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ORIGINAL ARTICLE

Evaluation of the healing effect of honey and colostrum in treatment of cutaneous wound in rat

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Abstract Wound healing is a dynamic and complex process of replacing devitalized and missing cellular structures and tissue layers. Due to the importance of wound healing, this investigation was designed to compare the effect of treatment using honey and colostrum on wound-healing process in rats. In this study, 80 adult female Sprague Dawley rats were randomly divided into four groups as negative control group, groups II, III, and IV were treated by honey, bovine colostrum, and honey + colostrum, respectively. Rats were anesthetized, hair was removed from the back, and then *a wound* was made on the back. Visual observation, histopathological examination, and biomechanical study were performed on days 3, 7, 14, and 21 after operation. Wound area in rats which taken colostrum and honey was lower than other groups. Promotion of wound contraction and epithelialization in rats that were treated with honey and colostrum was better than the others. Biomechanical parameters in animals were treated by colostrum and honey significantly more than other rats. A combination of honey and colostrum on wounds can enhance healing better than honey and colostrum separately.

Keywords Honey · Colostrum · Wound healing · Histopathological · Biomechanical

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Introduction

Wound healing is an important issue. There are several stages in *wound-healing process* including inflammatory, angiogenesis, proliferative, and remodeling. All stages may vary in length due to nutritional deficiencies, aging, steroids, wound infections, hypoxia, edema, vitamin deficiencies, diabetes, and radiation. A variety of agents have been used topically to treat wounds for many years such as Aloe vera (Ghasemi et al. 2012), glycerol (Stout and McKessor 2012), zinc (Voicu et al. 2013), tocopherol (Cheng et al. 2013), ascorbic acid (Kamer et al. 2010), lantana (Lantana camara) (Reddy 2013), honey (Jenkins et al. 2013; Cooke et al. 2015), and colostrum (Kshirsagar et al. 2015).

Honey with a long tradition of use in wound healing for ulcers, burns, and acute or chronic wounds has many accounts of the use for treating wounds in ancient scripts from Greece, Egypt, and India. And also the Koran praises the virtues of honey (Mohammed et al. 2015). Different studies have mentioned the effect of honey and also its higher efficacy compared with new wound-healing materials (Tan et al. 2012; Yaghoobi and Kazerouni 2013). Several studies in animal models demonstrated that honey reduced healing time,

decreased scarring, and improved healing process (Anyanechi and Saheeb 2015).

Colostrum—the first mammary secretion—with different clinical applications like the treatment of bacterial and viral diarrhea in adult and children, arthritis, pneumonia, sinusitis, and allergic (Thapa 2005) and also as an immunity boosting supplement has many growth and repair factors with antiviral, antibacterial, and antiinflammatory properties. The growth factors of colostrum-like transforming growth factors (TGF α and β) (Noda et al. 1984), fibroblast growth factors (FGF), and platelet-derived growth factors (PDGF), and epidermal growth factor (EGF) (Sanjay and Ritura 2015) are very effective in promoting wound healing that directly affect on DNA and RNA. They help in repair of tissues like the skin, muscles, bone, and cartilages. The immune factors such as IgG, IgA, neutrophils, macrophages, lactoferrin, defensins, and cathelicidins present in colostrum make it suitable for application topically on wounds (Stelwagen et al. 2009). In view of this problem, this study was developed, whose main objective was the clinical assessment of the effect of treatment using honey and colostrum on wound-healing process in rats.

Materials and methods

Experimental procedure

A total of 80 adult female Sprague Dawley rats, weighing 200 ± 20 g were randomly divided into four groups of 20 animals in each. Rats in group I received no treatment and served as negative control group. Group II, positive control group, was treated with honey. Group III was treated with bovine colostrum while group IV with honey and colostrum. Animals were maintained under controlled conditions of temperature (23 ± 1 °C) and light cycle of 12 h light and 12 h darkness. They were fed standard rat diet and were given water ad libitum. Animals were used in accordance with the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (registration number: 1274).

