

Histological evaluation of the effect of topical application of Curcumin powder and essential oil on skin wound healing

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ABSTRACT

Background: Herbal medicine can be called one of the branches of medicine in various forms. Turmeric curcumin has proved its efficiencies a coloring, flavoring agent and has been traditionally used in medicine, exhibiting remarkable anti-inflammatory and antioxidant properties. The varied biological properties of curcumin and lack of toxicity even when administered at higher doses makes it attractive to explore its use in various disorders like diseases of skin. It is good potential agent for wound healing.

Materials and methods: Sixty four new Zealand rabbits were used in this study ,they were divided into four groups,each group was subdivided as follows: Experimental groups(8 rabbits) right facial side of animals for essential oil application and left facial side animals for curcumin powder application, 8 animals as control group(right facial sides) .Histological assessment regarding the count of inflammatory cells was performed on all studied samples for the healing intervals (3, 7, 10, and 14 days).

Results: Histological findings of the study showed that re-epithelialization, wound contraction were accelerated after topical application of curcumin material especially the essential oil at wound site.

Conclusion: Topical application of curcumin essential oil was significantly effective in skin wound healing as compared to curcumin powder.

Key words: Curcumin, cutaneous wound-healing, topical application, essential oil. (J Bagh Coll Dentistry 2015; 27(3):58-63).

INTRODUCTION

Skin, is the largest soft outer covering organ in the body. It has several functions, the most important being to form a physical barrier to the environment, allowing and limiting the inward and outward passage of water, electrolytes, various substances and protection against micro-organisms ⁽¹⁾. Skin is composed of three primary layers. The epidermis; which provides water proofing and serves as a barrier to infection. The dermis; which serves as a location for the appendages of skin. The inner most layer is hypodermis ⁽²⁾.

Wound is generally a knowledge tissue damage resulting in the disruption of the original tissue architecture and homeostasis ⁽³⁾. Wound healing, as a normal biological process, achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal successfully, all four phases must occur in the proper sequence and time frame ⁽⁴⁾. Turmeric scientific name is *Curcuma longa*, is a plant that belongs to the ginger family Zingiberaceae. One of the most important components is a substance called Curcumin. Considerable interest has been focused on curcumin compound; mechanisms that drive wound repair are complex and have challenged wound-healing investigators for many years ⁽⁵⁾.

Curcumin treatment reduces wound-healing time, improves collagen deposition and increases fibroblast and vascular density in wounds thereby enhancing both normal and impaired wound-healing. The beneficial effects of curcumin and the potential of this compound to be developed as a potent nontoxic agent for treating skin diseases ⁽⁶⁾. Turmeric essential oil contains hundreds of molecules antimicrobial, effects, antioxidant effects, antifungal effect, inflammation and edema effects ⁽⁷⁾.

MATERIALS AND METHODS

Materials

- Curcumin powder (Fluka /Germany)
- Essential oil(Hemani/pakistan)
- Anesthetic solution: Ketamine Hydrochloride (Ketamin 50mg/ml) {1 ml/kg body weight}; Xylocain (10%) {1 ml/kg body weight}.
- Zylazine (20mg/ml).
- Formalin 10%, Ethanol alcohol 96%, Xylol, Paraffin wax.
- Hematoxylen and eosin (H&E).

Methods

Sixty four adult rabbits weighting average of (1.25-2.5kg). Experimental animals were divided into four groups, eight animals for each healing interval (3,7,10, and 14 days).

Each group consists of (16) animals, experimental group (8 rabbits) and control group (8 rabbits). The animals of experimental group

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were (8): animal ,right facial side for essential oil application, and animal left facial side for curcumin powder application. The animals were anesthetized, two centimeters (cm) length incisional wound were made on the cheek at both side (right and left) control and experimental (oil and powder application), then left to heal spontaneously.

