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Original Article

Effects of Topical Application of Clay and Honey on Cutaneous Wound Healing in Rabbits

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ABSTRACT

There are certain medications and procedures that can accelerate the healing of skin which have shown improvement and acceleration of wound healing with honey. Furthermore, based on studies of traditional medicine, clay therapy is effectiveness in accelerating wound healing. This study conducted to compare the efficacy of honey and clay in accelerating the healing process of the skin. 9 rabbits were used in this experiment and divided into 3 groups, first control group treated with a placebo, the second treated with a cataplasm of clay and the third treated with honey. Within 4 th and 7 th and 10 th days, 3 rabbits in each group were scarified and macroscopic and microscopic of the wound were studied and histopathological features. The results of our experiments showed that the formation of granulation tissue and the density of fibroblasts keratinisation in surface of wound in the treated group with clay were most than the group treated with honey but the two bioactives reduced inflammation, swelling and wound dehiscence in rabbits. The findings suggest that clay and honey applied topically on cutaneous wounds accelerates the healing processes and appears to have an important property that makes it ideal as a dressing on cutaneous wounds.

Keywords: Clay, Honey, Wound Healing, Rabbit.

INTRODUCTION

Wound healing is a complex and dynamic process. Analyzing the events of normal wound healing is a necessary prerequisite for understanding pathological processes. In this regard, animal and cellular models have proved to be quite useful, although there were significant differences in skin wound healing between experimental animals and humans (Graham, 2004). Maintaining skin integrity is vital for human and animals to protect the organism against dehydration, bleeding and ingress of microorganisms. In order to do this, animals evolved a sophisticated mechanism of wound healing to quickly plug the gap, re-epithelialize over the defect and rapidly replace the lost dermis with new matrix. Clearly, the speed of wound healing depends on many factors, including the size of the wound, blood supply to the area, presence of foreign bodies and microorganisms, age and health of the patient and nutritional status of the patient (Mcgrath and Breathnach, 2004). In other hand, in several reports the rapidity of healing seen with honey dressings is noted (Mcinerney, 1990; Adesunkanmi and Oyelami, 1994).

Recent studies in the rat model have demonstrated that as wounds healing, local concentrations of zinc, calcium, copper and magnesium change according to the phase in the wound healing cascade and associated biochemical events (Lansdown *et al.*, 1999). Clinical observations in human and experimental studies have demonstrated that deficiencies in the availability of these metals, imbalance in local concentrations, or defects in metabolism are potential causes of defective or non healing wounds (Moynahan, 1974). Many of the authors reporting the use of honey as a dressing on infected wounds attribute its effectiveness at least partly to its antibacterial properties (Ndayisaba *et al*., 1993). Others have reported that honey promotes the formation of clean healthy granulation tissue (Efem, 1988) and growth of epithelium over the wound (Subrahmanyam, 1994).Thus it helps skin regenerate and make the plastic surgery unnecessary (Hejase *et al.*, 1996).

Honey has been reported to reduce inflammation, edema and exudation (Subrahmanyam, 1996).The present study is designed to investigate the action of clay and honey on skin wound healing in rabbit.

MATERIAL AND METHODS

9 rabbits (Fig. 1) underwent removal of a skin portion and then they were divided into three groups: the control group (n=3), honey treated group (n=3) and clay-treated group (n=3) respectively. Under general anesthesia, the entire

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lateral surface of the thigh is shaved and disinfected with iodine antiseptic solution. A segment of 1.5 $Cm^2$of skin was removed (Fig. 2).

The control group received simple dressing with sterile gauze, whereas clay group received daily topical application of cataplasme of clay (mixture of Montmorillonite and kaolin, 1 gram/day) and honey group received daily topical applications of natural honey (1 g/day) (Ghaderi *et al.*, 2010).

On the 4^{th}, 7^{th}, and 10^{th} days of operation, 3 rabbit separate from each group and received an overdose of ether anesthesia. Afterward a piece of treated skin was removed and fixed with 10% formalin solution. After fixation, routine processes of tissue preparation including dehydration, clearing and infiltration was performed. Spicemens were embedded in paraffin blocks. The paraffin blocks were trimmed and thin serial sections (4-5µm) were cut with a rotary microtome. Sections were randomly selected and stained with Hematoxylin and Eosin (H&E).

The study of sections included gross pathology for the presence of infections, dehiscence and repair and histological evaluations of wound site for the degree of healing.

