

Full Length Research Paper

## Wound healing activity of flaxseed *Linum usitatissimum* L. in rats

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Wound healing is a complex process which requires special factors and condition. Drugs which accelerate wound healing are expanded, particularly herbal drugs which are mainly safe and effective. Hence, the present study was conducted to evaluate wound healing activity of flaxseed oil on experimentally induced incision wound. The therapeutic ointments were prepared according to two concentrations, 0.75 and 1.5%, and were applied to wound. 32 male rats were divided into 4 groups of 8 rats. Two circle-shapes, full thickness wounds with 7 mm<sup>2</sup> in diameters were made in both side of backbone. Tissue samples were obtained at the end of 3, 7, 14 and 21 days from all groups and were stained with hematoxylin and eosin, then were reviewed under light microscope. Treated animals showed significant reduction of inflammatory cells in the period of re-epithelization. Flaxseed oil significantly accelerates wound healing process and suggested flaxseed as an effective herbal drug for wound in skin.

**Key words:** Wound healing, inflammatory cell, flaxseed oil, epithelization.

### INTRODUCTION

Wounds are inevitable events of life which are made due to physical, chemical or microbiological infections. Normal wound healing response begins immediately after the tissue is injured. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production, later the epithelial tissue is regenerated (Souba and Wilmore, 1999). Research on drugs which accelerate wound healing is developing and it is a crucial subject in biomedical science. Several plants and herbs have been used experimentally to treat skin disorders including wound healing in traditional medicine (Nayak et al., 2009).

Flaxseed (*Linum usitatissimum*) is one of the oldest cultivated plants in the world and is cultivated for its fiber and oil. Flaxseed and its derivatives, flaxseed oil and in-seed oil, are rich source of the essential fatty acid, alpha-linolenic acid, which is a biological precursor to omega-3

fatty acids, such as eicosapentaenoic.

Several animal studies suggested that omega-3 fatty acids of this plant may have potential anti-cardiovascular disease activity, anti-renal disease, anti-inflammation and some autoimmune disease, such as inflammatory bowel disease and rheumatoid arthritis. The lignan found in flaxseed is called secoisolariciresinol diglucoside (SDG) which is a type of carbohydrate classed as a phenolic compound (polyphenol) and it is a powerful antioxidant and has been shown to enhance the immune system functioning and being effective against many different diseases, such as cancers. The present study has been conducted to evaluate probable wound healing characterization of flaxseed on experimentally included wounds in rats.

### MATERIALS AND METHODS

#### Oil material and preparation of the extract

The flaxseed seed found in Iran is *L. usitatissimum* L., in this study, flaxseed oil was extracted by cold pressure method, then it was blended with eucerin-vaselin to prepare treatment ointments with

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different doses (0.75 and 1.5%).

### Experimental animals

32 Male Wister rats (190 to 210 g) of approximately 3 months were used as experimental animals and were divided into four groups of eight rats. The animals were housed in standard environmental conditions of temperature ( $22 \pm 3^\circ\text{C}$ ), humidity ( $60 \pm 5\%$ ) and a 12 h light/dark cycle. During experimental time, rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*.

### Surgical procedures

After anesthesia induction with xylazine 2% and ketamine 10% (I.M. 60 mg/kg) rats were fixed in ventral posture on surgery table. Then, the dorsal area from scapula to ilium were scrubbed and prepared to surgery. Two circle-shapes, full thickness surgical wounds with 7 mm diameters in both side of the backbone, 1 cm away from backbone and 5 cm away from each other were made with biopsy punch 7 mm. With this excisional wounding method, epidermis, dermis, hypodermis and panniculus carnosus layers were removed completely (Luisa and DiPietro, 2003).

### Treatment

After surgical wounds were treated, all rats randomly were colored with none toxic color and divided into three groups. In group 1, ointment with 0.75% flaxseed oil and in group 2 with 1.5% flaxseed oil were administered. Group 3 as placebo was administrated with eucerin-vaseline and group 4 as control group did not receive any substance. All rats were treated for 21 days. Daily observation was performed and any wound fluid or any evidence of infection or other abnormalities were noted.