Firstly, all rats were checked up which were clinically healthy. Animals in each group were gently held and anesthetized using a combination of intramuscular ketamine (100 mg/kg, Alfasan Co., Netherlands) and xylazine (8 mg/kg, Alfasan Co., Netherlands) before wound creation. Then, hair was removed from the back with clippers. A *circular wound* in *2-cm diameter* with a sterile surgical blade was made on the back of their neck. Then wounds simply were washed with saline before dressing. Operation was performed under clean conditions. The progressive changes in wound are monitored by a camera on days 3, 7, 14, and 21 after operation. The *wound dressing* completely in groups II and III was performed with honey (0.5–1 ml) and colostrum (0.5–1 ml) every day, respectively. In group IV, wound was dressed daily at morning and afternoon with honey (0.5–1 ml) and colostrum (0.5–1 ml), respectively. And after recovery, the rats were kept in their individual cages (25 × 25 × 40 cm^3) under suitable ventilation until the end of the study (21 days).

Histopathological examination

Histopathological study was performed on days 3, 7, 14, and 21 after operation using healing markers like re-epithelization, collagenation, predominant inflammatory cells, and neovascularization. After operation, five rats on each day were euthanized with Thiopental sodium (100 mg/kg, IP, Reza-Daru Pars Co. Iran). Then, sections (1 × 2 cm^2) from each rat were taken for histopathological study. Sections (1 × 2 cm^2) were immediately fixed with 10 % formalin solution, dehydrated with 90 % ethanol and embedded in paraffin. Then, they were cut into thin slices and stained with Haemotoxyline-Eosin (H&E) and observed under a light microscope. Pathologic changes of the wound were assessed and reported.

Biomechanical study

The method used has been described previously by Oryan and Moshiri (2011). After operation, five rats on days 14 and 21 were euthanized with Thiopental sodium (100 mg/kg) IP. The skin of the back, including the wounds, was shaved, excised (2 cm in length and 1 cm in width), and immediately transferred to a Petri dish of normal saline to prevent drying. Samples (five wounds from each group) were wrapped in normal saline, aluminum foils, and plastic bags and kept in −70 °C freezer until tensile testing. On the day of the biomechanical test, the samples were defrosted at room temperature then specimens were kept moistened by immersing in 20 °C normal saline. Then samples were attached to tensiometer holders (Tensiometry®, Co. Zovic, Germany). The following parameters were measured: yield strength (yield point) (kg), ultimate strength (kg), and stiffness (kg cm^2).

Statistical analysis

The data were analyzed using computerized statistical program (SPSS version 16.0). *Analysis* of pathological findings was done by *the Kruskal-Wallis H test. The differences between the groups were determined using the Mann-Whitney U test,* whereas data of wound area was analyzed using one way ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

Results

The wound photos and visual observations on different days showed that wound area decreased in a time-dependent

manner in four groups. Wound area in the negative control group was more than those of in other groups on all days of experiment. In those which were given colostrum, wound area was more than that of in positive control group on days 14 and 21. Also, wound area in rats, which taken colostrum and honey, was lower than that of in positive control group on days 14 and 21 and it showed significant decrease in wound area ($P < 0.05$) (Fig. 1). Improvement of wound in treatment groups on different days was illustrated at Fig. 2.

Histopathological evaluations

Day 3

The number of lymphocytes and macrophages in the negative control group was lower than that of in other groups. Epithelialization in the negative control group was not observed but granulation tissue, epithelialization, angiogenesis, and collagen formation were detected in other groups especially in the group that was treated with colostrum and honey.

Day 7 and 14

The number of lymphocytes and macrophages in the negative control group was more than that of in other groups. Granulation tissue and epithelialization and also numerous blood vessels, fibroblasts, and collagenous fibers were observed in the treated groups especially in the group that was treated with colostrum and honey. On day 14 compared with day 7, the number of inflammatory cells was lower. Furthermore, the promotion of wound contraction and epithelialization as well as acceleration of granulation tissue formation was observed on day 14 better than day 7.

