Wound Healing Properties of Moringa Oleifera on Normal cell line

Doaa Abbas Aljeml[1], Rana A. Ghaleb[2], Nissren Jalal Mohammed[3]*
[1,3]Department of Pharmacology, College of Medicine, University of Babylon, Iraq
[2]Department of Anatomy and Histology, College of Medicine, University of Babylon, Iraq

Abstract
The quality of the patient's life is affected by the complex and difficult process of wound healing. Hemostasis, inflammation, proliferation, and remodeling are the four highly planned stages that it goes through to be accomplished. All four steps must take place in the right order and time period for a wound to heal successfully. Tropical and subtropical nations are home to the monogeneric plant species Moringa oleifera of the Moringaceae family. The purpose of the current study was to investigate M. oleifera's potential to heal wounds in vitro. Cell viability and wound scratch test experiments were performed in the study. The Madin-Darby Canine Kidney (MDCK) cells are mammalian cell line was used ; it was subjected to various concentrations of Moreingo oleifera After 24 hours of incubation, the MTT test was used to analyze their influence on the viability of MDCK cell line, The second experiment included: induce a wound in MDCK cell line then expose the scratched cells to the enhancement dose which was obtained from the first experiment to detect the more active one for wound healing
Keywords: Normal cell line; Moringa Oleifera; Wound Healing

1. Introduction

The largest organ of the body, the skin, acts as a barrier to defend against the outside world (7). The skin is also the organ most vulnerable to injury from foreign invaders. Treatment and care for cutaneous wounds are becoming major concerns in clinical settings. Human wounds that are extensive or open cannot heal via self-contraction, making the process extremely challenging (8).
Surgery, accidents, extrinsic factors (such as pressure, burns, and cuts), or pathologic illnesses like diabetes or vascular diseases are only a few of the causes of wounds. Depending on the underlying causes and effects, these types of injury are categorized as either acute or chronic wounds (15).
Acute wounds that take 8 to 12 weeks to heal, and the mechanical harm brought on by the sheering, blunting, and/or stabbing action of hard objects can result in these wounds. (17).
In addition, chronic wounds are divided into three primary subgroups: vascular ulcers, pressure ulcers, and diabetic ulcers.
Chronic wounds are characterized as ulcers or open wounds that do not heal within three months. (14) .
Homeostasis, inflammation, proliferation, and remodeling are the four steps that make up the natural healing process. In homeostasis, platelets clump together that make up the natural healing process, it is allowing fibroblasts to move to the wounded area and create a thrombus. (9).
Blood vessels are damaged then the inflammatory phase are started. At the same time, platelets release various chemotactic and vasoactive mediators that draw fibroblasts and endothelial cells to the site of the wound in addition to inflammatory cells like neutrophils and macrophages (2).
The third to tenth day mark the beginning of the proliferative phase, which includes mechanisms that lead to re-epithelialization, angiogenesis, collagen deposition, and the development of granulation tissues (12).
Herbal products for wound care or therapy include cleaning, debridement, and supplying a moist environment that encourages the establishment of an optimal natural healing climate. When the state of wounded tissues is comparable to that before the injury, remodeling takes place.dysregulation of any component of the wound healing process slows the healing process and can cause a variety of skin diseases, such as persistent ulceration or non-healing. The nutritional and medical properties of the plant Moringa oleifera, a member of the genus Moringaceae, are well known. The examination of the M. oleifera seed's phytochemical composition reveals that this plant has all the critical components required for the activity of wound healing (1).
Flavonoids and phenolic acids, which are polyphenolic substances, are abundant in the dried leaves of MO. A benzo- pyrone ring is a typical structural element of flavonoids, which plants produce in response to microbial diseases. Consumption of flavonoids has been found to defend against chronic conditions linked to oxidative stress, such as cancer and cardiovascular disease (16).

2. Material and Methods

Moreingo olifera Extract Preparation

Fresh leaves of Moringa oleifera plant were collected from small plant nursery, in Hilla city- Iraq. The plant leaves were washed with deionized water thoroughly and left to dry for 14 days in dark place at room temperature, then grinded to fine powder and stored in tight dry container for further use.
The plant leaves then soaked and macerated in concentration of 100 gm of leaf powder in 1 liter of

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DW and left for extraction at room temperature for 48 hours.

