4A:** Photomicrograph of the inferior surface of the tongue of subgroup 3A showing loss of epithelial coverage. Granulation tissue infiltrated by inflammatory cells (H&E stain, x100). **Fig. 4B:** Higher magnification of ulcer edge of fig. 4A showing apparently thin epithelial edge. The granulation tissue filled with inflammatory cells (yellow arrow) and fibroblasts (black arrows) (H&E stain, x400). **Fig. 4C:** Photomicrograph of the inferior surface of the tongue of subgroup 3B showing healed ulcer covered with apparently thin keratinized epithelium (black inset) (H&E stain, x100). **Fig. 4D:** Higher magnification of the black inset of fig. 4C showing the epithelium with nearly flat rete pegs, few mitotic figures (black arrow) and apoptotic cells (yellow arrows). Lamina propria shows few inflammatory cells (blue arrows) and some fibroblasts (red arrows) (H&E stain, x400).

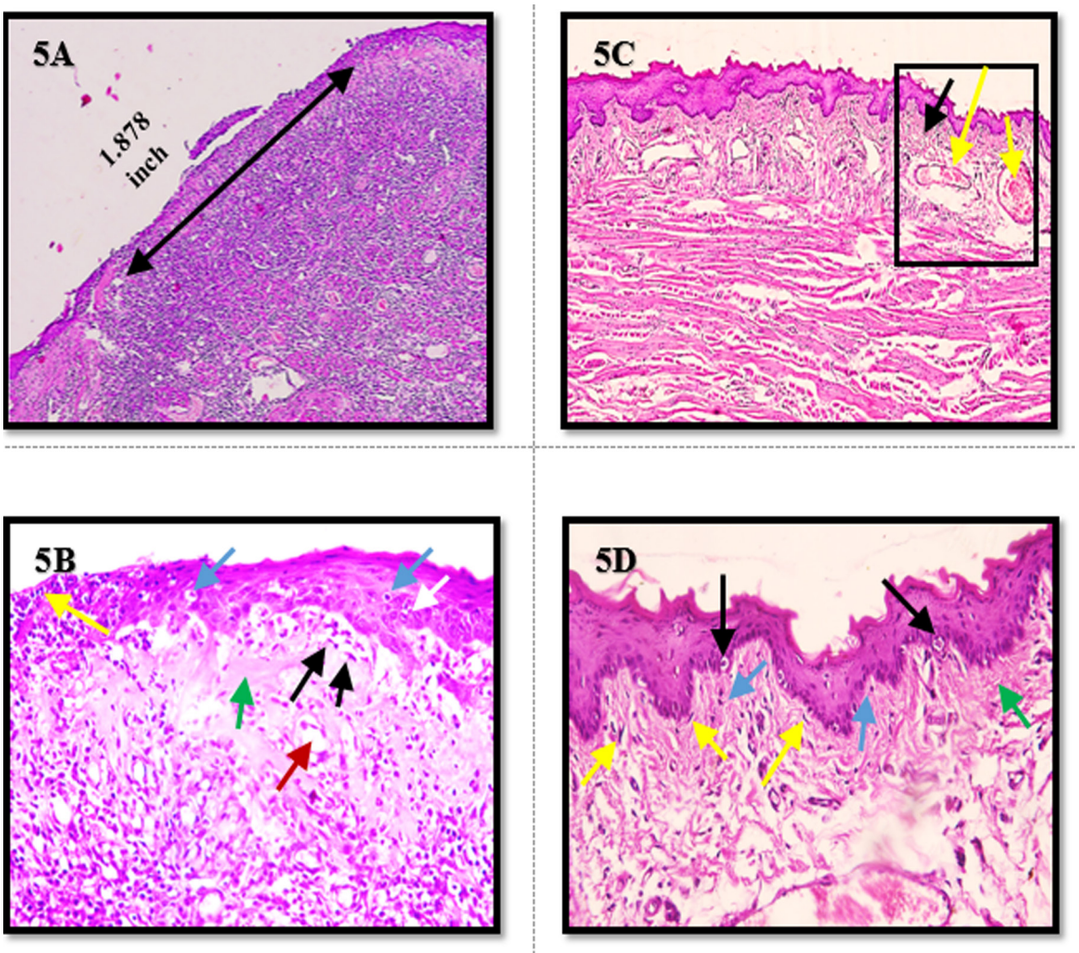


**Fig. 5A:** Photomicrograph of the inferior surface of the tongue of subgroup 4A showing loss of the epithelial coverage. Ulcer bed filled with granulation tissue with inflammatory cells infiltration (H&E stain, x100). **Fig. 5B:** Higher magnification of ulcer edge of fig. 5A showing apparently thin epithelial edge with apoptotic cells (blue arrows) and mitotic figures (white arrow). Granulation tissue with inflammatory cells (yellow arrow). Fibroblast (black arrows), blood vessels (red arrow) and condensed collagen fibers are seen in the lamina propria (green arrow) (H&E stain, x400). **Fig. 5C:** Photomicrograph of the inferior surface of the tongue of subgroup 4B showing keratinized epithelial coverage (black inset). Connective tissue showed dilated blood vessels filled with RBCs (yellow arrows) and collagen fibers (black arrow) (H&E stain, x100). **Fig. 5D:** Higher magnification of the black inset of fig. 5C showing apparently moderately thick keratinized stratified epithelium developing apoptotic cells (black arrows) and moderately long rete pegs. Connective tissue shows some fibroblasts (yellow arrows), scarce inflammatory cells (blue arrows) and collagen fibers (green arrow) (H&E stain, x400).

