



الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
بُونِي بَرَسِيْتِي اِمْلَاة اِنْبَارَا بِيْحْسَا مَلْدِيْسِيَا

Effect of Flaxseed on Genes Expression during Wound Healing in Animal Model

ASSOC.PROF. DR. BASMA EZZAT MUSTAFA AL-AHMAD

FUNDAMENTAL DENTAL AND MEDICAL SCIENCES DEPARTMENT

FACULTY OF DENTISTRY

INTERNATIONAL ISLAMIC UNIVERSITY

MALAYSIA

WOUND HEALING

- ▶ **Wounds** result in a series of social, physical, and psychosocial impacts that give rise to socio-economic burdens.
- ▶ The current healthcare system approaches to **wound** management are always accelerate wound healing to reduce the socioeconomic burden while lowering morbidity and mortality.
- ▶ **Wounds** can compromise an individual's independence, working capacity, and self-image, which may eventually affect one's quality of life.
- ▶ A chronic **wound** is a significant public health concern that takes up resources from the healthcare system globally.
- ▶ The incidence of the chronic **wound** has been developing in a fashion similar to the “silent epidemic.”



WOUND HEALING

- ▶ **The wound-healing** process is a series of events that attempts to restore the injured tissue to a normal state, preventing serious complications.
- ▶ The healing of the injured skin is an extremely complicated and dynamic process involving a series of sequential and overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. The process needs interactions between various cell types, multiple cytokines, and growth factors.
- ▶ It is one of the most complex biological processes in multicellular organisms.
- ▶ Growth factors, cytokines, and chemokines are key signaling mechanisms to coordinate the healing process.
- ▶ The activation of cellular proliferation is crucial in the tissue repair and regeneration stage.
- ▶ Impaired **wound healing** post-significant problems related to healthcare and expenditure.



FLAXSEED

- ▶ The flaxseed plant (*Linum usitatissimum*) is a member of the Linaceae family, originally native to West Asia and the Mediterranean countries.
- ▶ The oil extract from dried and ripened flaxseeds is one of the richest dietary sources of essential fatty acids
- ▶ Flaxseed is a natural product that enhances the immune system's functioning against different diseases.
- ▶ The omega-3 fatty acid in the flaxseed oil has been shown to influence pro-inflammatory cytokine production positively at wound sites.
- ▶ Linoleic acid conveys essential nutrients that assist in cellular respiration and membrane regeneration.



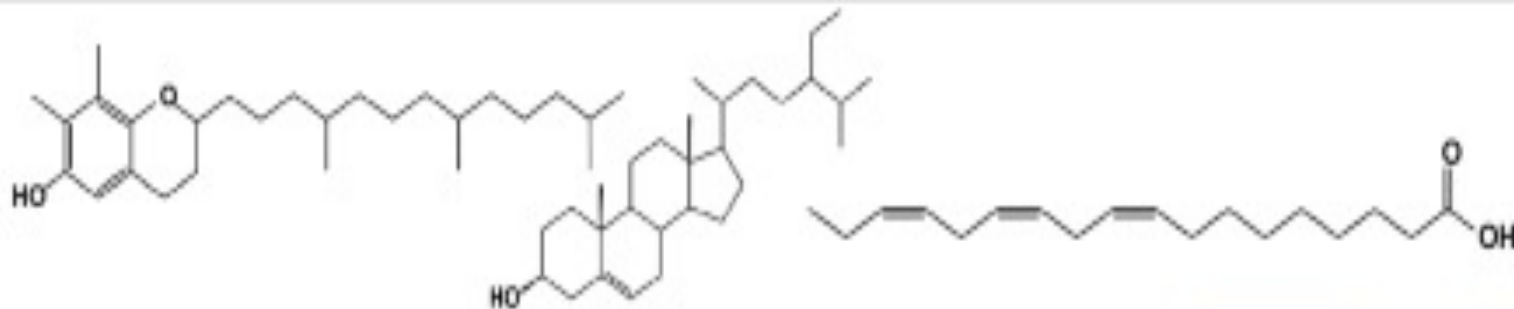
FLAXSEED

- ▶ Furthermore, its richness in flavonoids is believed to play an important role in the tissue regeneration phase of wound healing by improving the collagen fibers strength and minimizing cell damage by enhancing DNA synthesis.
- ▶ Flaxseed oil's popularity and affordable cost make it an attractive candidate for studies.
- ▶ Antioxidant molecules present in flaxseed can protect the healthy cells by sequestering the reactive oxygen species, hence preventing oxidative stress.
- ▶ The pharmacological properties of flaxseed are attributed to the presence of polyunsaturated fatty acids (PUFA) and mono-unsaturated fatty acids (MUFA) in its composition that acts to stimulate the production of growth factors, fibroplasia, and neovascularization.





