Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Turmeric extract ointment for treatment of open wound

Hajar Fadhil Abdul-zahra, MSc and Thaier Alwan Abid, PhD
Department of Surgery and Obstetric / College of Veterinary Medicine / University of Al-Qadisiyah / Ad Diwaniyah–IRAQ
Correspondence: Abid TA, Thaier.abid@qu.edu.iq

Abstract

Objective: The study was designed to investigate the effects of topical application of turmeric extract ointment in treatment of open wounds.

Methods: Thirty male adult mice weighing approximately (50 gm), and aged between (60-70 days) were utilized and randomly divided into two equal groups (n15), G1(control), and G2 (turmeric) groups. Two open parallel (vertical or horizontal) circular full-thickness (0.5 cm in diameter) skin incision on the back of the animals were made by using punch machine. One incision was treated and the other was left as control in the same animal. Wounds of G1 were left without treatment. Left wounds of G2 were treated with a topical application of 20% turmeric extract ointment, in a dose of 200mg/cm2 once a day for 7 successive days. Assessment of the wound healing was done grossly and microscopically. Specimens were taken for the two groups after 7, 14 and 21 days PW for histopathological evaluation of the healing process.

Results: Macroscopic results were shown significant $P < 0.05$ decreased of the size of wound in treated group in compare with control group and control wounds of the same animal at 7, and 14 days, while the readings become near each other at 21 days. Microscopic results of G1 show, thin early re-epithelialization of epidermal layers, hyperkeratosis, and presence of acantholysis due to inflammation. In the dermis there was newly formed blood vessels and infiltration of inflammatory cells, and vacuolization at the dermoepidermal junction at 7 day. At 14 days there was thin irregularly arranged re-epithelialized epidermis layers, with hyperkeratosis of stratum corneum, and a gap or acantholysis between epidermis and dermis later. The infiltrations of inflammatory cells and congested blood vessels were evident in the dermis. Also there was accumulation of the fibroblasts, and evenly arranged of thick collagen fibers in the dermis beneath the wound site. Microscopically G2;at 7 day PW, exhibit completely regenerated thick epidermis, severe infiltration of PMNCs and numerous multi-oriented new blood vessels, along with additional new collagen fibers. At 14 days PW well thickening of epidermis were seen upon deep area of granulation tissue. The field showed less cellular and more collagen, palely of hair follicles, swollen sebaceous glands, and the duct of sweat glands can be notified. Whereas at 21 days PW, well organized four layers of epidermis including a thick layer of keratin were seen in the skin wounds. The superficial dermis (SD) and the deep dermis (DD) are less cellular, contain more mature collagen fibers, hair follicles, sebaceous glands, and sweat gland.

Conclusions: The treatments of open skin wounds with turmeric extract ointment 20%, were seen improve the healing process and accelerate the proliferation, wound contraction, maturation and remodeling phases of wound healing.

Key Words: Turmeric, Curcumin, Wound healing.

Introduction

The skin, making up approximately15% of the total body weight of an adult, though it become the largest organ in the body (1). Skin open wounds are extensive tissue damage and are contaminated or infected, thus should provide optimal wound management to achieve

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

rapid and optimal wound healing (2), The wound healing is a multi-stage phenomenon that requires the activation, induction of numerous cell types as keratinocytes, endothelial cells, fibroblast, inflammatory cells, and macrophage which appear to be central to this process (3), The healing process depends on local wound factors, systemic mediators, the underlying disease, and the type of injury. These factors combine to determine if physiologic or acute wound healing occurs, or if there is an abnormal healing process, also called chronic wound healing (4, 5). Wound healing is a complex process, divided into at least three phases: Inflammatory phase, proliferative phase, and remodeling phase. The inflammatory stage begins with platelet aggregation and formation of the fibrin clot, which reestablishes hemostasis and provides an extra cellular matrix for cell migration (6). This phase is triggered by a variety of mediators released from injured tissue cells and capillaries, activated platelets and their cytokines and the by–products of hemostasis (7). The subsequent proliferative phase lasts for about 2 to 3 weeks after the inflammatory phase, dominated by days the formation of granulation tissue and epithelization (8, 9). Re-epithelization these phase also includes contraction of wound edges pull together to reduce the defect in the second step epithelial tissues are formed over wound site (3). Epithelization of open wound is different from that of closed wound. In open wounds epithelization occurs after a bed of granulation tissue has formed. There is a latent period of approximate 4 to 5 days before epithelization starts, (in closed wound epithelization may complete in 48 hours as the epithelium migrates through the fibrin clot) (10). The remodeling phase of wound healing is characterized by a decrease in cell population and an increase in collagen organization in granulation tissue which form a scar. During the remodeling phase myofibroblasts play an important role in wound contraction and scar formation (8, 9, 10, 11, 12, 13). Curcumin is the active substance found in turmeric; the root of *Curcuma longa* and a member of the ginger family, has long been used as a medicine and as food flavor (14). Curcumin despite its poor bioavailability offers potential therapeutic efficacy against a number of human disorders, including cancer, cardiovascular disease, diabetes, arthritis, and neurological diseases, (15, 16, 17, 18). Also it has an additional uses including anti-inflammatory, antioxidant, pro-apoptotic, chemo-preventive, chemotherapeutic, antinociceptive, anti-proliferative, anti-parasitic, and antimalarial effects, and anti-tumor properties (19, 20). Studying curcumin and its pharmacological effects has become more crucial in recent years (20). Curcumin has been found to have therapeutic effects in wound healing (21) because of its anti-infectious, anti-inflammatory, and antioxidant properties. Furthermore, it has been demonstrated that the presence of curcumin accelerates the development of granulation tissue, collagen deposition, and tissue remodeling. Again, curcumin can speed up the healing of wounds by boosting fibroblast growth, vascular density, and epithelial regeneration (19). Curcumin accelerates cutaneous wound healing by multiple biological actions: Such as modulating cytokines and transforming growth factor (22), involvement of TNF-α, MMP-9, α-SMA, and collagen (23).The study was aimed to evaluate the effect of topical application of turmeric extract in treatment of open skin wounds in mice.