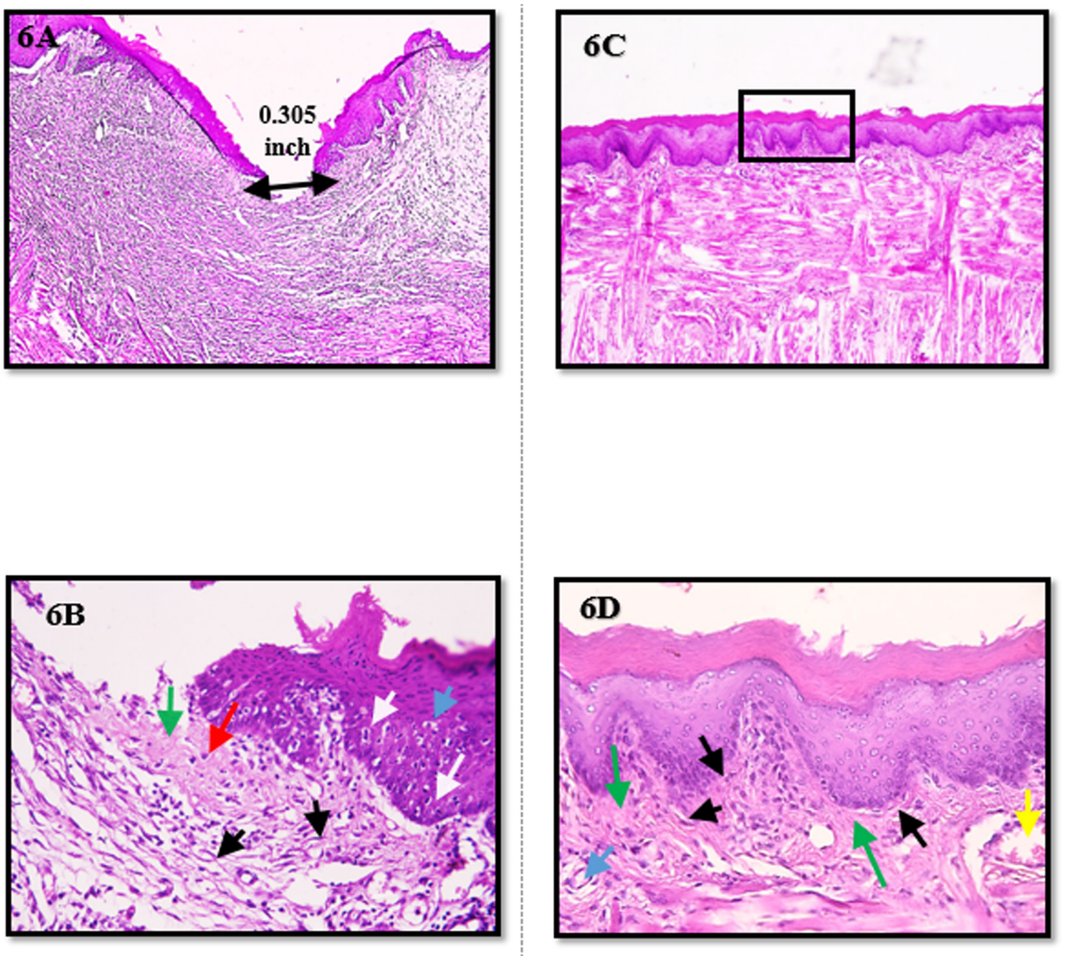


**Fig. 6A:** Photomicrograph of the inferior surface of the tongue of subgroup 5A showing creeping of both epithelial edges towards each other over connective tissue (H&E stain, x100). **Fig. 6B:** Higher magnification of ulcer edge of fig. 6A showing epithelial edge containing apoptotic cells (blue arrow) and mitotic figures (white arrows). Connective tissue showed few inflammatory cells (red arrows), many fibroblasts (black arrows) and collagen fibers (green arrow) (H&E stain, x400). **Fig. 6C:** Photomicrograph of the inferior surface of the tongue of subgroup 5B showing keratinized epithelium and lamina propria of almost normal thickness and architecture (H&E stain, x100). **Fig. 6D:** Higher magnification of the black inset of fig. 6C showing well-developed rete pegs of the epithelium. Many fibroblasts (black arrows) and organized collagen fibers (green arrows) are seen in lamina propria. Few blood vessels are dilated (yellow arrow) while others are normal (blue arrow) (H&E stain, x400).

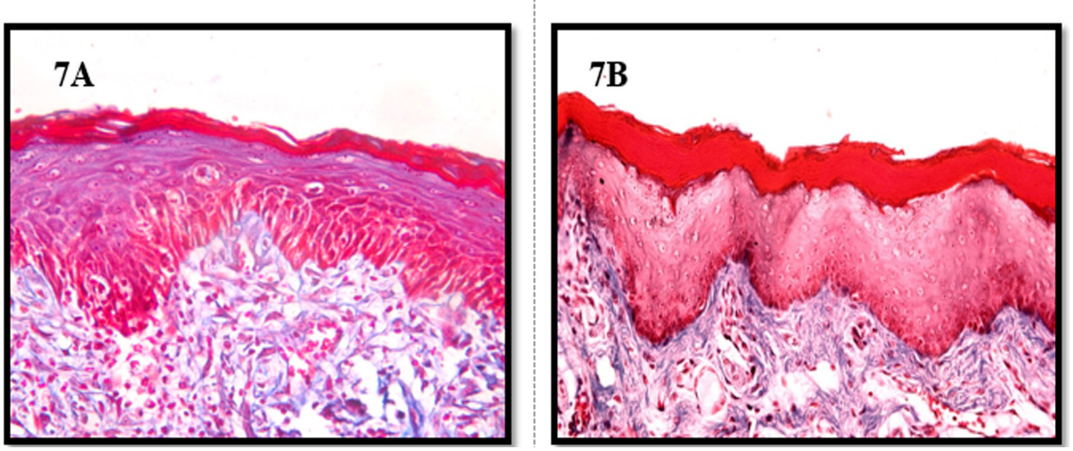


**Fig. 7A:** Photomicrograph of the normal section of inferior surface of the tongue tissue of subgroup 1A showing apparently almost equal amounts of mature red stained collagen fibers and immature blue stained collagen fibers in the lamina propria (MT, X400). **Fig. 7B:** Photomicrograph of normal section of inferior surface of the tongue of subgroup 1B showing apparently almost equal amounts of mature red stained collagen fibers and immature blue stained collagen fibers in the lamina propria (MT, X400).

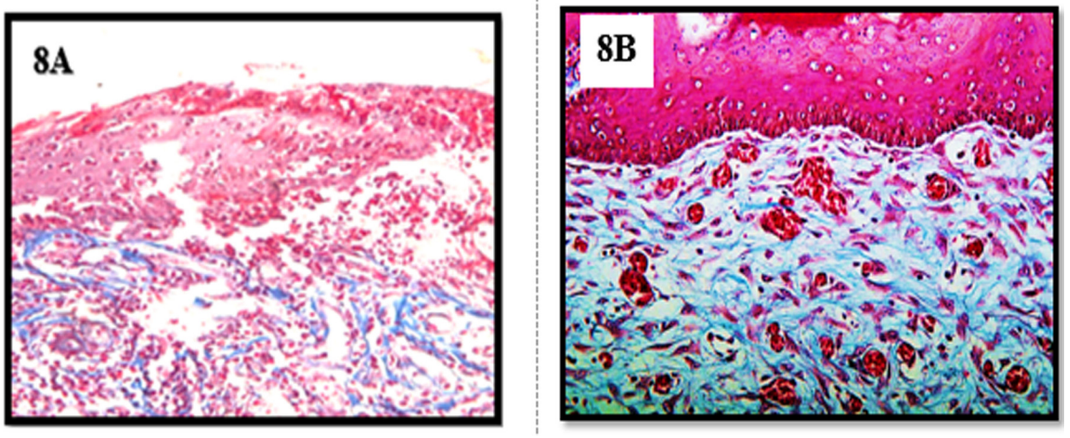


**Fig. 8A:** Photomicrograph of the ulcerated area of inferior surface of the tongue tissue of subgroup 2A showing dominance of mature red stained collagen fibers and focal distribution of immature blue stained fine collagen fibers in the lamina propria (MT, X400). **Fig. 8B:** Photomicrograph of the healed ulcer of subgroup 2B showing dominance of immature fine collagen fibers in the lamina propria (MT, X400).

