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HEALING EFFECT OF TOPICALLY APPLIED NANO-CURCUMIN ON BUCCAL TRAUMATIC ULCER IN ALBINO RATS (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Background: Curcumin possesses numerous biological advantages, such as antioxidant, anti-inflammatory, antimicrobial and anticancer properties. However, its practical application is hindered by its low solubility and short plasma half-life. Therefore, this study seeks to evaluate the impact of topically administered Nano-curcumin on the healing of experimentally induced traumatic ulcers in the buccal mucosa of albino rats.

Material and methods: Fifty-four adult albino rats were randomly assigned to five groups. The negative control group (n=12) received no ulcer induction or treatment and was euthanized at two time points: on the 7th day and the 14th day. **2-Positive control group:** (n=6), buccal traumatic ulcers were induced, and the rats were euthanized after 2 hours. **3-Self-healing group:** (n=12), induction of traumatic ulcer in buccal mucosa of rats, then euthanization after 7th and 14th days (6 rats for each intervals) for self-healing evaluation. 4-**Curcumin treated group:** (n=12), induction of buccal traumatic ulcer, curcumin gel was topically applied on ulcer twice daily, euthanization after 7th and 14th days (6 rats for each intervals). **Nano-Curcumin group:** (n=12), induction of buccal traumatic ulcer, Nano-curcumin emulsion was topically applied on ulcer twice daily, euthanization after 7th and 14th days (6 rats for each intervals). Ulcer healing was evaluated by Histopathological and immuno-histochemical assessments for TGF-βII and E-Cadherin.

Results: Nano-Curcumin treated group indicating potential for ulcer healing histopathologically through re-epithelization and collagen deposition without granulation tissues in short time, and immuno-histochemically through recording high immuno-expression of TGF-βII and E-Cadherin compared to other studying groups in albino rates.

Conclusion: Nano-curcumin represent effective alternative to conventional medication and has superiority on native curcumin regarding the acceleration of buccal ulcer healing due to better solubility and enhanced Nano-properties.

KEY WORDS: Curcumin, Nano Particles, Wound Healing, TGF-β, E-cadherin

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INTRODUCTION

Injuries to the oral mucosa that disrupt its continuity and causing pain can significantly affect both the normal physiological function of the oral cavity and the individual's quality of life. Thus, identifying a safe and effective treatment to address this issue and expedite the healing process is crucial. [1]. Oral mucosal ulceration is damaged oral epithelium and its underlying lamina propria, characterised by inflammation and necrosis of the connective tissue [2]. Wound healing is a multifaceted process characterized by a series of intricate cellular and biochemical events that work to restore the strength, structure, and functional integrity of damaged tissues [3]. The healing process includes three overlapping phases: the inflammatory phase (I), the proliferative phase (II) or new tissue formation (re-epithelialization, angiogenesis, and proliferation), and finally the tissue remodelling of extracellular matrix (ECM). These phases are caused by intricate signalling network that includes growth factors, chemokines, and cytokines [4].

Curcumin, also known as diferuloylmethane, is the primary curcuminoid that is extracted from the turmeric plant, also known as Curcuma longa turmeric. It possesses anti-inflammatory, hypoglycemic, and antioxidant effects, making it an exceptionally pleiotropic structure. On top of that, it contains antibacterial properties and the ability to cure wounds [5,6]. By acting on several stages of wound repair, Curcumin accelerate the healing process through its antioxidant activities [7] and anti-inflammatory actions [8]. Beneficial effects on the production of granulation tissue [9], collagen deposition [7] and wound contraction [10], as well as the induction of re-epithelization [11].

It has been proven that the therapeutic efficacy of curcumin is restricted due to its low bioavailability, which is caused by low absorption, rapid metabolism, and rapid removal from the body [12]. In order to overcome these challenges, researchers are currently concentrating their efforts on developing innovative curcumin delivery formulations and technologies that enhance stability, bioavailability, and targeted delivery [13]. A wide range of topical formulations of curcumin, including fibres, films, hydrogels, emulsion, and various nano-formulations, have been produced with the purpose of delivering curcumin to specific areas of the body that have been specifically injured [14]. The development of nano-range formulations of curcumin, which are commonly referred to as "Nano-curcumin," has provided conclusive evidence that this drug is capable of accomplishing all of its biological effects [15]. In light of this, the purpose of the present investigation was to determine whether or not the topical use of nano-curcumin may improve the healing process of buccal traumatic ulcers.

Null Hypothesis: There is no discernible impact that the topical administration of nano-curcumin has on the healing process of severe mouth ulcers.

MATERIALS AND METHODS

Ethical Approval

The experimental methodology adheres to the Guidelines that are utilised for Animal Experimentation and has been accepted by the ethical committee Faculty of Medicine, South Valley University, Qena, Egypt with the permission number (SVU 695/2023), the study methods were carried out in accordance with the recommendations of World Health Organisation (2011) for Experimental Animals.

Sample size determination:

According to Faul et al. (2007) [16], the computation of the sample size was carried out with the help of G*Power version 3.1.9.2. In previous studies, the effect size conventions (f) were found to be 0.61, which is considered to be a large value. This was determined by using an alpha (α) level of 0.05 and a beta (β) level of 0.05. The power of

the study was determined to be 95%. The estimated sample size (n) should be at least 54 samples, with each group consisting of 12 samples. Additionally, there should be 6 samples for the positive control group.

Sample selection and grouping

Fifty-four male albino rats, each weighing between 150 - 200 grams, were utilized. They were accommodated in groups of three per cage throughout the study duration, with unrestricted access to tap water and standard chow. The rats were maintained in climate-controlled environments, with room temperatures set between 21 to 23°C and relative humidity levels kept at 60-65%. Furthermore, the conditions of their dwelling consisted of a regular cycle consisting of twelve hours of light followed by twelve hours of darkness continuously.

All rats were numbered from 1 to 54, and then were randomly divided by the web site by https://www.randomizer.org/ into five groups as follows:

Negative control group: (n=12), no ulcer or any treatment was done. Rats were euthanized according to intervals 7th and 14th day (6 animals per each period)

Positive control group (Zero-day ulcer): (n=6), Buccal traumatic ulcers were induced, and the rats were euthanized after 2 hours.

Self-recovery group: (n=12) Buccal traumatic ulcer were induced as in positive control group, animals were euthanized according to healing intervals 7th and 14th day (6 animals per each period) [17].