Walnut
oil



Antioxidant

↑ AOPP, TBARS,
GSH in
diabetic rats



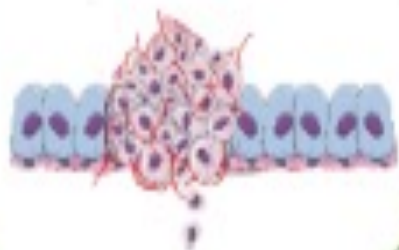
↑ LOX, SOD, CAT and
glutathione peroxidase

↑ cell membrane integrity



Anticancer

↑ Apoptosis
↓ proliferation
↓ metastasis



Anti-osteoporosis

Modulation of prostaglandin
biosynthesis in osteogenic cells

↑ ALP, TRAP
↓ IL-1 β , IL-6, TNF- α



Anti-inflammatory

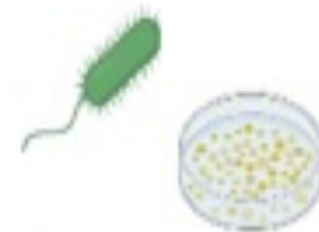
↓ NF- κ B, MAPK pathways



↓ proinflammatory cytokines
ALA converts into PGH₂,
PGE₂, LTB₄, and EPA

Anti-bacterial

↑ macrophage bacterial uptake
↓ inflammatory markers
↓ pneumonia



FLAXSEED

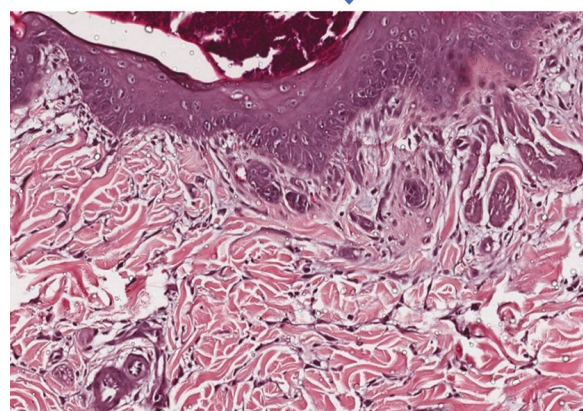
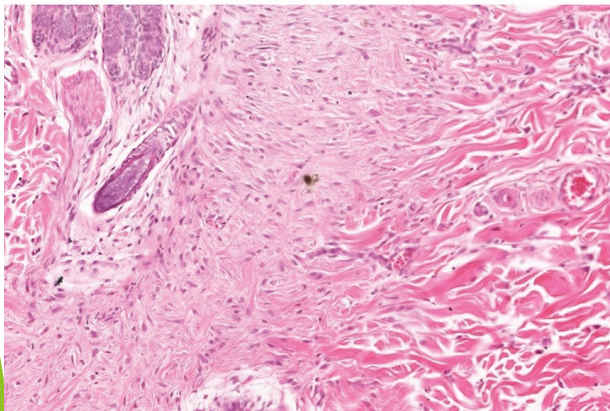
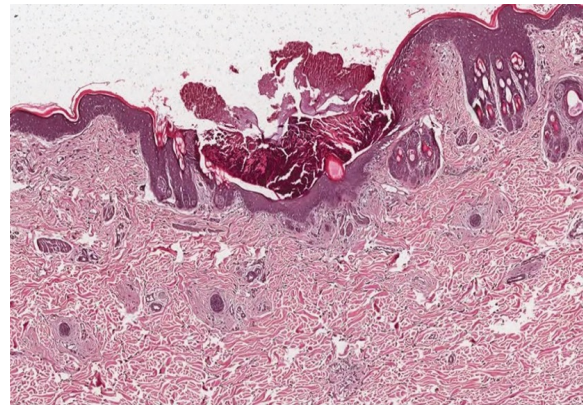
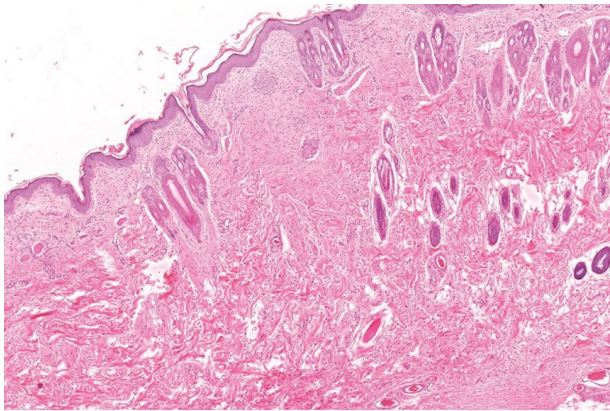
- ▶ Previously authors reported that linoleic acid exhibits proinflammatory characteristics and an anti-inflammatory effect.
- ▶ Therefore, in the process of tissue repair, specifically, as a proinflammatory agent throughout the inflammatory phase of the healing process, flaxseed serves as a substrate for the synthesis of eicosanoids such as prostaglandins, leukotrienes, and thromboxane, these, in turn, modulate immunological and inflammatory responses by altering leukocyte functions and accelerating the process of tissue granulation.
- ▶ Nevertheless, limited studies have been done looking into the response triggered by immune cells and the wound-healing-related genes using flaxseed extract onto the wounded skin for the healing process.



