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Histological Evaluation of Local Application of Licorice Oil on Facial Skin Wound Healing in Rats

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Abstract

Background: The biological process of wound healing involves four well organized stages: hemostasis, inflammation, proliferation, and remodeling. Licorice root oil is commonly found in skin care products and is said to have remarkable calming characteristics. Licorice Root Oil is also frequently used in serums due to its soothing effects. Objective: Histological evaluation of the effect of topical application of licorice oil on the healing of facial skin wounds in rats. Methodology: Sixty male healthy albino rats that weigh about 250- 300 gram were utilized in this research. The experimental part of this study done in a private veterinary clinic in Al-Najaf city from 10/12/2023 to 18/12/2023. An incisional surgical wound to the full thickness of skin of about 20 mm length were done in cheek skin of all. The animals were divided randomly into the following groups: Control group: thirty rats, surgical incision was created at cheek right side that left for spontaneous healing without any treatment. Experimental group: thirty rats, surgical was created at cheek left side, received once daily local application of 30 um of licorice oil. Wound contraction, epithelial thickness and the number of inflammatory cells were assessed. All animals were scarified according to the healing periods that 15 control and 15 experimental were sacrificed for each healing period (three days and seven days), and samples required for the histological section had been collected. Results: It was observed that there is rise in the wound contraction and epithelial thickness, and decrease in inflammatory cell count from 3 to 7 days having a significant difference which is higher in the experimental group as compared to that in the control group. Conclusion: Results showed that local application of licorice oil improves healing of wound in rats.

Introduction:

With an approximate surface area of 1.8 m2, the skin is the biggest organ in the human body and is the primary protective barrier of the human body. The skin consists of three layers: the outermost layer is the epidermis, which constitutes a thin layer of flattened squamous epithelial cells divided into four layers of keratinocytes which are in various levels of differentiation. It lacks its own blood supply, and fed through the blood vessels beneath. The second layer the dermis mostly comprises of an extracellular matrix including connective tissue like blood vessels, collagen, nerves, lymphatics, pilosebaceous units as well as sweat glands. The final layer is the subcutaneous layer, made up of fat and connective tissue(1).

A wound is a harm or interruption to the ordinary anatomical shape and characteristic(2). Wound restoration technique is based at the tissue's reparative potential(3). The process of wound healing is complicated. It includes several types of cells, including fibroblasts, keratinocytes, lymphocytes, neutrophils, endothelial cells and macrophages(4). Skin wounds are considered four main stages of acute wound healing, which are presented as overlapping: hemostasis, inflammation, proliferation and remodeling(5). Many biochemical materials and cells are participating in the healing route to complete repair of tissues(6). At different phases of the healing process, numerous vital cells and growth factors are involved. Multifaceted regulation controls those events(7). Animal models provide valuable information about pathological and biological processes, thus are valuable for the progress of biomedical science. Furthermore, they facilitate the research and experimentation of products which are essential in human and veterinary medicine such as drugs and vaccines, as well as surgical procedures(8). Rats were simple to work with, exhibited human-like metabolic reactions, and could be used for surgery as experimental animals(9).

Licorice oil is one of the most important medicated plants that have been used extensively and studied extensively on the globe. The word liquorice has been derived from Old Greek glykyrrhiza glykys means sweet and rhiza means root. One of the main active compounds is glycyrrhizin which possesses a cortisone effect (10).Glycyrrhiza belongs to Fabaceae which includes more than twenty species and the important one is glabra(11). It has thus attained a very high popularity; more especially because of its main medicinal compounds including glabridin 4, licochalcone E,and licochalcone A that could aid in the prevention of oxidative damage bioactive moiety, glycyrrhizin, a triterpenoid saponin glycoside. It exhibited gastroprotective, anti-inflammatory, antiviral, antitumoral, antifungal, antihepatotoxic, anticancer effects, antimicrobial, rheumatoid arthritis, ulcers and reduction of low-density lipoprotein and hot flashes of menopause. The natural antioxidants found in Licorice include saponins, phenols, isoglycyrrhizin, isoflavonoids, flavanones, liquiritigenin and 18β-glycyrrhetinic acid (12).

