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Comparison of the Efficacy of Honey and Animal Oil in Accelerating Healing of Full Thickness Wound of mice skin

Comparación de la Eficacia de la Miel y Aceite Animal en la Aceleración de Cicatrización de la Herida en Todo el Espesor de la Piel de Ratón

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SUMMARY: There are some medications and procedures that can be used to accelerate the healing of skin wounds. Some studies have demonstrated improvement of burn wound healing with honey treatment. In other hand, based on traditional medicine have improved wound healing with animal oil. This study was done to compare the efficacy of animal oil and honey in accelerating healing of full thickness wound of skin in mice. In this experimental study 36 male NMRI mice were subjected to full-thickness skin wounds under general anesthesia. Animals were randomly allocated to receive either single daily applications of placebo, animal oil and honey (n=12 for each group) respectively. On 4th, 7th and 10th days, four mice from each group were sacrificed using an overdose of anesthetic. Macroscopic and Microscopic characteristic of wounds were studied pathologically and histologically. The findings of this study showed that formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of wound and thickness of basement membrane and epidermis in Honey treatment group was more than animal oil group. Honey more than animal oil decreased inflammation, edema and dehiscence of wound in mice. The wound healing rate in honey group was higher than in animal oil group ($p<0.05$). This study showed that honey more than animal oil accelerates healing of full thickness wound of skin in mice.

KEY WORDS: Honey; Animal oil; Wound healing; Skin; Mice.

INTRODUCTION

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 upon 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 & Breathnach, 2004).

The increased interest in complementary therapies has led to the investigation of products traditionally believed to have a beneficial effect in wound healing. Several studies have demonstrated improved wound healing with traditional medicinal oils treatment (Dursun *et al.*, 2003; Kietzmann & Braun, 2006; Pei *et al.*, 2006; Wang, 2006).

In other hand, in several reports the rapidity of healing seen with honey dressings is noted (Adesunkanmi & Oyelami, 1994; McInerney, 1990). 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 (Farouk *et al.*, 1988; Ndayisaba *et al.*, 1993). Others have reported that honey promotes the formation of clean healthy granulation tissue (Efem, 1988; Wadi *et al.*, 1987) and growth of epithelium over the wound (Subrahmanyam, 1998; Subrahmanyam, 1994). Thus it helps skin regenerate, making plastic surgery unnecessary (Efem, 1993; Hejase *et al.*, 1996). Honey has been reported to reduce inflammation (Yang, 1944), edema and exudation (Subrahmanyam, 1996). However, there is a tendency for some practitioners to dismiss out of hand any suggestion that treatment with honey is worthy of consideration as a remedy in modern medicine. An editorial in Archives of Internal Medicine assigned honey to the category of "worthless

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but harmless substances" (Soffer, 1976).

Based on studies that have demonstrated improved burn wound healing with honey treatment and based on traditional medicine have demonstrated improved wound healing with traditional medicinal oil, we anticipated that honey and animal oil will accelerated healing of full thickness wound of skin.

The aim of this experimental study was to compare the efficacy of animal oil and honey in accelerating healing of full thickness wound of skin in mice.

MATERIAL AND METHOD

Thirty-six male NMRI mice were randomly allocated to the control group (n=12), animal oil –treated group (n=12) and honey-treated group (n=12) respectively. Under general anesthesia a 2-mm full thickness wound was made on the back of the skin of each animal in this group. The control group received simple dressing with sterile gauze, whereas animal oil group received daily topical applications of traditional animal oil (so called yellow oil = sheep butter, 1 gram/day) and honey group received daily topical applications of natural honey (1 g/day). On the 4^{th}, 7^{th}, and 10^{th} day of operation, four mice 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. Specimens were then embedded in paraffin blocks. The paraffin blocks were trimmed and thin serial sections (4-5µm) were cut with a 2145 rotary microtome (LICA Company, Germany). Some section were randomly selected and stained with hematoxylin and eosin (H&E).The section were studied by one dermatologist and two pathologist who were blind to the treatment of each groups. The study of sections included; 1) gross pathology for the presence of infections, dehiscence and repair; 2) histological evaluations of wound site for the degree of healing.

Finally Data were analyzed by using SPSS package and chi-square test ($P<0.05$).