Curcumin group: (n=12) In the same manner as the positive control group, a buccal traumatised ulcer was generated. A 1% curcumin oral gel was applied topically (twice day) to the location of the ulcer using mucoperiosteal elevators in three layers. This was done after the ulcer had been inducing for a period of twenty-four hours. Following the application of the initial layer to the ulcer, the substance was rubbed for a duration of one minute until it was completely absorbed. After applying the second layer to the ulcer, the substance was rubbed for an additional minute until it was completely absorbed by the surrounding tissue. In conclusion, the third layer was utilised as a dressing that had the effect of totally covering the ulcer [18]. Animals were euthanized according to healing intervals 7th and 14th day [17] (6 animals per each period)

Nano-curcumin group: (n=12), induction of buccal traumatic ulcer, after 24 hours of ulcer induction, Nano-curcumin emulsion was applied topically (twice daily) to ulcer area as in curcumin group, animals were euthanized according to healing intervals 7th and 14th day (6 animals per each period)

Materials

1% curcumin oral gel (Abbott Healthcare Pvt., Ltd., Mumbai, India) (Curenext oral gel® 10 mg/g) purchased from Sigma company, Egypt.

Curcumin Nano-emulsion was purchased from Nano-Gate company Egypt. According to manufacturer, the materials used are curcumin, Tween 80, methanol, purified water, ethanol 96%, and soybean oil. These additives to generally aims to improve the solubility, stability, and bioavailability of curcumin. Available evidence suggests that these additives do not negatively impact the action of curcumin. Instead, they often enhance its effectiveness [19].

In a beaker glass, a pre-emulsion solution was prepared by combining 1 milligram of curcumin, 62.5 milligram of Tween 80, 200 microliters of soybean oil, and 5 millilitres of filtered water. The vial bottle was then filled with 2.5 millilitres of milling beads and one magnetic stirrer once this stage was completed. After that, the pre-emulsion solution was added to the bottle of vials. In order to determine the optimal milling period for the solution, it was milled above the stirring plate for 1, 4, 8, and 24 hours. Between 200 and 300 microliters

of curcumin Nano-emulsion were extracted from the vial bottle and stored in an Eppendorf tube at each and every milling time that was calculated [20].

Characterization of Nano-Curcumin

The characterization methods of Nano-curcumin were carried out in Central laboratory unit Faculty of science, South Valley University, Qena, Egypt. Through the use of Transmission Electron Microscope (TEM), X-ray powder diffraction (XRD), and Ultra violet/ Visible spectrophotometry (UV/Vis), Nano-curcumin was analysed to determine the size of its particles as well as the surface morphology of its particles. In order to get XRD patterns, a D 5000 Siemens diffractometer from Germany was utilised. The radiation used was CuKα, with a wavelength of 0.154178 nm of wavelength. TEM (Joel Jem-1010, Japan) was utilised in order to investigate the dimensions and characteristics of the materials that were acquired. The optical properties were investigated using a computerised analytikjena SPECORD 200 Plus spectrophotometer from Germany. The wavelength range that was investigated was from 300 to 800 nm, and the temperature was kept at room temperature.

Buccal traumatic ulcer induction procedure

Initially, a clinical assessment was carried out to determine the integrity of the buccal mucosa in the area of interest. The albino rats assigned to the positive control, self-recovery, curcumin, and Nano-Curcumin groups were anesthetized using a freshly prepared mixture of ketamine (90 mg/kg, Medistar, Ascheberg, Germany) and xylazine (10 mg/kg, Riemser, Greifswald, Germany), administered intraperitoneally. Subsequently, each rat underwent an oral cavity antisepsis procedure using 0.12% chlorhexidine, applied with individual, single-use cotton swabs. Tissue punch biopsy was used for inducing traumatic circular ulcer of 4 mm diameter with 1 mm depth in buccal mucosa [21] (Figure 1).

Assessment methods

- **Histopathological evaluation**: buccal mucosal samples were obtained at the end of experiment (after euthanizing at 7th and 14th day) and fixed in 10% buffered formic acid. The collected specimens were subjected to standard processing techniques and sectioned into slices measuring 4–6 µm in thickness. The haematoxylin and eosin (H&E) staining technique was utilised for the purpose of histological examination of these sections.

- **The immuno-histochemical detection system of Transforming Growth factor-βII (TGF-βII) and E-Cadherin:** Adhering to the instructions provided by the manufacturer, the ultra-vision mouse tissue detection system was utilized for this

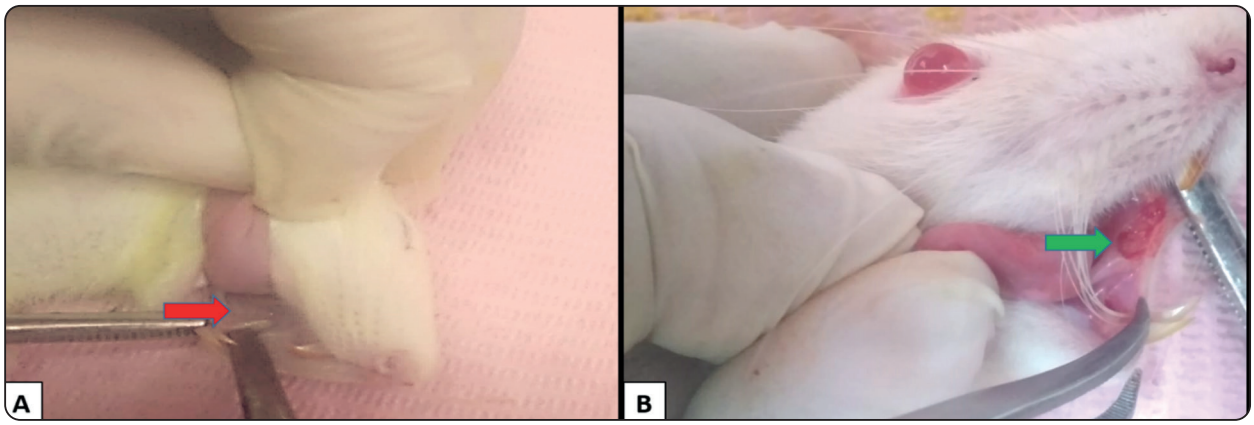

Fig. (1) A photograph showing (A) Normal intact Baccual mucosa (B) The buccal traumatic ulcer created by punch biopsy (Zero-day ulcer)

study. This system is inclusive of a monoclonal antibody designed to couple with the primary antibody, specifically the **TGF-βII** (Catalog #MA5-37505, Invitrogen, USA) and **E-cadherin** (Catalog #EP700Y, Cell Marque, USA) monoclonal antibody. The constituents of the kit together establish a streptavidin-biotin based immunoenzymatic antigen detection system (ultraView Universal DAB Detection Kit, Catalog #760-500, Roche, Switzerland) The process comprises incubation in a sequential fashion, beginning with a primary antibody that is not conjugated and specific to the target antigen, and then proceeding to the addition of a biotinylated secondary antibody that interacts with the first antibody. Subsequently, this complex is treated with an enzyme-conjugated streptavidin and DAB chromogen. A section's positivity or negativity has been determined by the absence or presence of brown staining (nuclear and cytoplasmic for TGF-βII and cytoplasmic for E-cadherin). To quantify markers staining, the percentage of area showing positive staining was determined using light microscopy at a standardized magnification (x200). An image analysis system was used to calculate the Positive Index (PI) (represent the proportion of individual cells that are positively stained), aiding in evaluating the percentage area of tissue area that stained positively. This analysis was carried out with the assistance of a computer system known as the Leica Quin 500, which is situated in Wetzlar, Germany. This system is equipped with a colour video camera, a colour display, and a central processing unit (CPU) from an IBM personal computer, all of which are connected to the microscope. An automatic calibration process was initially performed on the image analysis system in order to transform the pixel measurements that were provided by the programme into actual micrometres.

