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Research Article

Assessment of Skin Wound Healing Potentiality of the Topical Prosopis Juliflora Leaf Extract Vs. Sulphadiazine in Male Albino Rat; Histological and Immunohistochemical Study

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Abstract

Background: The wounded tissue may be repaired throughout three phases: inflammatory, proliferative and remodeling. Prosopis Juliflora (PJ) plant extract is used to speed up cutaneous wounds healing and improve its quality. **Aim of the work:** To evaluate the PJ wound healing potentiality versus sulphadiazine after topical application on experimentally induced cutaneous wounds. **Materials and methods:** After wound excision, rats were randomly allocated in three groups; positive control, SUL-treated and PJ-treated. The wound was covered with sulphadiazine and PJ leaf extract twice daily, for 21 days post injury (DPI). On the 12th and 21st DPI, full thickness skin specimens and wet granulation tissues were taken for hematoxylin and eosin, Masson's trichrome and TGF-β immunohistochemical staining and antioxidants assay. **Results:** Direct observations of the excised wounds on the 21st DPI showed complete wound healing and re-epithelization in SUL-treated and PJ-treated. However, the wound is still opened in positive control. On the 12th and 21st DPI, the mean wound diameter and surface area were significantly decreased in addition to significant increases in wound contraction percentage in both SUL-treated and PJ-treated groups. The mean values of antioxidants enzymes significantly increased however the malondialdehyde (MDA) levels were significantly decreased in treated groups. **Conclusion:** The topical application of PJ leave extract possesses significant positive roles in enhancing the process of skin wound healing, when compared to topical sulphadiazine. These positive impacts are attributed to alleviation of inflammation, attenuating oxidative stress, upgrading angiogenesis, collagen synthesis and TGF- β expression.

Keywords: Prosopis Juliflora, wound, healing, Sulphadiazine, TGF-β

Introduction

A wound is a sort of physical injury that causes skin tear (open wound) or contusion (closed wound), altering the normal skin structure and function [1]. Depending on the severity of the wound, the wounded tissue might be repaired totally or partially throughout a complicated and prolonged process [2]. Skin wound healing is a complicated process that enlists the collaboration of numerous tissues with diverse cell lineages [3] .

This entire process involves three overlapping phases: inflammatory, proliferative and remodeling. Immediately after injury, the inflammatory phase begins with vasoconstriction, which triggers homeostasis, and then with the release of inflammation mediators, which promotes inflammation. In the prolife-rative phase, the activation of the fibroblasts and the angiogenesis process are the main factors in formation and proliferation of granulation tissues and matrix formation. Finally, the remodeling phase is marked by reorganizations and improvement in the collagenous fiber components that enhance the wound tensile strength [4].

It has been documented that a variety of factors, including infections, inflammatory and immunological reactions might hinder and delay the healing process[5]. Moreover, Recurrent trauma, inadequate perfusion or oxygenation, and excessive inflammation are factors that cause and maintain the chronicity of wounds [6]. It has been noted that an imbalance between the formation of free radicals and antioxidants causes oxidative stress, tissue damage, and sluggish wound healing. Thus, eliminating ROS may be a crucial tactic in the process of healing [7].

Despite recent advances in understanding the underlying principles of wound healing, healing wound defects has encountered several challenges, including the creation of scar tissue and aesthetic issues. Research on agents that cure wounds is one of the emerging topics in modern medicine, and the search for more potent and effective medications is likely one of the key difficulties facing researchers [8] .

It is beyond dispute that medicinal plants are helpful in the treatment of various illnesses [9] . The efficacy, reliability, and safety of these therapeutic plants and other herbal items are being studied in both developed and developing nations [10] . According to estimates from the World Health Organization, 80% of people in several nations utilize plants as their primary source of medication [11]. The use of medicinal plants, for many years, in the treatment of cutaneous wounds to speed up and improve the quality of healing stems from their high concentrations of flavonoids, tannins, alkaloids, saponins, triterpenes and naphthoquinone [12].

These numerous healing ingredients have persuaded researchers to study them and determine the mechanisms behind their probable wound healing abilities. Prosopis Juliflora (PJ), also known as mesquite, is one of the various medicinal plant species belonging to the Leguminosae (Fabaceae) family and has been widely used in folk medicine. PJ is widespread in Saudi Arabia and is an evergreen tree species well adapted to arid and semi-arid zones [13]. It grows as small tree or shrub of variable sizes characterized by possessing prickles and thorns. [14]. These species are well known for their abilities to withstand wind, bind soil, and stabilize sand, as well as for their capacity to grow in the most difficult soil conditions and persist in environments where other trees cannot [14].

Historically, it is used as animal fodder and a source of wood in some regions [15]. A different part (bark, leaves, pods, and flower) of the plant has been used traditionally for the treatment of several diseases such as toothache, asthma, callouses, conjunctivitis, diabetes, diarrhea, dysentery, fever, flu, liver infection, malaria, skin inflammations, and spasm [16].

The plant extract reported to contain medicinal properties such as anti-oxidant [17], anti-inflammatory [18] antibacterial [19], antifungal [20], antimalarial [21] and anti-cancer [22]. Moreover, decoction and tea its extract from leaf and seed extracts are believed to have a healing properties for the skin wound and digestive disorders [23].

These medicinal have been mostly attributed to several phytoconstituents extracted from the PJ such as flavonoids, tannins, alkaloids, ellagic acid, glycosides, steroids, and quinones [24] .

The usefulness of medicinal plants in the treatment of diseases is unquestionable. Traditional herbal medicine practitioners have described the healing properties of various wild plants [25]. Various healing constituents in these plants have prompted researchers to examine them with a view to determine their potential wound healing activities. To share in this debate, the present study was designed to scientifically evaluate the dermal wound healing potentiality of PJ in comparison to Sulphadiazine after topical application on experimentally induced cutaneous wounds in male albino rat models.