In monitoring of postoperative day, we will noted the macroscopic evolution of skin healing animals treated with clay and honey, and evaluate possible effects on wound healing. Three periods of histological samples have been programmed (4^{th}, 7^{th}, and 10^{th} days). The dressing was prepared by honey after dilution with distilled water (1/3 honey and 2/3 of distilled water). The obtained dilution was applied to the wound in a homogeneous manner. Subsequently, the wound was covered by thin dry sterile gauze. The dressing was repeated every 24 hours. For preparation of clay cataplasm, distilled water was added to the mixture of clay powder (white and green) which was fresh and sterile, to compose a thick tab which were covered the entire wound. A sterile dressing was applied to the clay plaster. The cataplasm was changed every 24 hours.

Fig. 1: Individuals cages in Tiaret institute, Algeria.

Fig. 2: The wound after ablation of skin fragment in rabbit.

RESULTS

The general condition of the animal health was good and all rabbits survived until the end of 10 days.

Macrocopic results

Interpretation of macroscopic results for all items in days 4, 7 and 10 Include:

Day 4

In controls part A of Fig. 3 there was a lack of contraction of the wounds with the formation of a crust

That occupies half of the wounds. In animals treated with the clay (Part B of Fig. 4), there was an early contraction of the woundswith a slight erythema on the edges, and forming a thin layer at the bottom of the wounds .In rabbits treated with honey (Part C of Fig. 5) was observed a strong inflammatory reaction with a slight contraction, and the formation of a crust which holds a third of the periphery of the wound.

Day 7

After 7 days, there was an acceleration of wound healing in animals treated with honey (Part F of Fig. 8) compared to the control group (Part D of Fig. 6). It was observed that the phenomenon of contraction was very strong compared to 4th day. It was also noted the development of a crust that occupied half of the wound. The animal treated with clay (Part E of Fig. 7) showed a slight contraction and a greatly reduced (Part E of Fig. 7) erythema, which indicated the existence of an inflammatory reaction with less crusting which was always a barrier.

Day 10

In the control (Part G of Fig. 9), there was a slight contraction of the wounds, a rash in the skin around the wound with the formation of a crust on the entire wound .In the treated clay Animals (Part H of Fig. 10), it was noted that the phenomenon of wounds contraction was done under the crust, with an early detachment of the latter. At the time of sampling there was a restoration of the integrity of the subcutaneous tissue. In rabbits treated with the honey (Part I of

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Fig. 11), there was an increase of the inflammatory phenomenon in addition to suppuration, with an extension of the erythema and the high congestion of the surrounding skin of the wound.

