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Expression of IGF-1R in Skin Wound Healing Treated by Topical Application of Cinnamomum Zeylanicum

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Abstract

Objective: The aim of this study was to evaluate the immunohistochemical expression of IGF-1R on cutaneous wound healing. **Material and Methods**: A total of 18 male albino rats (Rattus norvegicus albinus) weighing nearly 300-400 gm were used in the present study. Two punch biopsies were done on the dorsum of each animal, and daily local application of cinnamon zeylanicum extract was performed on left side, while the right one was allowed to heal naturally (control measure). The process of wound skin healing was tracked for periods (1, 3, and 7 days), all specimens were processed for immunohistochemistry examination. **Results**: Based on immunohistochemical results, experimental groups had the highest mean values of IGF-1R expression in the stroma and epidermis when compared to control groups. These values increased with time, which was more noticeable on days three and seven of the experiments. **Conclusion**: Positive localization of IGFF-1R in rat's skin indicated the effectiveness of cinnamon zeylanicum extract in accelerating the healing process of cutaneous layers of skin.

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Introduction

Cutaneous wound healing is a complicated natural process. Attempts to repair the defect caused by significant damage start very early during inflammatory response [1]. Ultimately, they result in repair. Herbal therapies have been utilized to treat wounds and injuries for centuries [2]. Cinnamomum zeylanicum, an old botanical synonym for the cinnamon tree, comes from Sri Lanka's former name. Cinnamon has diverse biological properties, including anti-inflammatory [2], antioxidant, [3,4], and anti-microbial [4]. The wound repair process requires close regulation of degradative and regenerative processes, a wide variety of cell types, and complicated interactions between many biochemical cascades needed to repair process without complications [5,6].

Growth factors released in the injured area encouraging cell migration to the wound site by chemotaxis, stimulate the growth of fibroblasts and epithelial cells, initiate angiogenesis, and extracellular matrix ECM deposition and remodeling of the wounded area [1]. Growth hormone action, often mediated by (IGF-I) is predominantly seen in the dermis of skin, impacting the synthesis of collagen fiber, IGF activity has a significant impact on both epidermal and dermal compartments, that enhance the proliferation, migration and survival of stem cells [7].

The insulin-like growth factor 1 receptor (IGF-1R) is a small, multifunctional molecule like pro-insulin, it is acting in an autocrine or paracrine way to aid in different and multiple functions, including the early phases of wound healing [6,8]. Many physiological functions rely on the important balance between proliferation of mitotically active epidermal keratinocytes of the skin and differentiation of post-mitotic cells. This balance has a crucial role in skin formation and development [9]. The study aimed to evaluate the effects of locally applied cinnamon essential oil on expression of IGF-1R in cutaneous wound healing in rats.

Material and Methods

Experimental design

18 male albino rats of about (300–350gm) and aging from (4-8) weeks were used [10]. All rats were maintained under good conditions with a standard laboratory environment. Animals were housed in single, and separate cages for 7 days in equivalent settings.

The rats were arbitrarily divided into 3 main groups (six rats each), based on period of healing (1, 3, and 7 days). Two full-thickness punch biopsies of 2 mm depth [10] were made on the dorsum of each rat with a sterilized punch of 5 mm diameter [11]. The

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wound on the right side was left untreated. While the other side (experimental) treated with local cinnamon essential oil (100% purity) every day till scarifying.

Surgical procedures

All surgical instruments were sterilized by using the oven at 150ºC for one hour. A combination of 50 mg (40 mg/kg-B.W.). Ketamin HCL and 2% xylazine (0.4 mg/kg B.W.) was injected intramuscularly according to the animal's weight. Hair clippers were used for removal of dorsal hair first, followed by disinfection using ethyl alcohol (70%). Two punch biopsies of 5 mm in diameter were done with a rotary device. 3 cm distant from each other. The control wound was irrigated with distilled water while left experimental (left side) was topically treated with one drop of cinnamomum zylanicum essential oil (10 µl, U.S.A.), applied by using a micropipette. Each rat received general anesthesia and was scarified at the end of (1, 3 and 7 days). The operation site was demarcated with nearly 5 mm of normal tissue surrounding the wounded area, and then a complete excisional biopsy was obtained. For fixation purposes. the specimens were placed in a plastic biopsy container filled with ten percent formaldehyde and cut using a microtome to gain 5 µm thickness for immunohistochemical study; polyclonal IGF-1R were used according to manufacturer instructions. SPSS software version 24.0 was used for statistical analysis.