- Full-thickness linear wounds were made on the skin on both sides of the interscapular region on each rabbit's back.
- Incisions were 15 ± 5 mm in length determined by surgical marker and measured using digital caliber, 4 mm wound depth was to the full thickness of the skin.
- Tissue samples were obtained at the 4th, and 7th post-wounding for microscopical analysis, and histopathological parameters including inflammation, re-epithelialization, neovascularization, surface closure rates, and gene expression analysis.



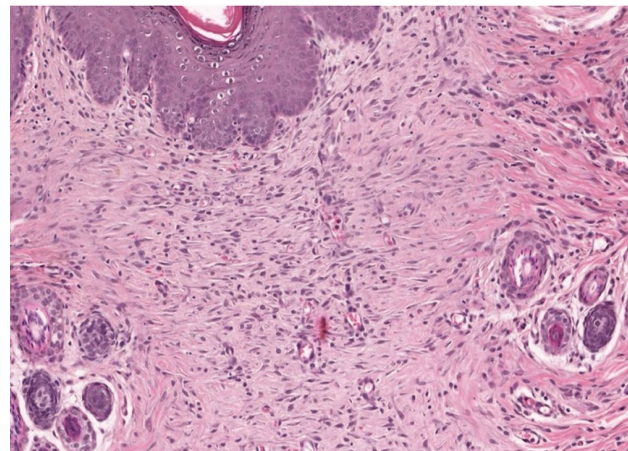
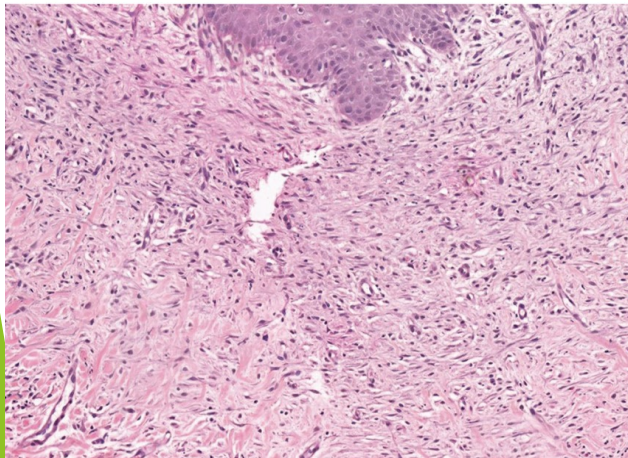
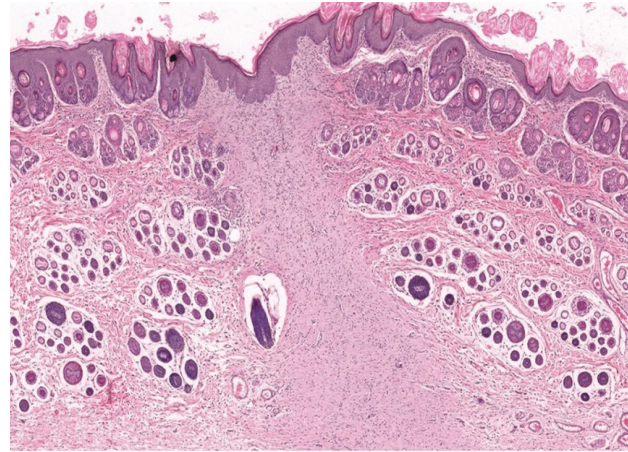
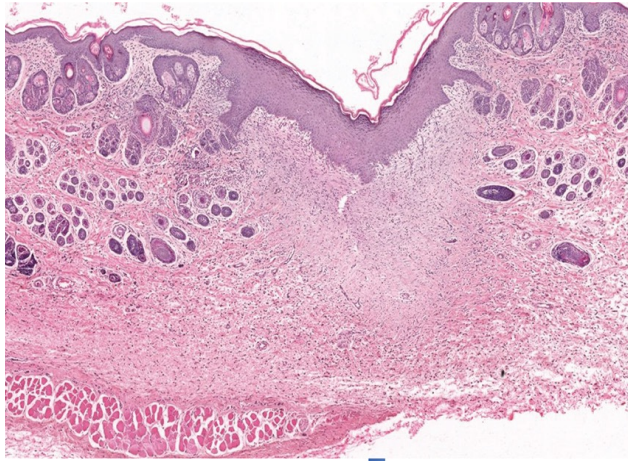
Four days after incision



- ▶ The histological findings show a moderate to heavy inflammatory cell infiltration from the base to the surface, the incision site was clean without any remnant necrotic tissue.
- ▶ The reepithelization, inflammatory cell infiltration, and surface closure rate parameters showed different histological scores at different intervals, presented at ($\times 40$ and scale bar $100 \mu\text{m}$).
- ▶ Skin wound section of rabbit treated with Flaxseed oil compared with untreated.
- ▶ It shows a higher rate of granulation tissue deposition by the presence of more organized collagen tissue



Seven days after incisions



- ▶ The histological findings show complete re-epithelization of the surface, the incision side showed the presence of collagen fibers, and a moderate amount of inflammatory cell infiltration is evidence.
- ▶ The margins of the wound did not show hyperpigmentation of melanin due to the absence of melanocytes in the basal cell layer of epithelium.
- ▶ The flaxseed-treated group showed a higher rate of epithelization and surface closure without sub-cutaneous edema compared to the untreated group.



