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Usefulness of Achillea Milefolium as healing topical agent in treating thermal burn (histological comparative study in Westar rats)

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Abstract:

Background: Wound can be defined as any disruption or any damage in tissue or anatomical function or structure. Achillea Milefolium extract has medicinal benefits for tissues, including the ability to repair wounds and act as an antioxidant and anti-inflammatory.

Aim of the study: The purpose of the study was to determine how Achillea Milefolium affected the healing of burn wounds.

Material and Methods: Twenty male rats had their right and left cheeks burned (aged around two months with average weight about 300 to 350 g). Rats with burned skin were assigned randomly into two equal groups. Achillea milefolium solution was applied locally to the burned areas on the right side once daily (experimental group), while the left part left without any kind of treatment as control group. Five rats from each group were euthanized after five and ten days, and tissue samples were taken from the burnt areas that had been injured for histological analysis.

Results: In comparison to the control group, rats given Achillea milefolium treatment had faster wound healing, according to histopathological analysis. Re-epithelialization, closure of the wound, and the infiltration of inflammatory cells were signs that the wound was healing.

Conclusions: Application of Achillea milefolium solution locally promotes healing of burn wounds.

Keywords: Achillea milefolium, burn, repair.

Introduction

The largest organ in every animal is the skin which serve many important functions and the most important one is the protection of the body from harmful effects of environment. The epidermis, dermis, and hypodermis are the three layers of skin.. [1]. The distinct keratinocyte-based layers that make up the epidermis are the stratum cornium., stratum basale, stratum spinosum, and stratum granulosum. All four of these layers serve as protective barriers against a variety of diseases, chemicals, and mechanical forces, and they also maintain fluid equilibrium The melanocytes are a specialized subset of keratinocytes that are in charge of pigmentation. This layer contains other types of specialized cells, including Langerhans cells, which are in charge of immunity. [2]

Achillea milefolium is an important type of Asteraceae family with widespread utilization in conventional medicine for the treatment of gynecological, hepatobiliary disorders, for wound healing and against inflammation. Extract of Achillea millefolium (yarrow) using supercritical carbon dioxide anti-solvent technique[3]. Multiple in-vivo and in-vitro models have confirmed the action of A. millefolium with anti-inflammatory, anticancer and antiulcer activities [4].

A wound is a damage or interruption to the normal anatomical structure and function. This damage may only involve a small rupture in the skin's epithelial continuity or it may go deeper, causing harm to the subcutaneous tissue as well as to various organs and tissues, including the muscles, arteries, tendons, bone and nerves,.[5]. Wounds can be grouped based on a variety of factors. Time plays a crucial role in the treatment of wounds and injuries. As a result, wounds can be clinically classed as acute or chronic depending on how long it takes for them to heal [6], Chronic wounds are unable to heal via the standard steps of the healing pathway, in contrast to acute wounds that do so[7, 8]. Hemostasis, inflammation, proliferation, and remodeling are the four stages of wound healing that can be disrupted by many variables, which can delay one or more steps in the process [8, 9]. Complicated wounds are tissue defects and infections that put the wound at risk [8, 10].

All tissues and organs recuperate from injuries. All tissues go through several of these healing processes in common. In order to comprehend the physiological processes occurring in the wound, wound healing is separated into many phases. A series of coordinated interactions between the biological and immune systems make up the intricate process of healing.[11].

Burns are injuries that, depending on their size and depth, call for special care. Regarding burn diagnosis and treatment, the depth and area of a burn are not constant, and burns can get worse over the first few days after an accident. increases in burn depth and body surface area. Oxidative damage, hypercoagulability, and cytokine buildup have all been linked to burn progression. Uncertainty exists on the local and systemic factors involved in the burn sequence of events. Three zones are used to illustrate the evolution of burn tissue. The tissue that was burned immediately and cannot be repaired is in the zone of coagulation [12]. The aim of the study was to assess how Achillea Milefolium affected the healing of burn wounds.

