In Vivo Histological Assessment of Local Application of Fenugreek Seed Oil on Cutaneous Wound Healing

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Abstract

Background: Healing is a process that restores the physical integrity of body structures. It is a dynamic, complex, multicellular process that involves the extracellular matrix, cytokines, blood cells, and growth factors. It includes hemostasis ,inflammatory phase, proliferative phase and maturation phase. Studies have shown fenugreek to be an anti-inflammatory agent, which supports its traditional use as a treatment for sore throat, arthritis, and wound healing.

Objective: Histological and histomorphometric assessment of potential activity of fenugreek oil on healing of experimentally induced cutaneous wound in rats.

Materials and methods: Thirty male albino rats (*Rattus norvegicus albinus*)weighing about 250-400gm were used in this study, two circular standardized wounds were performed on rat dorsum with a sterile biopsy punch(5mm) in diameter .The wounds at the right side were left to heal spontaneously as a control ,whereas the left side treated daily with fenugreek seeds oil(0.2ml). Sacrification of animals was done at the end of each healing periods (1^{st} ,3^{rd} , and 7^{th} day) to collect the specimens by cutting the skin about 5 mm around the edges of the wound for histological examination.

Results: Obtained findings showed decrease in mean values of wound contraction with time, as lowest mean value recorded at day 7 at experimental side. inflammatory cells count showed highest mean values recorded in experimental groups at day 1,however mean values of epithelial thickness increased values with time in all groups .

Conclusion: the present study showed that the fenugreek oil was effective for wound healing when applied locally.

Key words*: wound healing, fenugreek seed oil , rats.*

Introduction

Wound healing is a complex biological process that takes place in all tissues in all organs of the body. Various cell types, including keratinocytes, neutrophils, macrophages, lymphocytes, fibroblasts and endothelial cells, are involved in this process [(1),]including tissue inflammation, proliferation, and remodeling [(2).] During the first few days of healing the re-epithelialization of wounds is important for creating a barrier between the outer and inner

environment to allow an undisturbed continuation of nascent repair processes [3]. *Trigonella foenum-graecum* Linn., commonly known as fenugreek is a short annual plant from the Fabaceae family [4] fenugreek is used as a medicine to treat several diseases besides being used as antioxidant [5], against inflammation [6]. when applied to a wound ,it releases its anti-inflammatory properties and works to maintain the healing process and reduces the inflammation fenugreek seeds contain fatty acids which build collagen that promotes wound healing and maintains skin elasticity [7].

