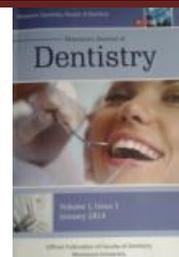




Impact of Curcumin on Tongue Ulcer Healing in Albino Rats



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Abstract:

Objectives: In this study, we evaluated the impact of curcumin, a natural product obtained from the rhizomes of *Curcuma-longo* on oral ulcer healing in rats.

Methods: 60 male rats were divided into 2 groups control group (CG) and curcumin treated group (CrG) given 200mg/kg Suspended in 0.5% CMC. Ulcer was induced using round filter papers 5.5 mm in diameter were soaked in 15 ml of 50% acetic acid. 6 random rats of each group were sacrificed on the day 3, 6, 9, 12 and 15 days post ulceration.

Results: CrG showed smaller ulcer size clinically and both histologically and immunohistochemically faster healing was observed.

Conclusions: curcumin administration accelerate oral ulcer healing through increase release of TGF- β and α -SMA.

keywords: Curcumin, tongue, ulcer healing.

Introduction

Oral mucosal wound or mouth ulcers are sores or open lesions in the mouth which are caused by various disorders [1]. Lesions are less common on the heavily keratinized palate or gingiva. In mild recurrent aphthous ulcers, the lesions reach a size of 0.3 to 1 cm and begin healing within a week [2].

Wound healing is a highly complex but orchestrated cascade of cellular and molecular events. Successful wound healing is the result of a sequence of several basic processes, including inflammation, cell proliferation, angiogenesis, wound contraction, epithelialization, and matrix remodeling. These processes, which depend on the appropriate and integrated functions of neutrophils, macrophages, fibroblasts, and endothelial cells, are thought to be organized by interactions among cells, extracellular matrix proteins, and growth factors. Any alteration of these healing processes will result in abnormal healing and adversely affect the surgical outcome [3].

Turmeric (*Curcuma longa* Linn) a tropical herb of the ginger family (*Zingiberaceae*), is a well-known culinary agent and food additive used to impart yellow color. Internal and local/topical use of turmeric has been advocated for several common ailments in the Indian and Chinese indigenous systems of medicine [4].

Much of this observed activity of turmeric seems to be due to the presence of an active agent called curcumin, which has undergone systemic research to unravel its useful/ valuable properties and to develop it as modern drug [5].

Curcumin has inhibitory activity against hydrogen peroxide-induced oxidative damage in human keratinocytes [6]. Curcumin also has been reported to have potent antioxidant activity. The other attractive feature of curcumin to explore as a vulnerary agent is that it is

nontoxic and has been consumed daily for centuries in Asian [7].

Materials and methods

Animals

60 adult male Albino rats weighting (200-250 g) were used in the study and divided into 2 groups: control group (CG) and curcumin treated group (CrG).

Curcumin administration

The animals of CrG were given 200mg/kg of curcumin suspended in 0.5% carboxymethylcellulose (CMC) delivered by gastric tube [8,9], starting from the day of ulcer induction. While animals of the CG were given CMC only as a vehicles of the drug.

Ulcer induction

Prior to the creation of the ulcers, all animals were anaesthetized with administration of pentobarbital (50 mg/kg). Round filter papers 5.5 mm in diameter were soaked in 15 ml of 50% acetic acid. In order to create round ulcer, an acid-soaked filter paper was pressed onto the inferior surface of the tongue for 60 seconds [10].

Clinical evaluation

Clinical photographs of the ulcers were recorded with a digital camera then tongue was taken for histological examination.

Histological and immunohistochemical analysis

Tongue specimens were fixed in 10% neutral buffered formalin for 24 hours then were trimmed and processed by standard paraffin-embedding methods. Sections were cut at 4 μ m, deparaffinized, and then stained with: H&E & Immunohistochemical staining using monoclonal antibodies to TGF- β and monoclonal antibodies to α -SMA.

Results

Clinical evaluation

Clinical observation showed that curcumin treatment resulted in a great reduction on the ulcer size. CrG showed

smaller size than CG, ulcer size at 9th day post ulceration was the smallest in all time periods (Fig.1).

Histological analysis

Multiple H&E stained sections were examined of both CG and CrG for epithelial regeneration, formation of granulation tissue, and fibrogenesis. In CG, at the 3rd day post ulceration; ulcer area was filled with inflammatory cells within the granulation tissue, fibroblasts started to appear at the 6th day post ulceration. At the 9th day the ulcer edges were apart with ulcer area filled with fibroblasts and collagen fibers (Fig.2, A). At the 12th epithelium over the ulcer was closed and became more organized at the 15th day post ulceration. In CrG; fibroblasts appeared at the 3rd day post ulceration and increased in number at the 6th day with marked migration of the epithelium and collagen deposition at the ulcer area. At the 9th day marked approximation of the ulcer edges, more organized collagen and increased number of fibroblasts (Fig.2, B). At the 12th day, the ulcer was covered by organized epithelium which became more stratified at the 15th day.