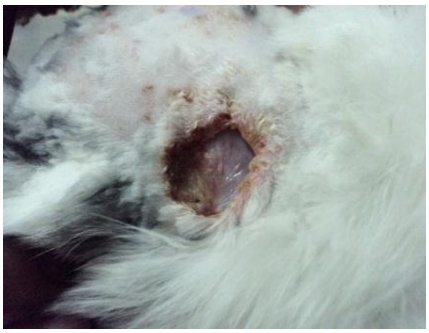

Fig. 3A: macroscopic view in Day 3

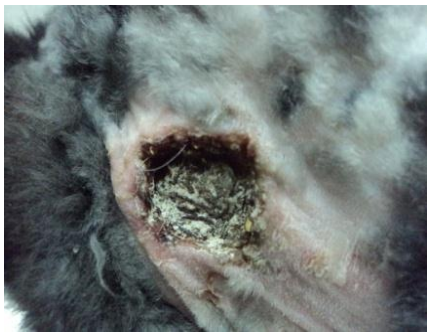

Fig. 4B: macroscopic view in Day 3

Fig. 5C: macroscopic view in Day 3

Fig. 6D: macroscopic view in Day 7

Fig. 7 E: macroscopic view in Day 7

Fig. 8F: macroscopic view in Day 7

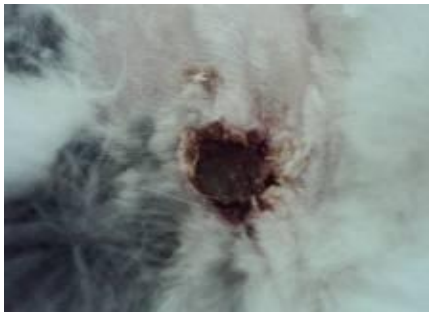

Fig. 9G: macroscopic view in Day 10

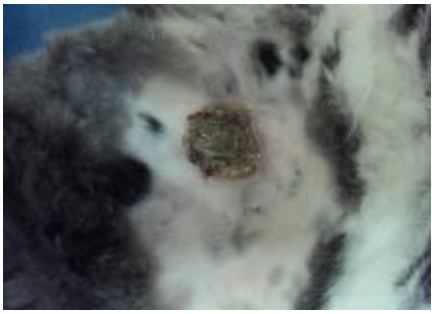

Fig. 10H: macroscopic view in Day 10

Fig. 11 I: macroscopic view in Day10

A, D, G: Rabbit control, **B, E, H**: Rabbit treated with clay, **C, F, I**: Rabbit treated with honey.
Experimental group in 4th day: (HE coloration, 10×), (A) Presence of an inflammatory granuloma, and predominance of mononuclear cells (macrophages) with vascular neoplasms. (B) Newly formed tissue adheres well to normal connective tissue. Connective tissue rich in vessels congested with a population of polymorphonuclear neutrophils. Figure very close to that of natural connective tissue. Condensation by migration or by multiplication of fibroblasts. (C) Wound region characterized by an abundance of dense fibrous tissue cells: fibroblasts.
Experimental group in 7th day: (D) region characterized by an abundance of dense fibrous tissue cells, fibroblasts, and a slight condensation conjunctive frame in the injured party.
(E) Dense connective tissue compared to controls. Less cell (inflammatory granuloma), they are condensed and not scattered. The presence of a detached crust (scab) it contains a fragment of scar tissue. It is a dense connective tissue rich in condensed cells; (F) Presence of subcutaneous oedema in inflammation and is the source of distance elements of connective tissue. The presence of a very condensed cell population.
Experimental group in 10th day: (G) The presence of all the components of a dense connective tissue. The number of cells is reduced compared with the observation at 7 day. Angiogenesis is observed and the presence of hair. It there's a well-organized training fibrous tissue, (H) oedema and predominance of newly formed blood vessels (angiogenesis), (I) intact healthy skin and important contraction of wound.

Microscopic results

The objective of the histological study of skin flaps was to evaluate the process of tissue repair (scarring), especially the proliferation of fibroblasts, angiogenesis and epithelialisation of wounds.

Assessment of histological analysis

We based on the following criteria in the study of histopathological sections: the hemorrhage, the arrangement of the fibrin, polynuclear cells and mononuclear cells, cells infiltration, necrosis, and angiogenesis.

The microscopic examination of skin samples taken in 4, 7 and 10 days, showed early onset of an inflammatory granuloma dice on day 4. The process becomes very important today 7, with a predominance of fibrous tissue rich in myofibroblasts especially for the sample treated with honey.

At Day 10, healing was almost completed for the control samples and clay latter showed an aspect of organization of advanced connective tissue.

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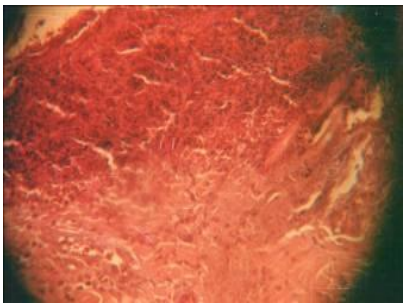