Materials and Methods

Thirty (30) adult male mice (50 gm weight), aged (60-70 days) were used and divided into two groups (n 15). After general anesthesia with Ketamine (80 mg/kg BW) and Xylazine (10 mg/kg BW) intramuscularly and preparing of animals for aseptic surgery, two open circular full-thickness (0.5 cm in diameter) skin incisions on the back of the animals were

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

made (24). One incision was treated and the other were left without treatment as control in the same animal. Animals were housed in standard laboratory settings (room temperature 20–24 C° and humidity 60%). Solid food was provided, and water was available at all times.The study was conducted according to the national guidelines for the Care and Use of Laboratory Animals. All protocols were approved by the High Committee for Review and Approval of Research Proposals of the Faculty in the University of Al-Qadisiyah College of Veterinary Medicine.

Control group (G1); did not receive any treatment and left to heal by second intension.

Turmeric treatment group (G2); The left wounds were treated with a topical application of 20% turmeric ointment (23, 25), in a dose of 200mg/cm^2 (26) once a day (27) for 7 successive days after the wound was created.

Turmeric extraction and ointment preparation

Turmeric extraction was done by conventional extraction technique using Soxhlet apparatus (28). The turmeric extract were grinded, powdered and sieved using 212 microns mesh size sieve to gain fine homogenized particles of the extract powder. Formulation of ointment was done by incorporating the extract powder in the base (Vaseline) by trituration using mortar and pestle (29, 30). To achieve 20% w/w turmeric ointment, five (5) grams of turmeric extract powder were mixed with 20 grams of Vaseline in glass mortar. Small amount of Vaseline were added and triturated with pestle, then additional quantities of the base are incorporated and triturated until all the 5 gm extract powder is mixed with the entire base (20gm Vaseline). Trituration was done gradually by increasing amounts of the base until uniform. The mixture transfer to a container and mixed thoroughly by inverting it several times use a vortex mixer for 10 minutes to increase the homogeneity of the mixture. Finally, the prepared ointment was kept in a dark container and was stored at room temperature to be used latter (29, 31, 32).

Morphometric assessment of the wound healing:

Wounds were observed for, alteration of skin color, presence of secretions, presence or not of infection (33).

Direct measurement of wound dimensions was determined at 7th, and 14th day post wounding (PW), using a graduated millimeter measurement ruler;

For measurement the progress changes of wound size (wound area), and wound contraction. Initially the diameter of the circular wound (zero day and 7th day) were taken. The circular wound surface area = (half diameter)^2 X 3.14. When the shape of wound were changed (at 14th day); the surface area = Length x Width (34, 35).

The wound contraction were measuring by the following formula; Percentage of wound contraction = (wound area on day 0 - wound area on day n / wound area on day 0 X 100) (34, 35). Morphometric data were statistically analyzed, using Least Significant Difference (LSD) test to find the significance between groups under the level of $P<0.05$ (36).

Preparation of specimens for histopathological examinations

Specimens of healed skin (wound biopsies) (1 cm^3) were taken at 7, 14, and 21 days PW, preserved in 10% neutral buffered formalin solution and send for processing, staining, and histopathological examination after sectioning in 5 µm and staining with Hematoxylin and Eosin stain to evaluate the progress of healing process (37, 38). The histopathologic assessment consist of; Presence of edema or not, vascular proliferation, vascular congestion, neutrophilic infiltrate (mild, moderate and severe), macrophage (mild, moderate and severe), lymphocytic infiltrate (mild, moderate and severe), young fibrosis, granulation tissue, and fibroblast proliferation (33, 39, 40, 41).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Results

Morphometric assessment of the wound healing:

Clinical evaluation: Throughout the time of experiment (21 days post wounding (P.W.)), all animals were seen, healthy, and active. No infection was seen in any of the animals' wounds, whether they were treated or not, and no deaths were reported. Within 4 hours of wounding, all wound regions swelled and erythematized, and continued to swell, erythema, and expand in size for the next 24 hours P.W. with amplification of inflammatory symptoms. The entire wound expanded, and the wound borders were elevated, red in color, and there was a thick scab owing to blood drying on the wound site from the second day P.W. onwards, which persisted longer than the seventh day P.W. The wound progressively reduced in size until it was transformed to a little scar tissue that was round or liner in shape on the 21st day.

Surface area (Wound area)(Size of wound): On day zero, the wound surface area (mm^2) in both control and treated animals was (19.62 mm^2). At 7, 14, and 21 days, the size of wound in treated groups was significantly reduced $P< 0.05$ when compared to control group and control readings of the same animal, whereas the readings of turmeric treated group at 21 days became significantly smaller than control and control readings of the same animal (table 1).