TOPICAL APPLICATION OF FLAXSEED



- ▶ Topical application of flaxseed extract on the full-thickness skin wounds in rabbits provided better epithelization, new vascularization, and collagenization than control groups.
- ▶ The flaxseed oil promoted biological activities that resulted in faster healing with a significant decrease in the severity of inflammation of non-diabetic animals at the 7th-day interval.
- ▶ This might be related to the antigen-masking activity of the flaxseed on lymphocyte proliferation.
- ▶ A significant increase in the closure rate of the surface area of the flaxseed exposed group on the 7th day compared to the control was observed.



TOPICAL APPLICATION OF FLAXSEED

- ▶ Enhanced therapeutic potential with the minimum formation of scabs subsequently.
- ▶ flaxseed oil showed more therapeutic potential when applied topically rather than being used systematically.
- ▶ Orally supplemented flaxseed oil showed a significant difference in the amount of fibrin in the rat model compared to the control group.
- ▶ Histological analysis disclosed that control group showed significant re-epithelialization compared to the flaxseed group.



TOPICAL APPLICATION OF FLAXSEED



- ▶ Another explanation for the absence of significant re-epithelialization in the flaxseed group can be attributed to the presence of a high concentration of ω -3 polyunsaturated fatty acids.
- ▶ The high molecular weight of flaxseed particles may adversely interfere with surfaced keratinocyte migration and proliferation.
- ▶ Flaxseed may increase the production of pro-inflammatory cytokines, including IL-1B at wound sites and thus, have non-invasive, therapeutic potential to effect cutaneous wound healing.



TOPICAL APPLICATION OF FLAXSEED



- ▶ Moreover, IL-1 expression increases keratinocyte growth, which assists in fibroblast proliferation and collagen synthesis.
- ▶ Angiogenesis is essential to carry oxygen and nutrients to newly formed healing tissue.
- ▶ New vascularization was highest in the flaxseed-exposed group compared to the control group.
- ▶ Flaxseed was found to induce the production of IL-6 and has a stimulatory effect on angiogenesis.



TOPICAL APPLICATION OF FLAXSEED



- ▶ Excessive PMN activity in the wound microenvironment successively ends up in the biosynthesis of protease and neutrophilic elastase which finally leads to tissue destruction and persistent inflammation.
- ▶ high flavonoid and phenolic acid contents of flaxseed oil offer a remarkable antioxidant and anti-inflammatory activity
- ▶ Flaxseed is a great source of polyunsaturated fatty acids. According to that flaxseed oil improves the immune status index and has a tendency to decrease the delayed-type hypersensitivity reactivity.
- ▶ Thus, suppressing cell-mediated immunity by flaxseed oil appears to be helpful in treating autoimmune diseases or inflammatory conditions.