Statistical analysis:

Each and every piece of information was computed, tabulated, and statistically analysed using appropriate statistical tests, as detailed below. For the purpose of determining whether or not the samples followed a normal distribution, a Shapiro-Wilk test was carried out. When calculating descriptive statistics, the mean and standard deviation (SD) were used as the units of measurement. A T-test with paired samples was utilised in order to compare the results of the different time periods (7 and 14 days for each group). To make a comparison between the groups that were being investigated, a one-way analysis of variance (ANOVA) was utilised. For the purpose of making pairwise comparisons between the groups, the Bonferroni post hoc test was carried out. A statistically significant P value is one that is less than or equal to 0.05. The statistical analysis was carried out by utilising the computer programme SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, New York: IBM Corporation) at significant levels of less than 0.05 (P-Value).

RESULTS

Nano-Curcumin characterization (Figure 2):

For the purpose of determining the crystalline nature and degree of purity of curcumin nanoparticles, XRD was utilised (Figure 2). At a diffraction angle of 2 θ, the following peaks were detected to be characteristic of the sample that had been prepared: 12.16°, 17.24°, 18.16°, 19.46°, 21.22°, 23.58°, 24.58°, 25.58°, 27.38°, 29.04°, 34.36°, 44.0°, and 77.76°. This demonstrated that the sample that was generated included crystalline curcumin [22], and the prevalence of broad peaks demonstrated that tthe obtained curcumin was of nano size. On top of that, the XRD pattern has not shown any other impurities, which is another evidence that our sample is completely free of any impurities.

In order to get the average crystal size (d), Scherer's formula was utilised, and the width of the peak was used as the basis for the calculation [23],

$$d=0.9\lambda/\beta \cos\theta$$

Given that λ represents the wavelength of the X-ray that is being utilised, β represents the full width at half maximum, and θ represents the Bragg's angle of reflection. A crystallite size of 23 nanometres was used as the average for the calculation of the peaks in the XRD pattern of curcumin nanoparticles.

TEM images confirm the production of spherical curcumin nanoparticles of around 17 nm in radius. The UV/Vis optical absorption spectra of the synthesised curcumin nanoparticles show a visible broad band centred at 310 nm, which is attributable to the production of curcumin in its nano-form. It is widely recognised that bulk curcumin exhibits a noticeable absorption band at 450 nm [24], indicating that Nano-curcumin typically displays a blue shift.

Histopathological (H&E) observations:

Negative control group:

Upon examination of the buccal mucosa in the negative control group on the 7th and 14th day, it was seen that the epithelium consisted of keratinized stratified squamous cells with rete ridges that were regular, broad, and short. Normal architecture of underlying lamina propria. Otherwise, minor salivary glands and muscle bundles in between well-organized collagen fibres were present in the submucosa (Figure 3.A, B).

Positive control group (Zero-day ulcer):

Within the positive control group, which corresponds to the zero-day group, the ulcerated area showed a total loss of epithelium. Inflammatory cells also begin to infiltrate along with the underlying connective tissues which appeared somehow teared. Margins of the ulcer are marked off by keratinized squamous epithelium (Figure 3.C).

Self-healing group:

On the 7th day after the ulceration, there was loss of the epithelial coverage over the ulcer area , but no significant reduction in the size of the ulcer. The epithelial cells along the edge of the ulcer exhibited enlargement, and certain nuclei displayed signs of cell division. The ulcer bed included an abundance of inflammatory cells and dilated blood arteries loaded with red blood cells. On the 14th day, the ulcer area was covered by a thin layer of keratinized epithelium, with mostly absent epithelial ridges. Upon increasing the magnification, the epithelium was observed to include both mitotic and many apoptotic cells. The lamina propria's connective tissue exhibited localised regions of granulation tissue, characterised by the infiltration of inflammatory cells, disorganised collagen fibres, and dilated blood vessels filled with red blood cells (Figure 4).

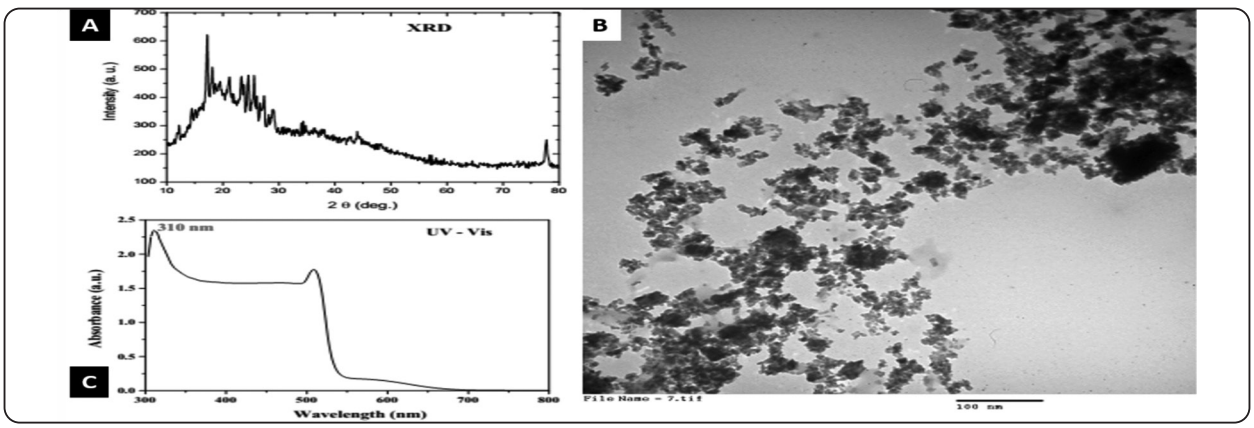

Fig. (2) Nano-Curcumin characterization, (A) XDR pattern (B) TEM (C) UV/Visible spectrophotometry.