### Histopathological study

The healing tissues samples obtained during days 3, 7, 14 and 21, from all four groups of animals were processed for histological study. The samples fixed in formalin and installed on slides, stained with hematoxylin and eosin, and were reviewed under light microscope. Recorded factors were scar, inflammatory cells, kind of inflammatory cells, angiogenesis, fibroplasia, congestion, collagen density fibroblast, fibrin and fibroblastic aggregation.

### Statistical analysis

All values are reported as mean  $\pm$  S.D, the statistical differences among groups were assessed using Duncan multiple range test and analysis of variance (ANOVA). A value of  $p < 0.05$  was considered significant. Statistical analysis was performed using SAS 9.1 for Windows.

## RESULTS

### Groups A (1.5%), B (0.75%), C (control groups) and placebo groups

In this study results of control groups and placebo groups were almost same; in fact some insignificant differences were seen.

### 3 days post-surgery

In group C, epithelization was very low. Whole tissue was swollen inflammatory cells, especially, neutrophils were highly seen but there was no sign of macrophages, neo-angiogenesis collagen and fibroblast.

In group B, epithelization and inflammatory cell rate were the same as C group but neo-angiogenesis was high and wound was highly congested. Presence of new collagen was acceptable and it was similar to group A. Fibroblasts were slightly in the place as well.

In group A, epithelization was more than both B and control groups. Macrophages were seen deep in the wound and the number of inflammatory cells was decreased. Neo-angiogenesis was expanded and congestion was lower than group B. Fibroblast rate increased as compare to group B.

### 7 days post-surgery

Presence of neutrophils in superficial layer was decreased in C group. Macrophages and fibroblasts were remarkably seen in the beneath layer. Collagenation was more than before.

In group B, the number of inflammatory cells decreased and was only seen in beneath layer. Neo-angiogenesis and collagenation is suitable. Epithelization was started slightly and fibroblasts were seen.

Wound healing activity was much better in group A as compared to groups B and C. Neo-angiogenesis in superficial layer was good and epithelial layer were about to form, even keratinized layer was being formed. Collagen filaments had started forming cross junctions and inflammatory cells were just under epithelial layer.

### 14 days post-surgery

Results on 14th day showed complete epithelization and thin keratinized layers. Mononuclear inflammatory cells were all over the wound. Collagen rate was acceptable, but collagen maturation was not complete. In the superficial area, neo-angiogenesis was not yet finished. Fibroblasts were more than fibrocytes.

There was no sign of scar tissue in group B and inflammatory cell rate was very low. Neo-angiogenesis which was just in superficial layer was lower than group C. Collagen formation in superficial layers was better as compared to beneath layers. Keratinized layers was visible and hair follicles were about to form, also, upper layers have a bit granulation tissue. Maturation collagen rate was much as compared to two mentioned groups. Granulation was better; neo-angiogenesis was slightly in upper layers. Collagen maturation was not completed only in the upper layer.

**Table 1.** Effect of flaxseed *L. usitatissimum* L. extract on percentage (%) wound healing in experimental rats.

S/N	Group	3 days	7 days	14 days	21 days
1	control	29.28 <sup>a</sup> ± 0.80	43.85 <sup>c</sup> ± 0.57	69.58 <sup>d</sup> ± 0.63	97.86 <sup>b</sup> ± 1.01
2	placebo	29.32 <sup>a</sup> ± 0.95	44.67 <sup>c</sup> ± 0.92	76.43 <sup>c</sup> ± 0.67	100 <sup>a</sup> ± 0.0
3	0.75%	29.82 <sup>a</sup> ± 0.29	54.35 <sup>b</sup> ± 1.09	88.81 <sup>b</sup> ± 1.91	100 <sup>a</sup> ± 0.0
4	1.5%	30.38 <sup>a</sup> ± 0.96	75.54 <sup>a</sup> ± 1.52	100 <sup>a</sup> ± 0.0	100 <sup>a</sup> ± 0.0
Significance	-	NS	**	*	**

\*All expressed as mean and standard deviation (S.D). Mean in columns with different letters were significantly different (NS, Not Significant; \*p < 0.05; \*\*p < 0.01).