Materials and methods

Experimental animals

Forty-two healthy albino rats (200–250 g), aged 1-2 months, were procured from the animal

breeding house Faculty of Pharmacy, Taibah University, Saudi Arabia. All the healthy pathogen free animals were housed individually in polypropylene cages in departmental animal house with standard conditions (23 ± 2°c temperature with 50–60% relative humidity, 12 h light/dark cycle). The rats were acclimatized to the new environment for one week prior to the experiments with free access to water and standard rodent pellet diet (70% carbohydrates, 25% proteins, 5% lipids). All animal experimental manipulations and postoperative care were conducted according to the guide for the care and use of laboratory animals (Institute for Laboratory Animal Research, National Research Council, Washington, Dc: National Academy Press, No. 85-23, Revised 1996).
Also, the study protocol was approved by Taibah College of Medicine Research Ethics Committee (CM-REC) (Study ID: TU-005-22).

Plant material and preparation of extract:
The PJ leaves were purchased and collected from a local farm in Medina, kingdom of Saudi Arabia (KSA). The plant was identified and authenticated by Prof. R.k. Asthana department of Botany and Microbiology, Faculty of Sciences Taibah University, KSA. Leaves were washed twice with tap water and distilled water then dried in 45°c oven for 48 hours. Dry leaves were ground finely in a mill to powder, and 200 gm were place in 500 ml of absolute ethanol and left in a shaking incubator for 48 hours at 35°c. The crude extract was filtrated twice through a cheesecloth and Whatman No.1 filter paper. Ethanolic filtrate was placed in a 35°c oven to evaporate for 72 hours [26].

Wound creation
In accordance to the method conducted by Ghashghaii et al.,, [12], the animals were anaesthetized by intramuscular injection of 1 mg/kg xylazine HCL (xylazine 2% ; Alfasan) as premedication, and 60 mg/kg ketamine HCL (ketamine 5% ; Trittau, Germany) for anesthesia. The back fur of the animals was depilated by shaving. The area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless-steel stencil. A circular full thickness incision of 2.5 cm diameter and 2 mm depth was made in the skin on the interscapular region of each animal, and the incised piece was removed. The procedure was carried out under clean aseptic conditions. Animals were allowed to breathe spontaneously during the surgery. A heating lamp was used to preserve the body temperature at approximately 37°c. The wound was left open, undressed and no local or systemic anti-microbial drugs were administrated. To minimize pain, we used light ether anesthesia by our expert technicians in dosing as the excision wound is raw and painful till 12 days post wound. Animals were closely observed for any infection and if they showed signs of infection were separated, excluded from the study and replaced [27].

Study design
After wound excision, animals were randomly allocated in three groups (14 animals in each group), the 1st group, served as positive control and received no topical treatment, the 2nd group, Sulphadiazine-treated (SUL-treated) group, (Flamazine cream, Riyadh Pharma, Saudi Arabia under license of SMITH & NEHEW pharmaceuticals LTD., England) was applied to the injury twice daily for 21 DPI until complete epithelialization and the 3rd group (PJ-treated) the wound was covered with PJ leaf extract cream 2% twice daily for 21 days post injury (DPI). Seven rats from each group were sacrificed on the 12th day DPI, full thickness skin specimens and wet granulation tissues were taken for histopathological study and assay of antioxidants respectively. On the 21st DPI, the remaining seven rats were euthanized, and the same procedures were repeated.

Measurement of wound area and percentage of wound contraction:
The progressive changes in wound contraction were monitored by determining the wound diameter macroscopically by naked eye on day 0,3,6,9,12,15,18, and 21 post-injury (PI). On the 12th and 21st DPI, the diameters of the wounds were measured using a ruler graduated in millimeters positioned at the level of the lesion, and the means of all wounds diameters were taken then the surface areas were calculated [28].

The Percentage (%) wound contraction was measured according to the following formula= (wound area on day 0 - wound area on day n) / wound area on day 0 × 100 [29].

Sample collection and histological evaluation:
On the 12th and 21st DPI, full thickness skin samples from the wound area including dermis,

epidermis and subcutaneous were carefully dissected and fixed in 10% neutral-buffered formalin, processed routinely, embedded and blocked in paraffin. Five µm - thickness sections were stained with hematoxylin and eosin, Masson's trichrome[30] and immune-histochemical staining for Transforming growth factor beta (TGF-β). Routine histological examinations were performed by three observers using a Nikon light microscope connected with Nikon camera (Nikon, Tokyo, Japan).

Estimation of antioxidants, free radicals

Homogenate of the wet granulation tissues (10%) was prepared in phosphate buffered saline (PBS) at 4°c and was used for the estimation of antioxidants, superoxide dismutase (SOD) and catalase (CAT) and reduced glutathione (GSH); and malondial-dehyde (MDA) as lipid peroxidation marker. The assay of SOD is based on the inhibition of the formation of NADH-phenazine metho-sulphate-nitro blue tetrazolium formazan. CAT measurement was done based on the ability of catalase to oxidize hydrogen peroxide. GSH activity in the homogenate was estimated by the ability to reduce DTNB within 5 min of its addition against blank. LPO levels were estimated in terms of malondialdehyde (MDA) released during lipid peroxidation [27].

Statistical analysis:

The obtained data were evaluated using SPSS software version 20 (SPSS Inc. Chicago, USA). One-way analysis of variance (ANOVA) and the post-hoc Tukey test were selected for multiple comparisons of the observed histomorphometric data in the different studied groups. Values gained were stated as means ± standard deviation (SD) and differences with $p < 0.05$ were considered statistically significant.

Immunohistochemistry staining procedures:

The sections were deparaffinized, washed with PBS three times, and blocked with 5% serum for 30 minutes. Then, the slides were treated with rabbit anti-CD68 primary antibody (1:100; Santa Cruz, USA), anti-TGF-β primary antibody (1:100; ABCAM, UK) at 4°c overnight. The slides were further incubated with goat-anti-rabbit secondary antibody (1:200; DAKO, CA, USA) at 37°c for 30 minutes, developed with 3,30-diaminobenzidine tetrahydrochloride (DAB) solution, and counterstained with hematoxylin. Brown color indicates positive staining under an optical microscope [31].