Wound contraction: The percentage of wound contraction was significantly increased ($P < 0.05$) in treated group at 7^{th}, 14^{th}, and 21^{st} days as compared with control group (G1). turmeric treated group (G2) recorded significant decrease of wound size (7.58, 4.5, and 3.5 mm^2 respectively), and a high significant percentage of wound contraction (61.3%, 77.07 and 82.16 % respectively) at 7, 14, and 21 days (table 1). The edges of the wounds initially at day 0 were seen clean, and marked sharp (Fig. 1), then all wounds become swell before it returns to the first level. The wounds of G1 at 7^{th} day show no signs of inflammation, little decrease in the size of wound in compare with day 0, and the wound was covered with thin scab. On day 14^{th} the scab was not found, more decrease in the size of wound were occurring, and scar tissue was developed. On 21 day PW, scar tissue were seen on wound site, and more decrease in the size of wound but it still large and obvious (Fig. 1). Treated wounds of G2 at the 7^{th} day PW show little reduction in size in compare with control in same animal, and control group at 7^{th} day. We notices the scab fall from two mice out of five mice. On the 14^{th} day PW, treated wounds of G2 show more reduction in size in compare with 7^{th} day of same group, while both incisions (control and treated) in this time (14 days) appears in same size. Complete epithelialization, scar tissue formation, and no scab cover the incisions also seen (Fig. 1). At the 21^{st} day PW, treated incisions of G2, show more reduction in size in compare with 14^{th} day, where the both incisions (control and treated) appears in same size (Fig. 1).

Histopathological assessment of the wound healing:

Histopathological outcomes of wounds of G1 at the 7^{th} day PW show thin early re-epithelialization of epidermal layers including startum corium, covered with crust over the wound site characterized by the extension of the epithelial projection from the wound's edges, hyperkeratosis, and presence of acantholysis due to inflammation. In the dermis there was newly formed blood vessels and infiltration of inflammatory cells (neutrophil and lymphocytes), and the vacuolar interface dermatitis (vacuolization at
the dermoepidermal junction). The detachment between re-epithelialized epidermis layers and dermis layers was clearly evident, with severe inflammatory reactions (Fig. 2A). At 14^{th} day G1 wounds exhibits un-covered (no scab) thin

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

irregularly arranged re-epithelialized epidermis layers rested on irregularly arranged startum basale layer, with hyperkeratosis of startum corneum, and a gap or acantholysis between epidermis and dermis. The infiltrations of inflammatory cells and congested blood vessels were evident in the dermis. Also there was accumulation of the fibroblasts, and unevenly arranged of thick collagen fibers in the dermis beneath the wound site (Fig. 2B). The histopathological finding of treated wounds of G2 at the 7th day PW show completely regenerated thick epidermis was seen connecting the two edigs of the wound, it compose from well differentiated four layers (including a heavy keratin layer) resting upon clear basement membrane. There were severe infiltration of PMNCs and numerous multi-oriented new blood vessels, along with additional new collagen fibers. Pink, brand-new collagen fiber, new blood vessels, a lot of PMNCs, and a lot of fibroblasts dominated the field (Fig.2D). At the 14th day the skin wounds shows thick well regenerated four conventional epidermal layers, starting at the base, are the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum, categorized as a thick covering of keratin. The superficial dermis (SD) and deep dermis (DD) tissues are less cellular and contain more mature collagen fibers and palely of hair follicles, swollen sebaceous glands, and the duct of sweat glands can be notified. There is little adipose tissue (panniculus adiposa) in the deep dermis. The panniculus carnosus (PC) muscle was found, and eosinophilic edema was also evident (Fig.2E). Treated wound of G2 at 21st days PW, show well organized four layers of epidermis including a thick layer of keratin were seen in the skin wounds. The superficial dermis (SD) and deep dermis (DD) are less cellular, contain more mature collagen fibers, and compose, hair follicles, sebaceous glands, and sweat gland. The panniculus adiposa, or adipose tissue, is scarce in the deep dermis. Eosinophilic edema and the panniculus carnosus (PC) muscle in the deep dermis were found (Fig. 4F).

Table (1); Surface area (wound area) and wound contraction.

Periods	Groups	Surface area (mm^2)		Wound contraction %	
		Treated	Control	Treated	Control
0 day	G1, , G2,	19.62Aa	19.62Aa	0Ja	0Ia
7 days	G1	15.43Ba	15.43Ba	21.3Ia	21.3Ha
	G2	7.58Eb	14.3Ca	61.3Fa	27.11Gb
14 days	G1	12.3Ca	12Da	37.3Ha	38.8Fa
	G2	4.5Gb	10.4EFa	77.07Da	46.9Db
21 days	G1	6.2Fa	6Ga	68.3Ea	69.4Ca
	G2	3.5HIb	5.1GHa	82.16Ca	74Bb
LSD(P<0.05)		1.04		3.16	

- **Means with different capital letters in the same column and small letters in the same row are significantly different**