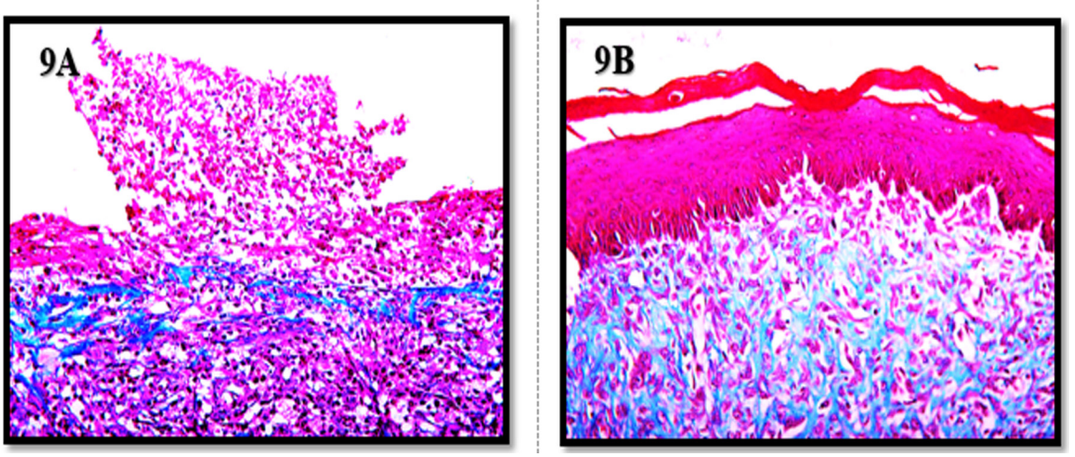


**Fig. 9A:** Photomicrograph of the ulcerated area of the inferior surface of the tongue of subgroup 3A showing horizontally arranged dense and thick immature collagen fibers in the lamina propria (MT, X400). **Fig. 9B:** Photomicrograph of the healed ulcer of subgroup 3B showing dense and thick randomly arranged immature collagen fibers in the lamina propria (MT, X400).

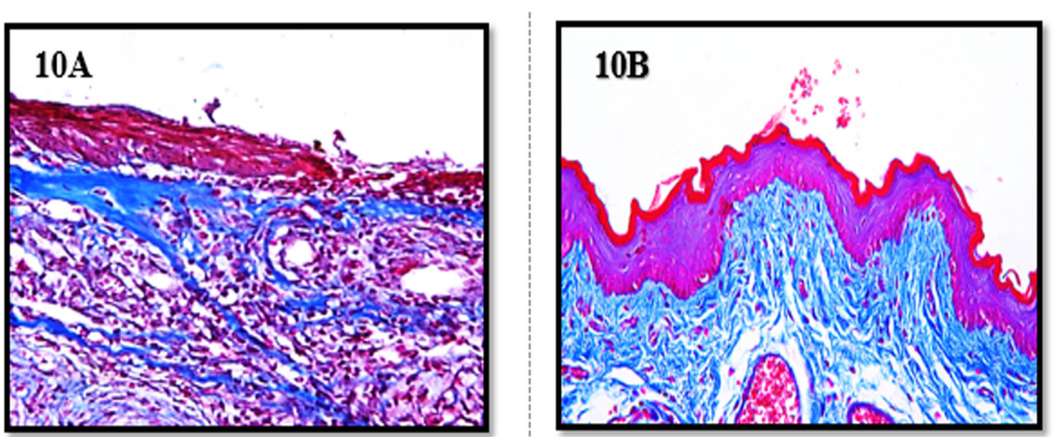


**Fig. 10A:** Photomicrograph of the ulcerated area of subgroup 4A showing thick scattered immature collagen fibers in the lamina propria (MT, X400). **Fig. 10B:** Photomicrograph of the healed ulcer of subgroup 4B showing coarse, radially organized bundles of immature collagen fibers in the lamina propria (MT, X400).

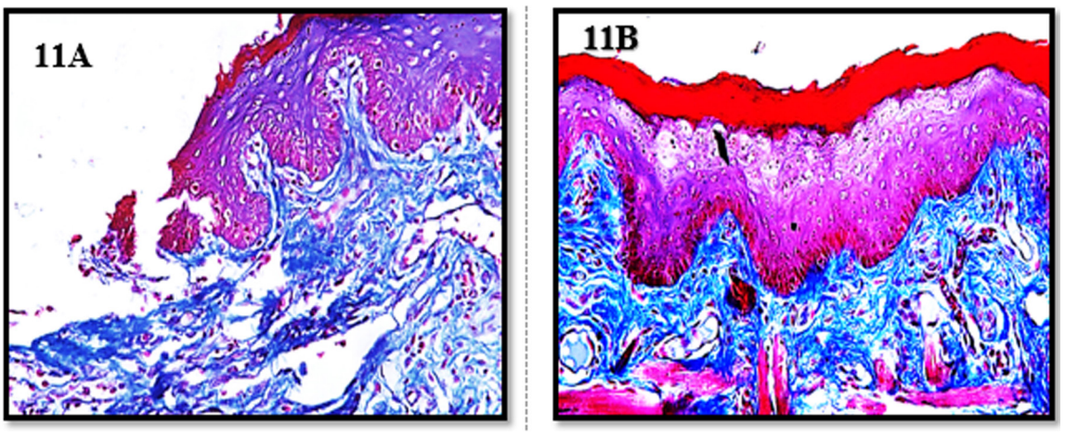


**Fig. 11A:** Photomicrograph of the ulcerated area of subgroup 5A showing dominance of wavy haphazardly arranged bundles of immature collagen fibers with areas of condensation in the lamina propria (MT, X400). **Fig. 11B:** Photomicrograph of the healed ulcer of subgroup 5B showing coarse and organized dense immature collagen fibers in the lamina propria (MT, X400).