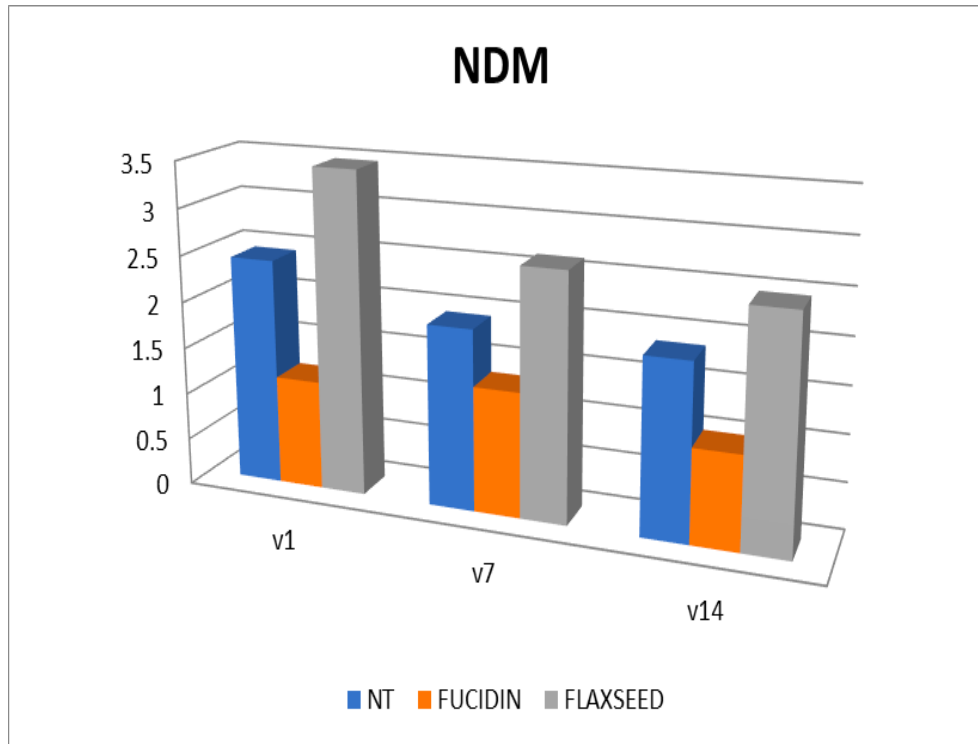




Effect of Flaxseed Extract on Skin Elasticity



Effect of Flaxseed Extract on Skin Elasticity



► the topical application of a flaxseed extract over 14 days of skin wound healing showed an additional effect on the skin as it improved elasticity as well as skin firmness.



Effect of Flaxseed Extract on Skin Elasticity

- This showed the therapeutic effect of flaxseed extract on biological tissue, including incitement of microcirculation and change of fibroblastic cell activity.
- Elasticity evaluation demonstrated increased density and firmness in the network of collagen/elastic fibers in the dermis and subcutis during the wound healing process, promising the production of therapeutic gel to be used in the wound healing process.

	Day 1	Day 3	Day 7	Day 10	Day 14
Flaxseed oil treated group (A)					
Fucidin cream treated group (B)					



MOLECULAR EFFECT OF FLAXSEED ON WOUND HEALING

In the wound area, several pro-inflammatory cytokines like IL-6 are secreted (by macrophages) and thus induce massive leukocyte infiltration from the bloodstream.

These leucocytes play an essential role around the wound site.

Anti-inflammatory markers such as Transforming growth factor-beta (TGF- β) aid in reducing inflammation and advancing the wound to the proliferation stage.

Flaxseed plays an important role in the tissue regeneration phase of wound healing by improving the collagen fibers' strength and minimizing cell damage by enhancing DNA synthesis.



EFFECT OF FLAXSEED ON GENE EXPRESSION DURING WOUND HEALING

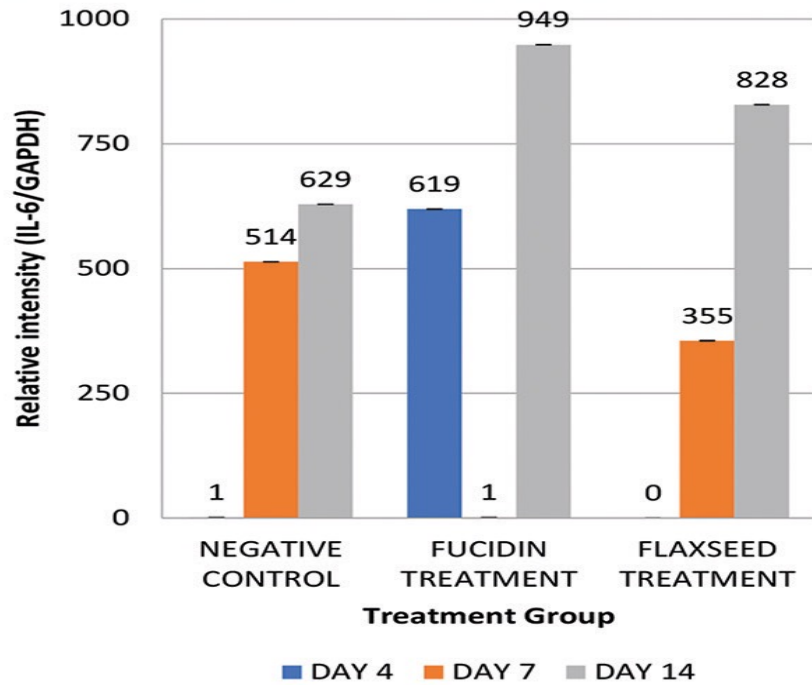
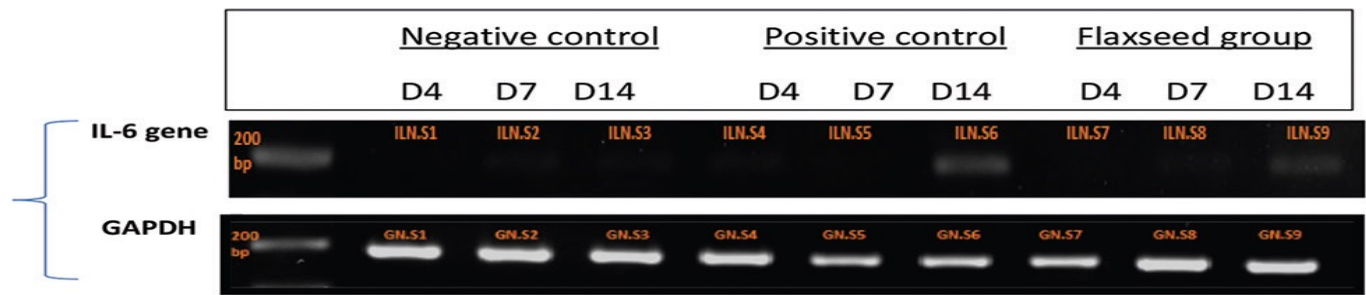
- ▶ The vascular inflammatory reaction takes place after the onset of the lesion. In the wound area, several pro-inflammatory cytokines like IL-6 are secreted (by macrophages) and thus induce massive leukocyte infiltration from the bloodstream.
- ▶ These leucocytes play an essential role in fighting infection. For instance, neutrophils destroy any harmful pathogens around the wound site.
- ▶ Tissue debris clearance by macrophages eventually provides a strong signal for the resolution of inflammation.



