ORIGINAL ARTICLE

EFFECT OF *CURCUMIN* ON EPITHELIALIZATION AND COLLAGENIZATION IN INFLAMMATION

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ABSTRACT

Mouth ulcers are common oral lesions. In Pakistan oral hygiene conditions are poor, standard facilities are not available, medications are relatively expensive and multi-drug resistance is a threat to public health. Turmeric is one of the nature's most powerful healer. An experimental study was designed and conducted in Post Graduate Medical Institute, Lahore to evaluate the role of curcumin on epithelialization and collagenization in inflammation of buccal mucosa of rabbits. Forty adult rabbits were used and divided into 2 groups as A and B of 20 each. Each group was further subdivided in two groups of 10 each depending on the day of sacrifice i.e. 5th and 7th day after infliction of wound on buccal mucosa. After anesthesia, a circular 3mm wide and 1mm deep excisional wound was created on the right buccal mucosa adjacent to maxillary molar teeth of each rabbit, using a punch biopsy tool. Group A animals were left without any treatment while the animals of group B were given 30mg / kg body weight of curcumin daily by oral gavage. The histological analysis revealed that animals treated with curcumin experienced faster epithelialization and collagenization than that in the control group, thereby demonstrating the effectiveness of curcumin as anti-inflammatory agent in inflammation.

Key Words: *Curcumin, wound healing, buccal mucosa, epithelialization, collagenization*

INTRODUCTION

Curcumin is a natural product isolated from the plant rhizome Curcuma Longa. It is the major component of turmeric.[1] Turmeric has been known for its medicinal properties in the past. This source of curcumin helps in management of arthiritis, hyperlipidemia, metabolic syndrome, oxidative and inflammatory conditions.[2,3]

Curcumin exhibits multiple phenotypic expressions that interacts with various mechanisms of action and molecular targets including cytokines, growth factors, proteins, enzymes, and receptors.[4]

The mechanism of anti-inflammatory action is due to inhibition of arachidonic acid metabolism NF-□B, LOX, cytokines (ILs and TNF) and COX.[5] Curcumin is reported to stabilize lysosomal membrane and causes uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity.[6] It down-regulates the activity of lipoxygenase and inducible iNOS.[7] It is also known to inhibit the production of TNF-alpha, migratory inhibitory protein and IL-1, 2, 6, 8 and 12.[8] It increases synthesis of collagen and proliferation of fibroblasts at the site of wound by increasing Collagen III, total protein and DNA.[6, 7] Inspite of being an anti-inflammatory agent, the distinctive feature is least gastrointestinal side effects.[3]

Therefore, this study was done to establish the role of curcumin in inflammation on epithelialization and collagenization of buccal mucosa of rabbits.

METHODS

After approval from ethical committee, study was conducted at Post Graduate Medical Institute, Lahore. Forty adult rabbits were included. After giving anesthesia (ketamine 40mg/kg body weight and xylazine hydrochloride 5-13 mg/kg body weight intramuscularly)[9], an excisional wound of 3 mm wide and 1 mm deep was created on buccal mucosa of right side lateral to maxillary molar of every rabbit by using a standard punch biopsy tool. Simple random sampling using balloting method, was done to divide them in two groups as A and B on the same day. Group A animals were not given any treatment while group B animals were

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Received for Publication: Oct 17, 2018
Revised: Feb 28, 2019
Approved: Mar 1, 2019

given 30mg/kg body weight of curcumin daily by oral gavage. Group A and B is further subdivided depending on the day on which they were sacrificed after infliction of wound into A1, A2 and B1, B2. Group A1 having 10 number of animals were sacrificed on day 5 and group A2 having 10 number of animals were sacrificed on day 7, same was done for Group B1 and B2.

They were sacrificed by decapitation after giving deep anesthesia (ketamine 40mg/kg body weight and xylazine hydrochloride 5-13 mg/kg body weight intramuscularly).[9] Buccal mucosa of each animal, 5mm around the wound was dissected with the help of scalpel, blade and dissecting scissors. Sample taken was immersed in 10 % neutral buffered formalin (NBF) (Merck Pharma®) for 48 hrs. Tissue processing, embedding, sectioning and mounting of 3-5micrometer sections of tissue were obtained and stained with hematoxylin and eosin and Masson's Trichrome and observed by a single observer under light microscope using 100X as magnifying power.

Statistical analysis

Data entry and analysis was done by using SPSS 20. Quantitative data was presented by using mean ± SD. Qualitative data was presented by using frequency table and percentages. As data was qualitative in nature so the comparison of all groups in relation to epithelialization and collagenization was done by using Chi-Square test.

