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Research Article

Biology

Studies of efficacy of *Achillea* species ethanol extract and honey on full thickness wound healing in diabetic albino rats

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Abstract: Wound healing is deemed a clinical issue in diabetic patients. This study aimed to investigate the efficiency of *Achillea* species ethanolic extract and honey on wound healing enhancement in streptozotocin-induced diabetic rats. Thirty adult male Albino rats (180- 230 gm) were used for creating full thickness skin wounds. Animals were grouped to control (GI) and diabetics (GII- GVI). Diabetes was induced by streptozotocin (45 mg/kg) and all animals received metformin (50 mg/kg orally for 14 days to control hyperglycemia). Full thickness wound (2X2 cm) was created on the back skin of all animals. Wounded diabetic animals were divided into 5 subgroups based on wound treatment into diabetic wounded untreated (GII), mebo cream (GШ), *Achillea* extract preparation (GIV), honey (GV), *Achillea* + honey (GVI). One ml of each treatment was used for daily wound painting by fine brush for 14 days. Wounds were observed daily for healing criteria or complications and photographed with a well-known scale to measure contraction rate. Smears were taken from wounds for microbiological study. Wounded area was dissected at 14 days, processed for paraffin sectioning and staining by hematoxylin and eosin, alpha –smooth actin (α-SA) and proliferating cell nuclear antigen (PCNA). *Achillea* extract, *Achillea* + honey, honey and mebo enhanced wound healing in diabetic rats. Most treatments modified microbial contents, blood profile and histological healing process. *Achillea* extracts, *Achillea* + honey and honey showed marked influence in enhancing wound healing in diabetic rats even better than mebo. So, it is recommended to use *Achillea* alone or mixed with honey for enhancing large a wound defects in diabetic patients.

Keyword: Diabetic, *Achillea*, Honey, Wound, Skin, Histology.

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1. INTRODUCTION

Diabetic patients suffering from vascular diseases are at risk of chronic wounds as chronic leg foot ulcers due to chronic inadequacy of blood supply or peripheral neuropathy and vasculopathy[1]. Wound managements are continuously advanced but in spite of this fact still there are problems in curing chronic large wounds. It is harder to treat chronic wounds than minor ones [2]. There are many methods available to treat chronic wounds, but few are effective [3]. Information regarding effectiveness of topical antimicrobials dressings in improving chronic wound healing is limited. Some of the used topical dressings are solutions of povidone-iodine, hypochlorous acid, cadexomer iodine, honey and collagenase [2]. Materials extracted from plants can efficiently be used in wound treatment, and many experiments were carried out for treatment of skin wounds in animals using plant extracts. Ashkani-Esfahani *et al.* [4] documented the effective use of Silymarin in wound healing due to its anti-inflammatory and antioxidant activities. Honey (Manuka and Tualang) was found very effective in healing and curing, when applied with success dd in management of wounds and ulcers [5, 6]. *Achillea* is one of the medical plants that contain many bioactive materials with enhanced biological activities [7]. *Achillea fragrantissima* is a desert plant that has antimicrobial, antiviral, antioxidant and anti-inflammatory activities due to the essential oils it contains. It was used for treatment of many diseases of liver, kidney, gastrointestinal tracts and heals wounds [8, 9]. Very limited description about clinical uses of *Achillea fragrantissima* was available [10].

This study focuses on synergistic effects of a combination of *Achillea* species ethanolic extract and honey on treatment of full thickness cutaneous wounds in streptozotocin induced diabetic rats' model.

2. MATERIALS AND METHODS

2.1 Materials

Achillea fragrantissima (Forssk.) Sch. Bip. is a flowering plant of the genus *Achillea L.* (yarrow) of the *Asteraceae* family. The plant was collected from the northern region of Saudi Arabia (Arar) in February 2019. The different parts of the plant were washed, dried and treated with methanol for extraction in the pharmaceutical lab King Fahd Medical Research Center (KFMRC), Jeddah, Saudi Arabia. Honey was obtained from the local market. Streptozotocin (STZ) was obtained from (Sigma, St Louis, Mo, USA). Metformin (N, N-dimethylbiguanide), anti-diabetic drug, and β-sitosterol (Mebo) creamd, was purchased from a local pharmacy.

2.2 Preparation of Achillea for topical application

Freshly collected *Achillea fragrantissima* (whole plant) was dried under shade for three days. It was then in powder form and its materials were then extracted with methanol (96%) by maceration method at room temperature for 3 days. The extract was filtered and evaporated and again filtered and stored in dark sterile bottles for further use.