Materials and methods

Assessment of burn severity

The clinician should be aware of the burn's extent and depth in order to assess its severity. It is essential to wait a couple days after the burn to mark the right burn degree since the depth of the burn may alter owing to infection and edema, Temperature, the kind of chemical that caused the burn, the thickness and vascularity of the burned skin, as well as other factors, all determine how deep the burn is. According to the depth, the

Burn was divided into three groups [13]:

An epidermal (superficial) burn of the first degree causes painful, edematous skin. In the same way as sunburns recover after one week with desquamation, discomfort is reduced by applying ice to erythematous skin for 12 to 24 hours. Second degree burn classified into superficial and deep. The epidermis, dermis and the sebaceous glands below the dermis are involved in the superficial type, edema and subepidermal bullae are noticed on the skin. Hair roots are not involved by burns. In the deep type, burn continue to the reticular part of dermis. The skin is thick and pale. Some places have redness, if the bullae are ruptured, the surface appears wet due to the leakage of plasma. But when the dryness of skin remains, the pain will progress, therefore, the clinician should put moist dressing to decrease the pain. A superficial type of second-degree burn cures without scar formation within 2–4 weeks. Hypo and hyperpigmentation may occur in the deep type, with healing process exceeding four weeks with scar formation and loss of function in hair and skin. If a second-degree burn covers more than 20% of the body's surface, fluid supply should be required. The entire skin, including the hypodermis, dermis, and epidermis, is infected with third-degree burns. Tendons, muscles, and bones may be affected by more severe burns. Erythema and dry skin are present. [14].

This study intended to determine the function of Achillea Milefolium in the healing of burn wounds by examining the thickness of the epithelium, observing wound contraction, and analyzing the inflammatory cells infiltration.

methodology

Design

A randomized clinical trial design of this investigation included ten Wester Albino rats, each weighing between 300 and 350 grams, and aged between 7-8 weeks. The research was conducted in conformity with the ethical standards for animal studies established by the college of dentistry at the University of Baghdad (No. 642722). Each rat received a thermal burn to the skin on both side of the cheek. The control group was assigned to the left side, and the experimental groups were given the right side. In order to compare the healing processes of the treatment groups to those of the control group, additional drugs were applied to the burned skin in the treatment groups.

Study grouping

1. A one ml solution of the Achillea Milefolium was administered to the rats in the Achillea Milefolium group (n = 10) after they had been thermally burned on the right cheek.

2. the left side if the rats were used as control group which received no treatment, total number was ten rats.

Experimental procedure

In this experiment we used diethyl ether as anesthetic agent, the chosen rat was put in plastic domestic container sized about two liters, then a cotton soaked with the diethyl ether solution was put in the container followed by closing the container to ensure the rat was inhaling adequate amount of the agent, few seconds later the animal would be completely anaesthetized.[15] the anaesthetized rat would be moved out the container to put on wooden sterilized table to remove fur from the face on both side of the cheek by putting hair removal gel (Veet, Bahrian) which is designed originally to human hair removal, that gel was put about one minute the plastic spatula was used to scrub the intended area and the fur was removed easily [16], while the animal was still under anesthesia (in few situation the rat was awake prematurely, thus it was essential to return it to the anesthetic container for two more minutes) the thermal damage or burn was conducted using Ash number 6 (2dental instrument used usually for dental filling) that Ash was heated to 100 centigrade though

soaking it in boiling water for about five minutes the immediately moved from water to rat skin to avoid cooling it, this heated Ash would left to ten seconds on rat skin to cause the burn, the exact procedure were repeated to opposite side and for all rats[17, 18].

Preparation of the treatment medication

The Achillea milefolium was the drug utilized in this investigation. The aforementioned medication was made by combining 1 g of the drug (Achillea M.) with 100 ml of ordinary saline for 1 minute in a hand liquid blender at the University of Baghdad's Faculty of Agricultural Engineering Sciences' Medicinal and Aromatic Plants Research Unit.

Treatment of skin burn

The skin that had been thermally burnt was then treated with the aforementioned medicine. Immediately following the skin-burning surgery, this drug was applied for 10 days, once daily. The burn wound area received one drop, which was thoroughly administered. After the skin-burning treatment, no medicine was applied to the control group.

Specimens' collection

Five rats were slaughtered after five days of drug administration, while the remaining rats were killed on day ten. Before the sacrifice operation, Diethyl ether was administered to the rats at the same concentration and with the same method as in the experiment to make them unconscious. After the sacrificial operation, a scalpel and blade no 15 were used to remove the charred wound region. The incision was made from the skin's surface to the underlying bone, and the excision border comprised the injured area as well as neighboring, healthy skin. Immediately following excision, the obtained samples were immersed in 10% formaldehyde.

Slides preparation

The typical process for preparing histology slides for staining with H&E was followed while working with the tissue that had been excised.