RESULTS

On day 5 in group A 70 % of the animals showed that 1/3 of the width of the ulcer was covered by epithelium. Only 30% animals showed 2/3rd epithelialization of the wound while no animal showed complete epithelialization. In group B the connective tissue showed signs of inflammation and the edges of epithelium were also proliferating towards the center demonstrating two third width of wound was epithelialized in 7 animals. Whereas no animal showed complete epithelialization.

On day 7, in group A 90% animals showed 2/3rd coverage by epithelium whereas only 1 animal showed complete epithelialization. None of the animal fell into 1/3rd epithelialized category. In group B complete epithelialization was observed in 70% rabbits as shown in Fig. 1, whereas none was included in the category of 1/3rd epithelialized tissue. The results show significant association between epithelialization and study groups on 7th day.

On day 5, in group A 70 % animals showed only few fibroblasts in the wound field, while none of the animals showed prominent fibroblasts which indicates that inflammation is in progression phase. In group B 60% animals showed invasion of predominant number

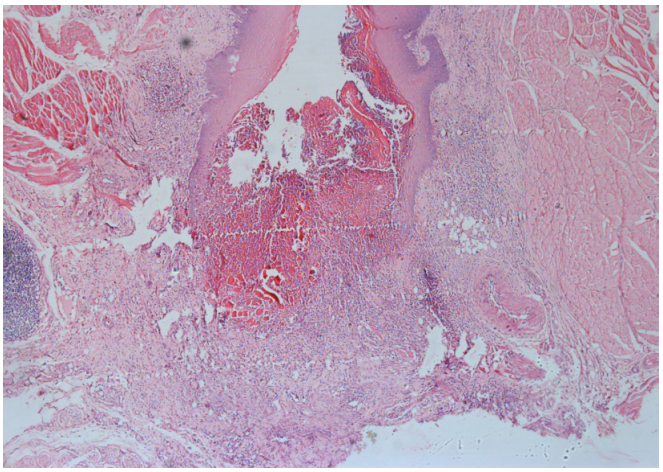

Fig 1: Photomicrograph showing 2/3rd of the wound covered by epithelium in control group (group A) on 7th day. H & E stain (magnification 50X).

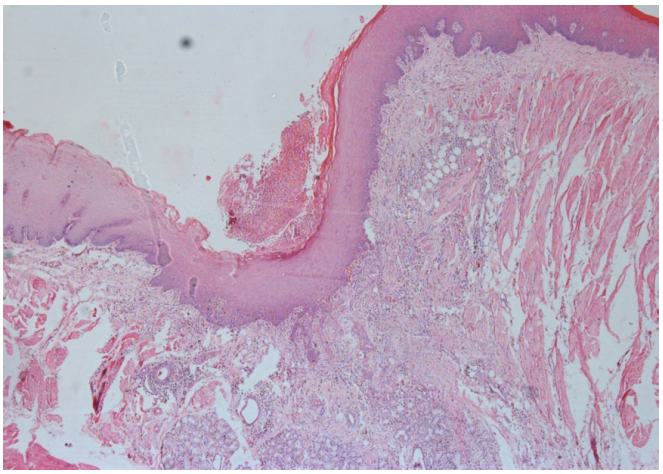

Fig 2: Photomicrograph showing complete width of the ulcer covered by epithelium in group B (given 30mg/kg body weight curcumin) on 7th day (50X).

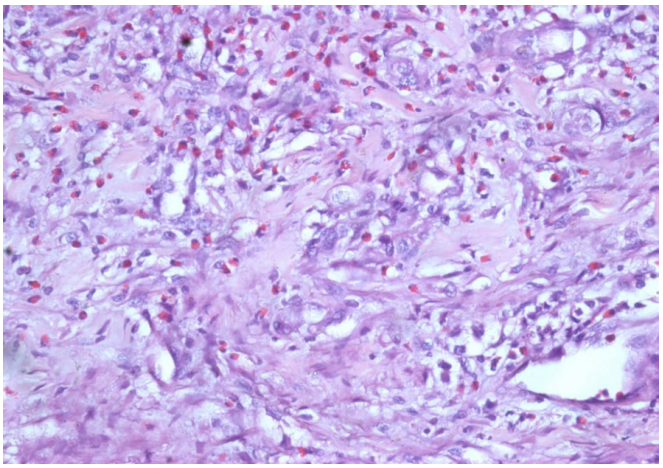

Fig 3: Photomicrograph showing collagenization in group A (control) on 7th day. (H & E stain 40X).