2.3 Induction of diabetes mellitus

The diabetic group of rats was given a single intraperitoneal (i.p) injection at 45 mg /kg STZ dissolved in a 0.01 M citrate buffer, pH 4.5. Blood from the tail vein was examined for fasting glucose concentration after 72 hours of STZ injection. Blood glucose levels of animals above 200 mg /dl were accepted as diabetics. [12]

2.4 Animal grouping and study design

Thirty adult male rats (180- 220gm) were used in this experimental study. The animals were placed in the laboratory for 3 days to acclimatize to the laboratory conditions, before initiation of experiments. They were kept in plastic cages in an air-conditioned room at 22±1°C and standard animal chow and water *ad libitum*. Ethical approval was obtained from KFMRC committee for animal care before start of the experiments (Number #108-19). All the experiments were carried according to the international – ARRIVE – guideline of experimental animal handling.

2.5 Creation of full thickens chronic wound in rats

After 4 weeks of induction of diabetes, a full thickness wound was created as follows: the animals were anesthetized using ether, followed by removal of the surface hairs on the dorsal area of the rat with an electric shaving machine, without damaging the stratum corneum. The site of the wound was marked as a specified area of 2X2 cm. Full thickness skin piece was removed by a sharp scalpel and homeostasis was ensured by sterile gauze. Animal drinking water was amended with analgesics for relieving pain [11]. Animals were divided into the following groups (n= 5 for each). GI: Control group, wound non-treated considered as normal non- diabetic rats. GII: Diabetic control group, wound non-treated. GШ: Diabetic + wound treated by mebo. GIV: Diabetic + wound treated by *Achillea*. GV: Diabetic + wound treated by honey (1g/day) [13]. GVI: Diabetic + wound treated by mix (*Achillea* + honey).

2.6 Topical treatment design

Wound was washed by sterile saline then daily covered by a constant amount (one ml) of the previous preparations for 14 days.

2.7 Blood Analysis

Blood collected at end experimental from retro-orbital venous plexus after anesthesia and complete blood count (CBC) that showed red blood cells (RBCs), white blood cells (WBCs), platelets (PLT) and hemoglobin (HGB) were tested and analyzed with an auto analyzer (Sysmex, Japan).

2.8 Microbiological assay

At the end of the experiment, microbiological swab from skin wounds was taken from all groups, for microbial culture and testing for positive and negative gram stained organisms.

2.9 Histological study

Skin wounded area was dissected at day 14, processed for paraffin sections stained by hematoxylin and eosin, alpha – smooth actin (α-SA) for myofibroblasts in order to evaluate wound contraction and proliferating cell nuclear antigen (PCNA) for examining the cell proliferation.

3. STATISTICAL ANALYSIS

The data were statistically analyzed and described as mean ±

standard deviations using statistical software (SPSS version 17). Comparisons between control and diabetic untreated groups with treated groups were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD). The *P* value <0.05 was considered statistically significant.

4. RESULTS

4.1 Wound healing

Wound healing evaluation in different studied groups was shown in Figure (1). There was a decrease in wound area in most diabetic treated groups especially in the mebo group followed by the mixed group after 14 days of treatment.