Results

Macroscopic assessment of the skin wound areas

Direct observations of the excised wounds on days 0,3,6,9,12,15,18 &21 were performed and recorded photographically (Fig.1). With passage of time, the activities of wound healing were clearly demarcated by naked eye and found to be increased in all the studies groups. On the 21st DPI, complete wound healing and re-epithelization were noticed in PJ-treated and SUL-treated. However, the wound is still opened in positive control group.

The wound diameters and surface areas:

The diameters of the wounds were measured, and its surface areas were calculated and expressed in cm^2 (Table.1 and Fig.1). On the 12th and 21st DPI, the mean wound diameter was significantly decreased in both the SUL-treated and PJ-treated groups when compared to the control. Also, the mean surface area of the wound in the SUL-treated and PJ-treated demonstrated a significant decrease in comparison with the untreated group (Table.1 and Fig.1). Additionally, the percentage of the wound contraction exhibited significant increases in SUL-treated and PJ-treated groups when compared to the control one and these were noticed in both the 12th and 21st DPI (Table.1).

Table (1): Representing the various measured and calculated parameters among different groups.

Parameter	Control		SUL Treated		PJ Treated	
	Day 12	Day 21	Day 12	Day 21	Day 12	Day 21
Diameter	2.0914±0.11596	0.8314±0.05757	1.5371±0.06157*	0.1971±0.29898**	1.7343±0.03952*	0.0943±0.11816**
Surface area	2.9829±0.92782	0.5457±0.07807	1.8171±0.13744*	0.0229±0.04855**	2.3557±0.10967*	0.0171±0.02215**
Wound contraction (%)	39.26 %	88.89%	63.00%*	99.53%**	52.03%*	99.65%**

All values are expressed as mean ± SD, n=7. $p \leq 0.05$ is significant. (*) significant difference versus the control on the 12th DPI, (**) indicates significant difference versus the control group on the 21st DPI.

Laboratories results (antioxidants, free radicals):
On the 12th and 21st DPI, the mean values of the MDA (lipid peroxidation marker) were significantly decreased in both the PJ-treated and SUL-treated groups when compared to the control. On the other hand, the levels of the antioxidants (GSH, SOD and CAT) demonstrated significant increases in the PJ-treated and SUL-treated groups as compared to the control and this was evident in both the 12th and 21st DPI (Table 2).

Table (2): The different levels of the antioxidant enzymes and lipid peroxidation marker

Enzyme	Control		SUL Treated		PJ Treated	
	Day 12	Day 21	Day 12	Day 21	Day 12	Day 21
MDA	4.9243 ± 0.19823	3.5671±0.29381	2.2057± o.17615a*	1.7243±0.05884b*	2.9000 ± 0.20849 a*	2.6486±0.21552 b*
GSH	0.6543 ± 0.05884	0.5300±0.06218	.2986±0.09668 a*	1.0200±0.07047 b*	1.1043±0.13758 a*	0.8729±0.05407 b*
SOD	28.2671±2.49563	40.9200±2.93345	78.5429±9.73179a*	55.4357±1.30311 b*	62.5800±4.07007 a*	49.6857±1.19102 b*
CAT	0.1301±0.00279	0.1089±0.00219	.2221±0.01367 a*	0.1686±0.00439 b*	0.1821±0.00620 a*	0.1417±0.00515 b*

All values are expressed as mean ± SD, n=7. * The mean difference ($p \leq 0.05$) is significant at the 0.05 level. (a*) significant difference versus the control on the 12th DPI, (b*) indicates significant difference when compared to the control group on the 21st DPI.

Histological results:

A. Hematoxylin and Eosin stain result:

1. Control group:
There was preserved histological architecture of the classic skin layers in the form of well-organized epidermis with epidermal rete-ridges. The epidermis was formed of 4-6 layers of stratified squamous keratinized epithelium. Stratum basale, the deepest layer formed of one layer of columnar cells containing oval basal nuclei that rest on the basement membrane. Polyhedral cells with centrally located nuclei arranged in 2-3 layers to form the stratum spinosum. The next 2-3 layers were formed of squamous cells with flat nuclei and deeply stained granular cytoplasm. The most superficial layer, the stratum corneum, was acellular including acidophilic keratin. Langerhans's cells were observed among the keratinocytes with characteristic cytoplasmic halos and deeply stained nuclei. A well-demarcated dermal-epidermal junction was also noticed. The dermis demonstrated normal thickness and comprised of papillary and reticular layers. The thin tightly packed collagenous fibers, connective tissues cells and capillaries were clearly observed in the papillary layer. Thick denser collagen bundles in superficial and deep reticular layers appeared well-organized with the present of hair follicles and sebaceous glands **(Fig. 2)**.

2. Positive control groups:
On the 12th DPI, a relatively thick regenerated epidermis, covered with thin keratin layer, at the site of the wound was noticed. The dermis demonstrated very thin collagen fibers in the papillary layer and thin fibers overlapped by fibroblasts in both superficial and deep reticular layers. On the 21st DPI, sections from the rats of the positive control group showed a relatively thin regenerated epidermis formed of

2-3 layers and thin covering keratin layer. No rete-ridges were observed. The dermis exhibited thick collagen bundles in superficial reticular layer and thin in the deep reticular. The blood capillaries and inflammatory cell infiltrates were clearly demonstrated. However, sebaceous glands and hair follicles were not detected **(Fig. 2).**

3. SUL-treated group:
After 12 days, the epidermis was regenerated and became thicker with no rete-ridges. The papillary layer of the dermis showed very thin collagen fibers, moreover the superficial and deep reticular layers contained thin collagen bundles. Multiple blood capillaries and polymorphonuclear cells were noticed. After 21 days, thinning of the epidermis and appearance of keratin layer with minimal rete ridges were observed. The dermis represented by newly formed disorganized, thick collagen fibers in the superficial and deep reticular layers with apparent very thin collagen in papillary layer. However, hair follicles and sebaceous glands were not seen **(Fig. 2).**