Day 21

In the treatment groups, epithelialization had completed especially in those who received honey and colostrum. And the number of inflammatory cells was the least, and also, hair follicles were seen in closer of the surgical line. Furthermore, collagenous fibers were observed regular and tight. These observations in the negative control group were not as well as in the treatment groups. Tissue repair in the treatment groups was significantly more mature than that in the negative control group ($P < 0.05$). Compared to the treated animals, the lesions of the untreated ones showed numerous neutrophils and macrophages infiltration (Figs. 3, 4, and 5).

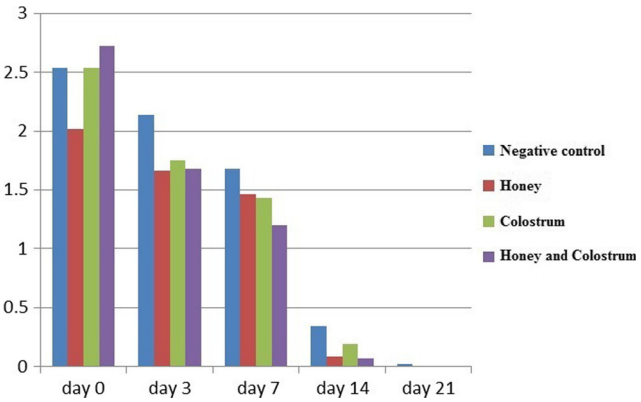

Fig. 1 Wound area for all groups on different days

Biomechanical study

The stiffness, yield point, and ultimate strength on day 21 in animals were treated by colostrum and honey significantly more than the negative control group ($P < 0.05$), which shows better biomechanical properties of the treated tissues and were more effective than the positive control group ($P < 0.05$).

Discussion

Honey and colostrum—two biological dressings—are studied by many researchers and also were used by ancient medicine in order to heal wound. But, today, their usages are transformed of an ancient remedy into modern therapy.

In this study, macroscopic evaluation and visual observations in treated groups showed that there were differences between wound areas on days of experiment in terms of contraction, color, and inflammation. Significant reduction in ulcer surface area in rats which taken a mix of honey and colostrum was observed. This finding was in agreement with Gauthier et al. (2006). and Sanjay and Ritura (2015) that they stated that colostrum dressings can be used as an adjunct in management of deep wound, and also, colostrum reduces the amount of discharge from wound and fastened the healing process. Because colostrum comprises a lot of growth factors which can reduce inflammatory cells and promote wound healing (Kshirsagar et al. 2015) and are important for healthy cell growth. Also, colostrum is a mediator of fibrosis and angiogenesis that helps in the healing of blood vessels. Colostrum due to epithelial (skin) growth hormone stimulates accelerated healing and powerful anti-inflammatory agents that can eliminate swelling and pain. Our study also is similar to Kshirsagar et al. (2015) that reported colostrum as very impressive at increasing healing of wounds when it is used topically to the injured area.

Sazegar et al. (2011) reported a considerable increase in the collagen fibers on wound healing in rats which were treated by honey that confirms our results. The numbers of fibroblasts during healing process increased. In the current study, on the first days post-operation, there were a large number of lymphocytes and tissue macrophages in the wound area but in the rest of healing and parallel by healing improvement, their