EFFECT OF FLAXSEED ON GENE EXPRESSION DURING WOUND HEALING

- ▶ *Inflammation is a double-edged sword. It presumably facilitates the progress of wound repair, but prolonged inflammation can lead to chronic wounds.*
- ▶ the gene expression of IL-6 in rabbits treated with **flaxseed** extract remained constant compared to those with the negative control.
- ▶ suggesting that the **flaxseed** extract could prevent the risk of chronic inflammation.





- ▶ RT-PCR analysis of gene expression for IL-6 at skin wound sites in rabbits. RT-PCR detected the mRNA of these molecules in flaxseed and controlled skin samples of rabbits. The fold change in the baseline gene expression of IL-6 represented the flaxseed influence on rabbit skin.



TGF- β :

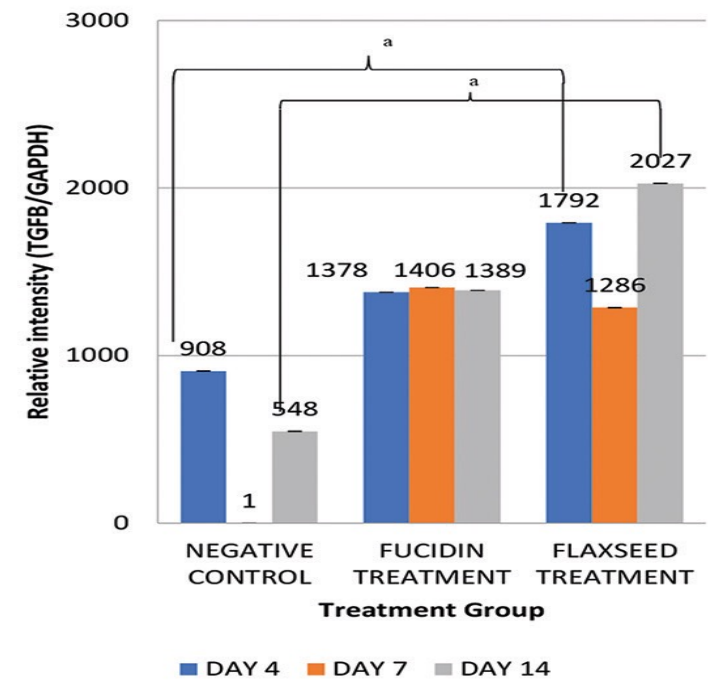
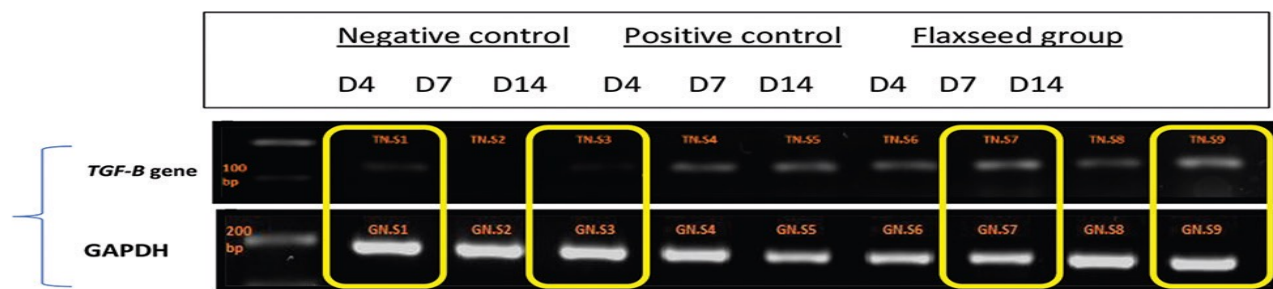
- ▶ TGF- β is a cytokine that regulates angiogenesis, cell proliferation, differentiation, extracellular matrix production, and immune modulation in wound healing.
- ▶ It is released by keratinocytes, platelets, and macrophages.
- ▶ The up-regulated TGF- β accelerates the recruitment of inflammatory cells into the wound site thus initiating inflammatory responses.
- ▶ At the proliferation phase, TGF- β 1 is critical for re-epithelialization and angiogenesis.
- ▶ Moreover, it involves in migration of fibroblasts, synthesis of extracellular matrix components, and differentiation of fibroblasts into myofibroblasts during the remodelling phase.