RESULTS

Microscopic features of wounds on day 4. Formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of wound and thickness of basal membrane and epidermis on day 4 Also Honey more than animal oil decreased inflammation, edema and dehiscence on day 4 (Table I, Fig. 1A). Degree of inflammation of wounds in animal oil group was more than honey group on day 4. Inflammation Degree of wounds in control group was more than animal oil group.

Microscopic features of wounds on day 7. Honey treatment more than animal oil treatment increased: 1) Formation of granulation tissue, 2) density and activation of fibroblasts 3) keratinization in surface of wound, 4) thickness of basal membrane and epidermis 5) Thickness of collagen fibers (Table II, Fig. 1B). Degree of inflammation of wounds in animal oil group was more than honey group on day 7. Also Degree of inflammation of wounds in control group was more than animal oil group.

Microscopic features of wounds on day 10. Density and activation of fibroblasts, keratinization in surface of wound, thickness of basement membrane and epidermis and thickness of collagen fibers increased in Honey group more than animal oil group on day 10. (Table III, Fig1C). Also inflammation of wounds was absent in honey and animal oil groups. Furthermore Degree of inflammation of wounds in control group on day 10 was less than on day 7. Most of inflammatory cells of wounds in control group were lymphocyte (85%) on day 10. Mean days to healing (complete closure) were 10, 13 and 14 for the honey, animal oil and control groups, respectively ($p<0.05$).

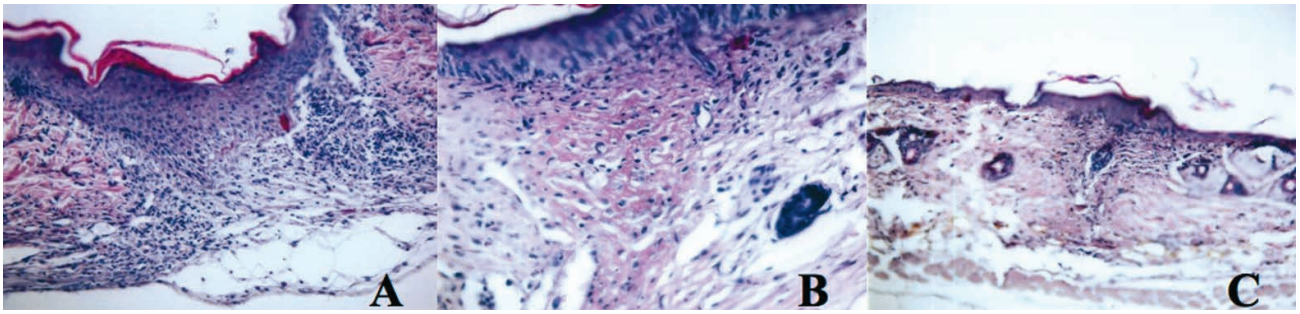

Fig. 1. A. decrease of inflammation, edema and dehiscence in the Honey group on day 4. B. thickness of collagen fiber on day 7 in the honey group. C. thickness of collagen fiber in the honey group on day 10 (H &E staining, X100).

Table I. Microscopic features of wounds in honey, animal oil and control groups on day 4.

Parameters	Honey group	Animal oil group	Control group
Inflammatory cells density	++	++	++++
Inflammatory cells type	PMN (40%) Lymph (60%)	PMN (65%) Lymph (35%)	PMN (98%) Lymph (2%)
Granulation tissue formation	+++	+/-	-
Edema	+	++	++
Fibroblasts density	++	+/-	-
Fibroblasts activation	++	+	-
Basal layer	5 layer	3 layer	2-3 layer
Increased thickness of epidermis in edge of wounds	9 layer	8 layer	7 layer
Keratinization in surface of wounds	++ Ortho and parakeratotic	+ Ortho and parakeratotic	+ Parakeratotic

PMN= Poly Morpho Nuclear; L= lymph; O= orthokeratotic; P= parakeratotic; N= normal; ABN= abnormal, - negative, +/- very mild, + mild, ++ mild to moderate, +++ moderate, ++++ severe

Table II- Microscopic features of wounds in Honey , Animal oil and Control groups on day 7.