All tissue specimens, samples and controls, were fixed in 10% neutral formalin and processed in a routine paraffin blocks. Each formalin-fixed paraffin-embedded specimen had serial sections were prepared as follows: 5µm thickness sections were mounted on clean glass slides for routine Haematoxylin and Eosin staining (H&E), from each block of the studied sample (experimental and the control groups) for histo- pathological re-examination.

Analysis of number of inflammatory cells

It was performed by counting inflammatory cells, in histological sections (H&E stained), for each animal and in four microscopic fields at x40 magnification. Scores for intensity of inflammatory reaction:

1. Absent or very few inflammatory cells.
2. Mild: average number less than 10 inflammatory cells
3. Moderate: average number 10-25 inflammatory cells
4. Severe: average number greater than 25 inflammatory cells⁽⁸⁾.

RESULTS

Three days duration;

Control group

After three days: histological view of skin section at wound site, showed the migration of keratinocytes the wound surface, developing hair follicles with adjacent granulation tissue (Figure 1).

Experimental group

A-Essential oil application

Microphotograph of facial skin section of 3days duration at wound site shows granulation tissue which is highly infiltrated with inflammatory cells (Figure 2).

B-Curcumin powder application

Histological view at wound site after 3days of curcumin powder (CP) application shows granulation tissue with congested blood capillaries, and large number of inflammatory cells can be detected in the dermis (Figure 3).

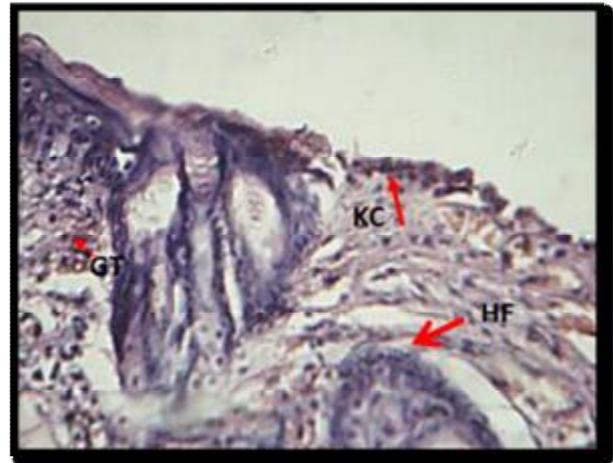


Figure 1: Microphotograph of control group of 3days duration, shows keratinocytes migration at wound surface (KC), granulation tissue (GT), and hair follicles (HF). H&EX20.

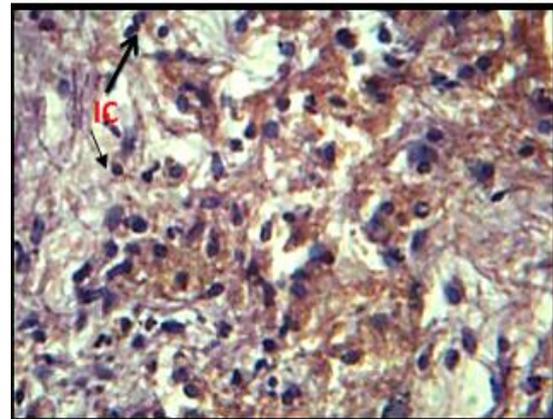


Figure 2: View of wound site of oil group, after 3days shows granulation tissue that is highly infiltrated with inflammatory cells (IC). H&EX40

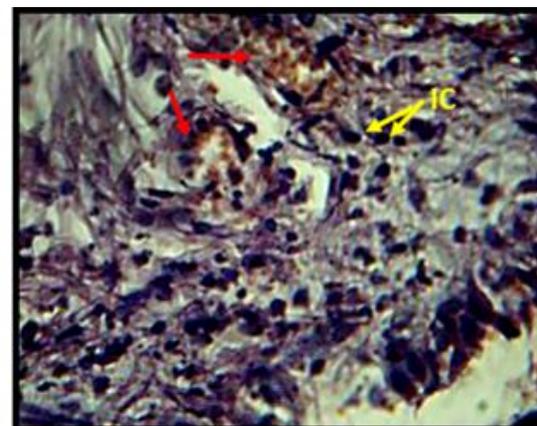


Figure 3: View of wound site after 3days of powder application, shows granulation tissue infiltrated by inflammatory cells (IC), and areas of blood congestion (arrow) H&EX40.