TGF- β :

- ▶ Several *in vivo* studies have revealed that impaired wound healing is associated with a reduction of TGF- β expression.
- ▶ We found that flaxseed treatment increased the expression of TGF- β on day 4 (1.7-fold) and day 14 (3.7-fold), suggesting its involvement in the inflammation and remodeling stage.
- ▶ The TGF- β upregulation on day 14 might promote tissue contraction and epithelial closure at the wounds.
- ▶ Moreover, an improved progression of tissue regeneration, including collagen bundle synthesis, vascular and hair follicle development, and fibroblast proliferation as well as fibroblast differentiation, has been reported on flaxseed oil-treated rabbits .





- ▶ RT-PCR analysis of gene expression for TGF-β at skin wound sites in rabbits. detected the mRNA of these molecules in flaxseed and controlled skin samples of rabbits. The fold change in the baseline gene expression of TGF-β that represented the flaxseed influence on rabbit skin .



CONCLUSION

- ▶ Flaxseed extract caused changes in the expression level of wound healing-related genes during different phases of the healing process.
- ▶ This suggests the potential role of flaxseed in a wound-healing treatment and highlights a new fundamental knowledge induced by a natural product (Flaxseed) which will be the base for introducing the potential of using this plant as one of the possible wound-healing medications.
- ▶ This study may have an impact on the future development of naturally based medication for improving the histopathological, immunological, and immunohistochemical response of chronic wound patients; and hence, improved quality of life.



RELATED PUBLICATIONS

Effect of flaxseed on TGF-B, IL-6, and MMP9 genes expression during wound healing process in rabbits. Open Access Macedonian Journal of Medical Sciences, 11 (A) pp. 41-46

Effects of flaxseed (linum usitatissimum) extract on osteoblast differentiation potential of stem cells derived from human exfoliated deciduous teeth. Makara Journal of Health Research, 25 (1) pp. 1-7

Flaxseed (Linum usitatissimum) extract activity on human oral fibroblasts (HOrF) cell line. Annals of Dentistry University of Malaya, 27 (8) pp. 50-54

Histopathological changes of the flaxseed extract on skin wound healing in diabetic rabbits. Open Access Macedonian Journal of Medical Sciences, 8 (A) pp. 881-892

The effect of flaxseed extract on skin elasticity of the healing wound in rabbits. The International Medical Journal Malaysia, 18 (1) pp. 5-12

The potential effect of different types of flaxseed (Linum usitatissimum) extract on the viability of oral fibroblasts human cell line. International Journal of Allied Health Sciences (3: Special Issue: Research Towards Sustainable Development Goals) pp. 829-829





Histopathological Changes of the Flaxseed Extract on Skin Wound Healing in Diabetic Rabbits

B. E. Mustafa Al-Ahmad¹, M. A. Kashmoola², Omar Abdul Jabbar^{2*}, K. I. Mokhtar¹, N. Mohamad³, R. Abdul Rahim⁴, Mustafa Nazih Shaban⁵

¹Department of Fundamental Dental and Medical Sciences, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; ²Department of Oral Maxillofacial Surgery and Oral Diagnosis, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; ³Department of Pathology and Laboratory Medicine, Kulliyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; ⁴Department of Basic Medical Science, Kulliyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

Abstract

BACKGROUND: Wound healing includes phases such as cell migration, extracellular matrix deposition, remodeling, and angiogenesis. There are growing medicines that accelerate wound healing, significantly herbal medications that mainly safe and reliable.

AIM: The aim of the study is to examine the histological changes induced by flaxseed oil during wound healing in diabetic animal model.

METHODS: Forty-five male white New Zealand rabbits divided into two main groups diabetic and non-diabetic each group is divided into three groups (n = 9). Diabetic animal group include: (1) Study group (adding Flaxseed), (2) positive control group (adding fucidin 2% cream), and (3) negative control group (no treatment) same distribution of non-diabetic animals groups. Four linearshape,full-thickness wounds were made in both sides of the backbone skin in each animal.

Edited by: Sinisa Stojanovski
Citation: Al-Ahmad BEH, Kashmoola MA, Jabbar OA, Mokhtar KI, Lestari W, Shaban M, Nazri AA. Effect of Flaxseed on TGF- β , IL-6, and MMP9 Genes Expression during Wound Healing Process in Rabbits. Open Access Maced J Med Sci. 2023 Jan 17; 11(A):414-46. https://doi.org/10.3889/oamjms.2023.10518
Keywords: Flaxseed, Wound healing, Diabetes, Rabbit, Histopathology
*Correspondence: Dr. Omar Abdul Jabbar, Department of Oral Maxillofacial Surgery and Oral Diagnosis, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang. E-mail: ojabbar@iium.edu.my
Received: 01-Jul-2023



Effect of Flaxseed on TGF-B, IL-6, and MMP9 Genes Expression during Wound Healing Process in Rabbits

Basma Ezzat Mustafa Al-Ahmad^{1*}, Nazih Shaban Mustafa², Khairani Idah Mokhtar¹, Widya Lestari¹, Munirah Sha'ban³, Aliah Ahmad Nazri¹, Omar Abdul Jabbar⁴