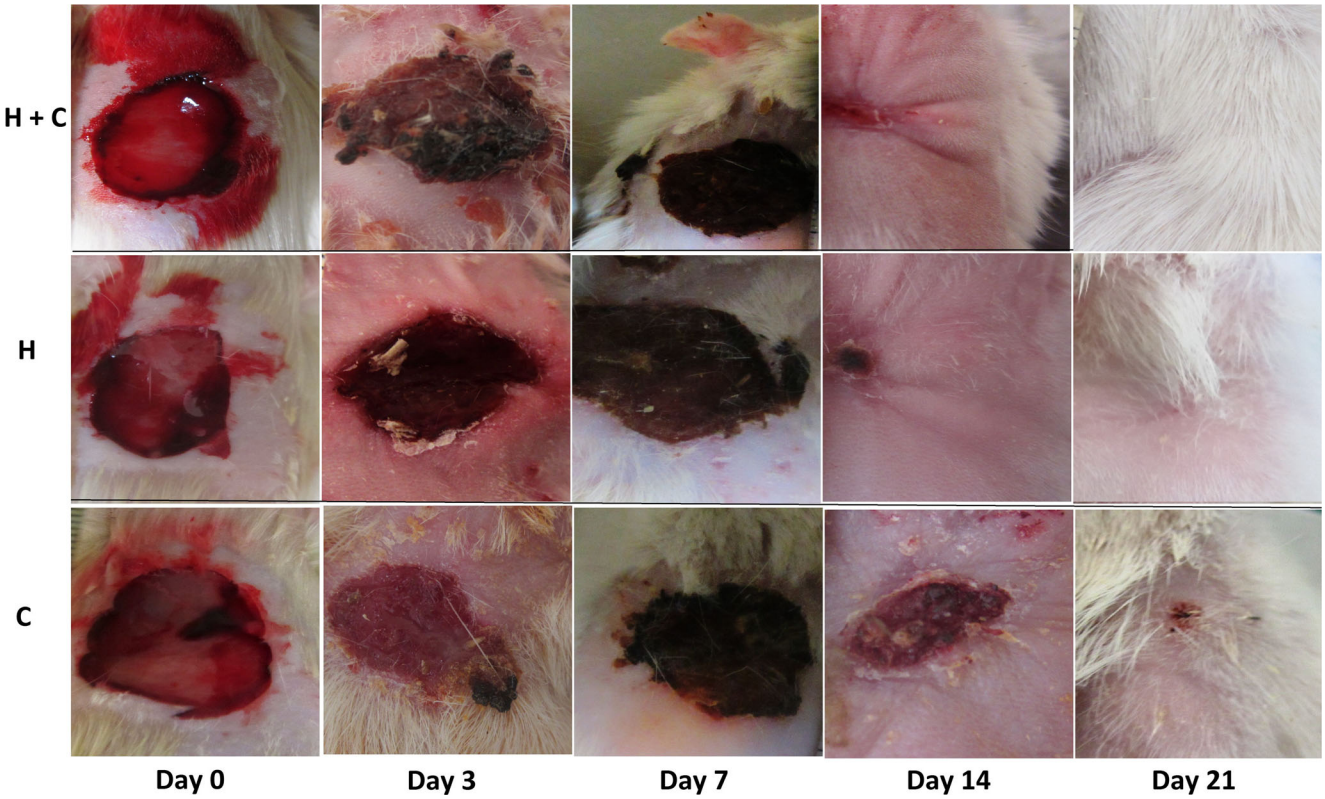

Fig. 2 Photographs of rats show various phases of wound healing in treatment groups. *H + C* honey and colostrum, *H* honey, *C* colostrum

numbers gradually decreased. On day 21, after operation, lymphocytes and tissue macrophages were absent. Macrophages by stimulating fibroplasias, collagen synthesis, and angiogenesis affect wound healing, while new collagen fibers, since operation day to end day, and closed wound became more and more, collagen fibers became more regulated, increased cross–linking and the fiber length, and improved fiber thickness. The collagen level was significantly higher in rats which were treated by mix of honey and colostrum on days 14 and 21. Honey contains a large number of biologically active peptides, including melittin, apamin, and adolapin, that increase membrane permeability which lead to the production of growth factors like VEGF and TGF-β1 and also collagen formation (Shin et al. 2013). Alizadeh et al. (2011) reported

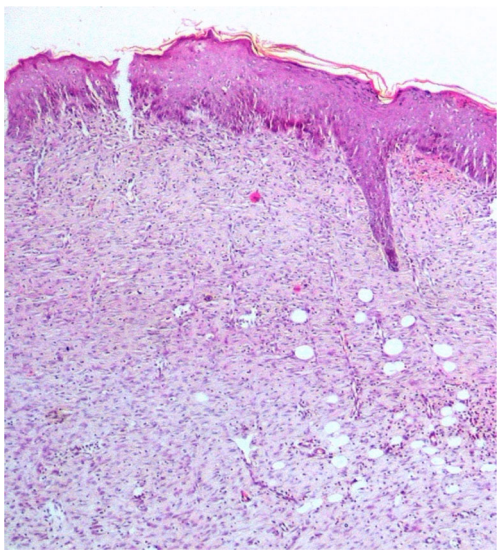

Fig. 3 Wound treated with honey and colostrum 21 days after operation. Epithelization had completed and third level epithelization is seen (H&E, ×400)