Seven days duration

Control group

Skin section of 7days duration, shows the new epithelium formation, fibroblasts and collagen fibers are noticed (Figure 4).

Experimental group

A-Essential oil application

Microphotograph of 7days duration at wound site shows the thin newly formed epithelium, loose fibrous connective tissue is detected in the dermis (Figure 5).

B-Curcumin powder application

Histological view of facial skin section in the dermis of 7 days durations, shows numerous blood capillaries, surrounded by number of inflammatory cells, fibroblasts and remodeling collagen fibers (Figure 6).

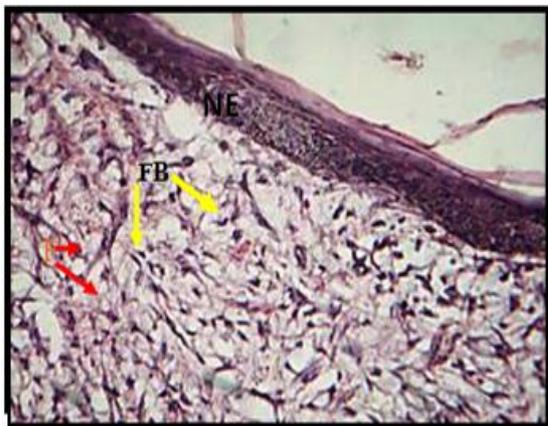


Figure 4: View of facial skin section of control group after 7days, shows, the new epithelium(NE),fibroblasts(FB), and fibers(arrows) in the dermis. H&EX 20.

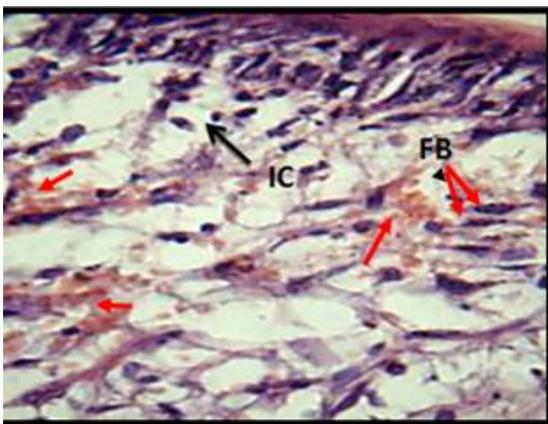


Figure 5: Microphotograph of 7days duration at wound site showsthe areas of blood congestion (arrows), few inflammatory cells(IC), collagen fibers and fibroblasts (FB) are illustrated. H&EX40.

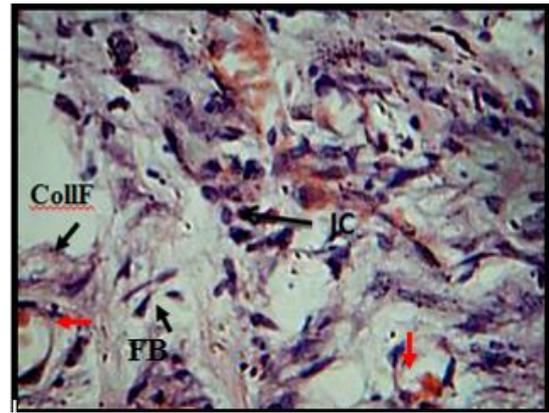


Figure 6: Histological view of section in the dermis after7days of powder application, shows blood capillaries (arrows), inflammatory cells(IC), fibroblasts (FB) and remodeling collagen fibers (CollF). H&EX40.