Materials and Methods:

The procedures carried out during the experiment adhered to the ethical principles of animal experimentation established by the Baghdad University College of Dentistry. The reference number for this approval is 876, dated 3/12/ 2023. The experiment was carried on 60 healthy male Albino rats that weighed approximately 250-300 gram. All the animals were evaluated by a veterinarian before starting the experiment to ensure their good health. The rats were housed in a dedicated animal room in conditions of controlled temperature and ventilation and were well-fed and well-hydrated. They were given one month to acclimate to their environment then the rats divided into two main groups 30 rats for each, and each group were divided into two sub groups 15 rats for each for the different healing periods, three and seven days. The animals were subjected to IM general anesthesia of 2% xylazine (0.4 mg/kg B.W.) and 50 mg of ketamine HCl (40 mg/kg B.W.). Surgical incisions approximately 20 mm long were made in the cheek of both experimental and control rats, with the full thickness of the skin and underlying tissues incised. The wound generated at day 0 then 15 rats from experimental group and 15 rats from control group were sacrificed for each healing period

(3days and 7 days), specimens were taken from the wound site on the day of sacrifice and put in fixative solution which is 10% fresh formalin for 24 hours and then follow the routine process in preparation of paraffin embedded tissue sections. This allowed us to count inflammatory cells and to analyze the thickness of the epithelium and the number of blood vessels. The percentage of wound closure was decided with the aid of the usage of the formulation: ((L1- L2) / L1) x 100, in which L1 is the length of wound at the day zero, while L2 is the length of wound at the day of sacrifice. The wound healing test determined the percentage of wound closure by examining the open wound area**(13).** Assessing the quantity of inflammatory cells in 5 fields The mean number of cells was then recorded for each healing period using a light microscope and a power (x40) lens(14). Using Image J software, the distance between the innermost layer of the basal layer of the epidermis and the outermost layer of keratin at the wound margins was measured in order to determine the epithelial thickness. The mean of the two measurements was then calculated**.**

Statistical analysis:

The statistical package for social sciences (SPSS) version 26 was used for statistical analysis. The results were with normality test, Mann Whitney test, independent T-test, averages standard deviations, and high and low values. Set the bar for significance at a 95% confidence level, with P-values of 0.05.

Results:

Clinical finding:

Descriptive information of the proportion wound contraction revealed that wound contraction on experimental group better than that of control group after three days with high significant difference (P< 0.000) also the wound contraction is better in licorice oil group than that of control after 7 days with statistical significant (p< o.1) Table (1).

Histological finding:

Three days duration:

Control group: Histological view of wound site showed infiltration of inflammatory cells, many blood vessels which are congested, necrotic tissue at wound surface, hair follicles, the area is bounded by granulation tissue with fibroblasts and irregularly arranged collagen fibers. As shown in Figure (1).

Experimental group: the photograph of wound site shows, new epithelium at surface underlying, fibroblast, collagen fibers, remnant necrotic tissue and few inflammatory cells with many blood vessels seen in the dermis,. As shown in Figure (2).

Seven days duration:

Control group: Histological examination of wound site after 7 days shows blood vessels, fibroblasts, granulation tissue with remodeling collagen fibers and sealing wound surface by new epithelium. As shown in Figure (3).

Experimental group: After seven days histological examination of wound reveals collagen fibers filling wound site, full epithelialization, fibroblasts, granulation tissue and blood vessels. As shown in Figure (4).

Epithelial thickness parameter

Mean values of epithelial thickness in control group are lower than that in experimental group after 3 days but with no significant difference (p> 0.05), while the thickness is higher in licorice oil group than that in control group after 7 days with high significant (P< 0.001) Table (3).

Inflammatory cell parameter

Mean values of inflammatory cell count in control group are higher than that in experimental group after 3 days but with no significant difference (p> 0.05) while mean values of the inflammatory cell count is lower in licorice oil group than that in control group after 7 days with highly significant difference (P< 0.000), in both groups there is a decrease in inflammatory cell count from 3 days to 7 days with significant change but this change is higher in licorice oil group than that in control group, these findings are demonstrated in Table (2).

Discussion

The complex process of wound healing includes the organized movement of cellular lineages and numerous specialized tissues. It needs tight coordination of cellular proliferation, migration, matrix deposition and remodeling, along with angiogenesis and inflammation. Whereas minor skin wounds heal in days, major accidents due to main surgical operation, trauma or acute contamination can take many weeks to heal, usually leaving behind a fibrotic scar which could affect characteristics of the tissue. Development of medications to avoid scarring and correctly repair chronic wounds needs a full understanding of the molecular and cellular mechanisms using wound recuperation(15).