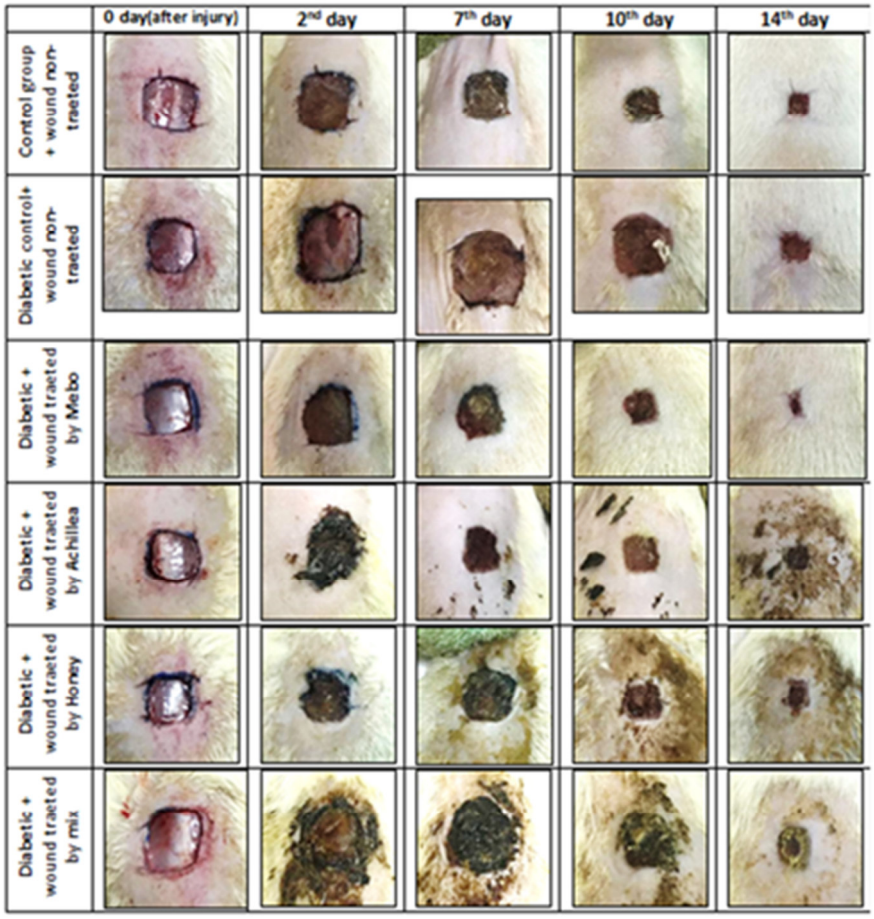
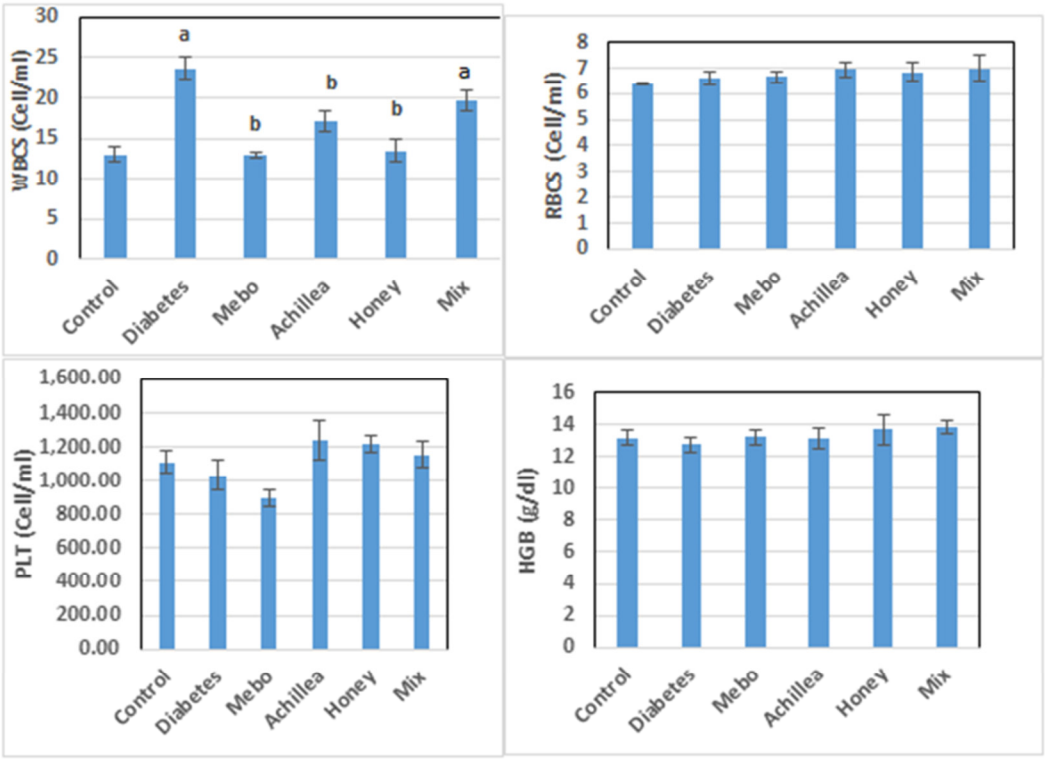


Fig (1): Sequences of wound closure (contraction) in all groups.

4.2 Blood Analysis

Graph (1) showed WBCs, RBCs, and PLT counts and HGB levels in different experimental groups at day 14th of wound healing. Wounded untreated diabetes rats and mixed treated groups showed significant increase in WBCs versus control, with reduction of WBCs in all other animals treated with other materials (GIII, GIV and GV groups) versus diabetic untreated groups. Insignificant differences in RBCs counts between different treatments were present, but GVI (*Achillea* + honey) recorded an insignificant increase in RBCs count, followed by GIV (*Achillea* extract), and honey GV compared to GII (diabetic untreated rats) with insignificant differences between groups. Insignificant differences in blood HGB levels between different treatments. Platelet counts showed insignificant differences between treated and untreated diabetic rats. Group treated with mebo (GIII) showed insignificant reduction in PLT count while other treatments GIV (*Achillea* extract treatment) and GVI (*Achillea* + honey mix) demonstrated an insignificant increase in PLT count compared to GII (untreated diabetic rats).



Graph (1): The white blood cells (WBCs), red blood cells (RBCs) and platelets (PLT) counts and hemoglobin (HBG) levels in different experimental groups at day 14 of wound healing. a: Significance versus control; b: significant versus diabetic untreated group.

4.3 Microbiological assay

Table (1) showed growth of gram-positive and negative bacteria as *Staphylococcus aureus* and *Escherichia coli* in GI (wounded non-treated) and *Staphylococcus aureus and Klebsiella spp.* & *Escherichia coli* in GII (diabetic wounded non-treated). No bacterial growth in all diabetic wounded animals treated with the different materials, mebo, *Achillea*, honey and *Achillea* + honey mix.

Table 1: Microbiological evaluation in skin in all experimental wounded (untreated / treated) diabetic rats.