Materials and Methods

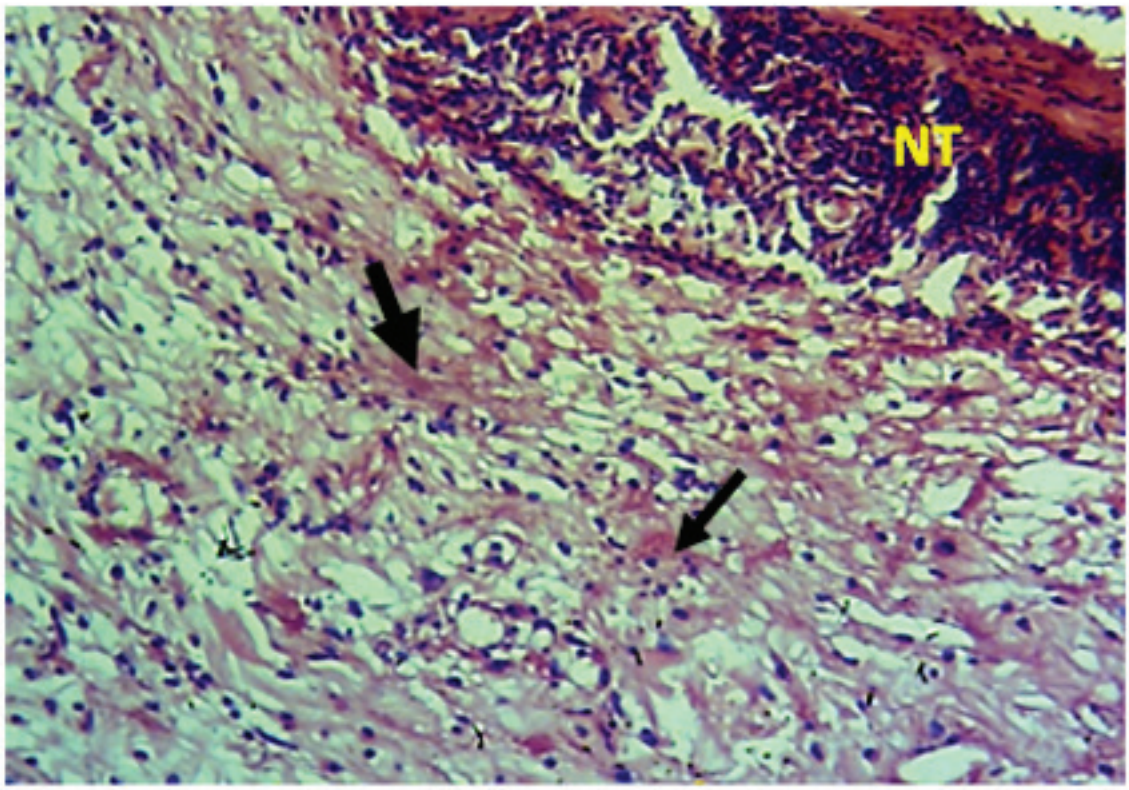
The practical part of this study begun at the animal house of Veterinary Medicine at Al-Kufa University .All of the procedures in the experiment were done in accordance to the animal experimentation ethical principles of (College of Dentistry – University of Baghdad) . Thirty male Albino rats with a body weight of (250–400gm) and aging from (6-9) months were used in this study [8]. All rats were maintained under controlled ventilation conditions, temperature, housing and feeding, and were given a standard diet (pellet) with an easy access to the tap water,theywere randomly divided into three main groups(10 rats in each group) according to the healing intervals (1,3 and7days). All surgical instruments were sterilized by using the oven at 150ºC for one hour. Each animal was weighed to determine the dose of anesthesia required , general anesthesia was induced by intra muscular injection with a mixture of ketamine and xylazine (100 mg/kg and 20 mg/kg, respectively) [9]. Removal of skin hair of the dorsal region was done by using hair removal lotion,then operation site swept by ethyl alcohol(70%)as disinfectant. Two excisional wounds were made at dorsal skin ,with approximately (1.5) cm distance from each other [9] with a biopsy punch of 5 mm of diameter .The two wounds were identified as right side (control) was left to heal spontaneously ,and the left side (experimental) was locally treated with fenugreek essential oil (0.2 ml) by a micropipette .Sacrification of animals was done at the end of each healing period (1st ,3rd , and 7th day) to collect the specimens by cutting the skin about 5 mm around the edges of the wound and were put in a 10% freshly prepared formalin