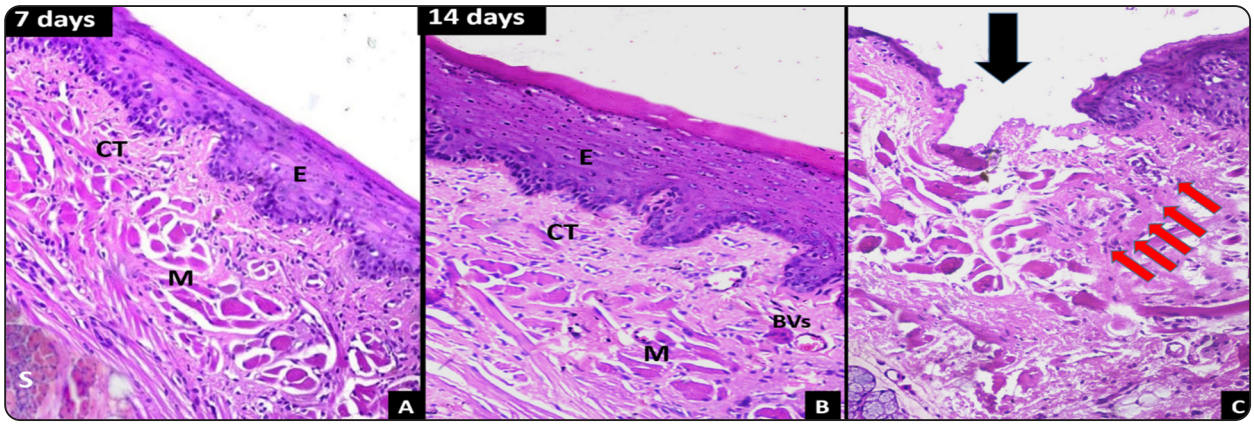

Fig. (3) Photomicrographs of H&E-stained sections of buccal mucosa showing (A, B) Negative control group at 7th and 14th days. (C) Positive control group (Zero-day group). (E) Epithelium, (CT) connective tissue, (M) muscle bundle, (BVs) Blood vessels (s) Minor salivary glands, (Black arrow) ulcer site, (Red arrows) inflammatory cells. (H&E mag. X200)

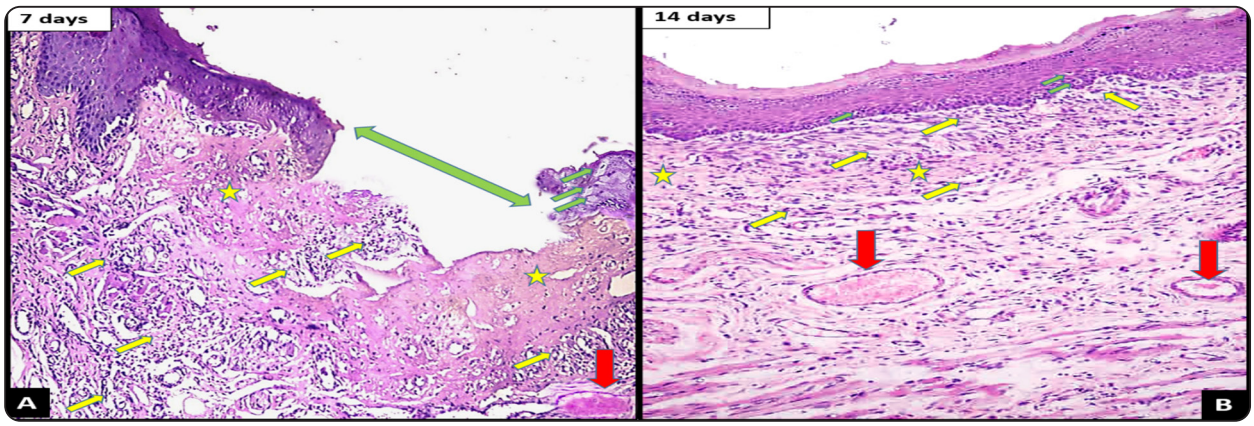

Fig. (4) Photomicrographs of H&E-stained sections of buccal mucosa showing ulcer area of Self-healing group at (A) 7th day(B) 14th day. (Yellow star) granulation tissue, (Yellow arrows) inflammatory cells, (Double head green arrow) ulcer size, (red arrow) dilated blood vessels, (green arrows) apoptotic epithelial cells) (H&E mag. X200)

Curcumin group:

On the 7th day after the ulceration, there is still a lack of epithelial coverage. There is some weak re-epithelization, with a thin layer of epithelium forming at the edge of the ulcer, causing a decrease in the size of the ulcer. The epithelial margin of the ulcer showed swollen and apoptotic cells. The area beneath the ulcer site, known as the lamina propria, was filled with granulation tissue that had been infiltrated by inflammatory cells. Additionally, blood vessels in this area were shown to be engorged with red blood cells (RBCs). Otherwise, at the 14th day, the ulcer of curcumin group showed complete coverage of ulcer with moderate thickness of keratinized epithelial layer with few short epithelial ridges. The underlying lamina propria showed organized collagen fibres with scattered inflammatory cells and newly formed blood vessels (Figure 5).

Nano-curcumin group:

Re-epithelialization via creeping of the two epithelial margins (showing apoptotic cells) across the ulcer bed was observed at the 7th day post-ulceration, resulting in a reduction of ulcer size. The lamina propria's connective tissue displayed many fibroblasts, disorganised collagen fibres, and a small number of dispersed inflammatory cells. On the 14th day, however, numerous well-developed epithelial ridges of the epithelium were visible along with the re-epithelization of keratinized epithelium over the lamina propria with nearly normal epithelial thickness and architecture. There were numerous fibroblasts and well-organized collagen fibres in the underlying connective tissue with dispersed inflammatory cells. Some blood vessels were normal, unless some dilated blood vessels were observed. (Figure 6)

Immuno-histochemical localization of TGF-βII

Positive and negative control group at the 7th and 14th intervals showed moderate immuno-reactivity in epithelium and weak immuno-reactivity in lamina propria to TGF-βII (Figure 7).

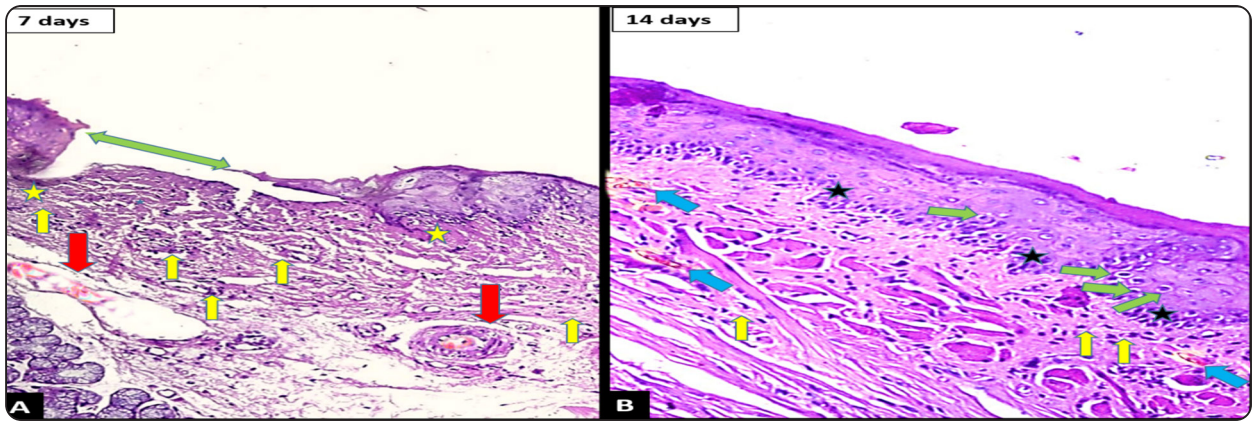

Fig. (5) Photomicrographs of H&E-stained sections of buccal mucosa showing ulcer area of Curcumin group at (A) 7th day (B) 14th day. (Yellow star) granulation tissue, (Yellow arrows) inflammatory cells, (Double head green arrow) ulcer size, (red arrow) dilated blood vessels, (green arrows) apoptotic epithelial cells, (Blue arrows) newly formed blood vessels, (Black star) short epithelial ridges (H&E mag. X200)