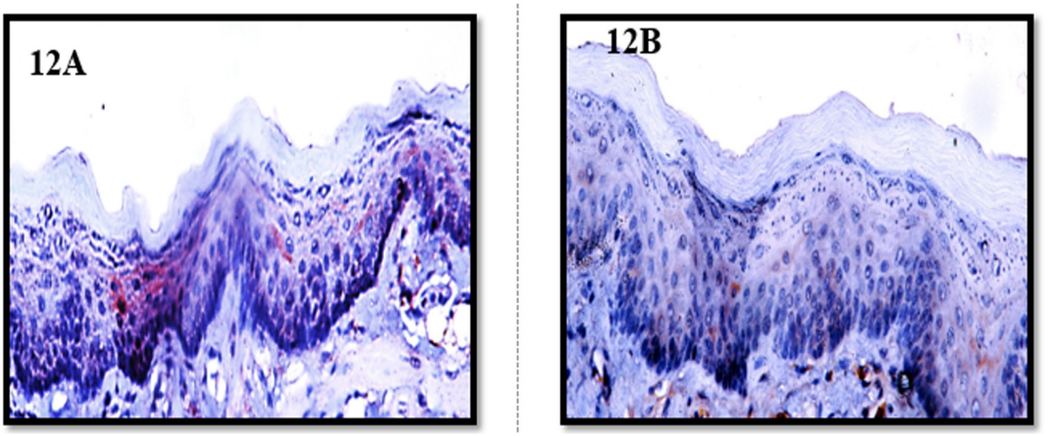


**Fig. 12A:** An immuno-stained photomicrograph of the normal section of the inferior surface of rat tongue of subgroup 1A showing negative immuno-staining of epithelium and the lamina propria (TGF-β, X400). **Fig. 12B:** An immuno-stained photomicrograph of the normal section of the inferior surface of rat tongue of subgroup 1B with undetectable expression of TGF-β stain in both epithelium and the lamina propria (TGF-β, X400).

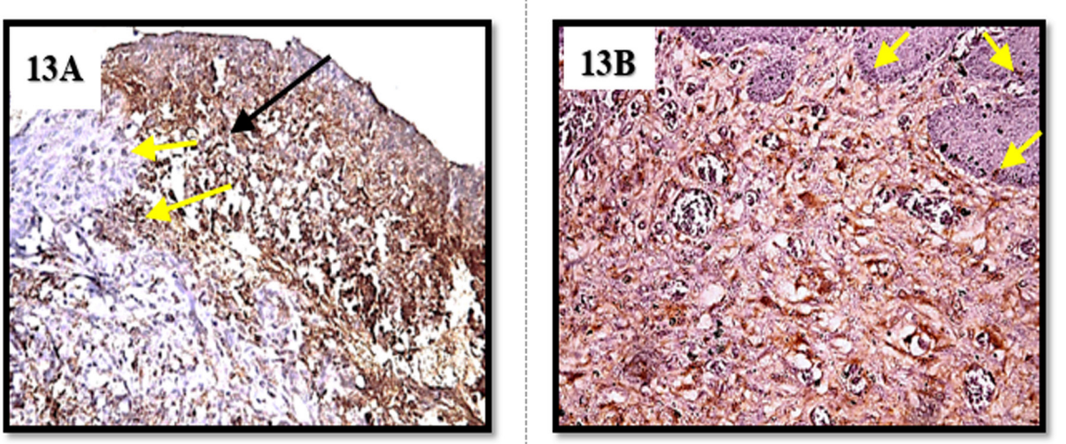


**Fig. 13A:** An immuno-stained photomicrograph of ulcer edge of subgroup 2A showing immuno-positively stained granulation tissue (black arrow). Few immunolabelled cells are seen in the epithelial edge and in the lamina propria (yellow arrows) (TGF-β, X400). **Fig. 13B:** An immuno-stained photomicrograph of subgroup 2B showing some basal immuno-positively stained cells in the healed epithelium (yellow arrows). The lamina propria showing generalized immuno-positivity (TGF-β, X400).

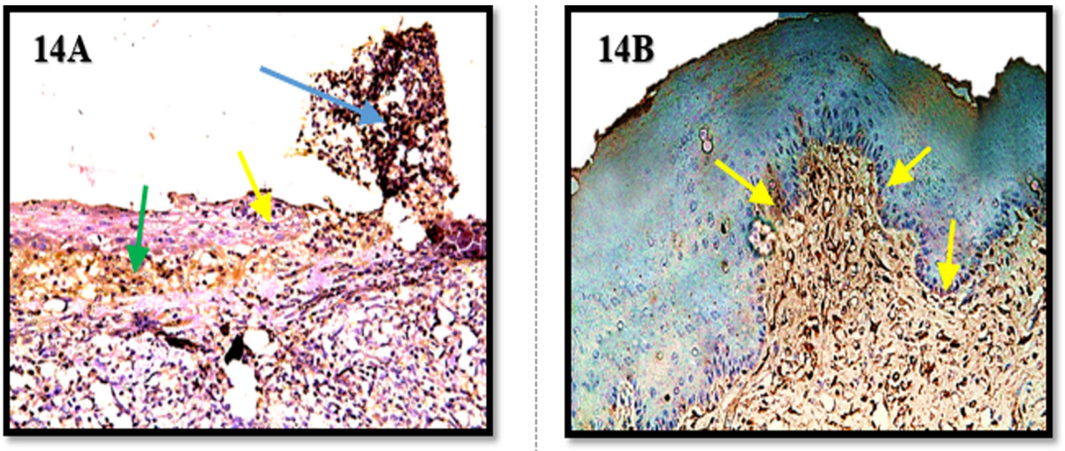


**Fig. 14A:** An immuno-stained photomicrograph of ulcer edge of subgroup 3A showing presence of immuno-positively stained cells in the basal and supra-basal layers of the epithelium (yellow arrow), the granulation tissue (blue arrow) and the lamina propria (green arrow) (TGF-β, X400). **Fig. 14B:** An immuno-stained photomicrograph of the healed ulcer of subgroup 3B showing some immuno-positively stained basal cells in the epithelium (yellow arrows) and generalized immuno-positivity in the lamina propria (TGF-β, X400).

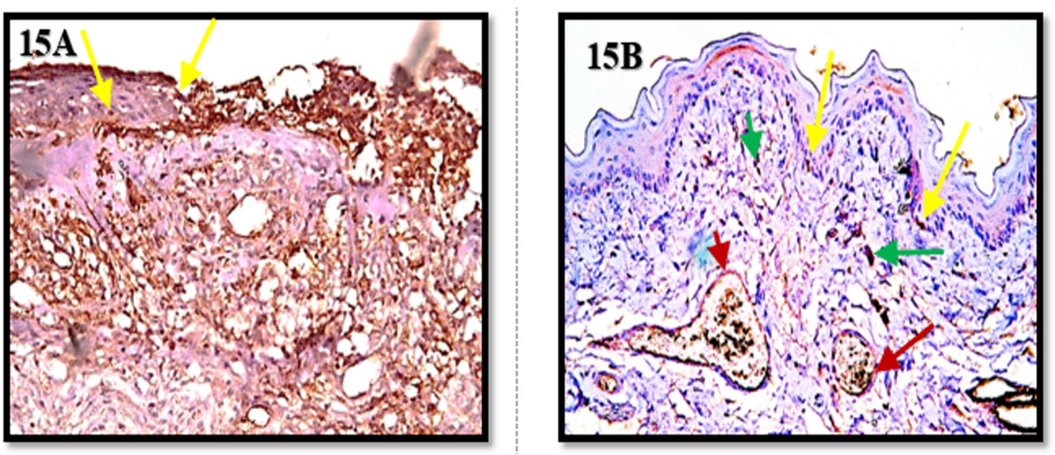


**Fig. 15A:** An immuno-stained photomicrograph of ulcer edge of subgroup 4A showing few immuno-positively stained cells in epithelial edge (yellow arrows). The granulation tissue and the lamina propria shows wide distribution of immuno-positively stained cells (TGF-β, X400). **Fig. 15B:** An immuno-stained photomicrograph of the healed ulcer of subgroup 4B showing scarce immuno-positively stained cells in the basal layer of the epithelium (yellow arrows), the lamina propria (green arrows) and the endothelial lining of blood vessels (red arrows) (TGF-β, X400).