Aqueous extract is filtered many times using medical gauze and the separated extract is then filtered using Whitman filter paper and then centrifuged at 1000 rpm for 10 min to dispose the settled waste and obtain clear liquid, left to dry to obtain solid extract. Preparation of stock solution for aqueous extract was made by dissolving 60 mg of aqueous extract in 30 ml DDW to obtain final concentration of 2000 mcg / ml then filtered using milipore filter syringe to discard any impurities.

Effect of Moringa oleifera on Cell viability of MDCK cell line

Three columns of Three replicate of 96 -well plate were seeded with MDCK cell line. first Column with 3 replicates was considered as a control group, and the three subsequent columns were treated with serial dilutions of Moringa oleifera aqueous extract (500,250,125,62.5, 31.25, 15.6) µg/ml Then the plate was covered and incubated once for 24 hours the wells were washed with 200 µl of sterile PBS. The effect of the Moringa oleifera on the growth of the MDCK line was assessed by MTT assay.

In vitro Wound Scratching

A sterile micropipette tip was used to make a wound in the middle row of cells on a disk.

3. Measurement of Wound

Using a digital camera (Sony), the morphological change in the wound area was documented. The plate was fixed under an inverted microscope during the capture process, and the wound area was photographed using a digital camera at 0, 3, 6, and 9 hours, respectively. 'Image J' software was used to calculate the size of the wound

4. Results

Cell viability Assays

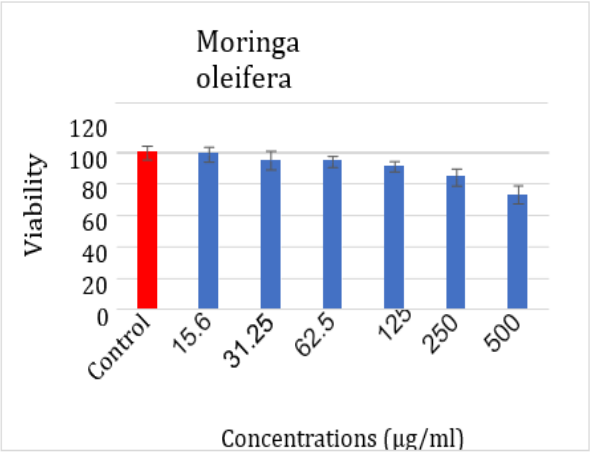

Fig 1. Effect of Moringa oleifera concentrations on viability of MDCK normal cell line after incubation for 24 hours

shows the different concentrations of aqueous leaf extract on MDCK normal cell line that there were no significant difference in cells viability between concentrations of Moringa Oleifera extract except the highest (500, 250, 125 µg/ml) which cause a significant decrease ($p \leq 0.001$) in the viability percentage.

Effect of Moringa oleifera on wound healing of MDCK cell line at different concentrations

Effect of Moringo olifera 7.8 µg /ml concentration on wound healing of MDCK cell line

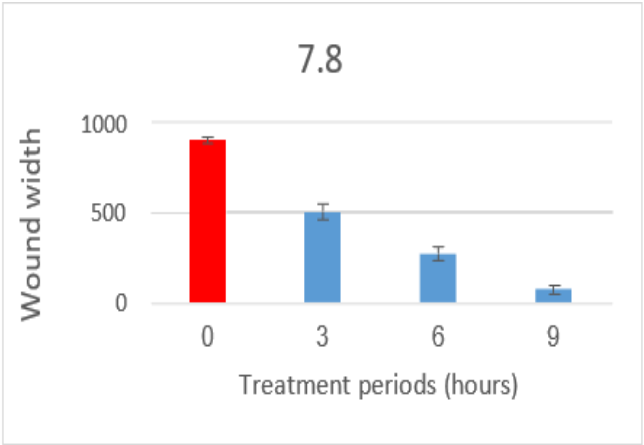

Fig 2. Effect of 7.8 µg/ ml concentration of Moringo olifera on wound healing of MDCK normal cell line after incubation for 12 hours