Counting inflammatory cells and measuring epithelial thickness

The thickness of the epithelium and the quantity of inflammatory cells were assessed using measuring software (ImageJ V.1.53K, National Institutes of Health, USA) at 10x magnification after histology slides were inspected and photographed under a light microscope. From the inner part to outer part of the slide, the epithelium's thickness was measured on both sides of the wound region under a light microscope. [19]. For the inflammatory cells count Counting number of inflammatory cells in five fields for each tissue specimen, by using a light microscope with a square net in one microscope eyepiece, cells were calculated under (power x40) lens by the mean number of cells [19].

Visual observation of wound contraction

On days 1 through 10 after burning, the rat animal's wound area was measured. The identical conditions were used each time a digital camera was used to record the size of the skin burn. To determine the ratio of wound contraction, measurements of the wound area were recorded daily from days 1 through 10. Image J 1.49v software was used to assess the wound area (National Institutes of Health, Bethesda, MD, USA). The following formula was used to calculate the wound contraction rate:

Wound contraction rate = (original wound area - particular day wound area) / Original wound area × 100%.

The wound site that was measured on that particular day was the day-specific wound, while the initial wound was the metal instrument's head area. [20].

Statistical analysis

The research statistics were computed using IBM SPSS V.23 (IBM Corp., Armonk, NY, USA). Calculated descriptive statistics included mean and standard deviation. Shapiro-test Wilk's was used to assess the group's normalcy. The effectiveness and significance of the treatment were examined using the analysis of variance (ANOVA). To determine whether there is a significant difference between the groups, the post hoc Tukey test was performed. T-tests were performed to measure the variation between groups over time. The threshold for significance was established at 0.05 or less. This research was self-funded by authors.

Results

Inflammatory cells

Table 1 Descriptive statistics of inflammatory cells number and groups' difference in each duration using the ANOVA test.

Duration	Groups	Descriptive statistics				Duration difference	
		Mean	St. deviation	Min.	Max.	F-test	*p*-value
5 Days	Control	751.333	63.656	645	860	41.699	0.000 (HS)
	Achillea Milefolium	814.000	43.932	770	880		
10 Days	Control	532.400	54.009	441	676	3.679	0.024 (S)
	Achillea Milefolium	497.600	62.364	400	560		

Table 2 Tukey test after ANOVA test.

Duration	Groups comparison		Mean Difference (I-J)	Sig.
5 Days	Control	Achillea Milefolium	62.67	0.436
10 Days	Control	Achillea Milefolium	34.80	0.740

Epithelial thickness

Table 3 Descriptive statistics of epithelium thickness in (µm) and groups' difference in each duration using the ANOVA test.

Duration	Groups	Descriptive statistics				Duration difference	
		Mean	St. deviation	Min.	Max.	F-test	*p*-value
5 Days	Control	20.842	2.955	16.236	25.47	17.683	0.000 (HS)
	Achillea Milefolium	23.127	4.413	18.123	28.550		
10 Days	Control	26.402	5.509	12.43	39.44	11.489	0.000 (HS)
	Achillea Milefolium	36.672	2.081	34.430	38.713		

Table 4 Tukey test after ANOVA test.

Period	Groups comparison		Mean Difference (I-J)	*p*-value
5 Days	Control	Achillea Milefolium	2.64	0.285
10 Days	Control	Achillea Milefolium	10.27	0.010 (S)

Wound contraction

Table. 5 Descriptive statistics of Wound contraction area in (cm2) and groups' difference in each duration using the ANOVA test.

Duration	Groups	Descriptive statistics				Duration difference	
		Mean	St. deviation	Min.	Max.	F-test	P-value
5 Days	Control	0.393	0.085	0.321	0.526	3.807	0.031 (S)
	Achillea Milefolium	0.388	0.033	0.337	0.425		
10 Days	Control	0.162	0.048	0.101	0.221	30.133	0.000 (HS)
	Achillea Milefolium	0.116	0.011	0.099	0.131		

Table. 6 Tukey test after ANOVA test.