4. PJ-treated group:
After 12 days, the epidermis regenerated with no rete-ridges. Thin collagen fibers were noticed in both the papillary and reticular layers of the dermis. After 21 days, almost normal epidermis with reformed rete-ridges were detected and the dermis showed reappearance of skin appendages in the form of newly formed hair microfollicles. Thin collagen fibers were seen in papillary layer. Thick relatively organized, tightly packed collagen bundles were also noticed in the reticular layer of the dermis. Newly formed blood vessels and mononuclear cellular infiltration were seen increasing after 12 days and decreased after 21 days **(Fig. 2).**

B. Masson's Trichrome (MT) staining:
1. Control group:
Sections from the control rats showed that the dermis exhibited thin interlacing collagen fibers in the papillary layer and tightly packed, well organized thick collagen bundles in superficial and deep reticular layers **(Fig. 3).**

2. Positive control group:
On the 12th DPI, thin collagen fibers were seen in all layers of the dermis including the papillary and both the superficial and deep reticular. On the 21st DPI, the dermis of the skin showed thick collagen bundles in superficial reticular layer and thin in the deep reticular of the dermis **(Fig. 3).**

3. SUL- treated group:
After 12 days, the dermis showed very thin collagen fibers in the papillary layer and thin collagen bundles in superficial and deep reticular layers. After 21 days, the dermis represented by newly formed disorganized, thick collagen bundles in the superficial and deep reticular layers with apparent thin collagen fibers in the papillary layer **(Fig. 3).**

4. PJ-treated group:
On the 12th DPI, very thin collagen fibers are seen in papillary layer also thin collagen bundles are seen in the superficial and deep reticular layers. After 21 days, the dermis showed reappearance of skin appendages in the form of newly formed hair follicles. Very thin collagen fibers were seen in papillary layer and thick relatively organized, tightly packed collagen bundles in the reticular layers of the dermis **(Fig. 3).**

C. TGF-β1 Immunohistochemical results
1. Control group:
Immunohistochemical stained sections showed mild expression of TGF-β1 within the epidermis and moderate expression in the endothelial cells of the blood vessels and fibroblasts **(Fig. 4).**

2. Positive control group:
Many cells showed moderate cytoplasmic immunoreactivity in the epidermis, endothelial cells lining the blood vessels and fibroblasts of the dermis **(Fig. 4).**

3. SUL- treated group:
After 12 days, mild positive reactions were seen in the epidermis with moderate reaction in the endothelial cells and fibroblasts of the dermis. After 21 days, moderate to strong positive reactions were noticed in the lower layers of the epidermis with strong positive reactions in the endothelial cells and fibroblasts of the dermis **(Fig. 4).**

4. PJ-treated group:
After 12 days, moderate positive reactions were noticed in the basal layers of the epidermis with strong positive reactions in the endothelial cells lining the blood vessels and fibroblasts of the

dermis. After 21 days, strong positive reactions were detected in the basal layers of the epidermis with marked strong positive reactions in the endothelial cells and fibroblasts of the dermis **(Fig. 4).**

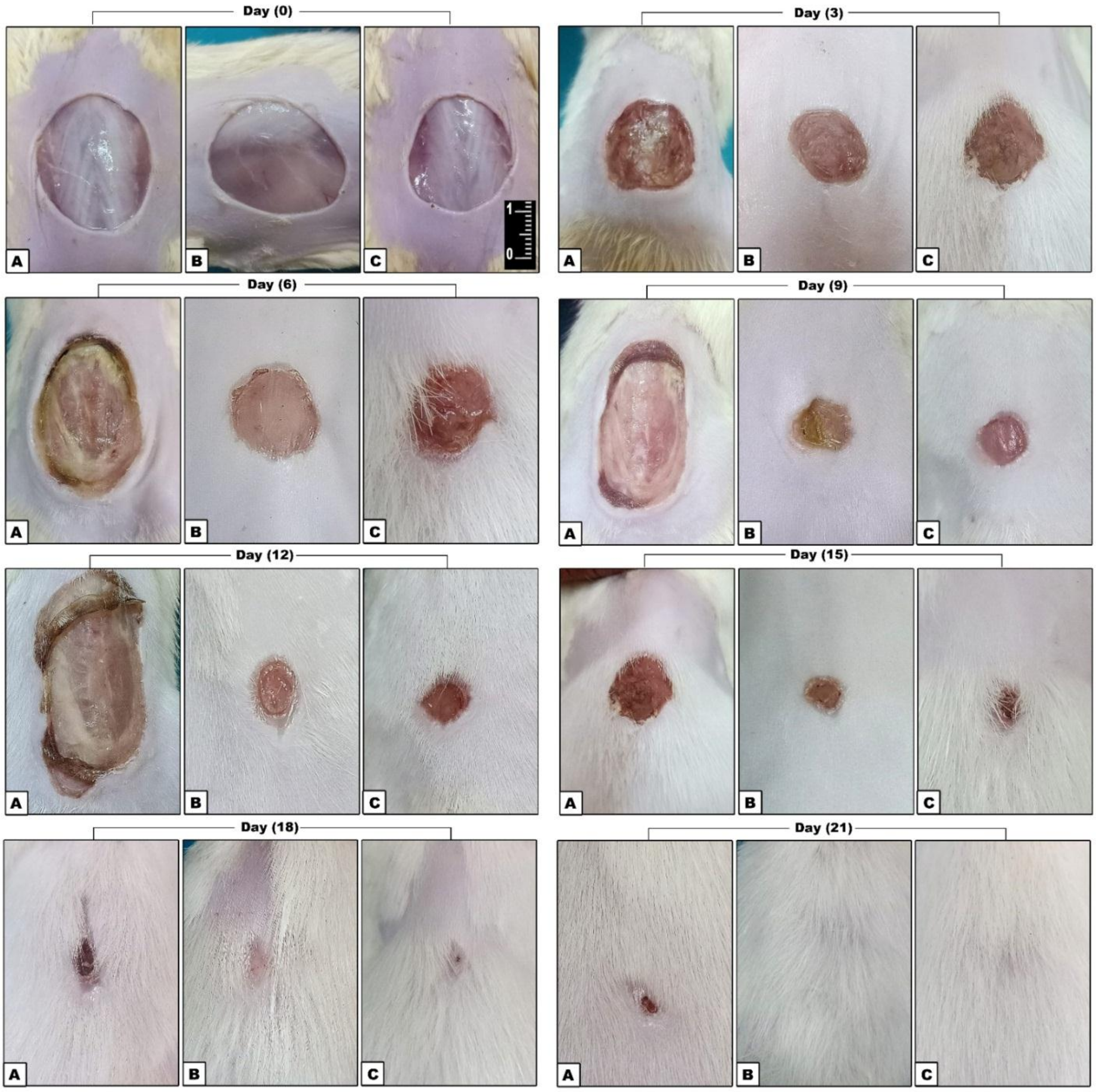

Fig. 1:
Images for macroscopic wound assessment of the different studied groups on days 0,3,6,9,12,15,18 &21 post-injury (**A.** control, **B.** SUL-treated lesions and **C.** PJ-treated).