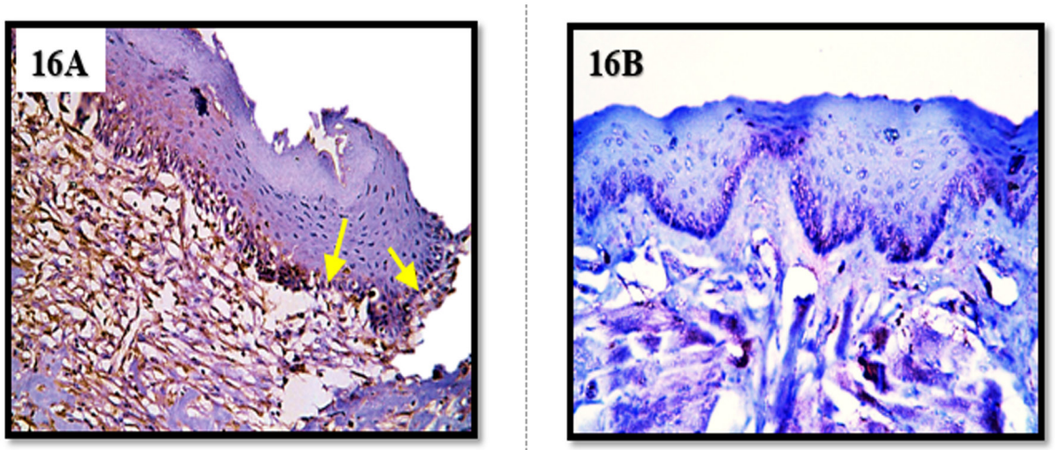


**Fig. 16A:** An immuno-stained photomicrograph of ulcer edge of subgroup 5A showing the epithelium with basal immuno-positively stained cells (yellow arrows). The lamina propria shows wide distribution of immuno-positively stained cells (TGF-β, X400). **Fig. 16B:** An immuno-stained photomicrograph of the healed ulcer of subgroup 5B showing negative immuno-staining of epithelium and the lamina propria (TGF-β, X400).

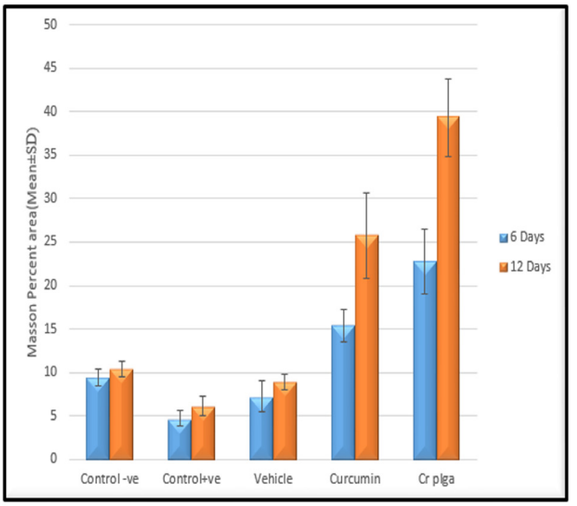


**Fig. 17:** Bar chart representing mean area percentage of immature blue stained collagen fibers in the different groups of the experiment.

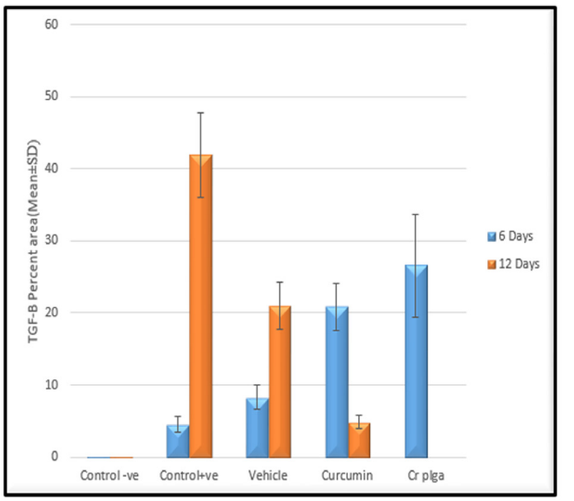


**Fig. 19:** Bar chart representing mean area percentage of TGF-β immunopositivity in the different groups of the experiment,

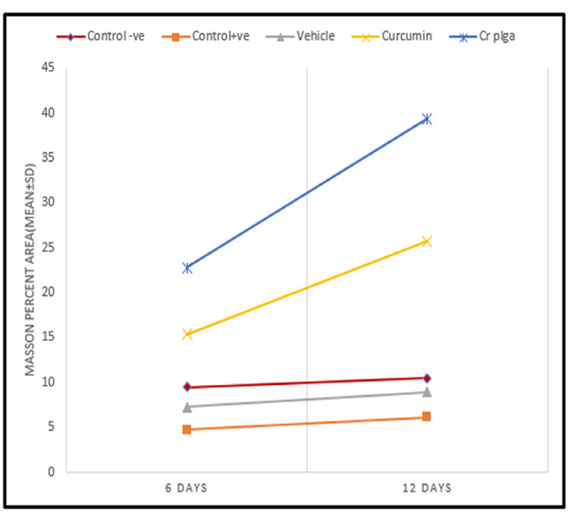


**Fig. 18:** Line chart representing mean area percentage of Masson's Trichrome stain at different time period in the same group of the experiment.

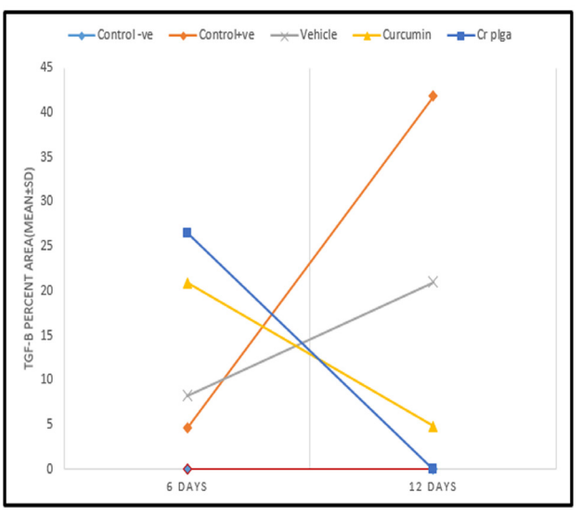


**Fig. 20:** Line chart representing mean area percentage of TGF-β immunostain at different time period of the same group of the experiment.