Ten days duration

Control group

View of facial skin section of 10days duration of control group, shows epithelium collagen fibers and fibroblasts (Figure7).

Experimental group

A-Curcumin oil application

Microphotograph of 10days duration at wound site shows maturing fibrous connective tissue, blood vessels (Figure 8).

B-Curcumin powder application

Histological view of facial skin section, of powder group after 10days, shows that the wound surface is covered by thin epithelium, numerouscongested blood vessels, remodeling fibers and fibroblasts (Figure9).

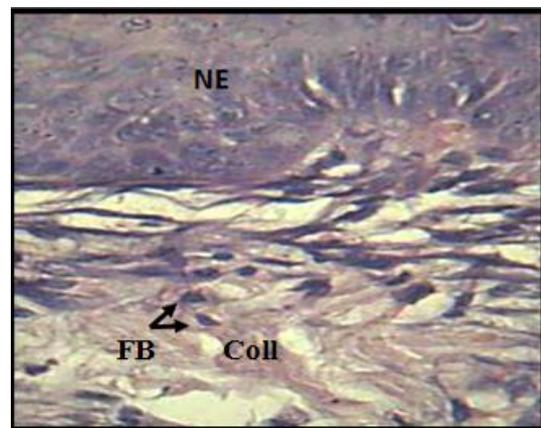


Figure 7: View of 10days duration of control group, shows the new epithelium(NE), collagen fibers (CollF) and fibroblasts(FB). H&Ex40.

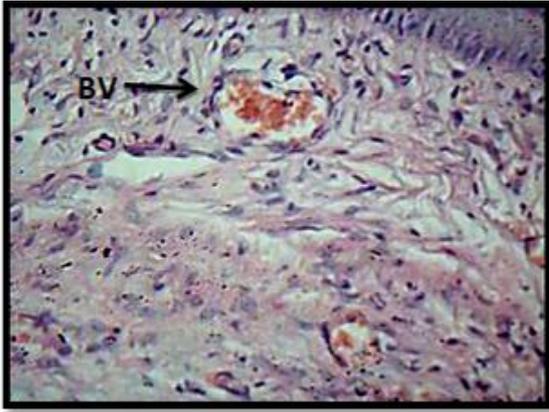


Figure 8: View of wound site, of oil group after 10days duration, shows fibrous connective tissue, blood vessels (BV), surrounded by remodeling fibers. H&EX10.

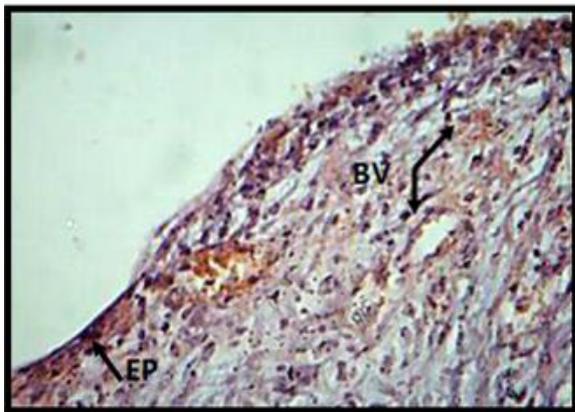


Figure 9: Histological view after 10days of powder application, shows wound surface is covered by thin epithelium, underlying mesenchyme shows numerous blood vessels (BV). H&EX20.

**Fourteen days duration
Control group**

Microphotograph of facial skin section of control group after 14 days, it is noticed that epithelium is thin without rete-ridges, fibrous connective tissue with decrease cellularity is shown, besides developing hair follicles are detected (Figure10).

**Experimental group
A-Curcumin oil application**

Microphotograph of 14 days duration, after oil application, shows complete healing of the wound, mature fibrous connective tissue, fibroblasts are arranged alongside the collagen fibers, proliferating hair follicles and blood vessels are seen (Figure11).