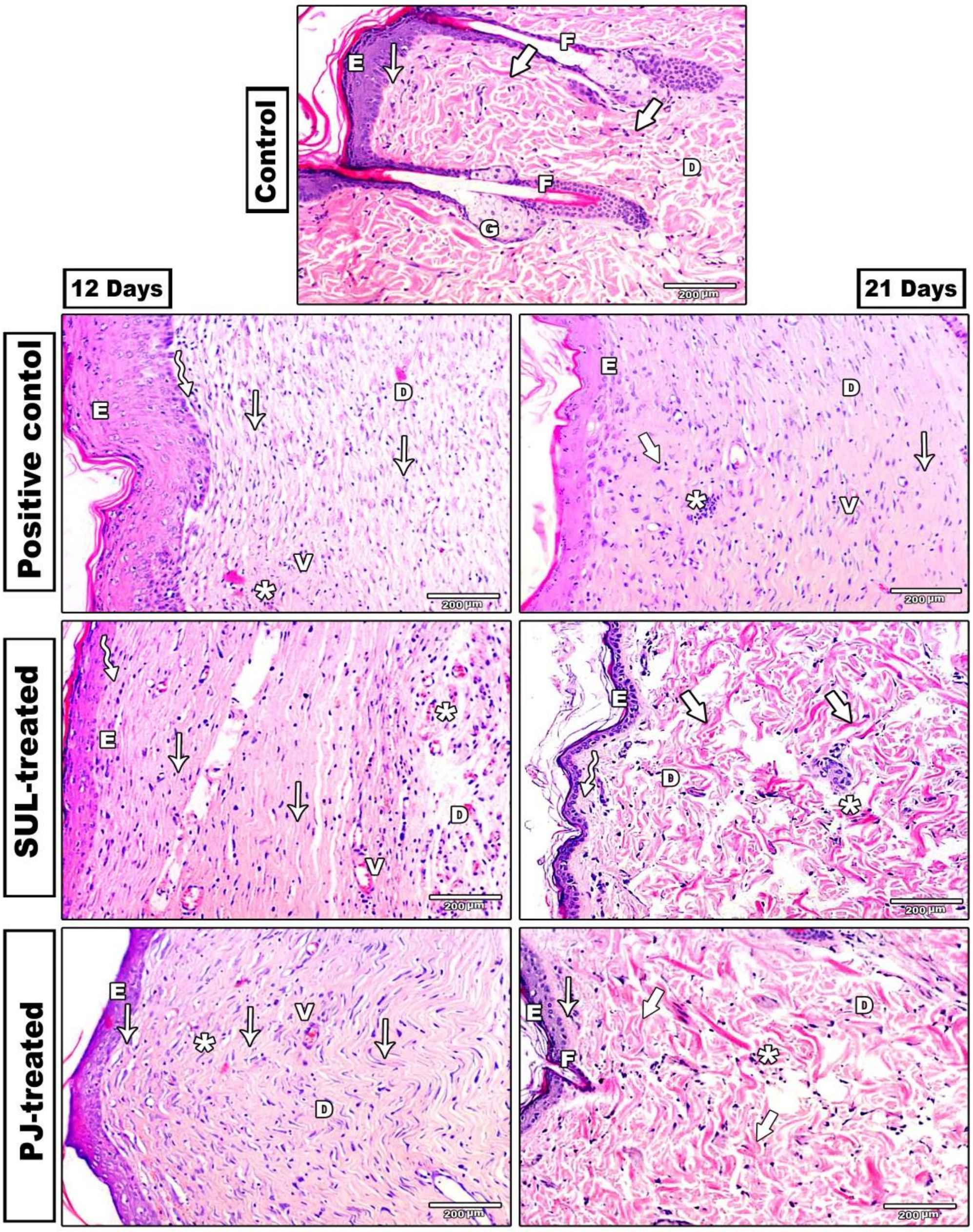

Fig.2:
Histopathological assessment of wound healing by H&E staining. In all the studied groups, the skin is formed of epidermis (E) and dermis (D). In the control group, thin (Thin arow) and thick (Thick arrow) collagenous bundles, hair follicles (F) and sebaceous glands (G) are well demarcated. On the 12th and 21st DPI, positive control sections show inflammatory cell infiltration (∗), blood vessels (V), thin collagen bundles (Thin arow). Very thin collagen fibers (wavy arrow) appear, on the 12th day, and are replaced by thick bundles (thick arrow) on the 21st day. In SUL-treated groups, inflammatory cell infiltration (∗), blood vessels (V), very thin collagen fibers (wavy arrow) appear in section on the 12th and 21st days DPI. Thin collagen bundles (Thin arow) and thick bundles (thick arrow) are noticed on the 12th and the 21st day DPI respectively. The PJ-treated groups on the 12th and 21st DPI show inflammatory cell infiltration (∗), blood vessels (V), thin collagen bundles (Thin arow). On the 21st day, the hair follicles (F), thick collagenous bundles are identified.

H&E stain, Scale bar 200 µm.