Parameters	Honey group	Animal oil group	Control group
Inflammatory cells density	-/+	-/+	++
Inflammatory cells type	PMN (1%) Lymph (99%)	PMN (10%) Lymph (90%)	PMN (20%) Lymph (80%)
Granulation tissue formation	++++	+++	++
Collagen fiber density	++++	+++	+
Fibroblasts density	++++	++	++
Fibroblasts activation	++++	++	++
Thickness of collagen fiber	60% of normal	50% of normal	30% of normal
Increased thickness of epidermis in	6 layer	6 layer	5 layer
Keratinization in surface of wounds	+++ Orthokeratotic	++ Orthokeratotic	++ Ortho and parakeratotic

PMN= Poly Morpho Nuclear; L= lymph; O= orthokeratotic; P= parakeratotic; N= normal; ABN= abnormal, - negative, +/- very mild, + mild, ++ mild to moderate, +++ moderate, ++++ severe

Table III- Microscopic features of wounds in Honey ,Animal oil and Control groups on day 10.

Parameters	Honey group	Animal oil group	Control group
Inflammatory cells density	-	-	+
Inflammatory cells type	-	-	PMN (15%) Lymph (85%)
Granulation tissue formation	-	-	+/-
Collagen fiber density	++++	+++/++	++
Fibroblasts density	+	++	+++
Fibroblasts activation	+	++	+++
Color of collagen fiber	deep	deep	light
Thickness of collagen fiber	85% of normal	55% of normal	50% of normal
Increased thickness of epidermis in edge	3 layer	3 -4 layer	4 layer
Keratinization in surface of wounds	+ Orthokeratic	++ Orthokeratotic	+++ Ortho and parakeratotic

PMN= Poly Morpho Nuclear; L= lymph; O= orthokeratotic; P= parakeratotic; N= normal; ABN= abnormal, - negative, +/- very mild, + mild, ++ mild to moderate, +++ moderate, ++++ severe.

DISCUSSION

This study showed that formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of wound, thickness of epidermis and thickness of collagen fiber increased in honey group in comparison with animal oil group. These results are similar to other studies (Efem, 1988; Subrahmanyam, 1998; Subrahmanyam, 1994; Wadi *et al*.).

According to our results honey decreased infection, inflammation, edema and dehiscence. These results are similar to other studies (Adesunkanmi & Oyelami; Ndayisaba *et al*.; Subrahmanyam, 1996; Yang). Our findings showed that the rapidity of healing is higher in honey group in comparison with animal oil group. This finding have noticed in other studies (Adesunkanmi & Oyelami; McInerney; Misirlioglu *et al*., 2003; Subrahmanyam *et al*., 2001; Tovey, 2000).

In a study, Lusby *et al.* (2006) with using excisional wound model in rats showed that L. xallardii honey, but not essential oil, has a beneficial action in wound healing. Our study showed similarfindings in animal model.

Also, in other reports the rapidity of healing with honey dressings was noted (Adesunkanmi & Oyelami; McInerney). In a study, Oryan & Zaker (1998) showed that treated lesions showed less oedema, fewer polymorphonuclear and mononuclear cell infiltration, less necrosis, better wound contraction, improved epithelialization and lower glycosaminoglycan and proteoglycan concentration on days 2 and 7 postoperatively and better tissue organization and consequently an improved tissue ultimate strength and yield strength on days 14 and 21 post operation. These findings suggest that honey applied topically on cutaneous wounds accelerates the healing processes and appears to have an important property that makes it ideal as a dressing for cutaneous wounds.

Furthermore, Staunton reported that honey accelerates healing because of its direct effects on tissue and antibacterial properties. In addition, dressings with honey can be changed relatively infrequently. Honey decreases inflammatory edema, hastens sloughing of devitalized tissue, attracts macrophages which cleanse the wound, provides a local cellular energy source, and protectively covers the wound. A high osmolarity, acidity, and hydrogen peroxide content confer honey with antibacterial properties. Here we describe the use of honey to manage a bite wound in a stumptail macaque (Staunton *et al.*, 2005).

Also Mphande *et al*. (2007) in a study on 40 patients revealed that Honey appears to be more effective than sugar in reducing bacterial contamination and promoting wound healing, and slightly less painful than sugar during dressing changes and motion.

Efem (1988) in a study reported that honey debrided wounds rapidly, replacing sloughs with granulation tissue. It also promoted rapid epithelialization, and absorption of oedema from around the ulcer margins.