Immunohistochemical analysis

TGF- β was localized in CrG and CG ulcers on the 3rd day in which less cells expressing the stain were detected in CG. TGF- β however, was significantly increased and found predominantly in the epithelium and granulation tissue of CrG ulcers. Migrating epithelium and granulation tissue showed maximum staining for TGF- β at the 6th day post ulceration (Fig.3 A & B).

CrG ulcers consistently showed a greater influx of fibroblasts, to determine if the infiltrating cells were myofibroblasts, sections were stained with α -SMA. At the 3rd day post ulceration, α -SMA stain showed positive results for the presence of myofibroblasts within the granulation tissue of CrG sections only. Starting from day 6th post ulceration myofibroblasts increased in number in sections of CrG. While sections of CG showed delayed infiltration of myofibroblasts as well as decreased number of these cells.

Discussion

The wound healing process can be categorized as follows: inflammation, fibroplasia, neovascularization, collagen deposition, epithelialization, and wound contraction. During the healing process, various growth factors are secreted to accelerate wound healing [11]. Curcumin is a popular traditional medicine was first used in Indian and traditional Chinese medicine; it has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemo-preventive and chemotherapeutic activity [12].

To determine the clinical healing in the present study, ulcer size was measured on each observation day. Ulcer size in CrG was considerably smaller at all time periods than CG. A similar marked reduction on wound size was observed in a full thickness wound in rats on the 7th day post wounding when curcumin incorporated in collagen

film [13]. Also in a randomized placebo-controlled clinical trial, curcumin gel showed marked decrease in aphthous ulcer size on the 4th and 7th day of the trial [14]. Samples of both 12th and 15th day post ulceration in all groups showed complete healing. Karavana et al, 2011 reported that oral mucosal ulceration in an animal model showed complete healing on the 12th day post ulceration [15].

Histological findings of sections stained with H&E in the present study showed faster ulcer healing in control group II (curcumin given) then control group I (ulcer without neither diabetes nor curcumin) marked by faster and more organized re-epithelialization, heavy inflammatory infiltration, neovascularization, early fibroblast infiltration and well aligned and organized collagen. Sidhu et al, 1998 reported that oral administration of curcumin in rats and guinea pigs with full thickness dorsal cutaneous wounds showed epithelial regeneration, neovascularization, organization and initiation of fibrogenesis and compact and well-aligned collagen [16]. Similar observation were reported by Xingyi et al, 2011 on applying curcumin- chitosan film to full thickness excisional wounds; an increase in inflammatory cells infiltration in granulation generation on the 3rd day while at the 7th complete re-epithelialization of wound occurred [17].

TGF- β is a critical peptide that controls repair, attracting cells to a wound, and promoting deposition of ground substance and collagen, and has been worthily called a "wound hormone" [18]. In the present study; immunohistochemical stain of TGF- β showed marked expression of the stain in CrG at the 3rd and 6th days post wounding, while CG showed expression of the stain to less extent. Results are in accordance with findings of Sidhu et al, 1998 that curcumin promotes healing by increasing biosynthesis of TGF- β [16].

There is a strong evidence of direct correlation between the level of α SMA expression and fibroblast contractility [19]. Immunohistochemical stain of α SMA in our study showed an earlier differentiation of myofibroblast cells at the 3rd day post wounding and ulcer contraction at the 9th day post wounding in CrG than CG. Roy et al, 2001 reported that addition of exogenous TGF- β 1 to a cultured lung fibroblast lead to increased expression of α SMA protein within the first 24-48 hours [20]. Also Denys et al, 2008 reported that TGF- β converts α SMA negative fibroblast into α SMA positive ones.

Conclusion

Based on the previous results we conclude that, administration of curcumin in normal rats with tongue ulcer lead to increase expression of TGF- β by curcumin lead to increased expression of α SMA and increased migration of epithelial cells; which lead to smaller ulcer size in the first 9 days.