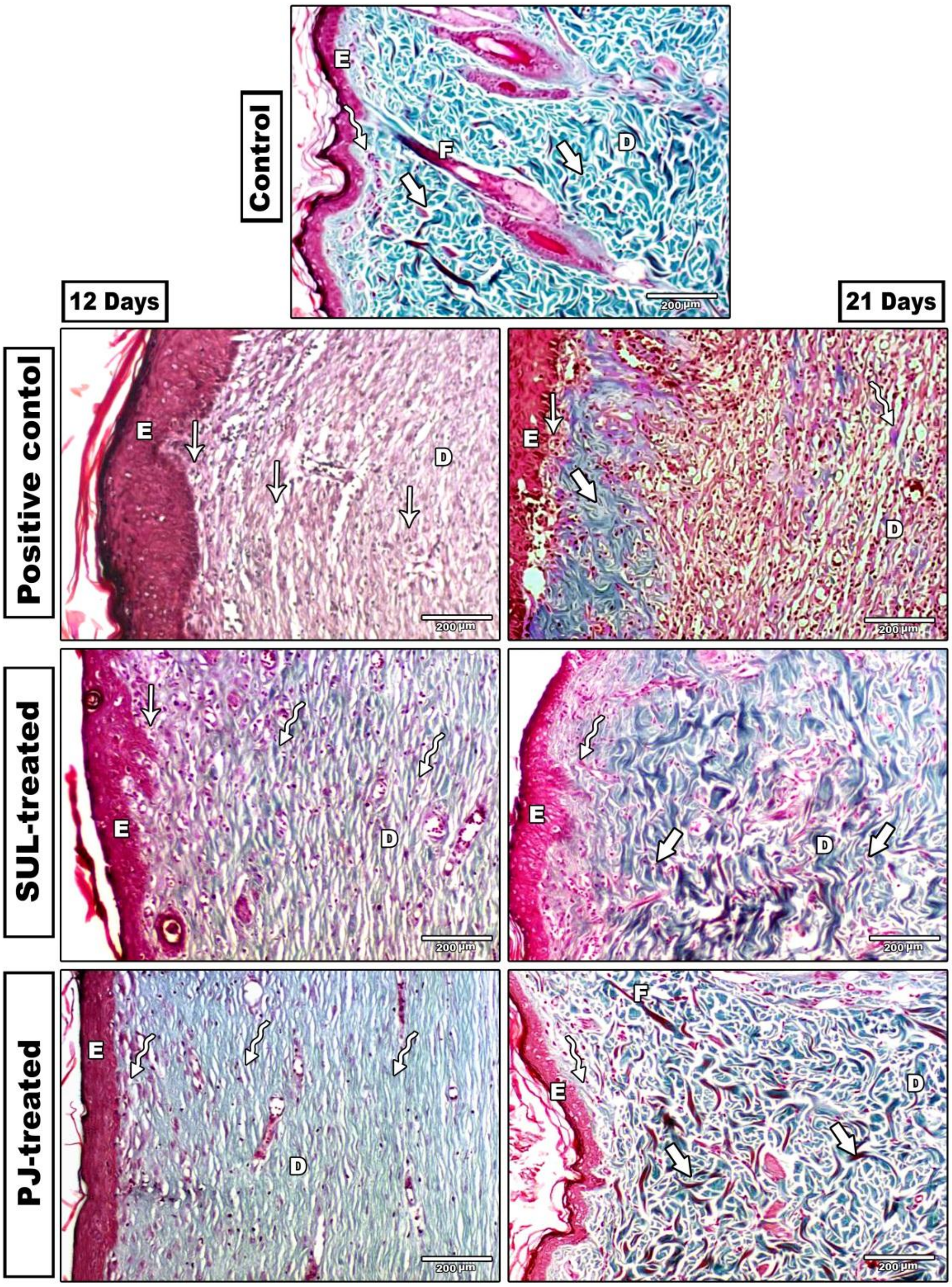

Fig.3:
Histopathological assessment of wound healing by Masson's Trichrome (MT) staining. The sections of all groups demonstrate that the skin is formed of epidermis (E) and dermis (D). The control group shows very thin collagen fibers (wavy arrow), thick collagen bundles (Thick arrow) and hair follicles (F).
In sections of the positive control, thin collagen bundles (Thin arow) are identified on the 12th and 21st day however, thick collagen bundles (Thick arrow) appear on the 21st day only. On the 12th day, sections of the SUL-treated groups demonstrate very thin (wavy arrow) and thin collagenous bundles (Thin arow) and on the 21st day, thick fibers (Thick arrow) start to appear next to the very thin collagen ones (wavy arrow). In PJ-treated groups, on the 12th only very thin (wavy arrow) collagen fibers are observed. On the 21st day, very thin (wavy arrow), thick bundles (Thick arrow) and hair follicles (F) are clearly noticed.

MT stain, Scale bar 200 µm.

