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Clinical and Histological Evaluation of Sesame Oil Effect on Skin Healing in Albino Rats. In Vivo Study

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Abstract

Aim: this study aims to evaluate the effect of sesame oil extract on skin healing clinically by measuring wound contraction and histologically by measuring epithelial thickness and vascularization.

Method: Twenty-eight male albino rats were subjected to general anesthesia, excisional wound was induced below the shoulder blades, then animals were divided into two groups: 1. Control group (cont.): fourteen rats were left to heal normally 2. Sesame oil group (SO): fourteen rats were treated with oil extract of sesamum indicum diary. Scarification was done in two periods: 7 and 14 days. Wounds were examined clinically and histologically.

Results: wounds treated with sesame oil showed significant faster wound contraction. The epithelial thickness and vascularization were significantly higher in sesame oil group when compared with control group.

Conclusion: treatment of wounds with sesame oil enhances wound healing and facilitate wound closure.

Introduction:

Skin is the largest organ in the body, which cover the whole exterior body surface. It made up of three layers, each layer have different architecture and function. The structure of skin serves as the body's first barrier against infictions, UV radiation, toxins and mechanical harm (1).

Histologically the skin composed from three major layers: 1. Epidermis: is the epithelial component of the skin, it's the body's outermost layer, although the epidermis is the smallest layer of skin, but it is work as a shield that protect the body from the outside world. 2. Dermis: is a fibrous connective tissue made up of collagen, elastic tissue, and extracellular elements such as blood vessels, nerve endings and hair follicles. The dermis help in supporting and protection of the epithelial layer, also it helps in regulation of body temprature, and aids with sensation. 3. Hypodermis: is the deepest layer of skin, it has adipose lobules, hair follicles, blood vessels and sensory neurons, it serves a variety of functions, including: connection, storing energy and insulation (2).

Wound healing is a regular dynamic process that include highly complex cellular, humeral, and molecular structure, that occurs following an epidermal layer injury. Wound healing is sometimes named as primary or secondary healing. The primary wound healing is the simple healing of a non-infected, well-approximated wound, its best illustrated by surgical wounds. But when the wound got infected, affected by hypoxia or immunological dysfunction, the secondary healing stage occurs, that include granulated tissue creation fallowed by epithelization of this new tissue (3).

There are three stages of wound healing: 1. **Inflammation:** activation of platelet seen during the early inflammatory phase (days 1-2), that influx inflammatory cells. During the late inflammatory phase (days 2-3), monocytes differentiate into macrophages, which play an important role in wound healing. this phase characterized by redness, swelling and heat.

2. **Proliferation**: stars at day 3 and lasts for two or four weeks. Type III collagen fibers and ground material produced by fibroblasts, that enhance wound's tensile strength. Fibroblasts can also differentiate into myofibroblasts (contractile cells), that facilitate wound contraction by bringing the wound edges together.

3. **Remodeling:** begun two to three weeks following wound creation and may last for a year or more. During remodeling, type III collagen is replaced by stronger type I collagen. Collagen maturation increases tensile strength in the wound, reaching its peak 12 weeks after damage (4).

There are many factors affecting wound healing include: 1. Intrinsic factors such as age, sex hormones and systemic disease, 2. Extrinsic factors such as drugs intake, smoking and bacterial biofilm (5).

Sesame oil is yields from *sesamum indicum* which contain various ingrediants with well-acknowledged cosmetic benefits. In addition the raw material also contains lignans, tocopherols, phytosterols, and bioactive compounds, including natural antioxidants (6).

Lignans found in sesame seeds in significant amounts such as sesamin, sesamolin, and sesaminol, that are considered as antioxident. Researches have been showed that the topical application of *sesamum indicum* extract lead to reduce the oxidative stress by inhibiting the production of xanthine oxidase and nitric oxide in rats (7).

Materials and Methods:

Sesame oil preparation:

Sesame oil was supplied as 100% cold-pressed virgin sesame oil. The Sesame seeds were collected from local market, then broken and damaged seeds were removed. Cold-pressing was performed through a komet single-screw oil presser. To prevent harmful effects caused by heating, the temperature was maintained at less than 45°c during cold pressing process (8).