Results

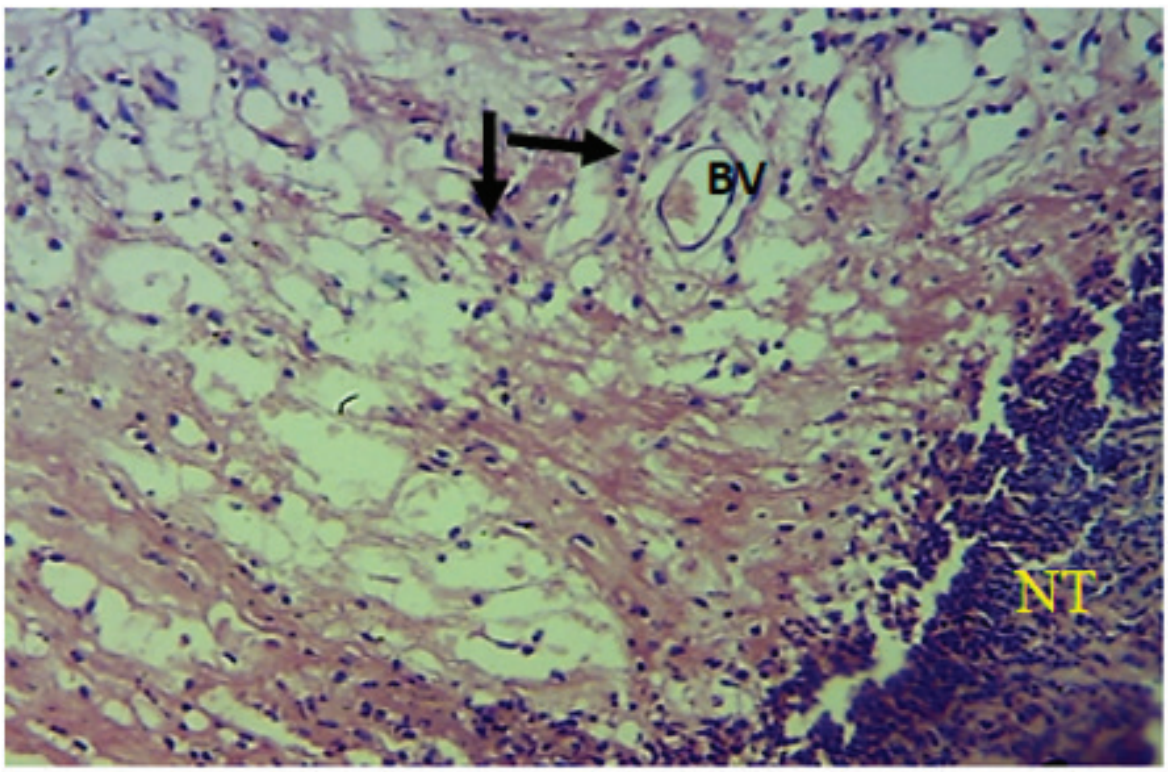
Histological findings

One day duration

Microphotograph of wound site of control group shows necrotic tissue at wound surface .Scattered blood islands and prominent inflammatory cells infiltration adjacent to blood vessels (figure1) ,and after addition of fenugreek oil, it shows wound surface covered by necrotic tissue ,extensive infiltration of inflammatory cells adjacent to congested blood vessels in the dermis(figure2)



Figure(1): Necrotic tissue seen at wound surface(NT) ,blood islands(arrows). H&Ex20



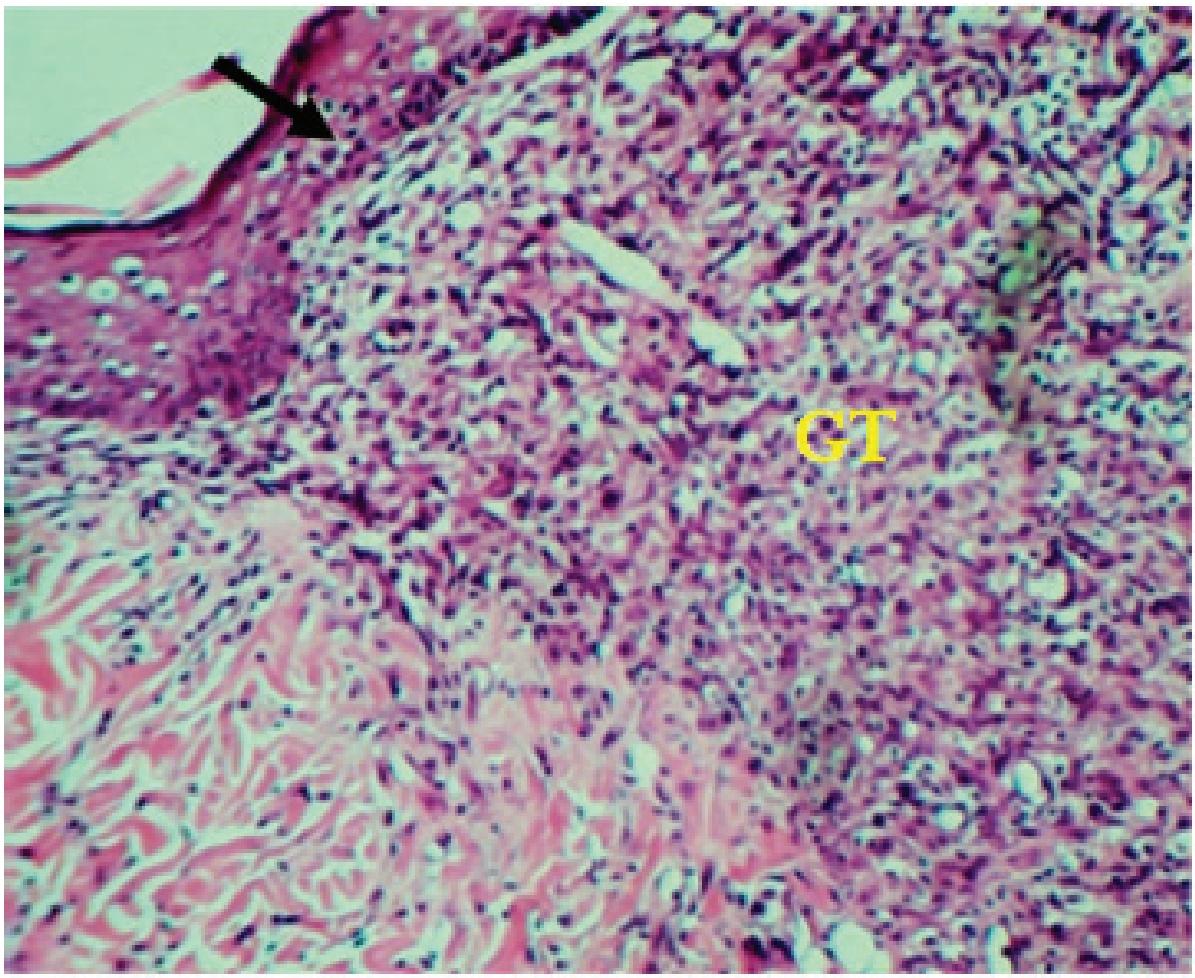
Figure(2):View of wound surface covered by necrotic tissue (NT),inflammatory cells (arrows),blood vessels(BV).H&Ex20