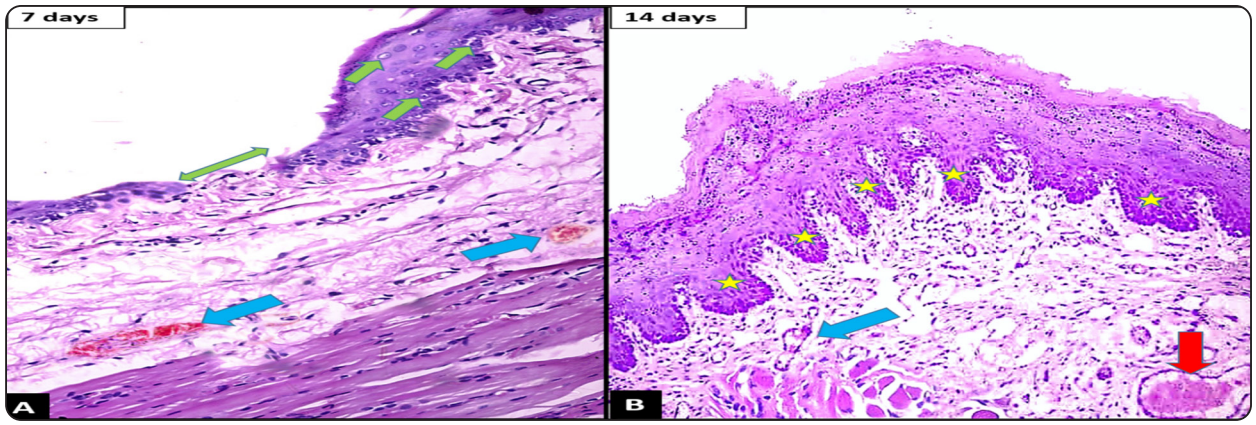

Fig. (6) Photomicrographs of H&E-stained sections of buccal mucosa showing ulcer area of Nano-curcumin group at (A) 7th day (B) 14th day. (Double head green arrow) ulcer size, (green arrows) apoptotic epithelial cells, (Yellow star) well developed epithelial ridges, (Blue arrows) newly formed blood vessels (Red arrow) dilated blood vessels (H&E mag. X200)

Otherwise, **self-healing group** showed weak immuno-reactivity in both epithelium and lamina propria to TGF-βII at 7th and 14th intervals. **Curcumin and Nano-curcumin groups** showed strong immuno-reactivity in epithelium with moderate immune- reactivity in lamina propria into TGF-βII at both 7th and 14th intervals (figure 8)

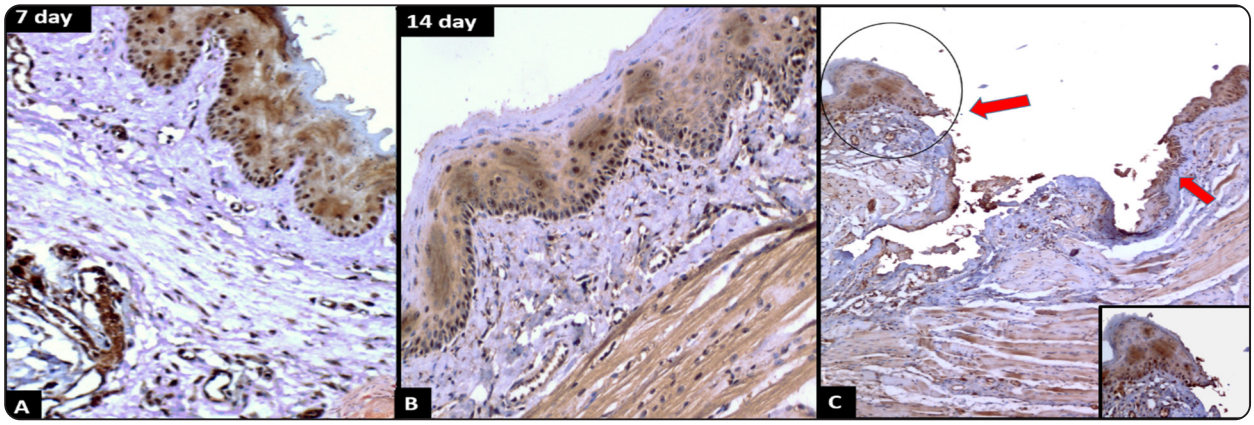

Fig. (7) Photomicrographs showing epithelial and lamina propria anti TGF-βII reaction. (A) Negative control at 7th day (B) Negative control at 14th day (C) Positive control (100 Magx.) with its high magnification. (red arrows) margin of ulcer (Mag. X. 200)