Shows the effect of Moringa oleifera at 7.8 concentration on wound healing of MDCK normal cell line that there were concentration on wound healing of MDCK normal cell line that there were significant healing ($p<0.001$) and decrease in wound diameter after (3, 6, 9 hr)

Effect of Moringa oleifera 15.6 µg /ml concentration on wound healing of MDCK cell line

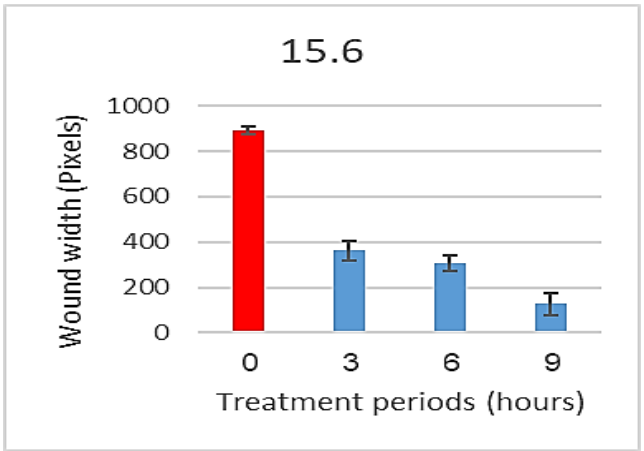

Fig 3. Effect of 15.6µg/ ml concentration of Moringa oleifera on wound healing of MDCK normal cell line after incubation for 12 hours

shows the effect of Moringa oleifera at 15.6 µg/ ml concentration on wound healing of MDCK normal cell line that there were significant healing ($p<0.001$) and decrease in wound diameter after (3, 6, 9 hr)

Effect of Moringa oleifera 31.25 µg /ml concentration on wound healing of MDCK cell linefor 12 hours

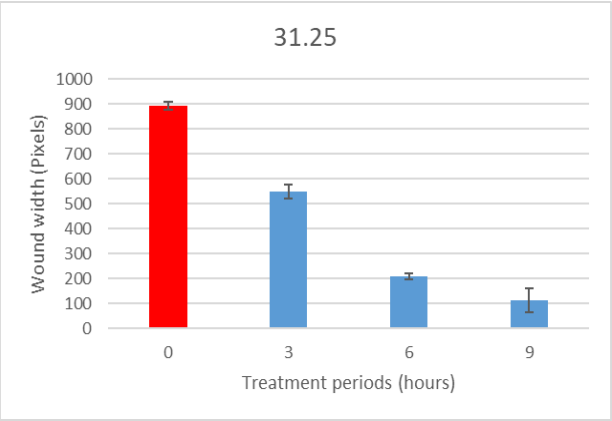

Fig 4. Effect of 31.25 µg/ ml concentration of Moringa oleifera on wound healing of MDCK normal cell line after incubation

shows the effect of Moreingo olifera at 31.25 µg/ ml concentration on wound healing of MDCK normal cell line that there were significant healing ($p<0.001$) and decrease in wound diameter after (3, 6, 9 hr)

Effect of Moringa oleifera 62.5 µg /ml concentration on wound healing of MDCK cell line

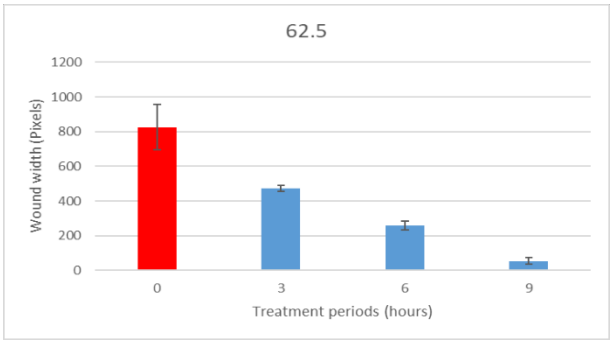

Fig 5. Effect of 62.5 µg/ ml concentration of Moringa oleifera on wound healing of MDCK normal cell line after incubation for 12 hours

shows the effect of Moringa oleifera at 62.5 µg/ ml concentration on wound healing of MDCK normal cell line that there were significant healing ($p<0.001$) and decrease in wound diameter after (3, 6, 9 hr)