Three days duration:

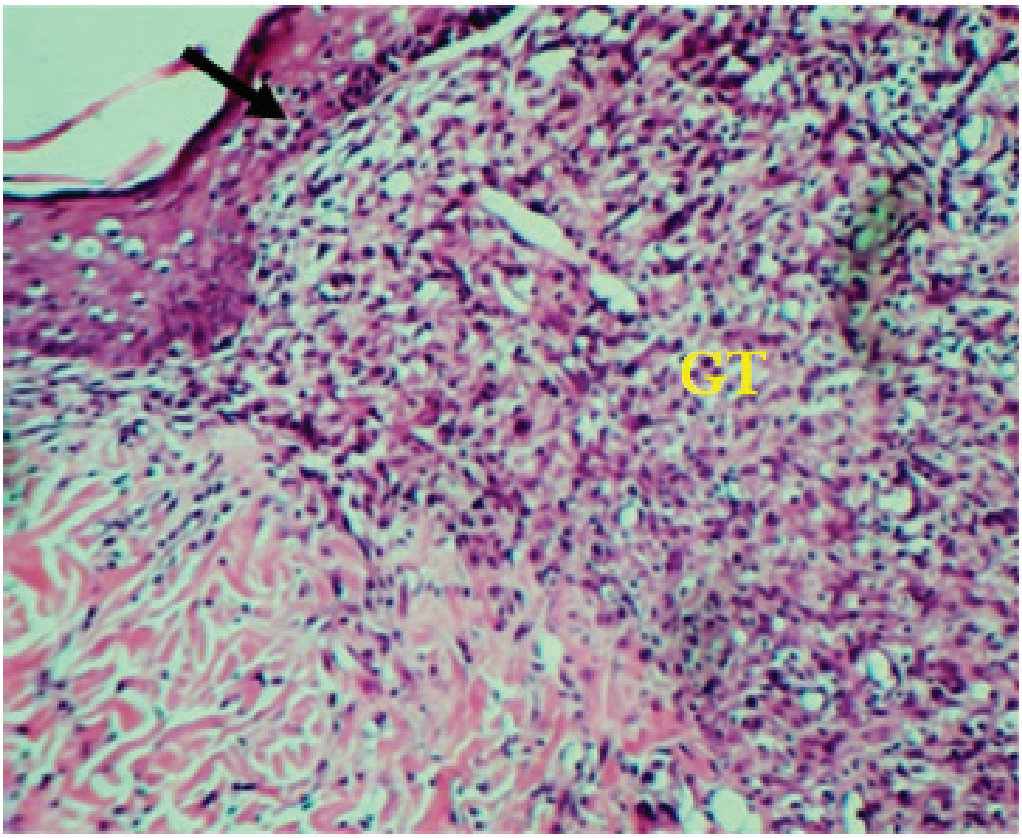
Microphotograph of wound site of control group shows surface sealed by new epithelium demarcated from remnant of necrotic tissue ,newly developing hair follicles in dermis .Other view shows granulation tissue ,fibroblasts. View of wound treated with fenugreek oil shows,new epithelium at surface underlying remnant necrotic tissue ,fibroblast and collagen fibers numerous blood vessels with adjacent few inflammatory cells seen in dermis.