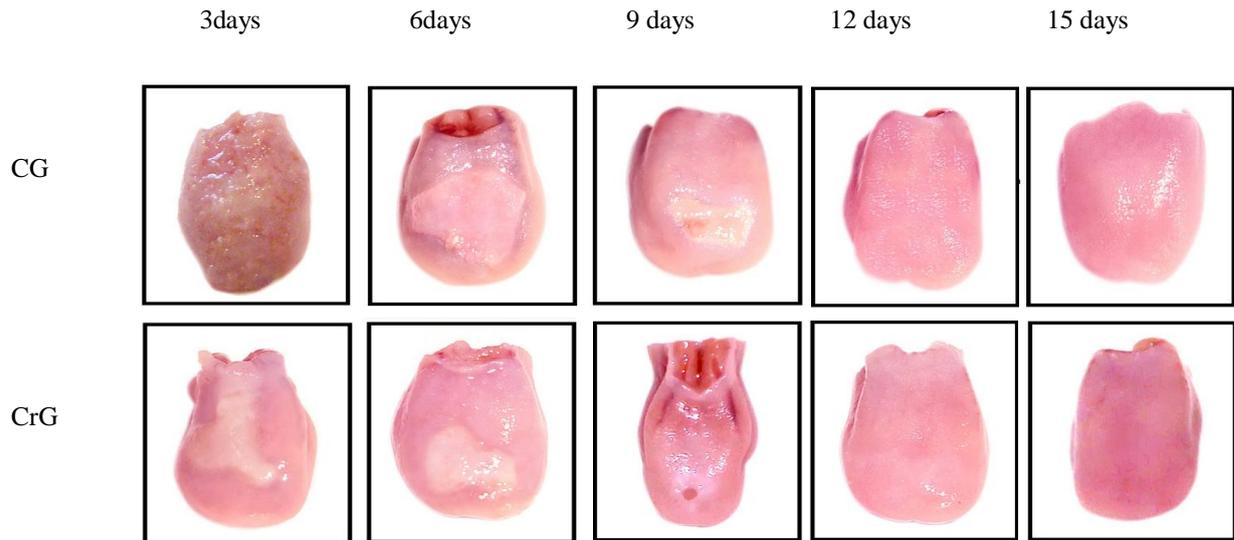


Figure 1: photograph showing samples of ulcers from both groups.

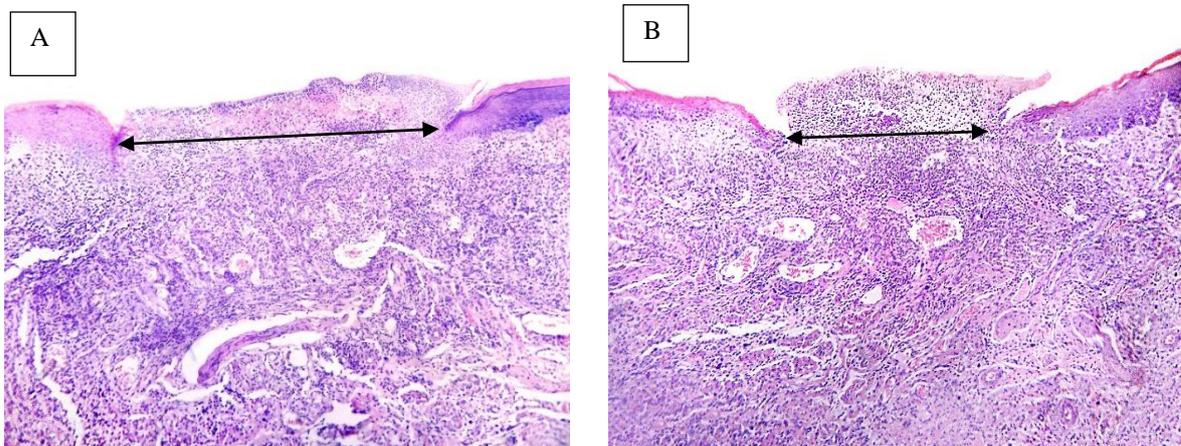


Figure 2: Photomicrograph at the 9th day showing distance between ulcer edges, control group (CG) A, curcumin group (CrG) B (H&E stain, x 40).

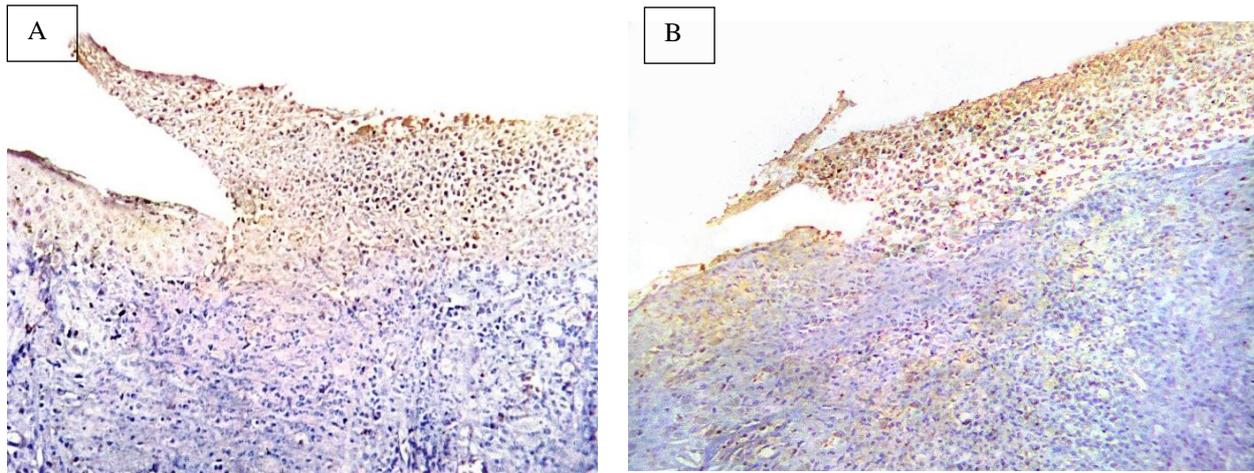


Figure 3: Photomicrograph showing expression of TGF- β stain in the cytoplasm of granulation tissue cells and marginal epithelium at the ulcer area at the 6th day post ulceration CG (A), CrG (B)(IHC staining, TGF- β DAB chromagen X100).

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