The use of herbal extracts as safe and effective therapeutic agents in polymeric systems has grown recently, and medicinal plants are now being considered as a new source of antifungal and antibacterial medications(16).

Medicinal plants play an excellent position inside the wound restoration procedure. In current many years, many researches have concentrated on the improvement of innovative wound dressings which contain medicinal plant extracts or their refined energetic compounds, that are capacity options to traditional wound dressings. Many researches have also examined the mechanism of action of several natural drugs in wound recovery method(17).

In licorice, the compound of saponin is likewise known as glycyrrhizin has the components glycyrrhetinic acid and glucuronic acid as its fundamental elements. The essential issue of glycyrrhizin is every so often famend for its cooling residences and licorice taste. This component is relatively thermally stable and stops the growth cytopathology of numerous viruses like DNA and RNA viruses. Furthermore, it's been generally used for the treatment of cough, sore throat, allergic reaction, ulcer and bronchitis. As a remedy for, osteoarthritis, rheumatoid arthritis, regulating low blood sugar and for arthritis and, also, fed on for Addison's disease as well. On the other hand, licorice root is utilized as the first line of treatment due to its highly therapeutic properties for numerous illnesses(18).

Nowadays, rodents account for the majority of the species used in biomedical research, as they may be deemed best fashions for investigating pathologies that have an effect on human populations because of their physiological similarity, which permits them to be hired to similarly our knowledge of such techniques as cancer, weight problems, sepsis, organic development, and organ transplants, amongst many others(19)

Rats were therefore chosen as the experimental animal for this study.

Wound contraction facilitates tissue repair. The right balance between very little contraction results in injuries that don't heal and very much contraction causes contractures is mandatory for optimal healing. The results of our present study, showed that the statistical analysis of epithelial thickness and wound contraction, increased with time and were highest in experimental group with significant difference at day 7, while inflammatory cell count decreased with time and was lowest in experimental group at day 7 in agreement with the findings of Zabihi who showed that extract of Glycyrrhiza glabra (Licorice) root has positive effects on the healing process(20).

Histological and histomorphometrical evaluation

Wound healing in a skin suggests a brilliant system of cellular feature this is extraordinary in nature. The restore method consists of the interplay of cells, cytokines and growth factors participated in closing the lesion(21).

The study's findings revealed that the mean values of inflammatory cell count were lower in the licorice oil group compared to the control group after 7 days, with a statistically significant difference, that is agree with finding of Kazemi et al study(22).

This observation suggests the effectiveness of licorice oil mostly because of their capacity to promote the production of granular tissue, extracellular matrix, and wound contraction(22). In which collagen formation is induced by isoflavan glabridin, which is produced from licorice roots(20).

In contrast, the control group exhibited more response of inflammatory cells, which may be attributed to invasion of bacteria and the absence of anti-inflammatory and immunomodulatory activities found in the licorice oil in agreement with the findings of study done by Shahid et al(23). The processes of granulation tissue formation and re-epithelialization is very important in the healing process, due to the restoration of skin

integrity, rendering it less susceptible to infection(24). The outcomes of our study indicate a higher thickness in the group treated with licorice oil compared to the control group after 7 days. This difference is attributed to the effect of the administered licorice extracts, this finding is in agreement with previous study by Zabihi(20). Our results ran in agreement with another study conducted by Zabihi et al, who showed that local application of licorice oil enhanced the healing process, re-epithelialization and angiogenesis(25).

Conclusions:

Findings obtained in this study showed that local application of licorice oil could be a potential source for promoting the acceleration of cutaneous wound healing through its anti-inflammatory and anti-oxidant properties.