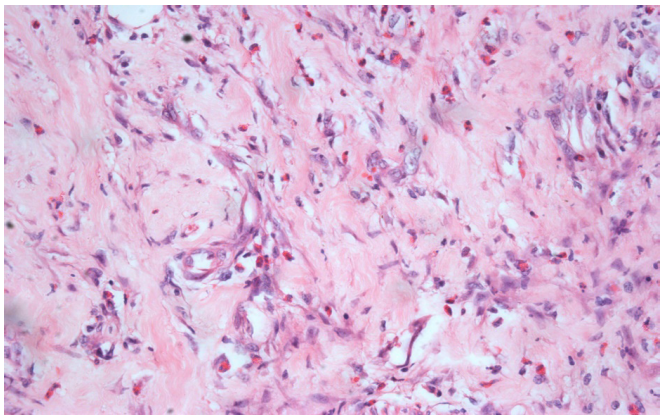

Fig 4: Photomicrograph showing collagenization in group B (30mg/kg body weight) on 7th day. (H & E stain 40X).

of fibroblasts, indicating increased proliferation of fibroblasts resulting in increased rate of collagenization. While none of the animal fell into the category of few fibroblasts. Significant association was found between study groups and fibroblasts on the 5th day. Whereas at day 7 association was not significant.

On day 7, in group A and B, 70% animals showed predominant fibroblasts. This association was found non-significant.

DISCUSSION

The histological observation revealed that in Group B the whole wound was covered with thin layer of stratified squamous epithelium on day 7 with very little or without fibrinous exudate. In group A epithelialization in still going along with the granulation tissue on day 7. So, the complete coverage by epithelium, resulting in closure of wound site demonstrates that the inflammation has recovered earlier in curcumin treated animals. Similar results were found in study conducted in 1998 and 1999 by Sidhu.[10] Complete re-epithelialization in 7 days in 72% of animals was observed in curcumin treated cutaneous wounds by Lopez.[11] Ledda et al.[12] depicted that curcumin when applied on the mucosa of tongue significantly enhanced epithelialization. Kumar et al. in 2009[13] presented the same results.

TABLE 1: EPITHELIALIZATION IN STUDY GROUPS AT 5TH& 7TH DAY

Epithelialization	5th Day			7th Day		
	A(Control)	B(Experimental)	Total	A(control)	B(experimental)	Total
Number of animals (n)	10	10	20	10	10	20
1/3 of the width of the ulcer	7(70%)	3(30%)	10	0	0	0
2/3 of the width of the ulcer	3(30%)	7(70%)	10	9(90%)	3(30%)	12
Complete width of the ulcer	0	0	0	1(10%)	7(70%)	8
Total	10(100%)	10(100%)	20	10(100%)	10(100%)	20

TABLE 1: EPITHELIALIZATION IN STUDY GROUPS AT 5TH& 7TH DAY

Fibroblasts	5th Day			7th Day		
	A(Control)	B(Experimental)	Total	A(control)	B(experimental)	Total
Number of animals(n)	10	10	20	10	10	20
Few Fibroblasts	7(70%)	0	7	0	1(10%)	1
More Fibroblasts	3(30%)	4(40%)	7	3(30%)	2(20%)	5
Predominant Fibroblasts	0	6(60%)	6	7(70%)	7(70%)	14
Total	10(100%)	10(100%)	20	10(100%)	10(100%)	20

Fibroblasts are the principal cells taking part in the healing process of wounds.[14,15] On histological observation it was seen that group B showed predominant fibroblasts at wound site in 60% of rabbits on day 5, depicting that fibroblasts has proliferated the site earlier to produce collagen earlier and subsided the inflammation earlier in curcumin treated group. Whereas in group A on 5th day number of fibroblasts are increasing but there are predominant in number on day 7, shows that the proliferation of fibroblasts is slow. In human study done by Pamula and Natalya[16] depicted that curcumin enhances proliferation due to spiked effect of curcumin on the cell signaling molecular tracks, which must have initiated the prolifertive phase. Krishnamoorthy et al.[17] likewise found that many herbal formulations which contain curcumin had a certain effect on enhancing division of fibroblasts, so it can be dynamically helps in the treatment of burns and wounds.

The histological examination exhibited that Curcumin has anti-inflammatory effects when it was used to treat rabbits wound inflammation as compared to control group.

CONCLUSION

There is significant potential in curcumin for epithelialization and collagenization in mucosa of rabbits after inflammation is induced.

Recommendation

Human trials are required so that the natural products may continue to furnish lone element of molecular divergence and biological functionality in research and drug advancements.

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3 Ayesha Ashraf:	Methodology, results and editing.
4 Tahira Naseem:	Methodology, analyzed the results.
5 Zain Ul Abdin:	Drafting of manuscript.
6 Sadia Iqbal:	Proof reading, expert research opinion in finalizing manuscript.