**Table 1:** Showing mean and SD values and results of One-way ANOVA followed by post-hoc tukey test for comparison between the mean area percentage of the blue stained immature collagen fibers in different examined groups.

| | -ve Control | +ve Control | PLGA NPs | NCr | Cr PLGA NPs | F | ANOVA *P value* |
|---|---|---|---|---|---|---|---|
| 6 days | 9.45±0.95 | 4.72±0.83 | 7.262±1.787 | 15.37±1.837 | 22.76±3.659 | 121.2 | <0.001* |
| Post-hoc | | P1=<0.001* | P1= 0.14<br>P2=0.064 | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001* | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001*<br>P4=<0.001* | | |
| 12 days | 10.45±0.91 | 6.106±1.090 | 8.9±0.92 | 25.76±4.94 | 39.3±4.4 | 211.7 | <0.001* |
| Post-hoc | | P1=0.02* | P1= 0.80<br>P2=0.25 | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001* | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001*<br>P4=<0.001* | | |

Data expressed as mean ± SD, SD: standard deviation, *P*: Probability, *:significance <0.05
P1: significance vs negative control group. P2: significance vs positive control group.
P3: significance vs PLGA NPs group. P4: significance vs NCr group.

**Table 2:** The mean area percentage (%), standard deviation (SD) values of the newly formed immature collagen fibers at different time period.

| | 6 days | 12 days | t | *P* |
|---|---|---|---|---|
| Negative control | 9.45±0.95 | 10.45±0.91 | 2.4 | 0.027* |
| Positive control | 4.72±0.83 | 6.106±1.090 | 3.18 | 0.005* |
| PLGA NPs | 7.262±1.787 | 8.9±0.92 | 2.63 | 0.016* |
| NCr | 15.37±1.837 | 25.76±4.94 | 6.22 | <0.001* |
| Cr PLGA NPs | 22.76±3.659 | 39.3±4.4 | 9.11 | <0.001* |

Data expressed as mean ± SD
SD: standard deviation *P*: Probability *: significance <0.05 Test used: Student's t-test (Unpaired)

**Table 3:** Showing mean and SD values and results of One-way ANOVA followed by post-hoc tukey test for comparison between the mean area percentage of the TGF-β immuno-positivity in different examined groups.

| | -ve Control | +ve Control | PLGA NPs | NCr | Cr PLGA NPs | F | ANOVA *P value* |
|---|---|---|---|---|---|---|---|
| 6 days | 0.0004±0.0001 | 4.6±1.06 | 8.27±1.67 | 20.9±3.26 | 26.5±7.16 | 95.04 | <0.001* |
| Post-hoc | | P1=<0.004* | P1= 0.1*<br>P2=17 | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001* | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001*<br>P4= 0.01* | | |
| 12 days | 0.00035±0.0001 | 41.86±5.885 | 20.99±3.227 | 4.85±0.9031 | 0±0 | 354.2 | <0.001* |
| Post-hoc | | P1=0.01* | P1= 0.1*<br>P2=0.1* | P1=<0.001*<br>P2=<0.001*<br>P3=<0.001* | P1=0.99<br>P2=<0.001*<br>P3=<0.001*<br>P4=0.007* | | |

Data expressed as mean ± SD, SD: standard deviation, *P*: Probability, *:significance <0.05
P1: significance vs negative control group. P2: significance vs positive control group.
P3: significance vs PLGA NPs group. P4: significance vs NCr group.

**Table 4:** The mean area percentage (%), standard deviation (SD) values of the immuno-positivity at different time period.

| | 6 days | 12 days | t | *P* |
|---|---|---|---|---|
| Negative control | 0.0004±0.0001 | 0.00035±0.0001 | 1.54 | 0.13 |
| Positive control | 4.6±1.06 | 41.86±5.885 | 19.7 | <0.001* |
| PLGA NPs | 8.27±1.67 | 20.99±3.227 | 11.06 | <0.001* |
| NCr | 20.9±3.26 | 4.85±0.9031 | 14.96 | <0.001* |
| Cr PLGA NPs | 26.5±7.16 | 0±0 | 11.7 | <0.001* |

Data expressed as mean ± SD
SD: standard deviation *P*: Probability *: significance <0.05 Test used: Student's t-test (Unpaired)

## DISCUSSION

Oral ulcer is a common oral disease that is very painful and may become infected, it can cause local tissue inflammation and necrosis elongating its course and increasing the patient's suffering[15].

In the current study, Albino rats were used because of their low cost and useful information they provide. The use of rats produced simple information but is still capable of inspiring further researches in this area of knowledge[16]. The rats weighed 200-250 gm that is considered as an ideal model for the study as they possess quite large tongues to enable the ulcer induction. The choice of male rats also cancelled the influence of sex hormones on the wound healing process[17] which is known to control oral mucosal wound healing[16]. The duration of six and twelve days post ulceration was chosen based on a previous study[9] reporting that there was histological changes at these times after ulcer induction. The treatment dose of 100mg / kg was chosen since it had been concluded that curcumin dose of 100 mg/kg daily by oral route was enough to induce cellular changes that aided in healing and in the same time provided a safety non-toxic dose[8].

In this study, Curcumin was the drug of choice because it was demonstrated to be an optimum wound dressing agent[4]. In the current study, PLGA was used as a carrier for curcumin since it had been reported that PLGA has the ability to accelerate the effect of curcumin in the healing process[7].

In the present study, light microscopic examination of the H&E-stained sections of the positive control group at six days post ulceration showed dilated blood vessels and dense inflammatory cells which infiltrated the extruded

granulation tissue. The epithelium at the ulcer edges showed mitotic and apoptotic activities. At twelve days post ulceration, ulcerated bed was covered by apparently moderate thickness of keratinized epithelium which showed shallow rete pegs, mitotic and apoptotic cells. Some inflammatory cells, fibroblasts infiltration and collagen fibers formation at the lamina propria were also seen. These results were in agreement with the authors[9] using acetic acid to induce ulcer at the tip of the inferior surface of the tongue of Albino rats. The investigators clarified that this model simulates what happens in the tongue ulcers in humans including epithelial loss and degradation, inflammatory infiltration, granulation tissue formation, collagen deposition and re-epithelialization. The apoptotic changes observed in the epithelium of the positive control group when compared to the negative one could be attributed to increased reactive oxygen species (ROS) production caused by the underlying inflammatory process[18].