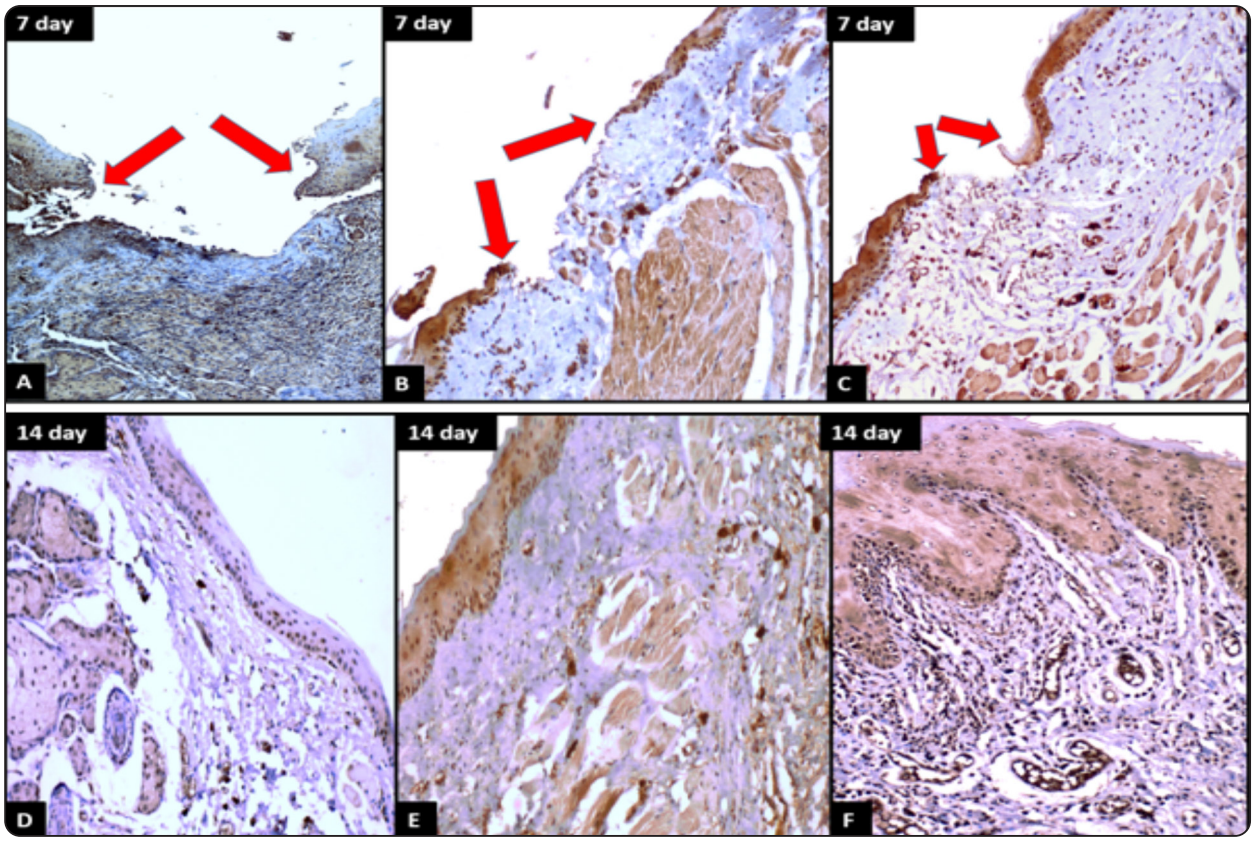

Fig. (8) Photomicrographs showing epithelial and lamina propria anti TGF-βII reaction (A,D) Self-healing group at 7th, , 14th day (B, E) Curcumin group at 7th, 14th day (C, F) Nano-curcumin group at 7th day, 14th day. (Red arrow) ulcer margin (Mag. X. 200)

Immuno-histochemical localization of E-Cadherin

Positive control and negative control group at 7th and 14th intervals showed moderate immuno-reactivity to E-Cadherin (figure 9).

Self-healing group showed weak immuno-reactivity at 7th day while at 14th day showed moderate immuno-reactivity to E-Cadherin. **Curcumin and Nano-curcumin groups** showed moderate immuno-reactivity at 7th day while at 14th day revealed strong immunoreactivity to E-cadherin (Figure 10).

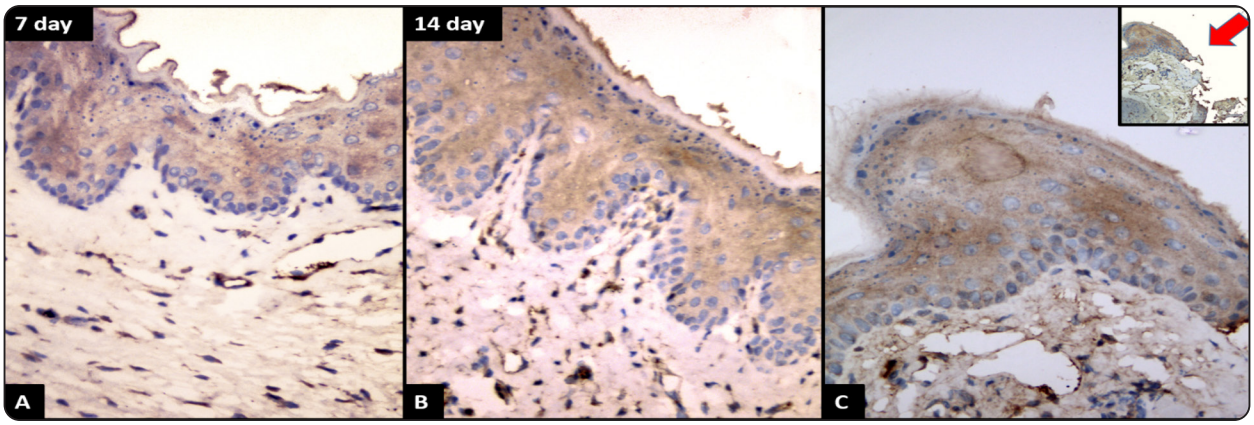

Fig. (9) Photomicrographs showing epithelial anti E-Cadherin reaction. (A) Negative control at 7th day (B) Negative control at 14th day (C) Positive control with its low magnification (Mag. X 200), (Red arrow) ulcer margin (Mag. X 400)