We used ANOVA test after using a paired sample t-test, which established the data's normal distribution. Alpha was defined as 0.05.

Results

One-day duration

Positive localization of IGF-1R expressed by fibroblasts and collagen fibers in control group is showed in Figure 1. Figure 2 shows the expression of experimental skin section group that positive reaction in hair follicles and fibrous connective tissue.

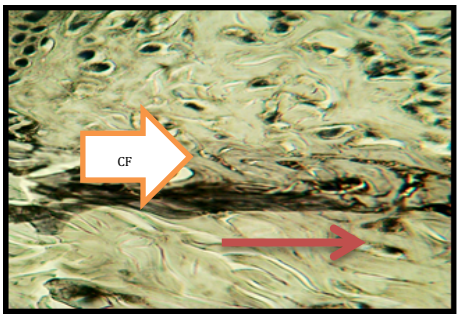

Figure 1. View of positive expression of IGF-1R by fibroblast (red arrow) and collagen fiber (arrow CF) in control group. DAB stain with counter stain hematoxylin, X40.

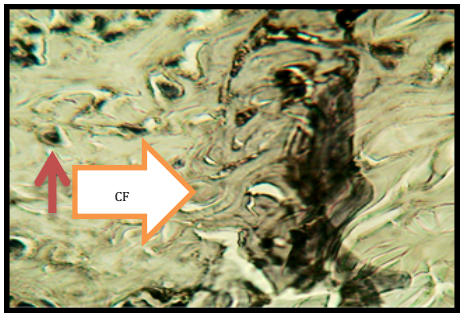

Figure 2. View of positive expression of IGF-1R by fibroblast (red arrow) and collagen fibers (CF). DAB stain with counter stain hematoxylin, X40.

Three days duration

Positive localization of IGF-R1 in the skin section of the control group is detected by epithelium, fibroblasts, and vascular endothelium as shown in Figure 3. Experimental skin section group shows positive localization of IGF-1R by new epithelium and fibroblasts (Figure 4).

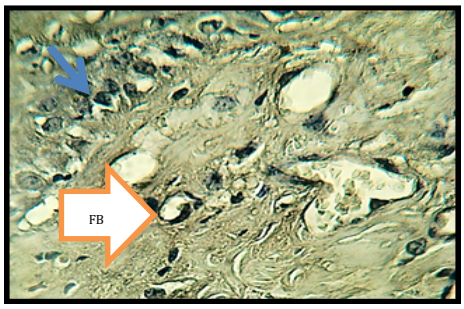

Figure 3. Magnified view of positive expression of IGF-1R by vascular endothelium (black arrow) and fibroblasts (FB), epithelium (blue arrow). DAB stain with counter stain hematoxylin, X40.

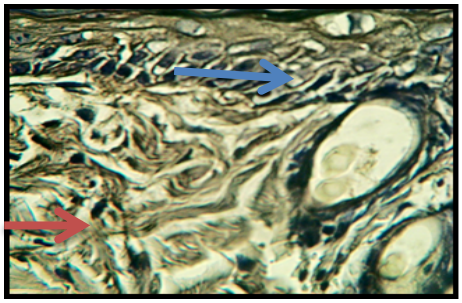

Figure 4. Positive localization of IGF-1R at new epithelium (blue arrow) and fibroblast (red arrow). DAB stain with counter stain hematoxylin, X40.