Groups	GI	GII	GIII	GIV	GV	GVI
Gram Positive Bacteria	*Staphylococcus aureua*	*Staphylococcus aureua*	No growth	No growth	No growth	No growth
Gram Negative Bacteria	*Escherichia coli*	*Klebsiella spp.*& *Escherichia coli*	No growth	No growth	No growth	No growth

4.4 Histological study

4.4.1 H&E stain

Figure (2) showed sections of rat dorsal back skin of G1 (control unwounded skin): The dorsal back skin was classified as thin skin. The epidermis is formed of a thin layer of stratified squamous keratinized epithelium, and the dermis contains collagenous connective tissue surrounding hair follicles and sebaceous glands. In GII (Diabetic wounded, non-treated): there was lack of epithelization at wound surface but the wound edge showed proliferating epidermal epithelium extended to cover granulation tissue at wounded area (A). The epithelium looked unhealthy with desquamated regions with marked inflammation in the underlying dermis (B). The wound bed (C) showed newly formed blood vessels oriented longitudinally with marked proliferating connective tissue cells with presence of mononuclear inflammatory resulting cells giving more cellularity to the wounded area. The deep layers showed damaged necrotic regions and inflammatory cells. GIII (Diabetic wound treated by mebo): showed partial covering of wounded area by hyperplastic epidermal growth. The epidermal layers looked organized, clean and healthy with less inflammatory cells in the underlying dermis (B). In (C) the connective tissue of the wound base lacked hairs and glands and showed less inflammatory cells and newly formed blood vessels which were still oriented longitudinally. GIV (Diabetic wound treated by *Achillea*): diabetic skin wound treated by *Achillea* was nearly completely covered by newly formed epidermis (A). The epidermis looked healthier and less hyperplastic with less inflammatory cells in underlying dermis compared to Mebo group (B). The wound base in (C) showed marked decrease in vascularization and inflammatory cells with well-developed collagenous fibers among fibroblasts with active oval vesicular nuclei but still without presence of skin appendages. GV (Diabetic wound treated by honey): perfect epithelization was observed (A), but also hyperplastic (B), dermis showed less inflammatory cells (C) than *Achillea* group. GVI (Diabetic wound treated by *Achillea* + honey mixture): wound edges distance was less than non-treated group but close to wound epithelial layer than mebo, *Achillea* and honey groups (A). The wound surface was filled with hyperplastic epithelial layer (B). The connective tissue dermis under the proliferating new epidermis showed few inflammatory cells and fibroblasts, and the wound granulation tissue lacking skin appendages wound base was formed by collagen, longitudinally oriented with many fibroblasts and blood capillaries (C).