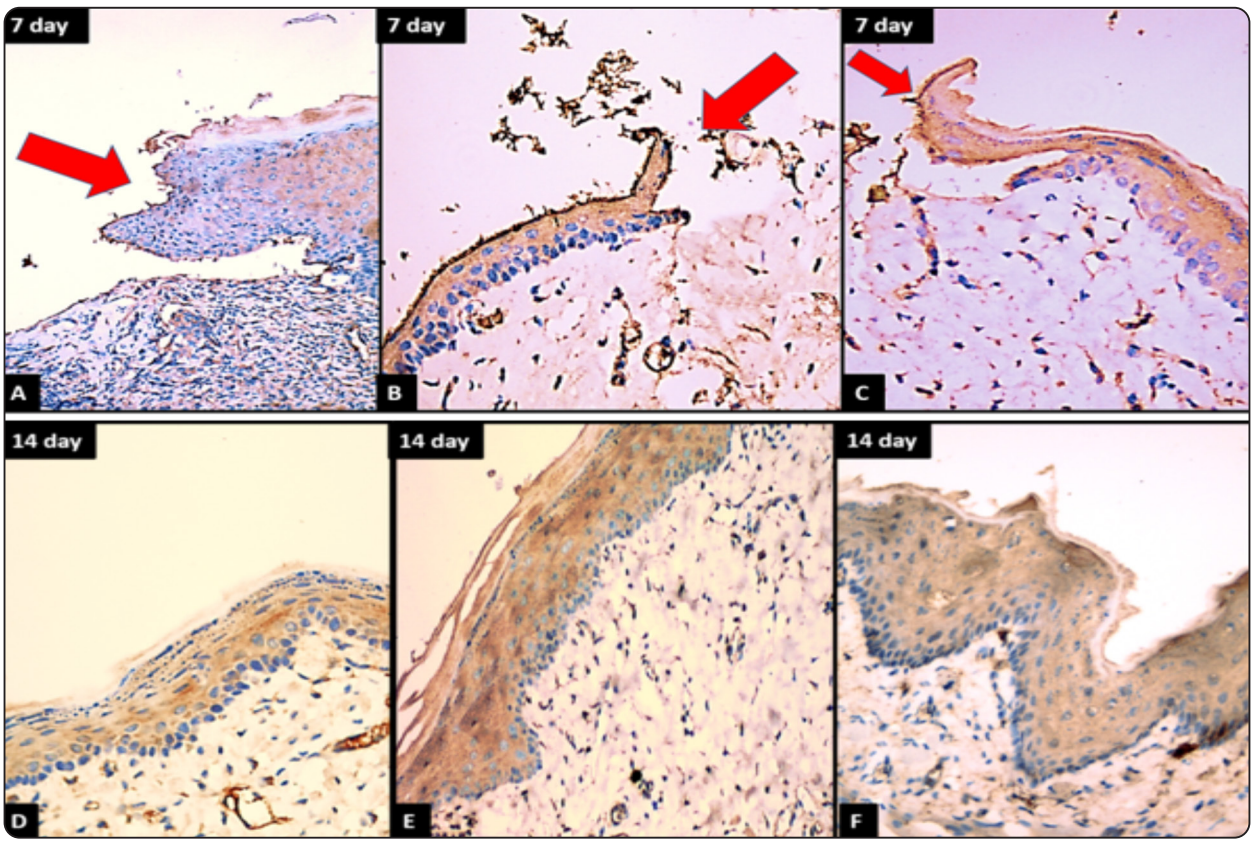

Fig. (10) Photomicrographs showing epithelial E-cadherin reaction (A,D) Self-healing group at 7th, , 14th day (B, E) Curcumin group at 7th, 14th day (C, F) Nano-curcumin group at 7th day, 14th day. (Red arrow) ulcer margin (Mag. X. 400)

Statistical results

Statistical analysis of Transforming growth factor-βII expression:

Mean and Standard deviation (SD) values for TGF-**βII** for positive, negative, **Self-healing, curcumin and** Nano-curcumin groups were presented in table 1.

A- Intergroups comparisons:

Concerning the expression of Transforming growth factor-βII in the epithelium, it showed highly significant difference among experimental groups. The mean TGF-βII was higher in nano- curcumin followed by curcumin and the lowest values were observed in self -healing group (table 1)

B- Intragroup comparisons:

Within each group, the values of TGF-βII at 14th day showed higher values compared to the 7th day values. This increase was statistically significant for nano-curcumin group and not for the rest of the groups.

Regarding the expression of TGF-βII in lamina propria**,** mean and standard deviation (SD) values for TGF-βII for negative, positive, Self-healing, curcumin and nano-curcumin groups were presented in table 2.

A- Intergroups comparisons:

There was a statistically significant difference in the expression of TGF-βII in the lamina propria of the buccal mucosa between different groups, both at 7th and 14th day (P<0.001). Pairwise comparison revealed significant differences between each group, except for Curcumin with Nano-Curcumin and Curcumin with the negative control at 7th day. Additionally, there was a significant difference between Curcumin and Nano-Curcumin at 14th day. The mean values of TGF-βII in Lamina propria were higher in Nano- Curcumin followed by Curcumin and Negative and positive control while the lowest values were recorded for self -healing at 7th and 14th day (table 2)

B- Intragroup comparisons:

Within each group, the values of TGF-βII in lamina propria showed statistically significant increase in mean value measured at 14th day for self-healing (P=0.002) and curcumin (P=0.001) while there is no significant increase for negative (P=0.368) and Nano-curcumin (P=0.175).

TABLE (1) Immunohistochemical expression of TGF-βII in Epithelium.

	7th day	14th day	% Change	*Paired T- test*	*P value*
Positive			***38.74***±0.97		
Negative	36.82±1.02[b]	38.61±1.16[b]	**10.29**	**2.50**	**0.169**
Self-healing	23.36±2.30[c]	24.36±1.18[c]	**4.28**	***1.36***	***0.229***
Curcumin	39.53±1.01[a]	41.75±1.92[b]	**5.62**	***2.084***	***0.092***
Nano- Curcumin	40.19±0.80[a]	44.09±0.62[a]	**9.70**	***8.69***	***<0.001*****
F test	***135.013***	***181.98***			
***P* value**	***<0.001*****	***<0.001*****			

*****; mean significant difference***

A,b,c (different supper script) mean significant difference between groups at the same time (column) using one way ANOVA at P<0.05

TABLE (2) Immunohistochemical expression of TGF-βII in Lamina propria

	7th day	14th day	% Change	*Paired T- test*	*P value*
Positive			10.49±1.10		
Negative	10.23±1.34[b]	11.71±1.20[b]	10.06	***0.990***	***0.368***
Self-healing	2.43±0.60[c]	3.07±0.58[c]	26.34	***5.960***	***<0.002****
Curcumin	10.64±0.61[ab]	13.37±0.77[a]	25.78	***7.722***	***<0.001****
Nano- Curcumin	11.65±1.02[a]	14.16±1.68[a]	21.55	***1.580***	***0.175***
F test	***120.78***	***113.68***			
***P* value**	***<0.001****	***<0.001****			

***; mean significant difference*

A,b,c (different supper script) mean significant difference between groups at the same time (raw) at P<0.05

Statistical analysis of E-Cadherin expression:

Mean and Standard deviation (SD) values of **E-Cadherin** for positive, negative, **Self-healing, curcumin and** Nano-curcumin groups were presented in table 3

A-Intergroups comparisons:

A statistically significant difference was seen between the various groups in relation to E-Cadherin, both at 7th ,14th day (P<0.001). Pairwise comparison revealed significance between each group, except for Curcumin and Nano-Curcumin after 7 days, as well as between Curcumin and the negative control after 14 days. The mean E-Cadherin was higher in Nano- Curcumin followed by Curcumin and Negative and positive control while the lowest values were recorded for self -healing after 7 days and 14 days (table 3).

B-Intragroup comparisons:

TABLE (3) Immunohistochemical expression of E-cadherin

	7th day	14th day	% change	*Paired T- test*	*P value*
Positive		***26.67±3.15***			
Negative	**35.87±2.44[b]**	**38.32±1.11[b]**	**6.83**	**2.52**	**0.083**
Self-healing	**19.78±0.65[c]**	**22.47±1.79[c]**	**13.60**	***3.75***	***<0.013****
Curcumin	**37.94±1.04[a]**	**41.64±2.01[b]**	**9.75**	***10.14***	***<0.001****
Nano- Curcumin	**39.48±1.92[a]**	**43.98±1.05[a]**	**11.40**	***5.37***	***<0.003****
F test	***200.55***	***239.36***			
P value	***<0.001****	***<0.001****			

***; mean significant difference*

A,b,c (different supper script) mean significant difference between groups at the same time (raw) at P<0.05

For all groups, there was a significant increase in mean value of E-cadherin measured at 14th day compared to 7th day for positive, negative, Self-healing, curcumin and Nano-curcumin groups with mean change 6.83%, 13.60%, 9.75% and 11.40 respectively.