However, there is a tendency for some practitioners to dismiss out of hand any suggestion that treatment with honey is worthy of consideration as a remedy in modern medicine. An editorial note assigned honey to the category of "worthless but harmless substances" (Soffer). In a study the effect on wound healing process of the topical application of honey in infected wound in an animal model was assessed (Gutiérrez-Vega *et al.*, 1995). Wister mice were used in which a block of tissue was excised, and were afterward infected with Escherichia coli. Once the wounds presented abscess collection, the animals were assigned to one of the groups of study: group I topical application of honey, group II use of hexachlorophene and group III topical application of honey and hexachlorophene. There was not difference among the groups of study with regard to the wound diameter, inflammatory response, formation of granulation tissue, epithelization and fibroblast population. Nevertheless the extra cellular matrix was more compact in the group I.

Pervious reports have shown usage of honey, focused on the treatment of shallow wounds such as from burn injury or superficial ulcers (Harris, 1994; Misirlioglu *et al*.; Subrahmanyam *et al.,* 2001; Tovey).

In this study we have shown that honey can be used in deeply wound and honey more than animal oil accelerates healing of full thickness wound of skin in mice. Three key ingredients in honey are responsible for its wound-healing capabilities:

I) Honey has high sugar content. Sugar absorbs moisture in wounds, making it difficult for bacteria to survive. Honey is mostly glucose and fructose. These sugars are strongly attracted to water, forming viscous syrup. When spread on a wound, honey absorbs water and body fluids, thus desiccating bacteria and fungi and inhibiting their growth.

II) Many kinds of honey are high in hydrogen peroxide, a common household disinfectant. Raw honey contains glucose oxidase, an enzyme that, in the presence of a little water, produces hydrogen peroxide.

III) Honey also contains bee pollen, enzymes and propolis, which can stimulate new tissue growth.

Honey can contain additional medicinal compounds, including essential oils, flavonoids, terpenes and polyphenols, depending on the plant from which the pollen was taken (Khristov & Mladenov, 1961).

We concluded that honey more than animal oil accelerates healing of full thickness wound of skin in mice. However, further clinical trial studies will need to be carried out on human wounds.

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GHADERI, R.; AFSHAR, M.; AKHBARIE, H. & GOLALIPOUR, M. J. Comparación de la eficacia de la miel y el aceite animal en la aceleración de cicatrización de la herida en todo el espesor de la piel de raton. ***Int. J. Morphol., 28(1)***:133-198, 2010.

RESUMEN: Existen algunos medicamentos y procedimientos que pueden ser utilizados para acelerar la cicatrización de las heridas de la piel. Algunos estudios han demostrado una mejora en la curación de heridas de quemaduras con el tratamiento de miel. Por otra parte, sobre la base de la medicina tradicional, se ha mejorado la curación de heridas con aceite animal. Este estudio se realizó para comparar la eficacia del aceite animal y la miel en la aceleración de la cicatrización de la herida en todo el espesor de la piel en ratones. En este estudio experimental 36 ratones NMRI machos fueron sometidos a heridas de piel de espesor total bajo anestesia general. Los animales fueron asignados aleatoriamente para recibir aplicaciones únicas diarias de placebo, aceite animal o miel (n=12 para cada grupo), respectivamente. En los días 4, 7 y 10, cuatro ratones de cada grupo fueron sacrificados mediante una sobredosis de anestesia. Las características macroscópicas y microscópicas de las heridas fueron estudiados patológica e histológicamente. Los resultados de este estudio mostraron que la formación de tejido de granulación, densidad y activación de fibroblastos, queratinización en la superficie de la herida, espesor de la membrana basal y la epidermis en el grupo de tratamiento con miel fue mayor que el grupo con aceite animal. La miel disminuyó más que el aceite animal la inflamación, edema y dehiscencia de la herida en los ratones. La tasa de cicatrización de la herida en el grupo con miel fue más alta que en el grupo de aceite animal ($p<0,05$). Este estudio demostró que la miel acelera más que el aceite animal la cicatrización de la herida en todo el espesor de la piel en ratones.

PALABRAS CLAVE: Miel; Aceite animal; Cicatrización de heridas; Piel; Ratón.

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