¹Fundamental Dental Medical Sciences, Kulliyah of Dentistry, International Islamic University, Kuala Lumpur, Malaysia; ²Department of Oral Maxillofacial Surgery and Oral Diagnosis, Kulliyah of Dentistry, International Islamic University, Kuala Lumpur, Malaysia; ³Department of Biomedical Sciences, Kulliyah of Allied Health Science, International Islamic University, Kuala Lumpur, Malaysia; ⁴Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Al Masheq University, Baghdad, Iraq

Abstract

Edited by: Mirko Spizacki
Citation: Al-Ahmad BEH, Jabbar OA, Mokhtar KI, Lestari W, Shaban M, Nazri AA. Effect of Flaxseed on TGF- β , IL-6, and MMP9 Genes Expression during Wound Healing Process in Rabbits. Open Access Maced J Med Sci. 2023 Jan 17; 11(A):414-46. https://doi.org/10.3889/oamjms.2023.10518
Keywords: Flaxseed, Gene expression, Wound healing, Rabbit
*Correspondence: Basma Ezzat Mustafa Al-Ahmad, Fundamental Dental Medical Sciences, Kulliyah of Dentistry, International Islamic University, Malaysia. E-mail: dbasma@iium.edu.my
Received: 24-Jun-2022
Revised: 14-Aug-2022
Accepted: 11-Jan-2023
Copyright: © 2023 Basma Ezzat Mustafa Al-Ahmad, Omar Abdul Jabbar, Khairani Idah Mokhtar, Widya Lestari, Munirah Sha'ban, Aliah Ahmad Nazri
Funding: This study was supported through research project (RACQ20-090-0090)
Conflict of Interests: The authors have declared that no

BACKGROUND: Wound healing is a natural restorative response to tissue injury, and it involves regulated order of cellular and biochemical actions to reinstate tissue after injury, which involves resurfacing reconstitution, and restoration of tensile strength of injured skin. Normal and impaired wound healing post-significant problems related to healthcare and expenditure. Most of the chemical medications which widely used for wound healing might cause unwanted side effects with prolonged use such as hyper scarring, thus studies using natural products are now deemed important. Flaxseed is a natural product that enhances the immune system functioning against different diseases. Nevertheless, limited studies have been done looking into the response triggered by immune cells and the wound-healing-related genes with the use of flaxseed extract onto the wounded skin for the healing process.

AIM: The main objective of this study is to analyze the expression of wound healing-related genes during different stages of the wound healing process induced by flaxseed *in vivo*.

METHODS: The effect of flaxseed oil in the early stages (day 4 and 7) and late stages (day 14) of wound healing was explored on New Zealand white rabbits by creating a longitudinal full thickness wound on their back. The gene expression profiles of transforming growth factor-beta (TGF- β), IL-6, and metalloproteinase (MMP9) genes which have roles in wound healing through inflammation, proliferation, and remodeling were studied by polymerase chain reaction method.



The Effect of Flaxseed Extract on Skin Elasticity of The Healing Wound In Rabbits

Abdul Jabbar O¹, Kashmoola MA², Mustafa Al-Ahmad BE³, Mokhtar KI⁴, Muhammad N⁵, Abdul Rahim R⁶, Qouta LA⁶

¹Department of Oral Maxillofacial Surgery & Oral Diagnosis, Kulliyah of Dentistry, International Islamic University Malaysia.

²Fundamental Dental & Medical Sciences, Kulliyah of Dentistry, International Islamic University Malaysia.

³Department of Pathology and Laboratory Medicine, Kulliyah of Medicine, International Islamic University Malaysia.

⁴Department of Basic Medical Sciences, Kulliyah of Medicine, International Islamic University Malaysia.

⁵Philadelphia University, Jordan, Amman.

ABSTRACT

Introduction: Management of disturbed wounds, large skin defects and the areas where skin tension precludes wound closure is of high clinical importance. Healing in wounds occurs through epithelization and contraction processes (second-intentions healing) that may result in certain undesirable complications including keloid and formation of a fragile epithelial layer. **Materials and methods:** 27 white New Zealand rabbit included in this study divided into 3 groups; one group of 9 rabbits received flaxseed gel topically for three time intervals (1, 7, and 14 days); a second group received Fucidin cream as positive control, while a third group has not received any treatment as negative control, Skin elasticity measurements were performed using the DermaLab system. **Results:** Throughout the study, skin elasticity was significantly greater in flaxseed group than in others. Flaxseed decrease elasticity value from (3.46 ± 2.05). Hence, Young's modulus of skin elasticity in flaxseed group was (2.46 ± 1.02) after 14 days (p = 0.003), while no significant differences were evident in both Fucidin group (1.16 ± 0.77) and non-treated group (1.86 ± 1.40)

PUBLICATION



الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
بِنِيَابِ رَبِّسْتِيْ اِمْلَانْ اِمْتَارَا اِيْحْسَانًا مِلْمَسَانَا

THANK YOU