Fig. 9A: microscopic view in Day 4

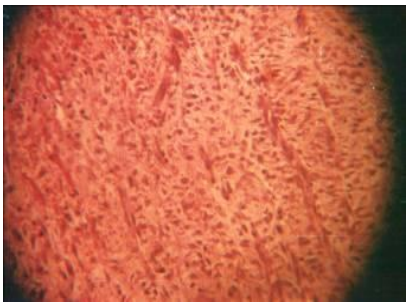

Fig. 10B: microscopic view in Day 4

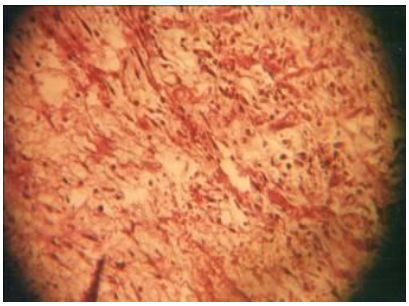

Fig. 11C:microscopic view in Day 4

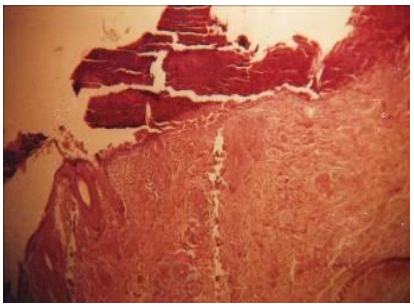

Fig. 15 G: microscopic view in Day 7

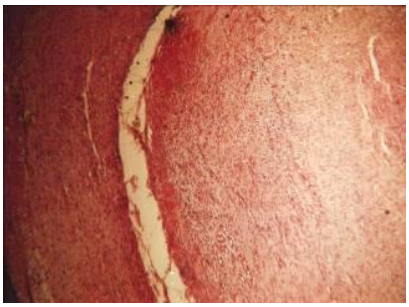

Fig. 16H:microscopic view in Day 7

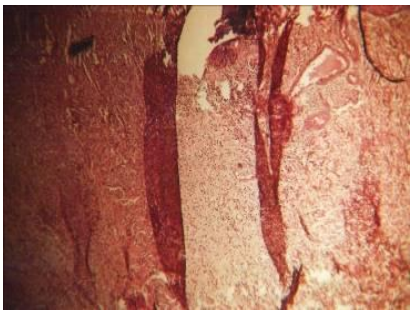

Fig. 17I: microscopic view in Day 7

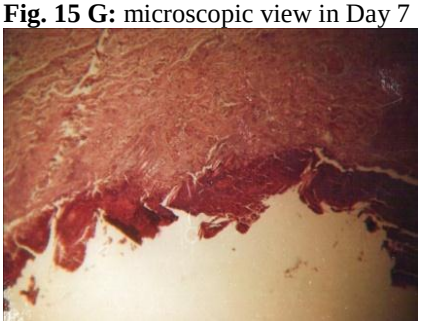

Fig. 17I: microscopic view in Day 10

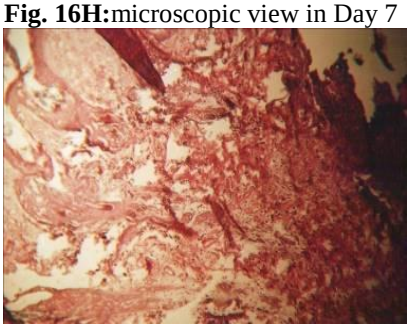

Fig. 17I: microscopic view in Day 10

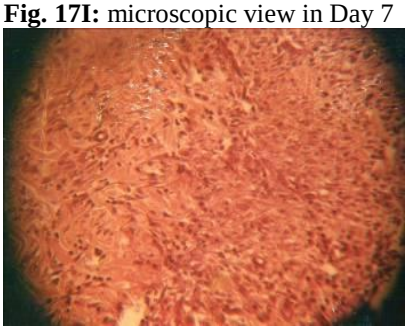

Fig. 17I: microscopic view in Day 10

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DISCUSSION

In this study, we investigated the effects of bioactive namely clay minerals and honey on the healing of skin wounds. The skin lesion immediately causes the formation of clots and local inflammation characterized by infiltration of neutrophils and macrophages in the wound site. These pathological changes are typical of the inflammatory phase of wound healing. The inflammatory reaction is considered instrumental in providing growth factors, cytokines and chemokines that orchestrate the movement necessary for wound repair cell (Singer and Clark, 1999). This study showed that the formation of the crust has played the role of a protective film and a protective barrier against external aggressions. While there has been forming a dense granulation tissue and activation of fibroblasts and an increase in the thickness of the collagen fibers in the group of animals treated with clay in comparison with the group of animals treated with honey. Recent studies have demonstrated that the effect of topical application of the clay due to its concentration of trace metals are essential to change their skin wounds reflecting metalloenzyme complexes requirements in essential events in the phases of wound healing (Lansdowne *et al*., 1999). The balance of traces of metals in the wound site is critical to the stage of healing, imbalances in the relative concentration of calcium , zinc Copper , etc. also the presence of ions xenobiotics such as cadmium are potential causes of bad healing (Vicanova *et al*., 1998) Clay is widely used in numerous industries. However, what make it unique for its consideration in natural medicine are the unique properties. When properly hydrated, it creates and sustains its own subtle electromagnetic field (negatively charged particles). It has very strong absorption properties, it attracts and holds on the surface of many toxic substances. When used immediately

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in a external severe trauma situations, clay packs significantly reduced tissue damage associated with swelling. The protection mechanism of clay is likely to be a combination of the absorption of the allergens and ameliorative of the skin barrier function. According to our results, the clay is a natural mineral product composed of hydrated silicates of aluminum, used as an external treatment for skin wounds has the effect such as rapid absorption of edema with removal of necrotic parts while respecting seines parties. In addition to these results, Clay also allows a rapid disappearance of the odor associated budding tissue and accelerated healing after transplantation. The clay absorbed even what appears to be off shoots of the main ulcer, sometimes very far from the original wound. This is very characteristic phenomenon probably through the lymphatic system Action on nodules and cup boards accelerated healing grafts .In the same way, a large number of in vivo research have confirmed that the use of honey in very inflamed wounds, allowed cleaning abscesses and reduced healing time antimicrobial effectiveness no side effects on tissues strong and powerful antiseptics stimulates tissue responsible for healing antiinflammatory and analgesic reduces edema and exudation reduces healing effect. Its PH has osmotic effects with play a antimicrobial efficacy .Healing properties of honey and explain its broad indications in the treatment of wounds. Several studies have demonstrated antimicrobial properties of honey and the effects of wound healing (Molan, 1992). It inhibits microbial growth due to high osmolarity (Chirife *et al*., 1982) but Cooper *et al.* (1999) showed that the antimicrobial effect of honey in a different way a secondary producers increase fibroblast collagen scar promote good quality. These healing properties of honey and explain its broad indications in the treatment of wounds. In our study, the results have a conflict of interest in relation to studies that were made by these authors.