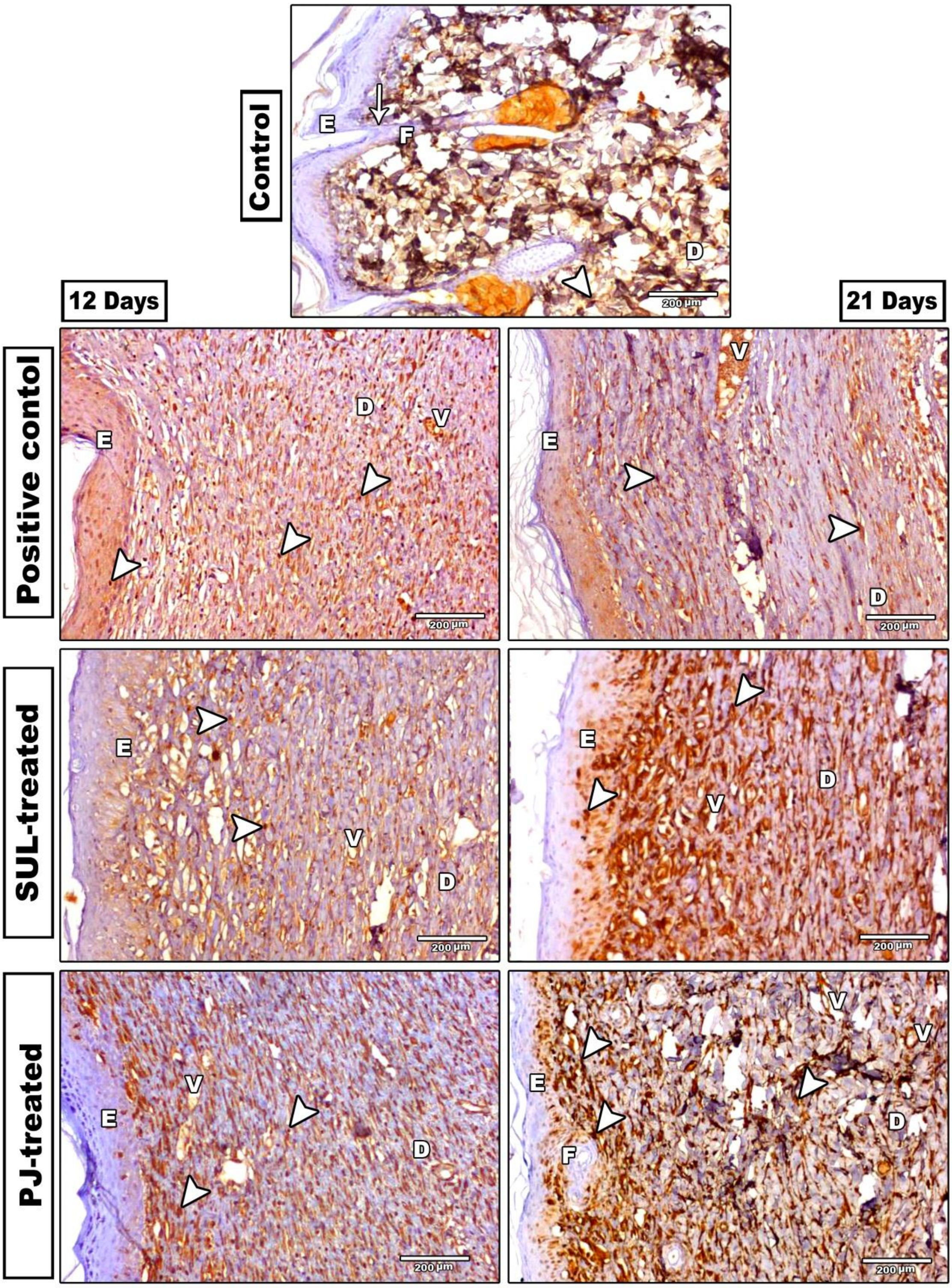

Fig.4:
TGF-β1 immunoexpression in the various studied groups; control, positive control, SUL- treated and PJ-treated on the 12th and 21st DPI. The skin comprises of two layers epidermis (E) and dermis (D). Immunoreactivities are well demarcated in the fibroblasts (arrowhead) and the endothelial cells of the blood vessels (V). Hair follicles are noticed in both the control and PJ-treated groups on the 21st day.

TGF-β1 immunoexpression, Scale bar 200 µm.

Discussion

The skin is the largest organ in both human and animal bodies, serving as a physical barrier against radiation and infections. [32]. Cutaneous wound healing involves a complex well-coordinated, dynamic integration of molecular and cellular biological activities [33]. These integrations not only boosted wound healing, but also are responsible for restoration of the functions of the wounded tissue[8]. Despite numerous ongoing therapeutic researches, there is still no effective cure for scarless wound healing [34]. Synthetic treatments carry a significant risk of side effects. Therefore natural mixtures are strongly proposed as powerful alternative medicines for wound healing [35]. These alternative herbal medicines possesses antimicrobial impacts preventing wound infection and does not need long standing dressing [36].

In the present study, direct observations of the excised wounds were performed and revealed increased activities of wound healing in all the groups. Complete wound healing and re-epithelization were demonstrated on the 21st DPI in SUL-treated and PJ-treated. On the 12th and 21st DPI, the mean wound diameters and surface areas were significantly decreased and the mean wound contraction percentages were significantly increased in both the SUL-treated and PJ-treated groups. On the 12th DPI, in SUL-treated and PJ-treated groups, the epidermis was regenerated and became thicker with no rete-ridges. The dermis demonstrated multiple blood capillaries and polymorphonuclear cells infiltrate. The arrangements of the different types of collagenous bundles were also noticed in MT-stained sections. Additionally, On the 21st DPI, in the SUL-treated group, thinning of the epidermis with minimal rete ridges was observed. The dermis represented by newly formed disorganized, thick collagen fibers in the superficial and deep reticular layers with apparent very thin collagen in papillary layer. However, hair follicles and sebaceous glands were not seen. In PJ-treated group, on the 21st DPI, almost normal epidermis with reformed rete-ridges were detected and the dermis showed reappearance of skin appendages in the form of newly formed hair microfollicles. Thick relatively organized, tightly packed collagen bundles were also noticed in the reticular layer of the dermis. Minimal newly formed blood vessels and mononuclear cellular infiltration were detected.

These improvements in the wound healing histologically could be attributed to angiogenesis enhancement[37], collagen fibers deposition[38], and subsequently increasing the wound tensile strength[39]. Collagen is one of the principal constituents of connective tissue that contributes to the tensile strength of the wounds during the healing process [40] . The remodeling phase of wound healing demonstrated an increase in the biomechanical resistance of tissues through replacing the granulation tissue rich in type III collagen by the stronger tissue rich in type I collagen [41], and these results goes in consistent with that obtained by various authors [45,46,47].

Diminishing blood flow to the wound site impinges the healing process through decreasing anabolic activity, impairing local immune and cellular defense mechanisms, protein malnutrition, inducing oxidative stress, and growth factors deficiency [45]. Therefore, angiogenesis is a compensatory mechanism for restoring blood flow to the wound area and hence improvement in oxygen and nutrients transport, which are essential for healing and re-epithelization[37]. Several literatures [49, 50] reported the same results.