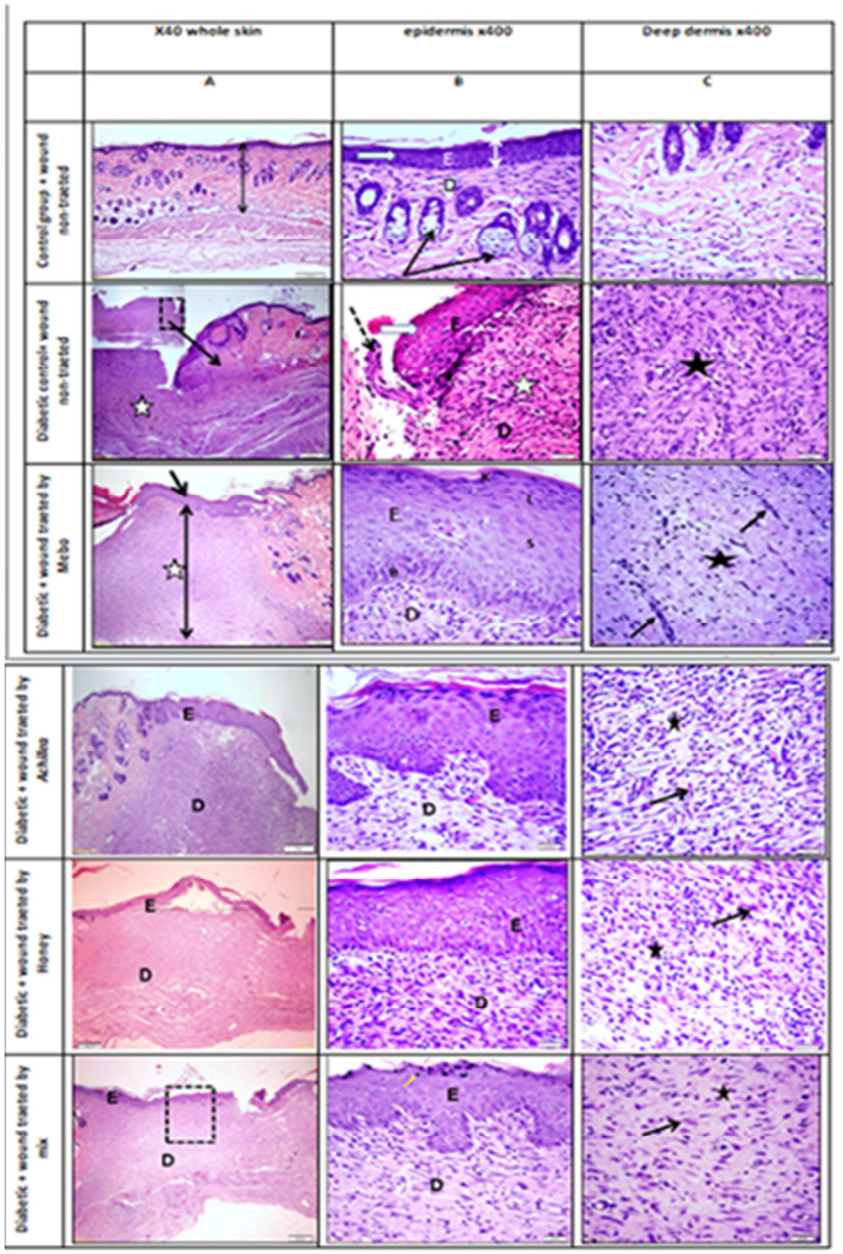


Fig (2): Light micrograph of diabetic wound treated by mebo, *Achillea*, honey and *Achillea* + honey mix.

G1- normal control

A. Light micrograph of normal skin of rats' low magnification (double head arrow). X40. H&E stain.

B-C The epidermis (E) is formed of thin layer of stratified squamous keratinized epithelium (white arrow), and dermis (D) that contains collagenous connective tissue surrounding skin appendages (hair follicles and sebaceous glands) (black arrow). X400. H&E stain.

GII- diabetic –untreated wound

A. Proliferating epithelium of the epidermis extended to cover the granulation tissue (growing collagenous connective tissue) (black arrow). Notice that the area of granulation tissue lacks its covering epidermis (white star). X40. H&E. stain.

B. Showing the epithelial proliferation of stratified type (white arrow) covering granulation tissue (white star) formed by proliferating fibroblasts and blood capillaries. Notice the presence of active fibroblasts and collagen fibers (arrow). X400 H&E stain.

C. Showing wound bed proliferating connective tissue cells resulting in more cellularity of the wounded area (black stare). X400. H&E stain.

GIII- Diabetic mebo –treated

A. Light micrograph of healed diabetic skin wound (at 14thday) treated by mebo showing regeneration by thick layer of connective tissue (double sided arrow lacking appendages (white stare) and covered with regenerated healthy stratified squamous epithelium (black arrow). X40. H&E stain.

B. Light micrograph of the regenerated epidermis showing formed by stratified squamous keratinized epithelium. The basal cells have hyperchromatic nucleus (B). The stratum spinosum (S) appeared polyhedral having a vesicular nucleus and closely attached to other stratum lucidum (L) and stratum corneum (k) X400 H&E. stain.

C. Regenerated dermis with increases of the collagen fiber and blood vessels (arrow).

GIV: Diabetic + Achillea treated

A. Light micrograph of healed diabetic skin wound treated by Achillea showing regeneration by granulation tissue. The wound is nearly completely covered by stratified squamous keratinized epithelium with absence of skin appendages. X40. H&E. stain.

B. Light micrograph of regenerated epidermis (E) showing stratified squamous keratinized epithelium with less str. Lucediume and corium compared to that observed in the mebo group with presence of black material in the surface area which seemed to be from the dressing Achillea material (arrow). Notice the well- developed collagenous connective tissue dermis (D) without presence of skin appendages. X400. H&E stain.
C. Dermal tissue showed collagenous fibers and fibroblast cells and small blood vessels (black stare and arrow). X400. H&E. stain.

GV: Diabetic + Honey treated
There was perfect epithelization (A) X40. H&E stain, but still hyperplastic (B), dermis showed less inflammatory cells, collagenous fibers and fibroblasts (black stare and arrow) (C) compared to Achilia alone. X400. H&E stain.

GVI: Diabetic + Mix of Achillea + honey treated
Wound edge gap was less compared to non -treated group but similar to that of mebo (A) X40. H&E stain. The wound area is covered by a hyperplastic epithelial layer but with less thickness compared to mebo Achillea and honey (B) X400. H&E stain. The connective tissue dermis under the proliferating new epidermis showed few inflammatory cells and proliferating fibroblasts nuclei. Wound granulation tissue showed lacking of skin appendages and is formed by collagenous fibers, fibroblasts and numerous blood capillaries oriented longitudinally (C) (black stare and arrow). X400. H&E. stain.