Study design:

Twenty eight male albino rats were used in this study, they were divided randomly into two groups:

1. Control group (CONT.): fourteen rats, their created wounds were left to heal normally
2. Sesame oil group (SO): fourteen rats, their created wounds were treated with sesame oil extract.

Seven rats from each group were scarified at seven days post operation day, the other seven rats from each group were scarified at fourteen days post operation day.

Surgical procedure:

Surgical procedure was done in animal house of Veterinary Medicine / Tikrit University in the periof from September-October, 2023. After 10 days of acclimation, rats were given intramuscular injection of general anesthesia (ketamine 10% (1 mg/kg) of body weight and xylazine 2% (0.2 ml / kg) of body weight), there dorsal aspect were shaved, cleaned with iodine, then a piece of cotton damped with alcohol where left on the shaved skin for about 10 minutes, excisional wound was created below the shoulder blades (this location was chosen for wound creation as it cannot be reached by the rats, thereby preventing self-licking and wound irritation), excisional biopsy wound of 10 mm diameter was created using a sterile, disposable biopsy punch. Half of the animals wound were left to heal normally, while the other half of animal wounds were treated topically once daily by sesame oil extract.

Scarification were done using overdose of general anesthesia in following manner:

1. Seven rats from control group and seven rats from sesame oil group were scarified 7 days after the surgical procedure.
2. Seven rats from control group and seven rats from sesame oil group were scarified 14 days after the surgical procedure.

Histopathological study:

On the days 7 and 14th, a tissue sample was collected from the wound site. 10% buffered formalin was used for samples fixation, then, tissue samples were subjected for dehydration, wax impregnation, and preparation of paraffin wax blocks. Tissue slices obtained by sectioning was done on a Microtome (3–5 micron thick), followed by staining with hematoxylin and eosin and examined under the light microscope.

The wound healing parameters which evaluated:

1. Wound contraction: the wounds were measured by electronic vernia in (mm) for the purpose of assessment of wound contraction in 4 periods (3, 7, 10 and 14 days). The wound contraction percentage was calculated by using the equation: [(d1-d2)/d1] x 100, whereas (d1) is the wound diameter on day 0, and (d2) is the wound diameter on day of measuring (3, 7, 10 or 14).
2. Histological analysis: light microscope was used with square grid in one eye piece, choosing five random fields, under power x40 to measure the followings:

A. Blood vessels: the numerical density of the blood vessels. Then the mean number of blood vessels registered.

B. Epithelial thickness: the epithelial thickness assessment was performed by using image j computer software, the distance from outermost layer of the keratin to the innermost basal layer of the epidermis at the wound edges was calculated, the mean of two readings was registered.

Statistical analysis

Data were statistically computerized using statistical package for social Sciences (SPSS) version 22 for windows. P-value of <0.05 was considered statistically significance.

Results:

1. Histological findings:

In 7 days duration the histological pictures shows re epithelization of the skin in both control and SO groups, new hair follicles, blood vessels, few distributed collagen fibers also noticed as showed in figure (1-a , 1-b). The epithelial lining in control group was underlines by granulated tissue. In SO group the blood vessels count were much higher than in control group also collagen fibers were dense packed fibers in SO group.

In 14 days duration , histological pictures of control group shows complete epithelization underlines by few inflammatory tissue. In SO group the histological pictures shows complete thick re epithelization of the wound site with

more regular distributed blood vessels and new hair follicles as showed in figure (1-c, 1-d).2. Comparing wound contraction:
Figure (2) reveal wound size in both control group and SO group at the days:0, 3, 7, 10 and 14. Table 1 showed that wounds contraction in SO group was significantly higher than that in control group in 7, 10 and 14 days.
3. Comparing epithelial thickness:
Epithelial covering was increased with time in both control and experimental groups but it was significantly higher in SO group in both healing periods as showed in table (1).
4. Comparing blood vessels number:
Table 1 revealed that blood vessels number was higher in SO group significantly when compared with control group in both 7 and 14 days healing periods, blood vessels count was higher and more irregular distributed in the 7 days healing period.