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

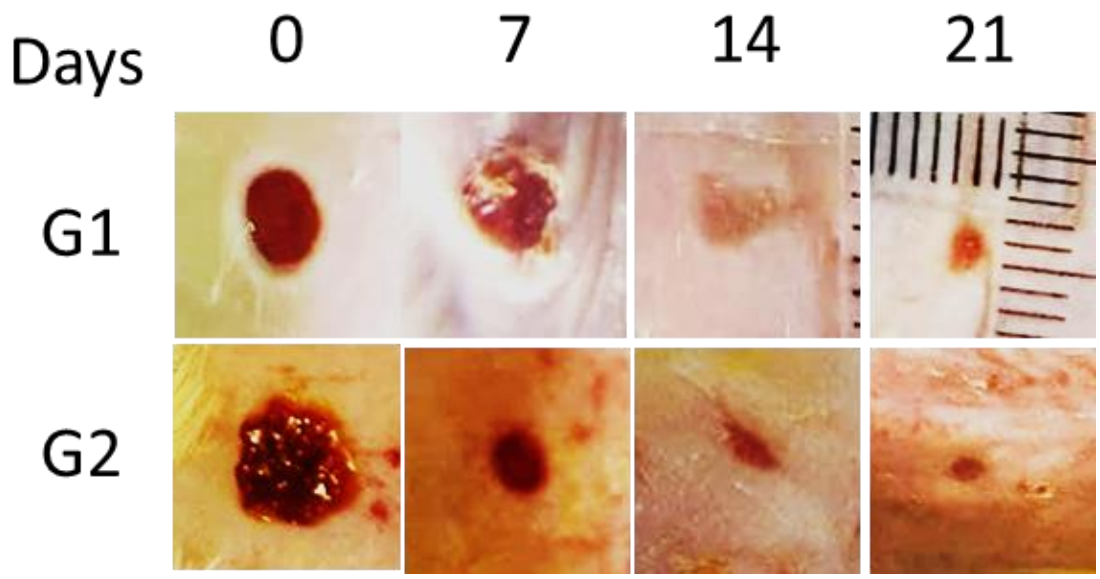

Fig. (4-1): Gross photographs of control (G1), and treated (G2) illustrate the open 5 mm circular full-thickness skin wounds of mice in various phases of wound healing at 7, 14, and 21days. On day 0 in both groups see the sharp edges of the circular wounds, On 7th day the wounds were covered with scab, also there was more reduction in size than the 0 day in treated group (G2) than the control (G1). At 14th day G2 show significant decrease in size in compare with control group, complete epithelialization was occur, more scar tissue, and the shape of wound was changed to liner shape. At 21st day more reduction in wound size in the treated group in compare with control. Complete epithelialization was occurring, and less scar tissue was seen.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