DISCUSSION

The current research used curcumin for its anti-inflammatory, anti-bacterial and anti-oxidant properties [25, 26] Moreover, curcumin in Nano-scales is better for solubility properties[27,28], otherwise using Tween80 as a surfactant to produce an emulsion for better application and retention on oral ulcer during treatment duration.

The present study employed Albino rats because to their cost-effectiveness and the valuable insights they offer. The use of rats yielded rudimentary data, although it remains potential of stimulating additional investigation in this field of expertise. Selecting male rats eliminated the impact of sex hormones on the process of wound healing, which is recognised as a regulator of oral mucosal wound healing [29]. The time intervals of 7 and 14 days after ulceration were selected based on a prior study that identified histological alterations over these specific periods [30, 4].

According to histopathological results of self-healing and curcumin treated rats in the current study, Curcumin-treated rats may show evidence of accelerated re-epithelialization with reduction in ulcer size at 7th days. While at 14th day, full coverage by epithelium with short few epithelial ridges and well organized lamina propria. This suggests that curcumin enhances the growth and movement of epithelial cells, resulting in quicker healing of wounds compared to ulcers that naturally heal and show no significant decrease in size by the 7th day.

On the 14th day, ulcers of self –healing group showed a thin layer of epithelium with dying and swelling cells, as well as a lamina propria with poorly defined collagen fibres and localised granulation tissue. There was also an enlargement of blood vessels and the presence of inflammatory cells. **Sideek et al. (2022)** determined that curcumin enhances the speed of wound healing by influencing all stages of the wound healing process, thanks to its antibacterial, antioxidant, and anti-inflammatory characteristics [31]. The previous study may provide an explanation for the outcomes observed in the curcumin group.

The histopathological results of curcumin and self-healing groups of current study come in agreement with results of **Shamsah and Zaidan 2020** that concluded that topical curcumin application in major oral mucosal ulcer showed more advanced healing features compared to the self-recovery group at 3rd, 7th and 14th day , including better epithelialization, reduced inflammation, and enhanced tissue remodelling [4]. Moreover, study of **Osman et al., 2022** that demonstrate that treatment of tongue ulcer with native curcumin at 12th day showed full coverage of ulcer with increase in newly formed collagen fibres comparing to the self –healing group. Otherwise, the ulcer bed at the 6th day was filled with granulation tissue infiltrated by inflammatory [32] cells which come in disagreement with results of current study at 7th day.

Unlikely, when comparing the current histopathological results of curcumin and nano-curcumin groups, nano-curcumin group demonstrated more accelerated healing rate at 7th day with marked contraction of ulcer size with no granulation tissue and moderate inflammatory cells infiltration. Moreover, at 14th day the ulcer was covered with almost normal epithelial thickness and well developed numerous epithelial ridges, well organized underlying collagen fibres with scattered inflammatory cells infiltrations. These results came in agreement with **Osman et al. 2022** investigation that demonstrated treatment with curcumin-loaded nanoparticles (Cr PLGA NPs) may promote the

formation of collagen fibres and potentially enhance the healing process of tongue ulcers in rats compared to curcumin group [32].

A comprehensive review of **Kumari et al, 2022** emphasizes the importance of Nano-formulations in enhancing the solubility, bioavailability, and controlled release of curcumin for improved wound healing [33], which give explanation about better histopathological picture of nano-curcumin group of present study comparing to curcumin group.

Transforming growth factor β (TGF-β) is a group of versatile cytokines that includes three isoforms: TGFβI, II, and III. Among them, TGFβ1 and TGFβ2 play a major role in the healing of skin wounds [34]. TGF-β plays a vital role in maintaining the balance of the outer layer of the skin and has been demonstrated to be a significant contributor in all stages of wound healing by controlling the activities of fibroblasts, keratinocytes, monocytes, endothelial cells, and other types of cells.

Based on current immuno-expression of TGF-βII in both epithelium and lamina propria, Nano-curcumin group at 7th day recorded the highest mean value comparing to other groups then curcumin group. The Increasing expression of TGF-βII in epithelium and lamina propria indicates re-epithelization [35] and collagen deposition [32] for regaining epithelial integrity and underlying lamina propria in the induced buccal ulcer.

The rate of increase in TGF-βII expression accompanying the healing process was high at 7th day for curcumin and nano-curcumin groups compared to the self-healing group. By reaching the 14th day the rate of increase in TGF- βII expression slowed down denoting the finalisation of the healing process.

The present immunohistochemistry findings align with those reported by **Mani et al. in 2002**. **Mani's group** observed that the wound bed exhibited a higher quantity of cells with positive staining and a stronger intensity of staining for TGF- β during the initial phase, in comparison to the positive control group. Their research shown that curcumin improved the healing of the skin by boosting the activation of TGF-β [35].

Furthermore, the group treated with nano-curcumin exhibited a higher level of immuno-positivity compared to the positive control group at 7th day after ulceration. However, the immuno-expression decreased in the curcumin treated group at 14th day after ulceration. Also, these results were compatible with those of **Shamsah and Zaidan 2020** who concluded that TGFβII-Rs expression was increased in all epithelial keratinocytes and lamina properia, after topical application of curcumin, moreover, Curcumin has a greater effect on TGFBII-R than on TGF-β itself, which increase expression with increase period of treatment [4].

E-cadherin is a glycoprotein located in the cell membrane that has a crucial function in preserving the integrity of cell-to-cell adhesion[36]. Curcumin regulates the activity of E-cadherin[37]. In the process of wound re-epithelialization, E-cadherin is crucial for managing the orientation of cells [38], as well as their specialisation, proliferation, movement [39], and ultimately, the development of connective tissue throughout the healing process. Furthermore, it assists in the prevention of scarring by facilitating the optimal deposition of collagen I and III in their appropriate proportions. Therefore, E-cadherin was used as the marker to assess the restoration of epithelial cells following the development of buccal ulcers.

Based on the E-Cadherin immunohistochemical results of current study, the highest mean values belonged to Nano-Curcumin then curcumin at 7th and 14th day when comparing to negative, positive and self-healing groups, which indicate regaining the epithelial integrity and ulcer sealing. These findings align with the research conducted by **Guo et al., 2021** which concluded that curcumin increases the production of E-cadherin via activating heat shock protein 40 (HLJ1), also known as DNAJB4. Up regulation of E-cadherin during the wound

healing indicates the re-epithelization and regaining epithelium integrity .

Based on histological and immuno-histochemical results, Nano-Curcumin and Curcumin have abilities to accelerate wound healing. Moreover, Nano-Curcumin appeared superior and accelerated healing more than Curcumin. The enhanced effect of Nano-Curcumin could be explained by its improved stability, bioavailability and better solubility gained at the nanoscale.

CONCLUSION

Nano-curcumin represent effective alternative to conventional medication and has superiority on native curcumin regarding the acceleration of buccal ulcer healing due to better solubility and enhanced Nano-properties.

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