Histochemical examination of the negative control group using Masson's Trichrome special stain showed apparently almost equal amounts of mature red stained collagen fibers and immature blue stained collagen fibers. While, the positive control specimens showed abundance of mature collagen fiber bundles with focal scarcely detected immature fibers at six days post ulceration. On other hand, at twelve days post ulceration, dominance of immature fine collagen fibers was seen in the lamina propria. These results were in agreement with findings of the previous study and were attributed to reduced regenerative capacity of the connective tissue fibroblasts because of the inflammatory changes in the tissue[19]. The previous investigators furtherly explained that following inflammation the activated macrophages produced many toxic substances as nitric oxide, ROS and matrix metalloproteinases destroying various types of collagen within the tissue affecting fibroblasts and hindering their capacity to synthesize new collagen. The present study was also parallel with another study which documented that after oral buccal ulceration, very few collagen fibers stained blue while the majority stained red[18]. Dominance of immature collagen fibers observed at twelve days post ulceration in histochemical photomicrographs of the positive control group were significantly confirmed in the statistical results when compared to the same group at six days.

Examination of anti TGF –β stained specimens of the negative control group showed negative expression. While, the positive control group at six days post ulceration revealed immuno-expression of TGF –β in the granulation tissue with few immuno-labelled cells in the epithelial edges of ulcer and in the lamina propria. At twelve days post ulceration, some of immuno-positively stained cells were detected at the basal layer of the epithelium and generalized immuno-positivity were seen in the lamina propria. These results were in agreement with authors revealing that at six days post ulceration there was a limited distribution of immuno-positive cells but at twelve days post ulceration, the positive control group showed wide distribution of immuno-positive cells[9]. The current results were supported with the previous study that showed limited and faint presence of immuno-expression of TGF –β at six days post ulceration in untreated wounds which was reflected to slowly healing compared to others groups[20]. In the present study, the statistical results of anti TGF –β immuno-stain revealed that at six days, the negative control group showed the statistically significant lowest mean area percentage of TGF-β immuno-positivity of all groups. While, at twelve days post ulceration the positive control subgroup was significantly higher than at six days post ulceration. Our results were also in parallel with another experiment which clarified that at seven days post ulceration, the buccal mucosa showed weak immuno-reactivity at the epithelial layers and strong reactivity at the granulation tissue but at fourteen days post ulceration the lamina propria showed strong positive reaction in the positive control group[21].

In this study, H&E-stained sections of the PLGA NPs group at six days post ulceration showed granulation tissue filled with inflammatory cells and fibroblast cells which were not observably seen in the positive control group. At twelve days post ulceration lamina propria showed few inflammatory cells and some fibroblasts. These results indicated that the effect of lactic acid on wound healing was better than non-treated positive control group at six and twelve days post ulceration. These results agree with previous work which showed that topical application of PLGA NPs in subcutaneous excisional wound showed better healing than the positive control group[22].

Statistical results of histochemical stain of the PLGA NPs group at six days and twelve days post ulceration demonstrated no significant difference compared to the negative control group and positive control group. This is not in parallel with another investigators who stated that PLGA NPs group showed more collagen formation than the positive control group at five and ten days post ulceration suggesting the early influx of fibroblasts[22]. The mismatching with our results may be due to the use of the authors to mice models in their experiment.

Statistical results of immuno-histochemical stain of TGF-β immuno-stained sections of PLGA NPs group at six days showed increased area percentage of immuno-positivity than the negative control group. Despite, it showed no significance when compared to positive control group which significantly increased at twelve days post ulceration. This came in accordance with Trabold *et al* who tested the effect of PLGA in the dorsal subcutaneous tissue of rats and found an increase in the production of TGF-β1 in the earlier stage of healing[23].

In the current study, the H&E-stained sections of the NCr treated group at twelve days post ulceration showed complete closure of the ulcer with keratinized stratified epithelium, the lamina propria contained collagen fibers

and many fibroblasts. This was in agreement with the results which confirmed that administration of curcumin in rats with tongue ulcer resulted in an organized re-epithelialization of the ulcer, fibroblast infiltration, well organized collagen fibers in curcumin group at twelve days post ulceration[9]. Basha explained that curcumin was a potent inhibitor of TNF-α production which mediated the expression of many inflammatory mediators and accelerated the healing of palatal ulcer when compared with the untreated group[24].

Statistical results of Masson's Trichrome stain of the NCr group showed a statistically significant increase in the area percentage of newly formed collagen fibers at six and twelve days post ulceration when compared to the PLGA NPs, negative control and positive control groups. This was parallel with the previous findings where curcumin group showed abundant thick collagen fibers than the positive control group at seven days in punch cutaneous wounds[14]. In addition, full thickness excisional cutaneous wound model treated by native curcumin showed greater collagen content than the positive control group or PLGA NPs group suggesting early fibroblast influx at five and ten days after wounding[22].

Immuno-histochemical results of NCr group at six days post ulceration showed wide TGF-β immuno-positivity distribution confirmed by statistical significant increase in the area percentage of TGF-β compared to PLGA NPs group, negative control or the positive control group. However, at twelve days post ulceration, it showed a significant increase compared to negative control group and Cr PLGA NPs group. In addition, there was significant reduction in area percentage of TGF-β in NCr group compared to PLGA NPs group and positive control group. This came in parallel with another authors[14] who reported that the wound bed showed more number of positively stained cells as well as a greater intensity of staining for TGF- β1 in the early stage compared to the positive control group which proved that curcumin enhanced the cutaneous healing by increasing the induction of TGF-β1. Also, more expression of immuno-positivity were found in the curcumin treated group compared to the positive control group at six days post ulceration then decreased in the immuno-expression at twelve days post ulceration in the curcumin treated group[9].

In the present study, the H&E-stained sections of the Cr PLGA NPs group at six days showed approximation of both edges of ulcer toward each other followed by complete closure of the ulcer at twelve days post ulceration and the epithelium was in its maximum thickness and showed almost normal keratinization and stratification with well-developed rete pegs which resembled the histological picture of the negative control group. Lamina propria showed well organized connective tissue papillae, fibroblasts and organized collagen fibers. These results were in accordance with earlier work which showed that after treatment with Cr PLGA NPs in excisional wounds, the dermal wounded area showed complete re-epithelialization with well-formed and differentiated epithelium and increased deposition of connective tissue at tenth day post wounding[22].

Histochemical results of Cr PLGA NPs group at six days post ulceration showed dominance of immature wavy haphazardly arranged collagen fibers and was statistically the group showing the highest significant increase of the area percentage of immatured collagen fibers compared to the rest of experimental groups at the same time period. At twelve days post ulceration, Cr PLGA NPs group showed dense bundles of immature collagen fibers and statistically showed the highest significant increase in area percentage of immature collagen fiber than the other groups at the same time period. This was in agreement with a previous experiment which proved that treatment of the subcutaneous tissue of rats with Cr PLGA NPs clearly unveiled a greater collagen content than the positive control group, PLGA NPs group and curcumin group suggesting the early influx of fibroblasts towards the wound and enhanced collagen deposition reported to be the mechanism of action of curcumin and lactic acid in wound healing[22].