Seven days duration

Histological examination of wound site showed new epithelium sealing wound surface ,granulation tissue with remodeling collagen fibers fibroblasts, and blood vessels(figure3) while after addition of fenugreek oil complete epithelialization noticed,wound site filled with granulation tisssue, blood vessels ,collagen fibers and fibroblasts(figure4) .



Figure(3):View shows surface sealed by new epithelium (arrow),granulation tissue(GT).H&Ex10.



Figure(4):wound site filled by granulation tissue (GT) ,new epithelium at surface(arrow).H&Ex10

Estimation of wound contraction

Results revealed that the recorded mean values of wound contraction decreased with time, the highest recorded values were noticed in experimental and control groups at day 1.Lowest mean value recorded at day 7.as seen in table1 .High significant difference was recorded between control and experimental groups among all durations(1,3 and 7days),these findings are illustrated in figure(5).

Table 3-1: Descriptive statistics of wound contraction in (mm) for both groups in each healing duration

Days	N	Studied groups		Range	T-test	P-Value
		Experimental Mean ± SD	Control Mean ± SD			
Day 1	10	4.26 ± 0.45	4.68 ± 0.29	3.15 – 5.0	4.178	0.001***
Day 3	10	3.72 ± 0.4	4.23 ± 0.31	3.1 – 4.9	4.588	0.001***
Day 7	10	2.82 ± 0.37	3.65 ± 0.43	2.08 – 4.21	4.56	0.001***

*P>0.05non Significant **P≤ 0.05 Significant ***p ≤ 0.01 highly Significant.

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Figure(5):Wound contraction among studied groups in different durations

Inflammatory cell parameter

The results showed that mean values were higher in experimental group than control group at day 1 with high significant difference(table2) Whereas no statistical significant difference recorded in means of inflammatory cells between both groups in days 3 and 7 ,these findings are demonstrated in (figure6) as well.

Table (2): Descriptive statistics of inflammatory cells count in each healing duration

Days	N	Inflammatory cells count		Range	T-test	P-Value
		Experiment Mean ± SD	Control Mean ± SD			
Day 1	**10**	137.75 ± 63.37	75.54 ± 18.07	53.25 – 240.5	3.126	***0.006
Day 3	**10**	65.42 ± 73.54	63.09 ± 19.19	10.5 – 224.75	0.110	**0.913**
Day 7	**10**	27.25 ± 5.4	24.27 ± 10.64	8.0 – 43.75	0.788	**0.441**