B-Curcumin powder application

Histological view of facial skin after 14 days of powder application, shows complete healing of wound, the cell layers of the new epithelium that covers wound surface are illustrated, it is obvious that there is reduction of cellular population, and establishment of dense mature collagen fibers (Figure 12).

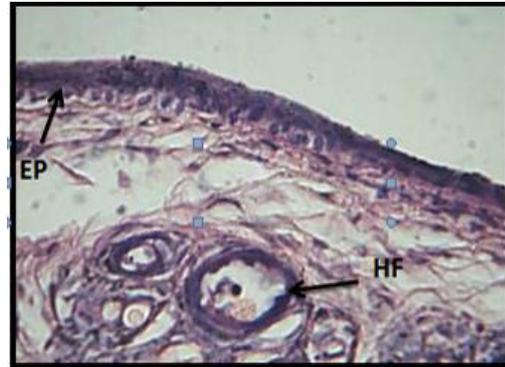


Figure 10: Microphotograph of control group after 14days, new epithelium (EP), fibrous connective tissue with decrease cellularity is shown, proliferating hair follicles(HF) are detected.H&EX40.

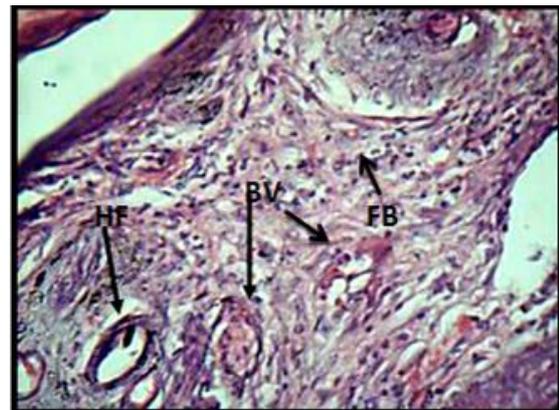


Figure 11: View of wound site of oil group after 14days, shows mature fibrous connective tissue, with multiple blood vessels (BV), fibroblasts (FB) and numerous hair follicles (HF).H&EX20.

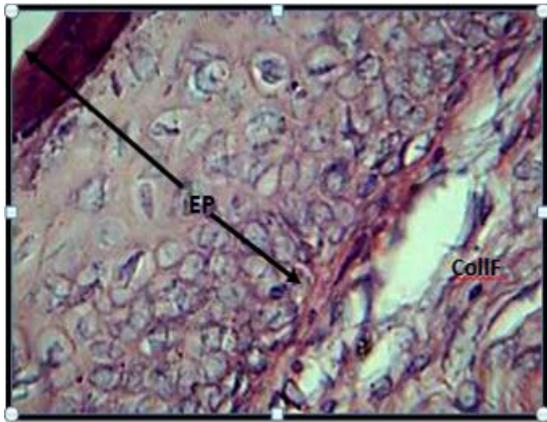


Figure 12: View of wound site of powder after 14 days, shows the new epithelial cell layers (EP), the underlying mesenchym shows dense mature collagen fibers (CollF). H&EX40.

Inflammatory cells assessment

The results of the present study have shown a higher count numbers of all estimated inflammatory cells for experimental group ,there was significant difference in the mean score of inflammatory cells at day 3, in which the control group significantly differ from other groups it had the lowest mean values.

At day 7, there was no significant difference between the groups. Whereas significant difference between the groups with $p < 0.05$ was shown after 10 days healing period. Highly significant difference was recorded between the groups at day 14 (Table1). Figure shows that mean values of inflammatory cells were decreasing throughout the four healing intervals which were well noticed in experimental oil group.

Table1: Comparison between different groups in different periods regarding score of inflammatory cells.