### 21 days post-surgery

Wound healing trend was almost finished in group C, collagens were in maturation phase but maturation was not ended. Granulation tissue also was formed. Fibrocytes were outnumbering as compared to fibroblasts which shows collagenation process. Thick layer of keratinized tissue had been made, but low rate of neo-angiogenesis showed that angiogenesis was not yet finished.

There was no sign of scar tissue in B group and neo-angiogenesis was rarely seen in middle and beneath layers. Collagen rate was much as compared to C group. Epithelization quality was lower than A group. Keratinization was acceptable and granulation tissue was low in upper layer.

In group A, collagenation had no significant difference with groups B and C, but the quality of epithelization and epithelium thickness were much more than the aforementioned groups. Keratinized layer was thicker than the other groups, but granulation differences were insignificant.

### Wound enclosure

Percentages of wound healing have been documented in Table 1. 3 days after drug application, no statistical significant differences were seen between groups (ns), although treated groups showed better wound enclosure percentage. Significant results were seen from day 7 in which treated groups showed higher closure of wound as compared to control and placebo, but there was no difference between control and placebo groups. Treated groups showed statistically significant difference as compared to each other as well. All groups demonstrated differences to each other, while group B had best wound closure percentage (100%) in 14th day. Complete closure of wounds was observed 21 days after the experiment.

### DISCUSSION

The results of the present study showed that the extract of *L. usitatissimum* accelerate the progression of wound healing activity. Wound healing consists of event roughly divided into three overlapping phases, such as inflama-

mation, granulation tissue formation and remodeling of the extra cellular matrix (Smith and Equist, 1967; Luisa and DiPietro, 2003). Different studies show that wound healing therapeutic agents which have anti-inflammatory antibacterial, antimycotic, insecticidal, antiseptic and anti-maggot properties should be externally applicable.

*L. usitatissimum* (flax) is an annual plant widely distributed in mediterranean and temperature climate zone. It is among the oldest crop plants cultivated for the purpose of oil and fiber (Millam et al., 2005). Furthermore, there is a report that flax products are also recommended for treating skin disease (e.g healing of chronic skin ulceration) (De Spirt et al., 2008). Flax oil is one of the richest sources of  $\alpha$ -linoleic acid ( $\alpha$ -LA), which includes about 44 to 57% of all fatty acids and it also contains 15 to 29% linolenic acid and 13 to 29% oleic acid (Muir and Westcott, 2003). Between them,  $\alpha$ -linolenic acid and linolenic acid are both required for cell membrane for the structural integrity (Flaxseed oil, 2003), so it can be effective in wound integrity as it was in the present study. Also, flaxseeds and oil (some extent) are a valuable source of secoisolariciresinol diglucoside (SDG) and other anti oxidative compounds, such as tocoferol, carotenoids, phenolic acids and anthocyanins. SDG also has anti-viral, anti-bacterial and anti-fungal properties (Muir and Westcott, 2003; Bozan and Temelli, 2008) which were proved to be major criteria to accelerate wound healing process in the present study. There is a report that flax fibers with high antioxidant level would be the perfect material for linen wound dressing. The high level of antioxidants (e.g. phenolic acids) can stimulate natural process of wound clarity by macrophages (Sen et al., 2002), and this bandage can assure the perfect milieu for effective healing by helping the natural process to progress. This can prevent fibroma formation, and it can also keep optimal humidity that facilitates epithelial cells migration (Dyson, 1988). It should be simultaneously used with fibers, transgenic oil emulsion and transgenic seedcake extract to promote healing of chronic skin ulceration (Magdalena et al., 2003).

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