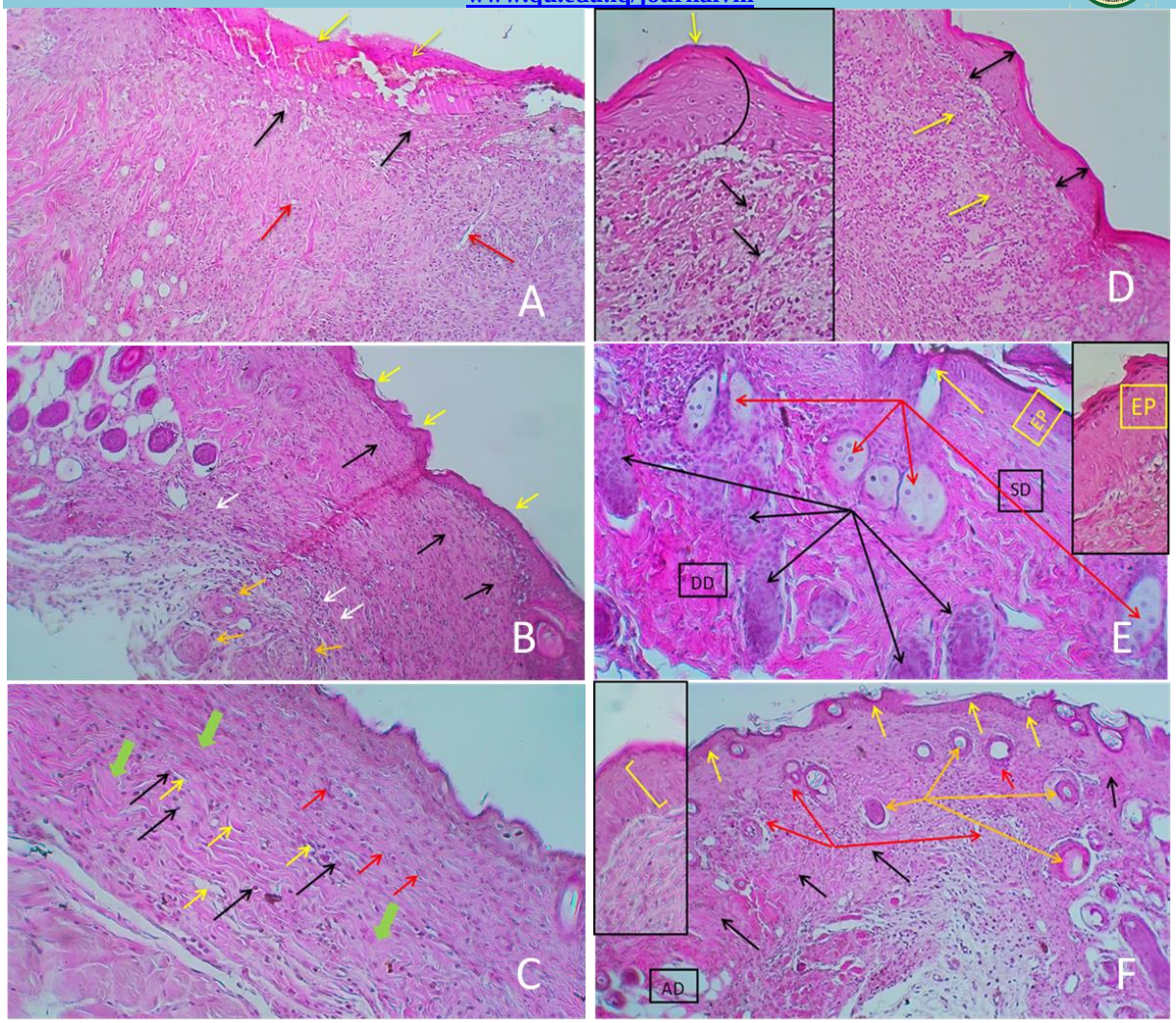

Fig. (2): (A)Untreated wound 7 days PW, the section exhibit thin, unstructured epithelial layers (black arrows) joining the two edges of wound was found under a thick layer of scab (yellow arrows). The epidermis thickened along the edge of the wound. New collagen fiber, and blood vessels were observed in the dermis(red arrows) H&E, 10X. (B): Untreated wound 14 days PW the section demonstrate regenerated thin epidermis (yellow arrows). The superficial dermis, filled with immature collagen fiber (black arrows), plenty of myofibroblast, less number of inflammatory cells, and less number of blood vessels, the deep dermis has more inflammatory cells (white arrows), and solitary combination of plump sebaceous glands (orang arrows), H&E 10X. (C): Untreated wound 21 days PW, demonstrate more mature collagen fibers in the dermis (thick green arrows), plenty of myofibroblast (red arrows), inflammatory cells (black arrows), and blood vessels (yellow arrows),H&E 20X. (D): Treated wound 7 days PW the section exhibit complete thick well organized epithelial layers (black arrows) connecting the two edges of the wound. The dermis involved with dense collagen fibers (yellow arrows), fibroblasts, and inflammatory cells (red arrows), H&E, 10X. The left box show the thickness of epidermis (black bracket) numerous multi-oriented blood vessels (black arrows) H&E 20X. (E): Treated wound 14 days PW, the section demonstrate well regenerated epidermis (EP). The superficial dermis (SD) and deep dermis (DD) tissues are less cellular and contain more mature collagen fibers and palely of hair follicules (black arrows), swollen sebaceous glands (red arrows), and the duct of sweat glands can be notified (yellow arrow), H&E 20X. The right box show the well regenerated and thick epidermis, H&E 20X. (F): Treated wound 21 days PW, the section demonstrate well organized epidermis (yellow arrows). The dermis is less cellular, contain more mature collagen fibers (black arrows), and compose, hair follicles (orange arrows), sebaceous glands, and sweat gland (red arrows). The adipose tissue (AD), is scarce in the deep dermis. Eosinophilic edema and the panniculus

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

carnosus (PC) muscle in the deep dermis were found. H&E 10X. The left box show the thick well regenerated epidermis, H&E 20X.

Discussion

The study was aimed to evaluate the effect of topical application of turmeric extract in treatment of open skin wounds in mice.Traditionally, many natural medicinal plants have been used to treat a variety of diseases since ancient times and are considered a potential source of phytochemicals for the development of new drugs. One of these is curcumin. Many natural medicinal plants have been used to treat a variety of diseases including wounds since ancient time (14). The pharmaceutical industry has made great strides in providing drugs that are able to stimulate the healing process, but only 1–3% of all drugs that are listed in Western pharmacopoeias are intended for use on the skin or cutaneous wounds. Of these, at least one third are obtained from plants (42). Herbal remedies for wound care include cleaning, debridement, and creation of an environment that will promote the healing process naturally (43). These plants are currently regarded to be a potential source of phytochemicals for the production of new drugs, they include curcumin (14). Curcumin is a wound healing agent (21). It decreases inflammation and induces cell proliferation to reconstruct the damaged tissue (44). When compared to control wounds on the same animal and control group at the seventh day PW, the treated wounds of G2 exhibit just a slight size reduction. Out of five mice, we only see two with the scab falling off. While both incisions (control and treated) appear to be the same size at 14th day PW, the turmeric-treated wounds exhibit more shrinkage in comparison to the 7th day of the same group. The incisions also show complete epithelialization, the development of scar tissue, and no scab. When compared to the 14th day, when both the control and turmeric-treated incisions were the same size, the turmeric-treated incisions are smaller at day 21 post-wound, that concur with (45) and (44) who reported curcumin was a powerful tissue repair drug that improved wound contraction at 4th, 8th, and 12th days post wound, respectively. However, curcumin also enhanced reduce wound size and, wounds can heal more quickly and scar less by regulating collagen and reducing reactive oxygen species.