5. Discussion

A unique combination of potentially bioactive compounds, including rhamnosyloxy benzyl isothiocyanate and its derivatives, niaziminins, niazinins, -sitosterol, niacin, phenolic acids, glucosinolate, flavonoids, gallic acid, coumarin, and caffeic acids in Moreingo Olifera, is likely the cause of the plant's pharmacological wide range M. oleifera. (14).

Muhammad reported that aqueous MO extract significantly increased the viability of fibroblast cells when compared to the untreated control, which may be justified by the fact that bioactive compounds responsible for the improvement of fibroblast cell proliferation and migration and show that aqueous fraction was safe and non - toxic to human dermal fibroblast cells even at 72 hours just because it did not affect the cell functions of fibroblast cells even at high concentration of 800 µg/mL (10).

There are several biocompounds in the extracts that target cancer cells only, little affecting normal cells. In particular, the unique monosaccharide d-allose, which is present in the leaves of the Moringa oleifera plant, stimulates the development of the protein thioredoxin interacting protein (TXNIP), a tumor suppressor and metastasis suppressor. Without significantly affecting normal cells, d-allose suppresses the development of cancer cells in the G1 phase (5).

The active ingredient was a Moringa oleifera leaf extract (MO E), which was made in an environmentally friendly way. Because of its abundance in quercetin-O-glucoside and quercetin-O-malonyl glucoside, which are responsible for the antioxidant, radical scavenging, and antibacterial activity, early analysis of MOE revealed (11).

Neutrophil control and the c-Jun N-terminal kinase pathway may be responsible for the anti-inflammatory activity. Tannins, phenols, alkaloids, flavonoids, carotenoids, and - sitosterol are active components that contribute to the anti-inflammatory function. Leaf extract increased glutathione levels and decreased malondialdehyde levels in a concentration-dependent manner (4).

Research on the effects of leaf extract on wound healing revealed enhanced tissue regeneration, reduced wound size, downregulated inflammatory mediators, upregulated vascular endothelial growth factor in wound tissues, and remarkable antiproliferative and antimigratory effects on normal human dermal fibroblasts (6).

6. Conclusion

Moringa oleifera effect was dose dependent, it has cytotoxic effect at high concentration. The low concentration (7.8, 15.6, 32.25, 62.5 µg/ml) enhanced wound healing after 12 hr incubation period. this result can be used for more research study to improve the wound healing in vitro and in vivo

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Self

7. Conflicts of Interest

The authors declare no conflicts of interest.

References

Aaliya, A. Garg, P. Goyal,R. et al (2021) 'A novel herbal hydrogel formulation of moringa oleifera for wound healing', Plants, 10(1), pp. 1–13. doi: 10.3390/plants10010025.

Amadeu, T. P, Seabra, Amedea B. de Oliveira, et al. (2008) 'Nitric Oxide Donor Improves Healing if Applied on Inflammatory and Proliferative Phase',

Journal of Surgical Research, 149(1), pp. 84–93. doi: 10.1016/j.jss.2007.10.015. 3-Arie Budovsky, Ludmila Yarmolinsky, S. B.-S. (2015) Effect of medicinal plants on wound healing.

Ayon Bhattacharya, Prashant Tiwari,1 Pratap K. Sahu,1 and Sanjay Kumar. (2018) cA review of the phytochemical and pharmacological characteristics of moringa oleifera', Journal of Pharmacy and Bioallied Sciences, pp. 181–191. Bartoccini, Ajpbs. JPBS_126_18.