Seven days duration

Positive localization of IGF-1R in the control group is detected by new epithelium; positive reaction is shown by dermal fibroblasts and vascular endothelial cells (Figure 5). While the microphotograph view after cinnamomum zylanicum application shows positive localization of IGF-1R by epithelial cells, hair follicles, collagen fibers, and fibroblasts (Figure 6).

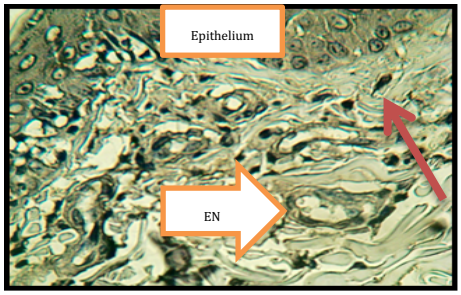

Figure 5. Photomicrograph shows positively stained endothelial cells (EN) and fibroblast (red arrow). DAB stain with counter stain hematoxylin X40.

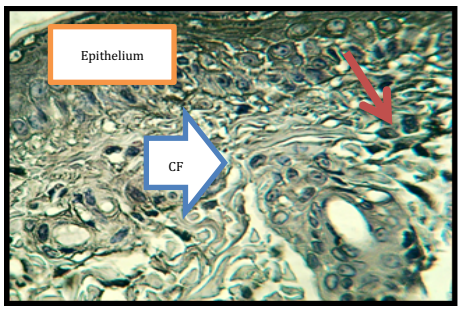

Figure 6. Positive expression of IGF-1R by epithelium (E), fibroblasts (red arrow). DAB stain with counter stain hematoxylin, X40.

Stromal expression

Statistical analysis of positively stained cells for immunohistochemical localization of IGF-1R as measured in different healing periods (1, 3, and 7 days) for studied groups is shown in Table 1. There was a decrease in mean values with time for the 1- and 3-day

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durations whereas values increased at day 7; the highest values were recorded at day 1 for the experimental group, the lowest value was detected at day 3 for the controls.
A significant difference (P <0.05) was observed between control and experimental groups on days 1 and 7. There was a non-significant difference (P>0.05) at day three. Those findings were illustrated in Figure 7.

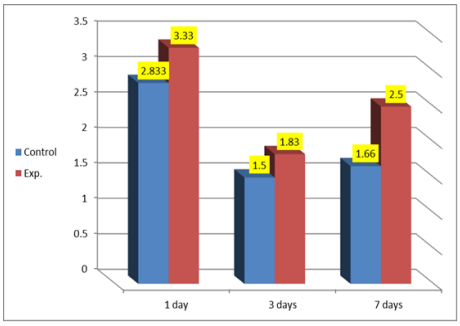

Figure 7. Experimental groups versus and controls regarding IGF-1R expression (stromal).

Epidermal expression
Statistical analysis of epidermal immunohistochemical localization of IGF-1R is demonstrated in Table 2. The mean value of positively stained cells increased with time at day 3 and 7 healing durations for control and experimental groups; the highest average value of positively stained cells was recorded at day (7) for the experimental group, whereas the lowest values were noticed at day 3 for the control groups Figure 8. Besides there was significant difference (p<0.05) between studied groups at 3,7 days as shown in Table 2.

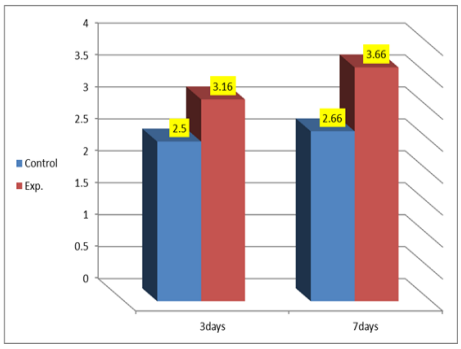

Figure 8. Comparison between experimental and control group regarding IGF-1R expression (epidermal).