Discusses

The present study discusses whether sesame oil can enhance the dermal wound healing which created experimentally in rats through monitoring the wound contraction process in association to histological assessment of the healing process. The results showed that sesame oil help in enhancing the healing process and facilitate wound contraction.
Kotade and Asad mentioned that wound contraction re epithelization, collage nation and inflammation processes are interlinked with each other, so any drug or outer intervention can affect the accuracy of skin healing, for example female hormones can affect healing process, for that reason only male rats were selected in the present study (9), also movement of the animals scratching or licking of wound area can lead to microbial contamination and delay the healing process (10), for that reason back of the rats was selected as area of wound creation.
In the present study, the effect of sesame oil on wound healing process was examined clinically by measuring the wound contraction rate. It was observed that sesame oil facilitate wound closure significantly, this findings agree with Mohammad *et al* (11).
Histological evaluation of sections at 7 days duration revealed complete epithelization in both control and animals treated with sesame oil but the epithelial layer was significantly thicker in SO group, this is because the sesame oil extract has possible antioxidant properties which aids in preventing oxidative damage and acceleratete the healing process as mentioned by Rekha *et al* (12).
Vascularization in wounds treated with sesame oil extract was significantly higher in 7 days SO group but it was reduced and became more regular in the 14 days duration this finding agree with Giuseppe *et al* (10).
In conclusion, wound treatment with sesame oil extract daily enhance healing process by promoting vascularization process and facilitate epithelization process these lead to facilitate wound closure by enhancing wound contraction process.