4.4.2 Immunohistochemistry study

4.4.2.1 1-Alpha-smooth actin (α -SA)

Figure (3) showed immunohistochemical staining of α -SA in skin of control and experimental rat groups. In GI: immunostaining was found only at blood capillaries walls and erector pili hair muscle. No expression was observed in the connective tissue fibroblasts In GII: α -SA staining was encountered mainly in newly formed capillaries and few proliferating fibroblast at wound base. Immuno-staining intensity and frequency of cells stained for α -SA was increased in all treated groups with marked staining in GIII, GIV and GV treated with mebo, *Achillea* and honey respectively. Honey and *Achillea* mixed (GVI) group showed decrease in actin expression compared to each alone.

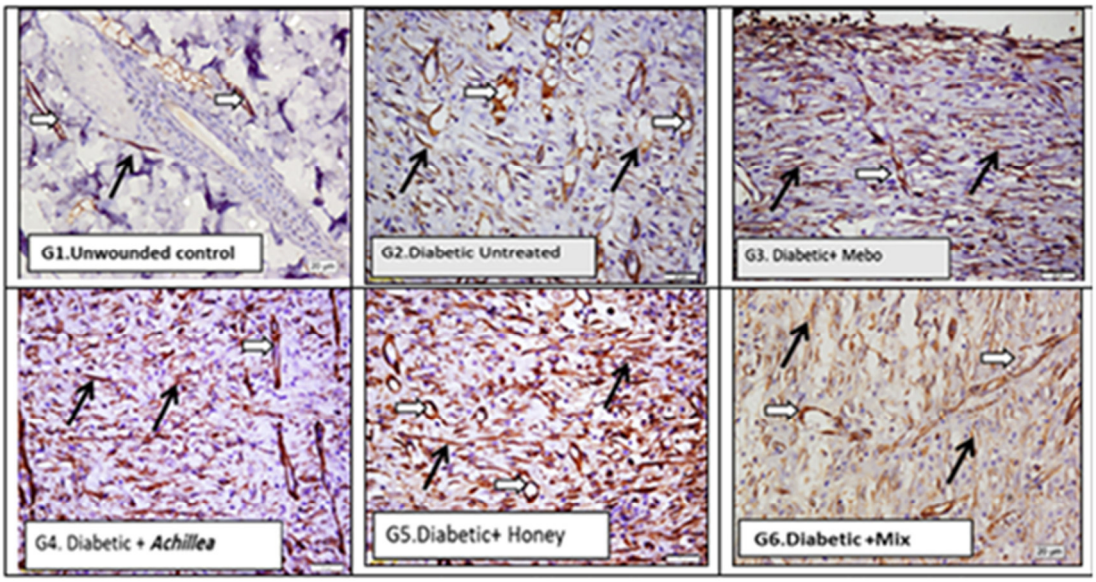


Fig (3): Photomicrographs for dorsal rat skin of normal control non-diabetic wounded, untreated and different treated wounds immuno-stained with alpha-smooth actin (α -SA) to show:

GI: normal control: showing staining around the tiny few dermal blood capillaries (white arrows), erector pili muscle (black arrow) near sebaceous gland (SG).
GII: diabetic –untreated wound: showing mild increase in staining at the bases of newly formed longitudinally oriented blood capillaries (white arrows) and proliferating irregularly arranged fibroblasts cytoplasm (black arrows).
GIII: Diabetic mebo –treated: showing increased immunostaining in the basal lamina of blood capillaries (white arrow) and the cytoplasm of horizontally arranged myofibroblast (black arrows).
GIV: Diabetic + Achillea treated: showing also marked increase in immunostaining α -SA smooth actin.
GV: Diabetic + Honey -treated: showing marked more increase in α -SA smooth actin immuno-staining in similar location of previous groups.
GVI: Diabetic + Mix of Achillea + honey treated: showing an increase in staining compared to untreated GII but less compared to other treated groups.

4.4.2.2 Proliferating cell nuclear antigen (PCNA)

Immunohistochemistry for PCNA showed that in GI: it was expressed in basal layers of epidermis and hair follicle sheathes and few scattered inactive fibrocyte nuclei. In GII: an increase in PCNA immunostaining was observed in proliferating fibroblasts with more stained inflammatory cell nuclei at wound area. More increases were observed in all treated groups especially GIV and GV groups that received *Achillea* and honey, less inflammatory cells compared to fibroblasts. In GVI: receiving mixture of both honey and *Achillea* less staining was observed (arrows) (Figure 4).