The benefits of topical drug administration are convenience, ease of application, and risk avoidance. Avoid changes in medication levels between and within patents, and assurance of ongoing medication release (46, 47, 48). Curcumin, when applied topically, is more easily accessible at the site of the wound, permitting long-term release of the drug at the dressing site, and delivering of curcumin in a sustained way at the wounded site to allowing wounds to constrict and heal more quickly (49). It can be administered safely and effectively through topical administration for local anti-inflammatory effects (50).The rate of wound contraction of topical treated curcumin wounds was noticeably higher. Additionally, in the treated wounds, the epithelialization period was shorter. These findings bolster curcumin's ability to speed up the healing process. The tensile strength of the tissue is increased when freshly generated collagens are deposited at the location of the wound (45). This improved centripetal movement of a full-thickness wound's edges to facilitate closure of the defect.Also At 7 days, the histopathological findings of G2 (20% turmeric ointment group) showing completely regenerated thick epidermis was seen connecting the two edges of the wound, it compose from well differentiated four layers (including a heavy keratin layer) resting upon clear basement membrane, The findings of the current study are consistent with those of (51), who reported that curcumin

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

treatment shortened the time required for epithelization by changing keratinocytes' phenotype from sedentary to migratory and proliferative, which sped up wound healing by re-epithelializing the epidermis. Additionally, (52) found that curcumin stimulates keratinocyte migration and proliferation, which restores the epithelial barrier of burn wounds more quickly than the control group. Also, according to (44), curcumin promotes epithelial keratinocyte to restore wound healing. This expedited epithelialization to start at day 4, mediate potent epithelialization at day 8, and result in full healing at day 12. Also at 7th days, there were severe infiltration of PMNCs and numerous multi-oriented new blood vessels, along with additional new collagen fibers which was clearly seen in experimental groups where pink, brand-new collagen fiber, new blood vessels, a lot of PMNCs, and a lot of fibroblasts dominated the field. This result is consistent with (53) who showed polymorph nuclear leucocyte (PMN) phagocytes bacteria and other foreign particles in the wound area after PMN arrived at the wound area. The macrophage M1converted into M2 macrophages, which are anti-inflammatory and encourage keratinocytes, fibroblasts, and angiogenesis necessary for tissue regeneration and proliferation, occurs after they have removed apoptotic cells. The final type of cell to enter wound blood during the inflammatory phase is the lymphocyte, which produces a variety of interleukins (IL). One of these is IL-1, which is thought to be essential for the regulation of collagenase, collagen repair, and extracellular matrix (ECM). IL-4 and IL-13, which lymphocytes also generate, suppress M1 activation and cause M2 activation. Also the findings was according with (51) who find, the topical administration of curcumin dramatically increased the mRNA and protein expression of transforming growth factor beta 1 (TGF-1) and vascular endothelial growth factor (VEGF) primarily from day 7 until day 14 of wound healing. It has been demonstrated that VEGF can promote endothelial cell proliferation and inhibit their apoptosis, while TGF-1 can promote angiogenesis by activating a variety of molecular signaling pathways, such as the Akt and ERK pathways, or by enlisting hematopoietic effector cells that express VEGF. Curcumin-induced angiogenesis hastens wound healing in diabetic rats (51). The turmeric extract ointment 20%, which was employed in this study, is biodegradable and may be entirely eliminated from the body once the lesion has healed. Given the characteristics, curcumin ointment acting as a controlled medication delivery system appears to be a potential choice for use as a wound dressing. Curcumin has anti-inflammatory, antioxidant, and antibacterial effects (54).

In Conclusions: The treatments of open skin wounds with turmeric extract ointment 20%, were seen improve the healing process and accelerate the proliferation, wound contraction, maturation and remodeling phases of wound healing in mice.

References

1-Kolarsick, P.A., Kolarsick, M.A. and Goodwin, C., (2011). Anatomy and physiology of the skin. *Journal of the Dermatology Nurses' Association*, 3(4), pp.203-213.

2-Atiyeh, B. S, Ioannovich, J., Al-Amm, C. A., and El-Musa, K .A. (2002) Management of Acute and Chronic Open Wounds: The Importance of Moist Environment in Optimal Wound Healing. *Current Pharmaceutical Biotechnology*, 179-195.

3-Rodero, M. P. and khosrotehrani, K. (2010). Skin wound healing modulation by macrophage. *J. of Inter. Clin and Exp. Patho*. 3 : 643- 653

4-Menke N, Ward K, Witten T, Bonchev D, Diegelmann R.. (2007). Impaired wound healing. *Clin Dermatol*; 25:19-25

5-Enoch, S. and Leaper, D.J., (2008). Basic science of wound healing. *Surgery (Oxford)*, 26(2), pp.31-37.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

6-Wilkinson, H.N. and Hardman, M.J., (2020). Wound healing: Cellular mechanisms and pathological outcomes. *Open biology*, 10(9), p.200223.

7-Gonzalez, A.C.D.O., Costa, T.F., Andrade, Z.D.A. and Medrado, A.R.A.P., (2016). Wound healing-A literature review. *Anais brasileiros de dermatologia*, 91, pp.614-620.

8-Broughton G.I., Janis, J.E. and Attinger, C.E., (2006a). The basic science of wound healing. *Plastic and reconstructive surgery*, 117(7S), pp.12S-34S.

9-Broughton GI, Janis JE, Attinger CE. (2006b). Wound healing: an overview. *Plast. Reconstruct. Surg*. 117, 1e-S–32e-S. (doi:10.1097/01.prs.0000222562.60260.f9).

10-Beldon, P., (2010). Basic science of wound healing. *Surgery (Oxford)*, 28(9), pp.409-412.

11-Li, B. and Wang, J. (2011). Fibroblast and myofibroblasts in wound healing: force generation and measurement. *J. of Tissue Viability*. 20: 108 – 120.

12-Janis, J.E. and Harrison, B., (2014). Wound healing: part I. Basic science. *Plastic and reconstructive surgery*, 133(2), pp.199e-207e.

13-Goldberg, S.R. and Diegelmann, R.F., (2017). Basic science of wound healing. In Critical Limb Ischemia (pp. 131-136). *Springer, Cham*.