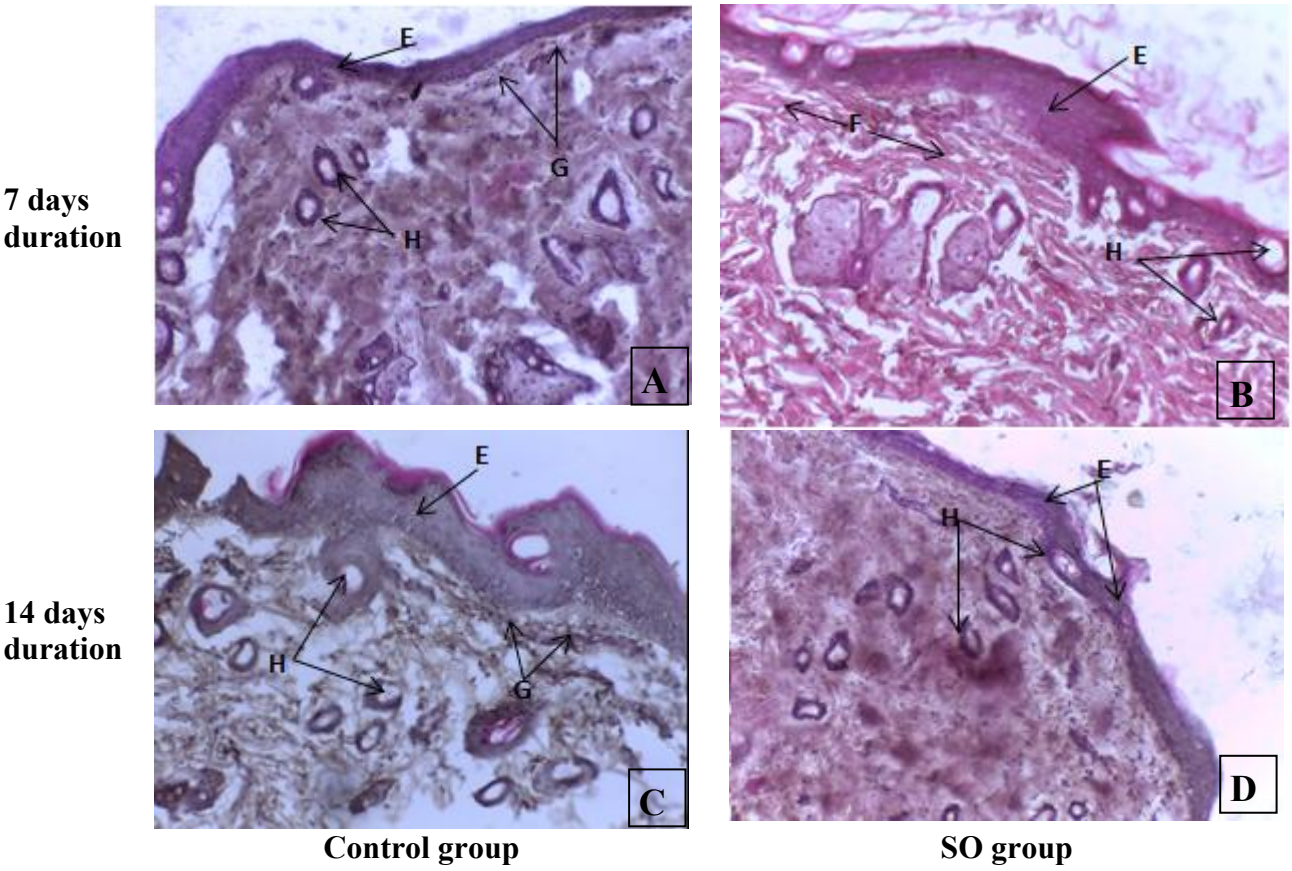

Figure (1): histological sections under light microscope 40x, E:epithelial layer, H: hair follicle, G: granulated tissue, F: collagen fibers

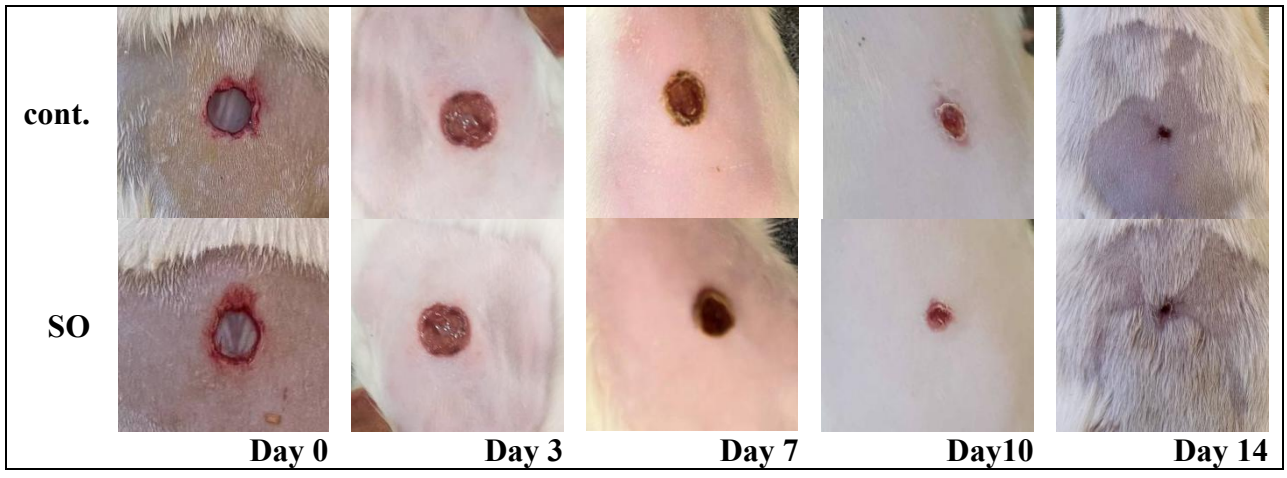

Figure (2): clinical evaluation of wound contraction

Table (1): statistical results of wound contraction rate, epithelial thickness and number of blood vessels

	Day	Mean ± sd		T test	P value
		Cont.	So		
Wound contraction	**Day 3**	5.3±2	6.2±1.2	-3.2	0.211
	Day 7	11.1±3.2	17.1±3.5	-3.4	0.05*
	Day 10	31.3±7.6	43.2±5.3	-4.3	0.001**
	Day 14	46.2±6.7	68.2±3.9	-5.6	0.000**
Epithelial thickness	**Day**	**Mean ± sd**		**T test**	**P value**
		Cont.	**So**		
	Day 7	13.7±1.9	15.3±3.7	-3.1	0.012*
	Day 14	28.2±2.9	33.7±4.3	-3.6	0.003**
Blood vessels count	**Day**	**Mean ± sd**		**T test**	**P value**
		Cont.	**So**		
	Day 7	2.5±0.5	4.5±1.2	-3.6	0.05*
	Day 14	1.4±0.4	2.7±1	-4	0.08*
*** (p<0.05) significant, ** (p<0.01) high significant**					

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