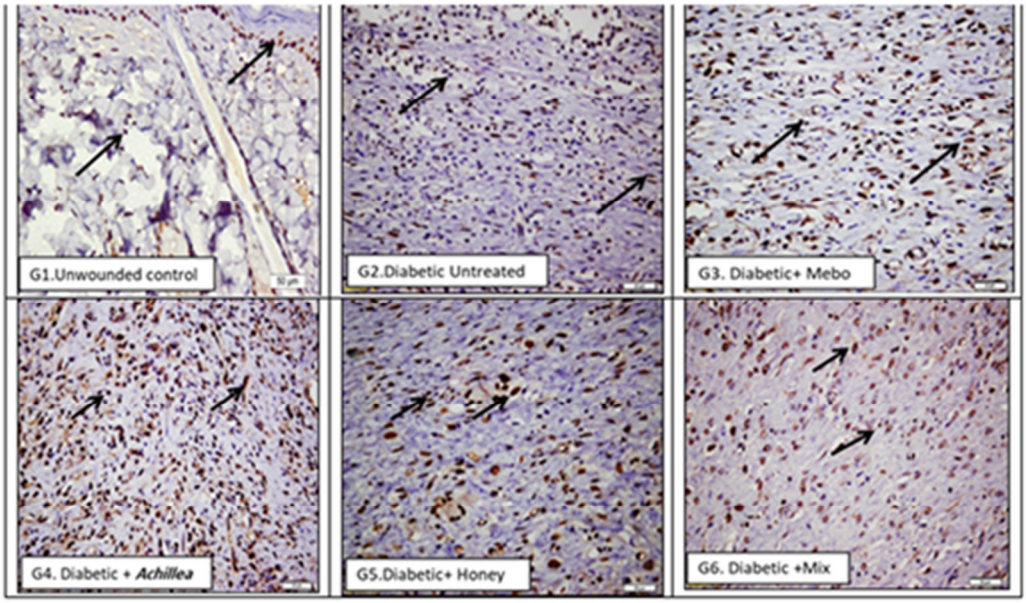


Fig 4: Sections from rat back skin immuno-stained for PCNA to show

GI: normal control: PCNA staining in basal epidermal cells and few nuclei of dermal inactive fibrocytes (arrows).
GII: Diabetic untreated: increased nuclei (fibroblasts & inflammatory cells) stained for PCNA (arrows) with more inflammatory cells than fibroblasts.
GIII: Diabetic mebo –treated: more increase in nuclei stained for PCNA (arrows) was observed compared to GII: untreated wound.
GIV: Diabetic + Achillea treated: Also increase in nuclei stained for PCNA stained nuclei (arrows) was observed compared to GII: untreated wound.
GV: Diabetic+ honey treated: Also increase in nuclei stained for PCNA stained nuclei (arrows) was observed compared to GII: untreated wound but less inflammatory cells.
GVI: Diabetic + Mix of Achillea + honey treated: decrease in nuclei stained for PCNA especially inflammatory cell nuclei compared to other treated groups (arrows).

5. DISCUSSION

Diabetes mellitus is a well-known clinical disease that imposes a health burden worldwide due to alteration of most body functions and lifestyle [14] including wound healing. Others [15,16] reported that transient hyperglycemia may be responsible for delay wound healing in diabetic patients. Thus in the present study, local management of full thickness wound using natural antioxidant herb extract (*Achillea*) was evaluated in metformin controlled –diabetic rat. Results obtained in this study indicated significant changes in wounds of diabetic rats compared to non-diabetics. At the end of the experiment, CBC showed significant elevation in WBCs in untreated diabetic animals and mixed treated groups versus control non-diabetic rats. WBCs counts were significantly decreased in diabetic groups treated with mebo, *Achillea* and honey groups versus diabetic untreated groups. The elevated in WBCs count could be due to secondary infection in diabetic rats. Wang *et al.* [17] reported reduction in leucocyte counts in early phases of wound due to their migration into wounded tissue, and returned to normal level after completion of wound healing. Meanwhile, the results of this study showed insignificant changes in RBCs and platelets counts and HGB levels in different studied groups versus control or diabetic untreated rats. In this respect, Mallick *et al.* [18] reported insignificant changes in RBCs count in wounded animals treated with Neem leaf glycoprotein treated animals compared to phosphate buffer treated controls. The antioxidant effects of both honey and *Achillea* extract and their antioxidant and anti-bacterial effects were tested herein. Growth of gram-positive and negative bacteria as *Staphylococcus aureus* and *Escherichia coli* in GI (wounded non-treated) and *Staphylococcus aureus and Klebsiella spp*. & *Escherichia coli* in GII (diabetic wounded non-treated) were noticed and the results revealed inhibition of bacteria in culture done form diabetic wounded treated animals with different materials, mebo, *Achillea*, honey and *Achillea* + honey mix. These results revealed the antimicrobial effects of *Achillea* extract and honey that could be also involved as an underlying mechanism for promoting wound healing observed in the present study. *Achillea millefolium,* one of *Achillea* species was proved to prevent biofilms formation of Staphylococcus genus bacteria, the most common cutaneous bacterial commensals [19]. Antimicrobial property of *Achillea* was also demonstrated by others Benali *et al.* [20] using gas chromatography-mass spectrometry. Herman and Herman [21] reported that *Achillea* species were among herbal substances used for burn wounds management due to its antimicrobial effect. Study of the histological characteristics of wound healing events showed delayed wound healing in uncontrolled diabetic rats compared to rats with metformin controlled hyperglycemic animals. The processes during wound healing in this study do not differ from what have been described by others [22, 23] that untreated diabetic wounded rats condition was characterized by blood clotting, swelling, tenderness, while treated animals showed epithelialization, precipitation of collagen, and finally appearance of smaller amount of scar tissue coincide with wound contraction. Bolajoko *et al.* [24] reported that oxidative stress in diabetic status underlies the high incidence of diabetic foot ulcer in most diabetic patients. The authors attributed vascular injury and alteration of wound perfusion to the delay of ulcer healing and their progress to diabetic foot presentation especially in those with uncontrolled hyperglycemia. Local application of recent anti- bacterial, anti-inflammatory and antioxidant dressings was proved to be effective in speeding the wound healing rate. Most of the action of such dressing is related to balancing biochemical processes associated with inflammation of large chronic wounds and aimed to improve healing [25]. Complementary treatment using natural substances were tried in clinical and experimental fields to enhance wound healing [26]. Plant extracts had been used as antibacterial, antifungal treatments since ancient times. Nayak *et al.,* [27] found that antimicrobial properties of *Jasminum auriculatum (J.*