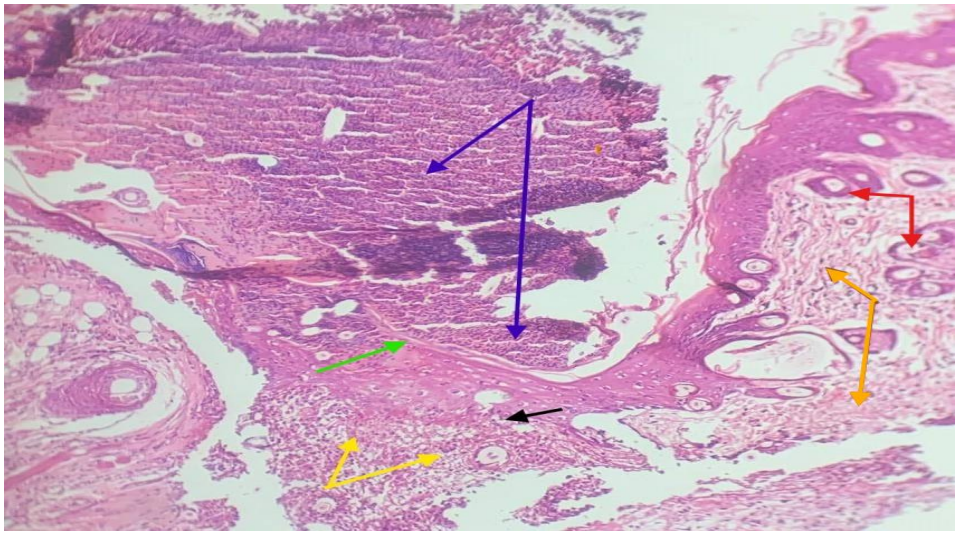

Figure 1: Histological view of control group's wound site at day 3 shows: necrotic tissue (Dark blue arrows), inflammatory cells (yellow arrow), wound epithelial edge (green arrow), hair follicles (red arrows), irregularly arranged collagen fibers and fibroblasts (orange arrows) and blood vessel (black arrow). H&E X10.

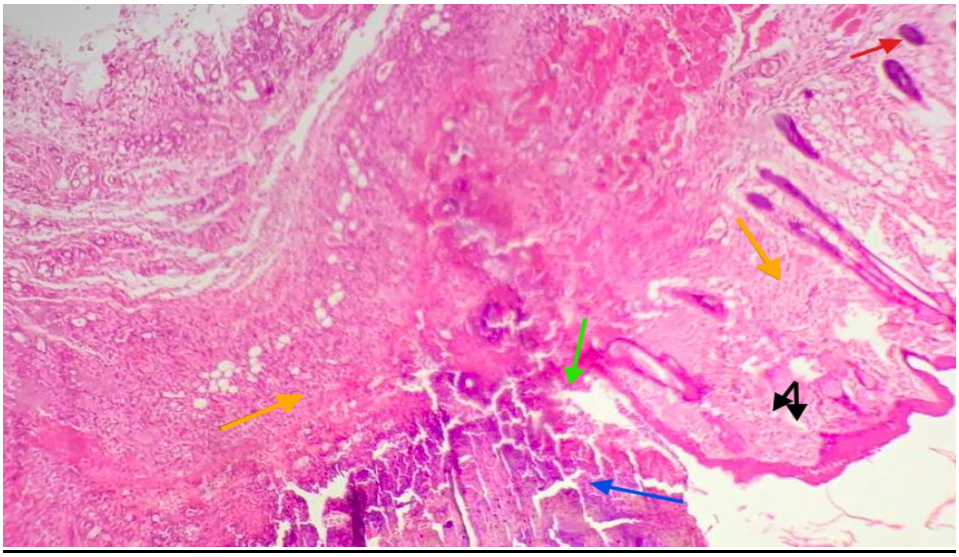

Figure 2: Histological view of wound site of experimental group at day 3 shows: wound surface and newly formed epithelium by migration of new epithelial cells (green arrow), granulation tissue (blue arrow), hair follicle (red arrow), collagen fibers and fibroblasts (orange arrows) and newly formed blood vessels (black arrows). H&E x10.