14-Akbar, M.U., Rehman, K., Zia, K.M., Qadir, M.I., Akash, M.S.H. and Ibrahim, M., (2018). Critical review on curcumin as a therapeutic agent: From traditional herbal medicine to an ideal therapeutic agent. *Critical Reviews™ in Eukaryotic Gene Expression*, 28(1).

15-Joe B., Vijaykumar M., Lokesh B. R. (2004). Biological properties of curcumin-cellular and molecular mechanisms of action. *Critical Reviews in Food Science and Nutrition*.;44(2):97–111. doi: 10.1080/10408690490424702.

16-Aggarwal, B.B. and Sung, B., (2009). Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends in pharmacological sciences*, 30(2), pp.85-94.

17-Agrawal, S. and Goel, R.K., (2016). Curcumin and its protective and therapeutic uses. *National Journal of Physiology*, Pharmacy and Pharmacology, 6(1), p.1.

18-Fadus M. C., Lau C., Bikhchandani J., Lynch H. T. (2017). Curcumin: an age-old anti-inflammatory and anti-neoplastic agent. *Journal of Traditional and Complementary Medicine*.;7(3):339–346. doi: 10.1016/j.jtcme.2016.08.002.

19-Noorafshan, A. and Ashkani-Esfahani, S., (2013). A review of therapeutic effects of curcumin. *Current pharmaceutical design*, 19(11), pp.2032-2046.

20-Urosevic, M., Nikolic, L., Gajic, I., Nikolic, V., Dinic, A. and Miljkovic, V., (2022). Curcumin: Biological activities and modern pharmaceutical forms. Antibiotics, 11(2), p.135.

21-Akbik, D., Ghadiri, M., Chrzanowski, W. and Rohanizadeh, R., (2014). Curcumin as a wound healing agent. *Life sciences*, 116(1), pp.1-7.

22-Prasad, R., Kumar, D., Kant, V., Tandan, S.K. and Kumar, D., (2017). Curcumin enhanced cutaneous wound healing by modulating cytokines and transforming growth factor in excision wound model in rats. Int. *J. Curr. Microbiol*. Appl. Sci, 6, pp.2263-2273.

23-Yen, Y.H., Pu, C.M., Liu, C.W., Chen, Y.C., Chen, Y.C., Liang, C.J., Hsieh, J.H., Huang, H.F. and Chen, Y.L., (2018). Curcumin accelerates cutaneous wound healing via multiple biological actions: the involvement of TNF-α, MMP-9, α-SMA, and collagen. *International wound journal*, 15(4), pp.605-617.

24-Masson-Meyers, DS, Andrade, TAM, Caetano, GF, Guimaraes, FR, Leite, MN, | Saulo N. Leite, SN, Frade, MAC (2020). Experimental models and methods for cutaneous wound healing assessment. Int. J. Exp. Path.. 2020;00:1–17.

25-Durgaprasad, S., Reetesh, R., Hareesh, K. and Rajput, R., (2011). Effect of a topical curcumin preparation (BIOCURCUMAX) on burn wound healing in rats. *J Pharm Biomed Sci*, 8(23), pp.1-3.

26-Kim, J., Park, S., Jeon, B.S., Jang, W.S., Lee, S.J., Son, Y., Rhim, K.J., Lee, S.I. and Lee, S.S., (2016). Therapeutic effect of topical application of curcumin during treatment of radiation burns in a mini-pig model. *Journal of Veterinary Science*, 17(4), pp.435-444

27-Prasad, R., Kumar, D., Kant, V., Tandan, S.K. and Kumar, D., (2017). Curcumin enhanced cutaneous wound healing by modulating cytokines and transforming growth factor in excision wound model in rats. Int. *J. Curr. Microbiol*. Appl. Sci, 6, pp.2263-2273.

28-Patil, S.S., Bhasarkar, S. and Rathod, V.K., (2019). Extraction of curcuminoids from Curcuma longa: comparative study between batch extraction and novel three phase partitioning. *Preparative biochemistry and biotechnology*, 49(4), pp.407-418.

29-Chhetri, H.P., Yogol, N.S., Sherchan, J., Anupa, K.C., Mansoor, S. and Thapa, P. (2010). Formulation and evaluation of antimicrobial herbal ointment. *Kathmandu university journal of science, engineering and technology*, 6(1), pp.102-107.

30-Sharma, S. and Singh, S.P., (2014). Dermatological preparations, formulation and evaluation of various semi-solid dosage form. Asian *Journal of Pharmaceutical Research and Development*, pp.10-25.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

31-Marriott, J.F., Wilson, K.A., Langley, C.A., and Belcher, D. (2010). Pharmaceutical Compounding and Dispensing. (2nd edition). *Pharmaceutical Press*. pp 163

32-Lane M.E. (2022). Ointments, pastes, gels, cutaneous patches and topical sprays (Chapter 28). In Taylor, K.M. and Aulton, M.E. eds., 2022. Aulton's Pharmaceutics E-Book: The Design and Manufacture of Medicines. (6th edition) *Elsevier Health Sciences*. Pp 453-462

33-d'Acampora, A. J., Vieira, D. S. C., Silva, M. T., Farias, D. C. D., and Tramonte, R. (2006). Morphological analysis of three wound-cleaning processes on potentially contamined wounds in rats. *Acta cirurgica brasileira*, 21, 332-340.