Duration	Groups comparison		Mean Difference (I-J)	*p*-value
5 Days	Control	Achillea Milefolium	0.005	0.999
10 Days	Control	Achillea Milefolium	0.046	0.048 (S)

Histological finding (Hematoxylin & eosin) stain

Control group

Fifth day

The histology findings at the burn site for this period revealed scab formation at the epidermis area with heavy infiltration of inflammatory cells, irregular arrangement of

collagen fibers and newly formed blood vessels. Figure (1)

Tenth day

The histology findings at the burn site for this period revealed re-epithelization at the epidermis, better arrangement of collagen fibers, inflammatory cells infiltration, multiple hair follicles and new formed blood vessels. Figure (2)

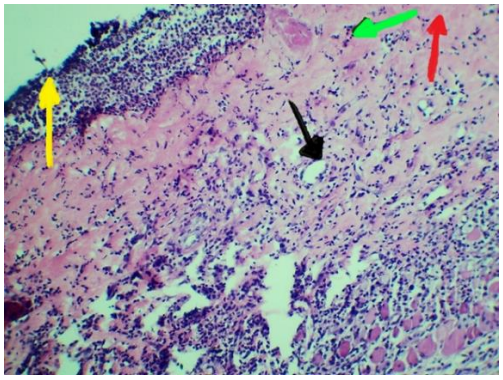

Figure (1) Microscopic view of control group at 5 days duration at burn site shows: scab (yellow arrow), blood vessel (black arrow), collagen fibers (red arrow), inflammatory cells (green arrow), H&E 20x

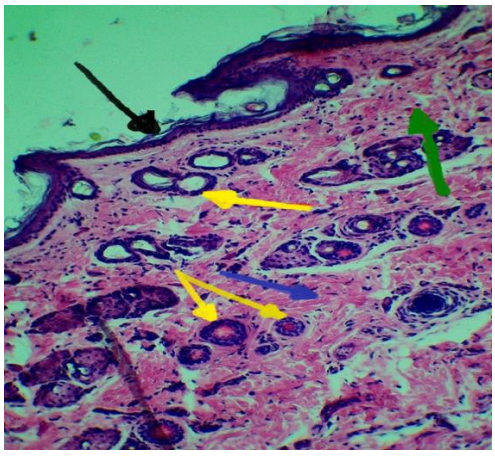

Figure (2) Microscopic view of control group at 10 days duration of burn site shows: re - epithelization (black arrow), collagen fibers (blue arrow), hair follicle (yellow arrows), inflammatory cells (green arrow), H&E 10x.

Experimental group

5 days duration

Histological view of skin section at burn site shows re-epithelization of the epidermis, inflammatory cells in the dermis with irregular arrangement of collagen fibers, hair follicles and formation of new blood vessels. Figure (3).

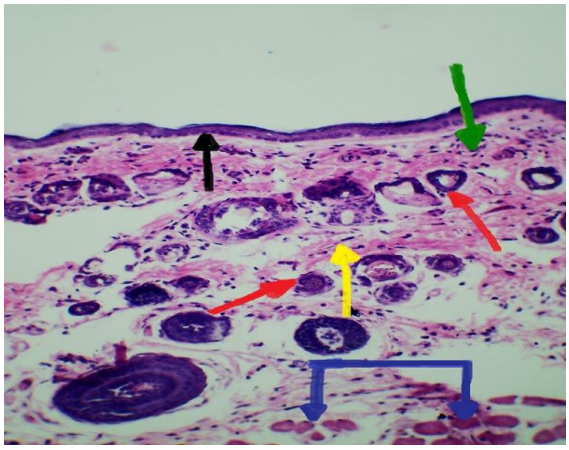

Figure (3) Microscopic view of Achillea group at fifth duration of burn site shows: newly formed epithelium (black arrow), blood vessels (blue arrows), fibroblasts (yellow arrow), hair follicle (red arrows), inflammatory cells (green arrow), H&E stain 10x

Tenth day

Histological finding at burn site shows re-epithelialization at the epidermis area, inflammatory cells less than 5 days duration with irregular arrangement of collagen fibers in dermis, new blood vessels, and hair follicles. Figure (4).

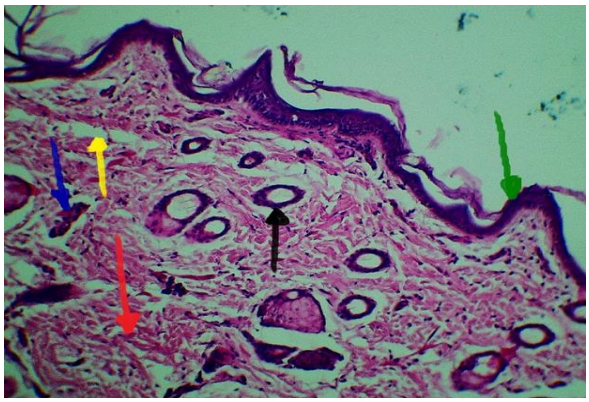

Figure (4) Microscopic view of Achillea group at 10 days duration of burn site shows: complete epithelization (green arrow), hair

follicle (black arrow), adipose tissue (yellow arrow), fibroblasts (Red arrow), blood vessel (blue arrow), H&E stain 10x.