***p ≤ 0.01 highly Significant

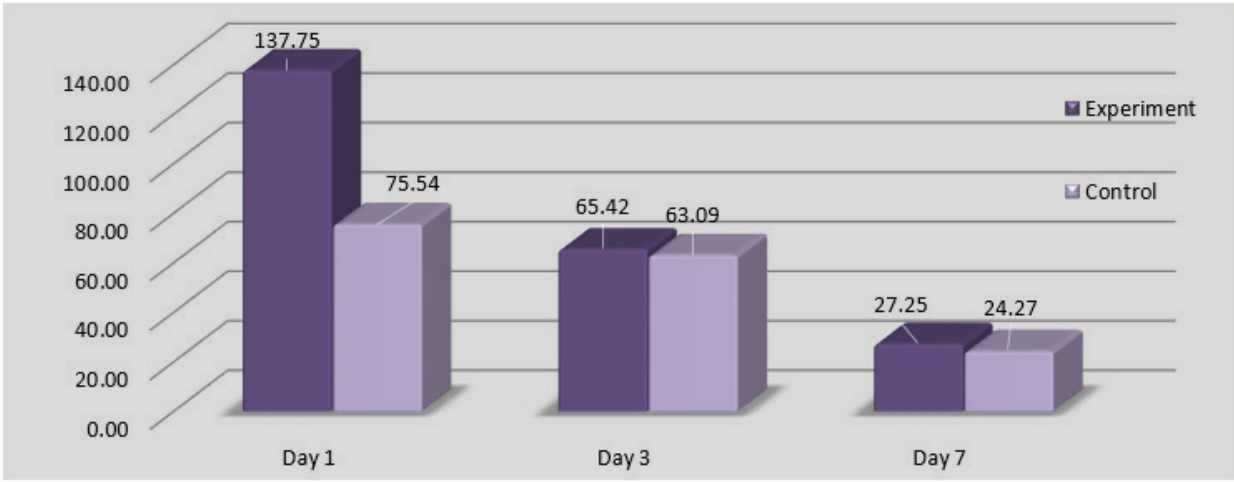

Figure (6): Mean of inflammatory cells in different

Epithelial thickness parameter

Mean values increased with time for all studied groups in different durations.

(table 3) shows comparison in epithelial thickness between experimental and control groups at days 1, 3, and 7with high significant difference between both groups.

Discussion

Wound healing is a complex biological process that takes place in all tissues in all organs of the body. Various cell types, including keratinocytes, neutrophils, macrophages, lymphocytes, fibroblasts and endothelial cells, are involved in this process[(1)]. It consists of tissue inflammation, proliferation, and remodeling[(2)].Fenugreek, when applied to a wound, releases its anti-inflammatory properties and works to maintain the healing process and reduces the inflammation also fenugreek seeds contain fatty acids which build collagen that promotes wound healing and maintains skin elasticity (**Dixit *et al*.,2005)**[(7)].The fatty-acid components of fenugreek oil used in this study in particular poly unsaturated fatty acids such as linoleic acid are effective in wound healing as reported by (**Poljšak *et al*.,2019)** [(10)].Incisional and excisional models commonly use rat's dorsum as the wound site since such site keeps the animal from reaching and manipulating the wound, besides rat's ready availability, low cost, and small size, with more economical and efficient use of limited laboratory space and housing facilities**(Wanda and Wysocki ,2008)**[(11)].

Wound contraction is the centripetal or concentric reduction in size of an open wound, is essential to second-intention healing, caused by movement of fibroblasts in granulation tissue collagen and pulling forces of granulation tissue myofibroblasts on the skin edges (Swaim *et al*.,2001) [(12)].However wound contraction, is usually more rapid than epithelialization and since new tissue is not created, causes a decrease in the overall healing time of rat wounds (Mogford and Mustoe,2001) [(13)].Wound healing begins with hemostasis at the site of injury, progresses to an inflammatory phase

followed by proliferation of the epithelial and matrix components, and ends with laying down of a highly organized collagn matrix(**Velnar *et al.,* 2009)** **(14)**.During inflammation phase, the granulation tissue is composed predominantly of inflammatory cells, mainly neutrophils that are recruited to the wound site and removed during the progression of the repair process **(Gurtner *et al*.,2008)(15).**In this study results showed marked increase in the inflammatory cells in the experimental group at day 1 as compared to control group, and remained slightly higher at experimental side at remaining durations in agreement with results concerned with inflammatory cells scoring which were obtained by **Marcos *et al*., ,2011(16)** who studied the effect of Brazilian green propolis in tissue repair of cutaneous wounds inWistar rats, and they found that at days1 and 3 the treated wounds demonstrated significant bigger means for inflammatory cells **.**The decrease in inflammatory cell mean values with time could be explained according to studies by **Kaur and Kapoor in2002(17)** who reported that fenugreek have antioxidant properties which can accelerate process of wound healing . Epidermal healing, or re epithelialization, is the renewal of the epidermis that has been damaged by a wound or a burn**(Rittié,2016)(18)** Epithelialization is the process by which cells from the epidermis at a wound's edge proliferate and migrate to cover the surface of the cutaneous defect **(Swaim *et al*.,2001)** **(12).**

The results of this study revealed that epithelialization was more accelerated in experimental group as the mean values of epithelial thickness was higher than that in control ones with high significant difference between studied groups and they increased with time which indicates rapid epithelialization and collagenation of proliferation phase in agreement with **(19)** who applied different concentrations of hydroalcoholic extract of *Trigonella foenum graecum* to evaluateit's proliferative action of skin wound of healing in albino rats which showed faster rate of epithelization in treated groups when compared with control ones.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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