auriculatum) accelerated wound healing and *Nigella sativa* extracts had been documented antimicrobial [28]. In the present study, hyperglycemia was controlled by metformin. *Achillea* extract and *Achillea* + honey mix were proved to enhance wound healing based on increased contraction rate, epithelization and decreased inflammatory cells. Serarslan *et al.*[29] mentioned that reactive oxygen species were produced due to skin damage and reduction in various antioxidant enzymes responsible for delayed wound healing. Both honey and *Achillea* were previously reported to possess wound healing promotion effects based on their effective antioxidant properties. Honey was tried with effective results in promoting healing of oral ulcer in experimental animal models [30] and clinical trials [2].The effects of most types of honeys used were mostly related to their antimicrobial and antioxidant properties [31]. As was documented by Dons and Soosairaj [32] who reported that honey enhanced wound healing by stimulated tissue growth, enhanced epithelial growth and reduced scar formation, and this was because honey is acidic, containing hydrogen peroxide, nutrients and antioxidants, and enhanced immunity. Healing of wounds requires agents that supply energy, and honey helps in formation of granulated tissue, and this may be due to the supply of more oxygen, which is considered as an important factor needed in tissue modulation and regrowth [33]. Synergistic effects of honey by addition to other food supplementation were found to be effective against diabetes and promotion of wound healing [34]. It was well known that perfect wound repair and closure depends on the presence of numerous biochemical and structural phenomena like keratinocytes, fibroblasts [35] and growth factors [36]. Epidermal re- epithelization and fibroblast proliferation and its transformation to myofibroblasts played a significant role in wound contraction and enhanced healing process [37]. In this study, α -SA immunohistochemistry was applied to wounded skin to explain the underlying mechanism by which *Achillea* extract and its mixture with honey improved wound contraction in diabetic rat wound. De novo expression of α-smooth muscle action was reported in proliferating fibroblasts which termed myofibroblasts during healing processes in many organs [38]. Transformations of fibroblasts into contractile myofibroblasts were described by Gabbiani [39] to be an important element in healing processes. Shinde *et al.* [40] described the role of fibroblasts accumulation of α-SA in enhancement wound contraction and remodeling and pointed to myofibroblasts ability to contract in a similar way to smooth muscle cells pulling cutaneous wounds edges and thus fasten wound closure and healing. In the present study, it was observed that in animals treated with a mixture of honey and *Achillea* extract, α-SMA immunostaining although stronger than untreated wound, it was less than honey and *Achillea* extract alone and this in favor of developing optimum wound healing without any scar formation. Fibrosis and scar formation were reported upon excessive or prolonged myofibroblast activity [41]. In the present study cellular proliferation was studied histologically using PCNA immunostaining. Topical application of different treatments was found to increase PCNA positive staining in proliferating keratinocytes at wound edge as well as fibroblasts at wounded areas of diabetic rats. Most positive reactions were found in wounds of animals treated with honey, followed by *Achillea* extract then by the mix of both when compared with non-treated wounds. The effect matches that obtained by mebo medicinal treatment. Re-epithelialization and epidermal cell proliferation were confirmed using PCNA staining in many animal models of cutaneous wounds [42, 43]. An increase in PCNA immune-expression was reported in many models of herbal medication of cutaneous wounds and burns like curcumin [44] and *Nigella sativa* [45]. The enhancement of cellular proliferation was attributed to antioxidants activity of those herbs, that could be also the underlying cause of what was observed with honey and *Achillea* extract [28, 31].

6. CONCLUSION

All the above results indicated the effectiveness of *Achillea* extract, *Achillea* + honey mix, honey and mebo in enhancing wound healing in diabetic wounded rats. The different materials modified the blood biochemistry, histological distortions and treated microbial infections in wounds of diabetic rats. The herbal extract of *Achillea*, *Achillea* + honey mix and honey could be used in treating wounded diabetic rats in a way matching that or even better than mebo. So, we advise to use *Achillea* extract and its mixture with honey for treatment of large wide gaped wounds in diabetics.

7. FUNDING ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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