HP	Score	Control		MS	SD	E .Oil		MS	SD	Powder		MS	SD	P value
		No	%			No	%			No.	%			
3 Days	1	0	0	19.2	3.81	0	0	34	8.56	0	0	27.5	8.05	0.001**
	2	3	37.5			0	0							
	3	5	62.5			1	12.5			4	50			
	4	0	0			7	78.5			4	50			
7 Days	1	0	0	14.5	4.95	0	0	23.5	6.73	0	0	17.7	5.05	0.429
	2	3	37.5			1	12.5			3	37.5			
	3	5	62.5			6	75			4	50			
	4	0	0			1	12.5			1	12.5			
10 Days	1	0	0	16	5.2	0	0	11.2	4	0	0	14.2	4.7	0.044*
	2	2	25			7	87.5			5	62.5			
	3	6	75			1	12.5			3	37.5			
	4	0	0			0	0			0	0			
14 Days	1	0	0	9.7	3.2	5	62.50	2.2	0.3	3	37.5	5.7	1.7	0.009**
	2	4	50			3	37.50			4	50			
	3	4	50			0	0			1	12.5			
	4	0	0			0	0			0	0			

*significant, **highly significant

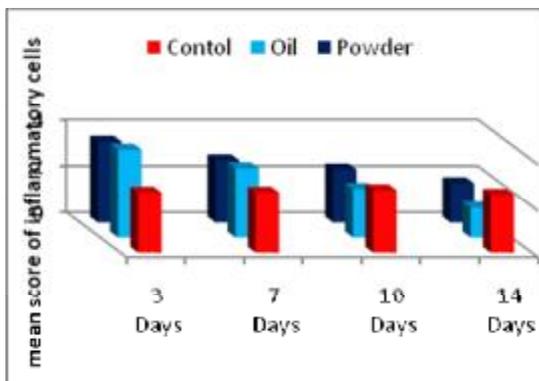


Figure 13: Mean score of inflammatory cells in different periods in the studied groups.

DISCUSSION

Wound healing is a complex process that involves inflammation, granulation and tissue remodeling. Interactions of different cells, extracellular matrix proteins and their receptors are involved in wound healing, and are mediated by cytokines and growth factors⁽⁹⁾

The use of herbal therapies for caring of wounds and injuries has been popular since ancient civilizations. In contrast to only 1–3% of modern drugs being used for the treatment of wounds and skin disorders⁽¹⁰⁾.

The results of this study showed clear promotion of healing in the experimental groups with (curcumin oil and powder)in comparison with the control groups ,mean values of

inflammatory cells were higher in experimental groups with (oil and powder), than in controls, then decreased with time, throughout the four healing intervals, indicating accelerated inflammatory reaction with application of curumin.

The histopathological examination observed that the good response of these groups may be related to stimulation of inflammatory cell or activation of the chemotactic factor,

At 3 days period, the wound site filled with a highly vascularized and proliferating granulation tissue. Also confined by study conducted by Hussein et al where histopathological findings showed hemorrhage with inflammatory cell infiltration, as well as congested blood vessels⁽¹¹⁾.

At 7 days, histological findings showed, thin new epidermis covering wound surface in studied groups, and fibrous connective tissue ,with fibroblasts and remodeling collagen fibers with areas of blood congestions, which was obviously seen in experimental groups where complete re-epithelialization of the surface, presence of collagen fibers ,inflammatory cell infiltration was evident ,this agreement with Lemo et al.⁽¹²⁾

10 days, re-epithelialization was complete. The underlying dermis showed remodeling immature collagen fibers, inflammatory cells are few, agreed with findings of Hussein et al.⁽¹¹⁾

Cellular fibrous connective tissue with congested blood vessels and infiltration of few inflammatory cells covered by thick, larger cellular epidermis was detected in the present study after 14 days, in disagreement with Jawad et al. who studied the histological healing response of the soft tissue incisions created by scalpel prepared on rabbit's skin specimens⁽¹³⁾.

As conclusions; topical curcumin represents simple and inexpensive model of wound healing enhancement and curcumin essential oil is more effective in enhancement of wound healing regarding histological assessment. Inflammatory cells had highest mean values, especially with oil group, and these values decreased with time.

Besides highly significant difference was recorded between the studied groups at day 14.

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