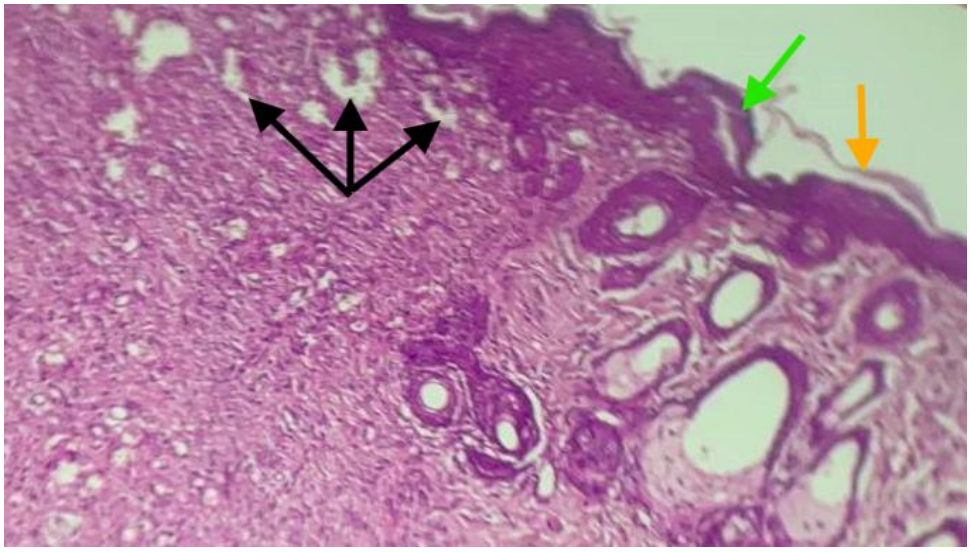

Figure 3: Histological view of control group wound site at day 7 shows: the wound surface sealed by new complete epithelium (green arrows), blood vessels in the dermis (black arrows), keratin layer (orange arrow).H&E x10.

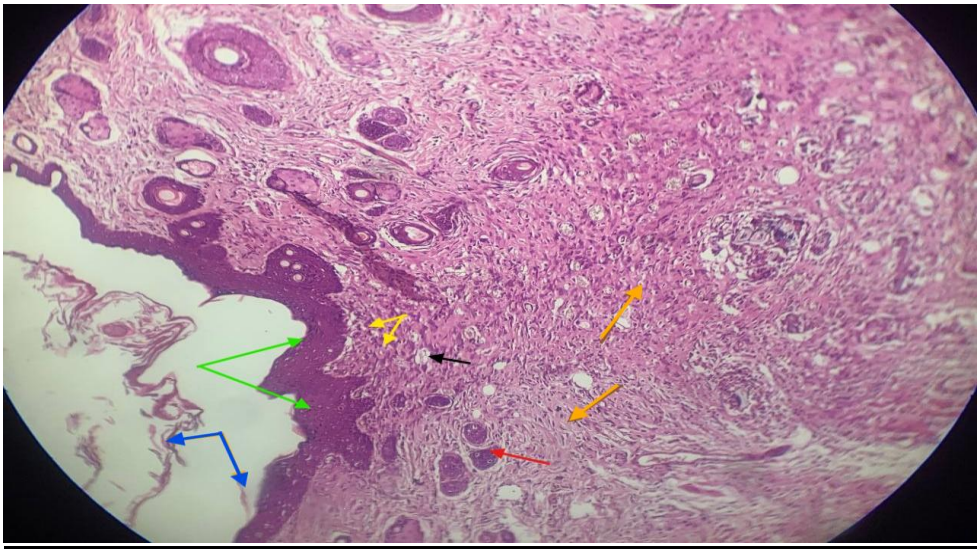

Figure 4: Histological view of experimental group wound site at day 7 shows: new thick complete epithelium at the surface (green arrows), keratin layer (blue arrows), collagen fibers and fibroblasts (orange arrows), hair follicle (red arrow), numerous newly formed blood vessels (black arrow) and few inflammatory cells (yellow arrows) H&E x10.

Table (1): The percentage of wound contraction in both groups for each healing period

Day	Type	N	Mean	St. Deviation	Std.Error.mean	P-values
3	Control	15	15.333333	6.784085	1.751643	.000
	experimental	15	23.933333	4.799057	1.239111	
7	Control	15	80.066667	13.796825	3.562325	.017
	experimental	15	91.513333	11.261303	2.907656	

Table (2): The inflammatory cells count for both groups in each healing period

Day	Type	N	Mean	St. Deviation	Std.Error.mean	P-values
3	Control	15	87.200000	9.064846	2.340533	.836
	experimental	15	86.533333	8.416537	2.173141	
7	Control	15	63.800000	7.532785	1.944957	.000
	Experimental	15	38.133333	11.885565	3.068840	

Table (3): The epithelial thickness for both groups in each healing period

Day	Type	N	Mean	St. Deviation	Std.Error.mean	P-values
3	Control	15	2.650000	0.533198	0.137671	.251
	experimental	15	2.873333	0.509337	0.131510	
7	Control	15	4.599333	0.533392	0.137721	.001
	experimental	15	5.790000	1.162841	0.300244	

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