Bich Hang Do1,2 · Thi Phuong Thao Nguyen1,2 · Nguyen Quynh Chi Ho1,2 · Thanh Long Le1,2 Nghia Son Hoang1,2 Chinh Chung Doan (2020) 'Mitochondria-mediated Caspase- dependent and Caspase-independent apoptosis induced by aqueous extract from Moringa oleifera leaves in human melanoma cells', Molecular Biology Reports, 47(5), pp. 3675– 3689. doi: 10.1007/s11033-020-05462-y.

Sivapragasam Gothai, Palanisamy Arulselvan1, Woan Sean Tan1, Sharida Fakurazi1. (2016) 'Wound healing properties of ethyl acetate fraction of Moringa oleifera in normal human dermal fibroblasts', Journal of Intercultural Ethnopharmacology, 5(1), pp. 1–6. doi: 10.5455/jice.20160201055629.

Kalachaveedu, M. et al. (2020) 'Fabrication and characterization of herbal drug enriched Guar galactomannan healing applications', International Journal of Biological Macromolecules, 148, pp. 737–749. doi: 10.1016/j.ijbiomac.2020.01.188. healing applications', International Journal of Biological Macromolecules, 148, pp. 737–749. doi: 10.1016/j.ijbiomac.2020.01.188.

Meng Li, Yongping Liang, Jiahui He, Hualei Zhang, and Baolin Guo. (2020) 'Two-Pronged Strategy of Biomechanically Active and Biochemically Multifunctional Hydrogel Wound Dressing to Accelerate Wound Closure and Wound Healing', Chemistry of Materials, 32(23), pp. 9937– 9953. doi: 10.1021/acs.chemmater.0c02823.

Mehwish,H,M . Liu,G . Rajoka M, S. Cai,H . Zhong,H .Song,H . Xia, L. (2021) 'Therapeutic potential of Moringa oleifera seed polysaccharide embedded silver nanoparticles in wound healing', International Journal of Biological Macromolecules, 184(June), pp. 144–158. doi: 10.1016/j.ijbiomac.2021.05.202.

Muhammad, A, Pauzi N, Arulselvan p, Faridah Abas, and Sharida Fakurazi. (2013) 'In Vitro Wound Healing Potential and Identification of Bioactive Compounds from Moringa oleifera Lam', 2013.

Pagano,C. Perioli,L . Baiocchi, C. Bartoccini,A . Beccari, T. Blasi,F . et al. (2020) 'Preparation and characterization of polymeric microparticles loaded with Moringa oleifera leaf extract for exuding wound treatment', International Journal of Pharmaceutics,587, p.119700.doi: 10.1016/j.ijpharm.2020.119700.

12- Pang, C. Ka Siu F,Wei, Kolar W, Mallappa K. l. (2020) 'Gene therapy in wound healing using nanotechnology', Wound Repair and Regeneration, 29(2), pp. 225–239. doi: 10.1111/wrr.12881.

13- Stohs, S. J. and Hartman, M. J. (2015) 'Review of the safety and efficacy of Moringa oleifera', Phytotherapy Research, 29(6), pp. 796–804. doi: 10.1002/ptr.5325.

14- Sylakowski, K., Bradshaw, A. and Wells, A. (2020) 'Mesenchymal Stem Cell/Multipotent Stromal Cell Augmentation of Wound Healing: Lessons from the Physiology of Matrix and Hypoxia Support', American Journal of Pathology, 190(7), pp. 1370–1381. doi: 10.1016/j.ajpath.2020.03.017.

15- Tottoli, E. M. Dorati, R . Conti,B . Genta, I. Chiesa,E . Pisani, S. (2020) 'Skin wound healing process and new emerging technologies for skin wound care and regeneration', Pharmaceutics, 12(8), pp. 1–30. doi: 10.3390/pharmaceutics12080735.

16- Vergara-Jimenez, Manal Mused Almatrafi and Maria Luz Fernandez. (2017) 'Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease',Antioxidant 6, 91; doi: 10.3390/antiox6040091

17- Zahedi P, Rezaeoam O, Ranaei-Siadat S, Jafari S, Supaphol P (2010) A review on wound dressings with an emphasis on electrospun nanofbrous polymeric bandages. Polym Adv Technol 21: 77–95