Discussion

Histological finding:

Hemostasis, inflammation, proliferation, and modification of the tissue, which results in the creation of scar tissue, are some of the stages of tissue healing that occur during wound healing. Growth factors generated by platelets during the coagulation phase activate in the area of the wound.[21].

Inflammatory cells count

As a result of the inflammatory phase of wound healing beginning one day after burn incidence, the Achillea milefolium group's infiltration of inflammatory cells into the wound site was slightly higher in the Achillea milefolium group's wound site over the course of five days than it was in the control group.[8]. In 10 days-duration the number of inflammatory cells during the histological scoring system in the Achillea milefolium group was less than control group because of anti-inflammatory effect of Achillea milefolium.[22] . This result is consistent with a study by Allahverdi et al. [23] using lanolin extract to treat burn injuries in rats. They found that after 10 days, there was a reduction in the amount of inflammatory cells.

Epithelial thickness

The proliferative stage of wound healing known as epithelialization involves the secretion of extracellular matrix and newly created capillaries to fill burn-induced deficiencies. Keratinocytes multiply close to the wound's edge, and stem cells and apocrine glands start to rearrange themselves into the basal cell layer and develop into keratinocytes surrounding the hair follicle bulbs. Then start to cross the edge of the wound. Near the inner wound edge, new basement membrane starts to form via fusion once they have enclosed the mesenchyme of ECM. Once the newly laid epithelial cells have settled, a second row of keratinocytes migrates over them to bridge the gap [24]. Within 24 hours of injury, the area of the epidermis closest to the edge of the wound starts to thicken. Keratinocytes form from stratum basale stem cells and begin migrating toward one another across the wound to close the gap [25-27] . After an ANOVA comparison between the control and Achillea milefolium groups indicated highly significant differences on days 5 and 10, a post hoc test was conducted using the Tukey test to confirm the specific differences between the two groups, with a p value of less than 0.010 during a period of 10 days, the Achillea Milefolium group was the primary contributor to the difference, while over a period of 5 days, there was no statistically significant difference between the two groups. The topical application of Achillea Milefolium kept the wound wet, concurring with [28] who postulate that the pace of epithelialization will be faster if the lesion is maintained moist. This assisted in re-epithelialization. In conclusion, this study showed that epithelial thickness increased gradually in both groups, but this rise dramatically accelerated from day 5 through the end of the trial; in Achillea groups, the enhancement was only apparent on day 10.

Wound contraction

A healing process called wound contraction reduces the extent of the damaged tissue and the amount of it that has to be repaired. Myofibroblasts, which are present near the wound's margins, are a part of this process. These myofibroblasts pull freshly generated collagen fibers in the injured tissues toward the wound defect, thereby minimizing its size. The two primary proteins involved in this process, actin and myosin, interact with freshly generated collagen fibers in the extracellular matrix to create a network that serves as the foundation for wound closure. [29]. Due to the mesenchymal origins of fibroblasts and their ability to display either a non-contractile or highly contractile phenotype, wound healing

has the advantage of reducing the wound borders, which leads to wound closure. Unwanted contracture and scarring are the results of excessive contraction, which can have negative functional and aesthetic effects. [30][31]. fibroblasts play a major role in the healing of wounds. Scar development brought on by excessive myofibroblast activity might hinder function and cause localized immobility.[26].

The anti-inflammatory and antioxidant properties of osteopontin and krill oil were discovered by Bruemmer and Law to be useful in the treatment of wounds. In this study, the scientists reported a major increase in the Achillea group that was subjected to this experiment after five days ($p = 0.031$), however a considerably large increase was detected after 10 days ($p = 0.001$) [32] The specific difference between the two groups was verified using a post hoc test utilizing the Tukey test. There were no notable differences between the two groups during the course of the first five days, but there were for the subsequent ten days. This is due to the fact that the wound begins to contract almost a week after the initial damage, and a post hoc test was carried out to confirm the specific difference between the two groups. [33].

It was discovered in the current study that the Achillea group performed better than the control group in terms of the advancement of wound contraction.

Conclusion

In this study, topical application of Achillea milefolium on damaged skin accelerated and enhanced the healing of burn wounds.

Conflicts of Interest

The authors reported that they have no conflicts of interest.

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