In the present work, in the SUL-treated and PJ-treated groups, the mean values of the MDA were significantly decreased and the mean values of the GSH, SOD and CAT increased significantly and these results go in agreement with several studies [28, 47, 59] . The first cells to arrive at the site of the wound are the neutrophils, that induce microorganisms elimination and initiation of inflammation [47]. Recruited neutrophils and other inflammatory cells generate ROS and induce oxidative stress in the wound area [48]. Excessive ROS production at the wound site persuades several structural alterations including damage to mitochondrial DNA, lipids and proteins that ultimately lead to apoptosis of surrounding cells as keratinocytes [51,54]. One of these ROS is the hydroxyl radicals that induces lipid peroxidation [50] and subsequently impairment in the metabolic functions of collagen, fibroblast and endothelial cells and keratinocyte capillary permeability. Therefore, lipid peroxidation can harmfully influence the process of the wound healing [51]. One of the most abundant carbonyl products of this process and considered as an important marker for lipid peroxidation is MDA [47]. Moreover, in the human body the antioxidants enzymes (GSH, CAT and SOD) adjust ROS homeostasis by controlling the vascular system [52] and this balance is extremely crucial for efficient wound healing [53]. Thus, the key strategy for better healing of the chronic wound is elimination of the ROS overproduction [54]. Therefore, crude extracts with high antioxidant capability may augment and boost the process of wound healing [55]. via inhibiting inflammatory reactions and subsequent oxidative stress [27].

Sulphadiazine has been reported to have a protective impact by inhibiting the growth of most microorganisms [56] . It also enhanced the wound healing by inhibiting the activities of matrix metalloproteinases and stimulates the re-epithelialization[57]. However, several studies countered these results[58,59]. Moreover, Sulphadiazine slowed down the scar separation in deep wounds. Atrophic and hypertrophic scars were noticed in cases treated with Sulphadiazine especially if the healing process lasted more than three weeks [60]. Numerous side effects were linked to the use of Sulphadiazine such as renal toxicity and transient leukopenia [61]. In the light of such data, the topical use of Sulphadiazine in treating wound healing should be limited [56]. Therefore, the needs are increasing for developing effective novel natural drugs in treating and accelerating the process of wound healing with minimal side effects [62,63].

In brief, the Prosopis species have been used in traditional medicine for the treatment of several diseases[13, 64,65]. Moreover, PJ tea, leaf and seed extracts are reported to have healing properties during management of skin wounds and digestive disorders. PJ different parts are very rich in pharmacological active substrates as piperidine alkaloids. These alkaloids include juliflorine, julifloridine julifloricine, juliprosine, juliflorinine and juliprosinene, sceojuliprosopinol [23]. The two alkaloids, juliflorinine and juliprosinene exhibited better antibacterial activities counter to strains of Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, Shigella sonnei and Staphylococcus aureus[24, 66,67]. PJ leaves derived alkaloid mixture demonstrated antimicrobial activities better than gentamycin, bacitracin, trimethoprim and chloromycetin against various Staphylococcus species (aureus, lactis, faecalis, and pyogenes) [14], E. coli, Salmonella typhi, Shigella flexneri, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae, Bacillus cereus, Listeria monocytogenes and Enterococcus faecalis. All the screened bacteria were sensitive to it and the most sensitive ones were Pseudomonas aeruginosa and Listeria monocytogenes [68].

Also, Juliflorine owns dose dependent immunomodulating, cytotoxic and anticancer activities mainly of catechin [69]. In addition, PJ exhibited considerable dose dependent anti-inflammatory effects in acute and chronic inflammation through inhibiting histamine and serotonin [67, 70]. In addition, The ethanol extract of PJ leaves extract was investigated and proved to have antioxidant and remarkable radical scavenging activities (RSA) and effective anti-pyretic and antiulcer activity [67,68, 69].

In this study, sections from the control group showed mild and moderate TGF-β1 immunoexpression within the epidermis and moderate expression in the endothelial cells of the blood vessels and fibroblasts respectively. Also, in positive control group moderate TGF-β1 Immunoexpression was detected. After 12 days, the SUL-treated group demonstrated mild positive reactions in the epidermis with moderate reaction in the endothelial cells and fibroblasts of the dermis. Moreover, on the 21st DPI, moderate to strong positive reactions were noticed in the lower layers of the epidermis with strong positive reactions in the endothelial cells and fibroblasts of the dermis. In PJ-treated group, moderate positive reactions were noticed in the basal layers of the epidermis with strong positive reactions in the endothelial cells lining the blood vessels and fibroblasts of the dermis on the 12th DPI. After 21 days, strong and marked strong positive reactions were detected in the basal layers of the epidermis and in the endothelial cells and fibroblasts of the dermis respectively.

Recently, numerous cytokines and growth factors are identified to be responsible for healing process including inflammation, re-epithelization, granulation tissue formation and angiogenesis[71]. The transforming growth factor (TGF-β1) improves the healing process in poorly vascularized and unhealed wounds in diabetic, immunocompromised, or elderly patients[72]. TGF-β1, derived from the degranulated platelets, macrophages and lymphocytes at the wound site, promotes the inflammatory phase of wound healing through inducing early activation and infiltration of neutrophils and macrophages [73]. Moreover, TGF-β1 endorses fibroblasts differentiation and maturation to myofibroblasts [74]. Several studies reported that the expression levels of TGF-β1 peaked in the early stage of healing then gradually decreased. The early increase in TGF-β1 expression is associated and could be attributed to several factors such as early inflammation, clot formation, infiltration of inflammatory cells and manifest angiogenesis. Later in the stage of scar maturity, these factors are gradually exhausted or even vanish, explaining the decrease in TGF-β1 expression levels at this stage [73]. In addition, several scholars [75] reported that binding to TGF-β receptors and then modifying its signal transduction lead to well-organization and normal alignment of new collagen in the treated groups [76].

Conclusion:

Our results indicate that the topical application of PJ leave extract possesses significant positive roles in enhancing the process of skin wound healing, when compared to topical Sulphadiazine. These positive impacts are attributed to modulation in the inflammatory phase of wound healing, attenuating oxidative stress, increase angiogenesis, stimulating collagen synthesis and upgrading the TGF- β expression. Therefore, we recommend the use of PJ leave extract in treating skin wounds for efficient and faster repair.

Conflicts of Interest:

The authors declare that there are no conflicts of interest**.**

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