Immuno-histochemical results at six days post ulceration showed Cr PLGA NPs group with wide distribution of immuno-positivity which was confirmed by a statistically significant increase in the area percentage among all sub-groups at the same time period. Whereas at twelve days a negative immuno-staining was detected which is almost similar to the negative control group. This suggests early healing of ulcer and downregulation of TGF-β1 to prevent scar formation. Our results were in accordance with another study documented that curcumin loaded PLGA NPs enhanced the biosynthesis of TGF-β1 in the earlier stages of healing which attribute for the rapidity of wound healing and reflecting in apparently decrease in the ulcer size due to the contractile activity of fibroblasts[25]. Also, our results were in agreement with Zhang *et al* who found that treatment of deep tissue injury in mouse model by curcumin loaded PLGA NPs led to upregulation of TGF-β1 and exhibited a smaller wound area and less tissue damage than the positive control group, PLGA NPs group and curcumin group on day seven post ulceration and faster ulcer closure at fourteen days post ulceration[26]. It can be proposed that Cr PLGA NPs-treated wounds achieved superior anti-inflammation and faster healing outcomes in the earlier stages of healing due to the synergistic mechanisms of action from both curcumin and lactate within wound sites resulting in down regulation of TGF-β1 as demonstrated in the present study by negative reaction at twelve days.

From the histochemical, immuno-histochemical and statistical results at six days post ulceration, it was observed that the increased immuno-expression of TGF-β in the groups of this study was accompanied by high expression of the collagen fibers deposition. This was also reported by[17,23,25] and was attributed to fibroblasts activation by TGF-β.

Based on the histological, histochemical, immuno-histochemical and statistical findings of this study, it was revealed that Cr PLGA NPs treatment showed enhanced and accelerated tongue ulcer healing than NCr.

## CONCLUSIONS

Administration of Cr PLGA NPs showed higher wound healing activity compared to that of native curcumin and PLGA NPs at the equivalent dose level and at the same duration.

Cr PLGA NPs showed the highest significant increase in collagen fibers content in ulcerated area at six and twelve days post ulceration and the highest significant increase in the expression of TGF- β at six days post ulceration which was reflected histologically by approximation of the edges of the ulcer.

## ABBREVIATIONS

**PLGA:** poly lactic-co-glycolic acid; **Cr:** curcumin; **NCr:** native curcumin; **NPs:** nanoparticles; **Cr PLGA NPs:** curcumin loaded poly lactic-co-glycolic acid; **TGF-β:** transforming growth factor- beta; **CMC:** carboxymethylcellulose; **S/O/W:** solid in oil in water; **gm:** grams; **mg:** milligram; **kg:** kilogram; **W:** watt; **°C:** Celsius; **mm:** millimeter; **H&E:** Hematoxylin and Eosin; **SD:** standard deviation; **SPSS:** Statistical Package for Social Science; **PBS:** phosphate buffered saline; ***P:*** probability; **RBCs:** red blood cells; **ROS:** reactive oxygen species; **TNF-α:** tumor necrosis factor- alpha.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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**الملخص العربى**

# تأثير الكركم المحمل على الجزيئات النانونية لحمض البولي لاكتيك كوجليكول مقارنة بالكركم الخام على إلتئام القرحة اللسانية في الفئران البيضاء - دراسة هستولوجية وهستوكيميائية وهستوكيميائية مناعية

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**[١]طبيب مقيم بمستشفى بنها التعليمي**

**[٢]قسم بيولوجيا الفم- كلية طب الاسنان-جامعة عين شمس**

**نبذة مختصرة:** الكركمين هو مكون يوجد في مسحوق الكاري وهو من الاعشاب القابلة للأكل ذات خصائص دوائية. يستخدم كعلاج مضاد للالتهابات ولمعالجة القرح الفموية. لكنه محدود الفاعلية لعدة اسباب مثل التوافر الحيوي الفموي المنخفض للغاية مما يعيق تطبيقه كعامل علاجي. لذلك ، تم تطوير الكركم المحمل على الجزيئات النانونية لحمض البولي لاكتيك كوجليكول لزيادة التوافرالحيوي الفموي للكركم.

**الهدف من التجربة:** دراسة تأثير الكركم المحمل على الجزيئات النانونية لحمض البولي لاكتيك كوجليكول مقارنة بالكركم الخام على إلتئام القرحة المحدثة كيميائيا في طرف السطح الباطني للسان في الفئران البيضاء.

**طرق ومواد البحث:** تم تقسيم سبعين من الفئران البيضاء الذكور البالغين إلى خمس مجموعات متساوية جميعهم تعرضوا لإحداث قرحة في اللسان ماعدا المجموعة الاولى ثم قسموا كالاتي:

المجموعه الضابطة السالبة: لم يتعرضوا لاحداث قرحة لسانية ولم يتلقوا علاج. المجموعة الضابطة الموجبة: تعرضوا لاحداث قرحة لسانية ولم تتم معالجتهم. ومجموعه البولي لاكتيك كوجليكول النانوني: تم اعطائهم ١٠٠ مجم /كجم من حمض البولي لاكتيك كوجليكول النانوني المعلق عن طريق الفم يوميا . مجموعة الكركم الخام: تم اعطائهم ١٠٠ مجم / كجم من الكركم الخام المعلق في ٥. ٠ ٪ من الكربوكسي مثيل سيليلوز عن طريق الفم يوميا. مجموعة الكركم المحمل على جزيئات حمض البولي لاكتيك كوجليكول النانوني: تم اعطائهم ١٠٠ مجم /كجم من الكركم المحمل على جزيئات حمض البولي لاكتيك كوجليكوليك النانوني عن طريق الفم يوميا. ثم تمت التضحية بالفئران عند ستة ايام و اثنى عشر يوما. ثم تحضير العينات وصبغها بصبغة الهيماتوكسلين والإيوسين للفحص الهيستولوجي الروتيني والصبغة الهيستوكيميائية ( الماسون ترايكروم ) لتقييم الكولاجين المتكون جديدا اثناء التئام القرحة والتمييز بين الكولاجين الناضج و الغير ناضج والصبغة الهيستوكيميائية المناعية لدراسة مستوى عامل النمو المتحول β.

**النتائج:** أكد الفحص الهستولوجي أن مجموعة الكركم المحمل على جزيئات حمض البولي لاكتيك كوجليكول النانوني أظهرت إعادة تشكيل للظهارة الحرشفية ، وفاعلية عالية في الالتئام مقارنة بالمجموعات الأخرى. وأظهرت النتائج الهستوكيميائية والهستوكيميائية المناعية زيادة في محتوى الكولاجين و مستوى عامل النمو المتحول β وذلك بسبب القدرة على التئام القرح افضل من الكركم الخام.

**الاستنتاج:** قدمت الجسيمات النانوية البولي لاكتيك كوجليكول العديد من الفوائد للكركمين المغلف وحسنت بشكل عام من الخصائص البيولوجية للكركمين كما اظهرت نتائج متقدمة في التئام الجروح عند استخدام نفس الجرعة والتوقيت.