34-Sardari, K., Dehgan, M. M., Mohri, M., Emami, M. R., Mirshahi, A., Maleki, M., and Aslani, M. R. (2006). Macroscopic aspects of wound healing (contraction and epithelialization) after topical administration of allicin in dogs. *Comparative Clinical Pathology*, 15(4), 231-235.

35-Patil, M. V. K., Kandhare, A. D.,andBhise, S. D. (2012). Pharmacological evaluation of ethanolic extract of Daucuscarota Linn root formulated cream on wound healing using excision and incision wound model. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), S646-S655.

36-Weinberg, S. L., and Abramowitz, S. K. (2008). Statistics using SPSS: An integrative approach. *Cambridge University Press*.

37-Luna, I.G. (1968). Manual of histology staining methods of the armed force institute & pathology . 3ed. McGraw . Hill book company . New York . pp: 34-215

38-Estevao, L. D, Vieira, P., Leite, A. G. B., Bulhoes, A. V., Barcelos, L. S., and Neto, J. (2019). Morphological Evaluation of Wound Healing Events in the Excisional Wound Healing Model in Rats, *Bio-protocol* 9 (13): e3285. https://doi.org/10.21769/BioProtoc.3285

39-Gal, P., Kilik, R., Mokry, M., et. al. (2008). Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semi-quantitative and quantitative histological assessments. *Vet. Med*. (Praha) 53,652–659.

40-Dancáková, L., Vasilenko, T., Kováč, I., Jakubčová, K., Hollý, M., Revajová, V., and Bjordal, J. M. (2014). Low-level laser therapy with 810 nm wavelength improves skin wound healing in rats with streptozotocin-induced diabetes. *Photomedicine and Laser Surgery*, 32(4), 198-204.

41-Gupta, A., and Kumar, P. (2015). Assessment of the histological state of the healing wound. *Plastic and Aesthetic Research*, 2, 239-242.

42-Lordani, T.V.A., de Lara, C.E., Ferreira, F.B.P., de Souza Terron Monich, M., Mesquita da Silva, C., Felicetti Lordani, C.R., Giacomini Bueno, F., Vieira Teixeira, J.J. and Lonardoni, M.V.C., (2018). Therapeutic effects of medicinal plants on cutaneous wound healing in humans: a systematic review. Mediators of inflammation, Volume 2018, Article ID 7354250, 12 pages https://doi.org/10.1155/2018/7354250

43-Maver, T., Maver, U., Stana Kleinschek, K., Smrke, D.M. and Kreft, S., (2015). A review of herbal medicines in wound healing. *International journal of dermatology*, 54(7), pp.740-751.

44-Kulac, M., Aktas, C., Tulubas, F., Uygur, R., Kanter, M., Erboga, M., Ceber, M., Topcu, B. and Ozen, O.A., (2013). The effects of topical treatment with curcumin on burn wound healing in rats. *Journal of molecular histology*, 44(1), pp.83-90.

45-Panchatcharam M, Miriyala S, Gayathri VS and Suguna L. (2006) Curcumin improve wound healing by modulating collagen and decreasing reactive oxygen species. *Molecular and Cellular Biochemistry*. 290(1-2):87-96.

46-Sahu, T., Patel, T., Sahu, S. and Gidwani, B., (2016). Skin cream as topical drug delivery system: a review. *Journal of Pharmaceutical and Biological Sciences*, 4(5), p.149.

47-Sunnetha, B.V., Chiranjeevi, S., Jayanthi, V., Akanksha, N.N., Sravani, P.K.R. and Raju, S., (2019). Formulation and evaluation of Aloe Vera herbal ointment [anti-inflammatory & anti-oxidant activity]. *World J. Pharm. Res*., 8, pp.688-699.

48-Chauhan, L. and Gupta, S., (2020). Creams: a review on classification, preparation methods, evaluation and its applications. *Journal of Drug Delivery and Therapeutics*, 10(5-s), pp.281-289.

49-Mohanty, C., and Sahoo, S. K. (2017). Curcumin and its topical formulations for wound healing applications. *Drug Discovery Today*, 22(10), 1582–1592. doi:10.1016/j.drudis.2017.07.001

50-Patel, N.A., Patel, N.J. and Patel, R.P., (2009). Formulation and evaluation of curcumin gel for topical application. *Pharmaceutical Development and Technology*, 14(1), pp.83-92.

51-Kant, V., Gopal, A., Kumar, D., Pathak, N.N., Ram, M., Jangir, B.L., Tandan, S.K. and Kumar, D. (2015). Curcumin-induced angiogenesis hastens wound healing in diabetic rats. *Journal of Surgical Research*, 193(2), pp.978-988

52-Partoazar A, Kianvash N, Darvishi M H, Nasoohi S, Rezayat S M, Bahador A, (2016). Ethosomal curcumin promoted wound healing and reduced bacterial flora in second degree burn in rat. *Drug Research*. 66(12):660–665.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

53-Berbudi, A., Fadlillah, A.A., Afni, M. and Atik, N., 2021. Effect of Curcuma longa Crude Extract, Curcumin and Nano Curcumin-based Gel Topical Administration on Excised Skin Wound Healing in Swiss-Webster Mice. *Biomedical and Pharmacology Journal*, 14(2), pp.635-641.

54-Gupta SC, Patchva S, Koh W, Aggarwal BB (2012) Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clin Exp Pharmacol Physiol* 39:283–299. https://doi.org/10.1111/j.1440-1681.2011.05648.x..