Discussion
One of the major concerns for medical staff in skin wounds that have a significant impact on people's health and lives is increasing morbidity and death rates [12]. The healing process is a complicated dynamic process including four overlapped phases: it starts with hemostasis, inflammation, and proliferation, and ends with tissue remodeling (maturation). The most important goal of the healing process is accelerating recovery of normal condition of tissues without scarring [13,14].
Growth factors are important regulators taking part in orchestrating and promote cellular responses, including: growth, migration, differentiation, and deposition of extracellular matrix needed for successful wound healing [5]. Insulin-like growth factor 1 acts as a regulator of vascular endothelial function adjusted vasoconstriction and /or vasodilation, and modulates inflammatory actins to conserve skin homeostasis [16].
The exact mechanism of the healing process of a wound is not clearly understood. There are several parameters that are involved in the healing of wounds, including histological results (re-epithelialization, granulation tissue, collagen accumulation, inflammatory reaction, angiogenesis, ulcer formation). The results showed a significantly increased level of IGF-1R expression during advances of the skin healing process as it stimulates the keratinocytes and fibroblast migration, especially during wound epithelialization [17]. While these cells express only small amounts of this protein. Thus, optimum healing is related to IGF-1R levels.
A few cells in the dermis and epidermis in normal skin express IGF-1R receptors (basal epidermal keratinocytes and undifferentiated epithelial cells related to the outer root sheath of hair follicle, and sebaceous glands. All epidermal cells, macrophages, and other cells of the skin showed positive expression of IGF-1R at day one to three after the injury in coincidence with [18].
In the present study, the highest mean values of positive expression for stromal (IGF-1R) were detected on day 1, when the process of re-epithelization is usually taking place. This is in agreement with Póvoa and Diniz (2011) [19], who studied growth hormone system: skin interactions and stated that the maximum levels of IGF-1 in body fluids and local tissues occur early in the first hours or days following the injury since it acts as a chemoattractant for endothelial cells correlated with the proliferation and migration of keratinocytes and fibroblasts which play a major role in the healing of wounds [20,21], by stimulating collagen synthesis and secretion, thus accelerating wound healing and increasing the strength of the wound. It may originate from migrating keratinocytes from epithelial cells of adjacent hair follicles, fibroblasts from granulation tissue, inflammatory cells, or from plasma in accordance with Seeger and Paller (2015) [22].

Conclusion
The present study concluded that using local cinnamomum zylanicum essential oil accelerates wound healing as demonstrated in IGF-1R expression, which is involved in the healing process in different durations.

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Conflict of Interest
The authors have no conflicts of interest to declare.

Regulatory Statement
This study was approved by the Research Ethical Committee of the College of Dentistry, Mustansiriyah University (study number: MUOPA9).

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Table 1. Comparison between experimental and control regarding IGF-1R expression (stromal).

Periods	Score	Control		MS	SD	Exp.		MS	SD	P-value
		No.	%			No.	%			
1 Day	1	0	0	2.833	0.408	0	0	3.33	0.516	0.023
	2	1	16.66			0	0			
	3	5	83.33			4	66.66			
	4	0	0			2	33.33			
3 Days	1	3	50	1.5	0.547	3	50	1.83	0.983	0.097
	2	3	50			1	16.66			
	3	0	0			2	33.33			
	4	0	0			0	0			
7 Days	1	3	50	1.66	0.816	0	0	2.5	0.547	0.039
	2	2	33.33			3	50			
	3	1	1.66			3	50			
	4	0	0			0	0			

Table 2. Control groups in comparison to experimental groups regarding IGF-1R expression (epidermal).

Period	Score	Control		MS	SD	Exp.		MS	SD	P-value
		No.	%			No.	%			
3 Days	1	0	0	2.5	0.547	0	0	3.16	0.752	0.036
	2	3	50			1	16.66			
	3	3	50			3	50			
	4	0	0			2	33.33			
7 Days	1	0	0	2.66	0.516	0	0	3.66	0.516	0.041
	2	2	33.33			0	0			
	3	4	66.66			2	33.33			
	4	0	0			4	66.66			