In our study, treatment with honey showed a delay in the installation of scar tissue compared to treated clay with a purulent infection of a single rabbit among the group 3 groups. The use of honey did not significantly decrease infection rates in other types of wounds. The accurate identification of wound infection is a difficult clinical issue in other types of wounds, particularly chronic wounds. Clinical presentation was an important indicator, but presentation may vary with wound type (Cutting, 2007). In our study the delayed healing compared with clay groups can be explained by the wrong choice of the quality of honey and the poor dilution of honey .In our study diluted honey, reduced its effectiveness.

CONCLUSION

Natural's biologics products offer an ideal wound healing in an optimal delay, without resorting to surgery, and we are now convinced that a serious study in rabbits could open promising therapeutic perspectives.

REFERENCES

Adesunkanmi K and Oyelami OA, (1994). The pattern and outcome of burn injuries at Wesley Guild Hospital, Ilesha, Nigeria: a review of 156 cases. The American Journal of Tropical Medicine and hygiene, 97(2):108-12.

Chirife J, Scarmato G and Herszage L, (1982). Scientific basis for use of granulated sugar in treatment of infected wounds. Lancet 1, (8271): 560–1.

Cooper RA, Molan PC and Harding KG, (1999). Antibacterial activity of honey against strains of staphylococcus aureus from infected wounds .Journal of the Royal Society of Medicine, 92 (6): 283-285.

Cutting KF, (2007). Honey and contemporary wound care: an overview, Ostomy wound manage, 53 (11): 49-54.

Farouk A, Hassan T, Kashif H, Khalid SA, Mutawali I and Wadi M, (1988). Studies on Sudanese bee honey: laboratory and clinical evaluation. Pharmaceutical Biol, 26(3): 161- 168.

Ghaderi R, Afshar M, Akhbarie H, and Golalipour MJ, (2010). Comparison of the efficacy of honey and animal oïl in Accelerating Healing of full thickness wound of mice skin. Int. J. Morphol, 28(1):193-198.

Graham JE, (2004). Rabbit wound management. Vet. Clin. North Am. Exot. Anim. Pract. 7, 37-55.

Hejase MJ, Bihrle R and Coogan CL, (1996). Genital Fournier's gangrene: experience with 38 patients. Urology, 47(5): 734-79.

Lansdown AB, Sampson B and Rowe A, (1999). Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds. Journal of anatomy, 195 (3): 375-386.

Mcgrath JA and Breathnach SM, (2004). Wound healing. In: Burns, T.; Breathnach, S, Cox, N. & Griffiths, C. (Eds). Rook's textbook of Dermatology. 7th Ed. London, Blackwell Science.

Mcinerney RJ, (1990). Honey a remedy rediscovered. J. R. Soc. Med, 83: 127.

Molan PC, (1992). The antimicrobial activity of honey: 2- variation in the potency of the antimicrobial activity. Bee wourld, 73: 59-76.

Moynahan EJ, (1974). Letter: Acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. Lancet. Aug 17, 2(7877): 399-400.

Ndayisaba G, Bazira L, Habonimana E and Muteganya D, (1993). Clinical and bacteriological results in wounds treated with honey. J. Orthopaedic. Surg., 7(2): 202-204.

Singer, AJ and R A. and Clark, (1999). Cutaneous wound Healing. The New England Journal of Medicine, 341(10): 738-46.

Subrahmanyam M, (1996). Honey dressing versus boiled potato peel in the treatment of burns: a prospective randomized study. Burns, 22(6): 491-493.

Vicanova J, Boelsma E, Mommaas AM, Kempenaar JA, Forslind B, Pallon J, Egelrud T, Koerten HK and Ponec M, (1998). Normalisation of epidermal calcium distribution profile in reconstructed human epidermis is Related to improvement of terminal différentiation and Stratum corneum barrier formation ; the Journal of Investigative Dermatology, 111(1): 97-106.