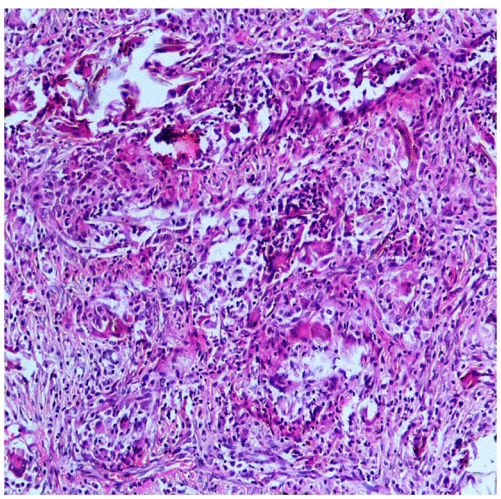

Fig. 4 Wound treated with honey and colostrum 21 days after operation. The fibroblast and collagen fibers with angiogenesis are present (H&E, ×400)

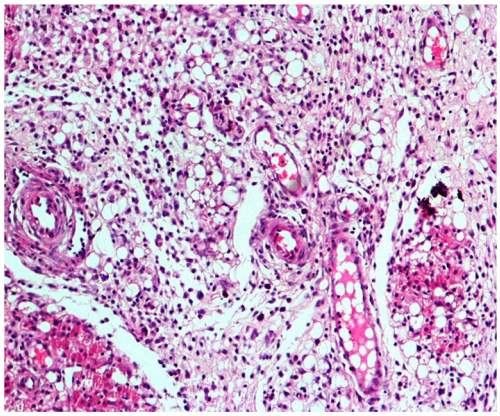

Fig. 5 Wound treated with honey and colostrum 21 days after operation. The tissue is well vascularized and also inflammatory cells are still present in the tissue (H&E, ×400)

that tissues treated with honey revealed relative epithelial proliferation and improved angiogenesis, which confirms our findings. Also, Thapa (2005) reported that colostrum due to its growth factors stimulates the repair and regeneration of collagen. And also, Esmaeili et al. (2014) showed that colostrum accelerates collagen formation and increases the number of fibroblasts.

Visual observations that showed wound areas in rats which taken a combination of honey and colostrum were significantly decreased especially on 21 days after operation. The evidences for the effectiveness of honey and colostrum in wound healing are in numerous reports published on case studies, animal studies, and randomized controlled trials. The healing effect of honey could be due to various physical and chemical properties such as low water content, the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey, acidity, and high sugar content (Kwakman and Zaat 2012; Mahendran and Kumarasamy 2015) that provide unsuitable place to prevent the growth of microorganisms so that it help to wound healing. Colostrum due to its factors like TGF and EGF accelerates wound healing and decreases wound area. These results are similar to Kshirsagar et al.(2015) that used colostrum in patients with deep wound and finally reported it promoted wound healing and reduced size of ulcer.

Histological evaluation especially in group IV revealed the inflammatory phase during the first 3 days after incision. Blood cells and a fibrin network filled the incisional space, creating a scaffold for migrating fibroblasts, and were increased in number near the incisional space. Proliferation of fibroblasts and new endothelial cells was found. On the superficial part of the dermis, necrosis was observed as a consequence of mechanical damage.

On the last experimental days, especially near day 21, epithelialization became completed, wound closed, and hair follicle was observed. These changes were observed in group IV more than the others. Honey dressing as compared to colostrum dressing is very effective in wound healing with faster coagulation, increasing angiogenesis, high anti-inflammatory, antimicrobial (due to its low water activity of 0.6) (Dluya 2015) and antioxidant activities (Molan 2011), increment *collagen* production, quicker fibroblast growth, high osmotic gradient (Mohammed et al. 2015), better epithelization, contraction, and remodeling. Our results are in agreement with Allen et al. (2000) that reported honey is suitable for wound dressing and useful in clearing wounds from infection. And also, the current study is similar with Oryan and Zaker (1998) that mentioned that honey accelerates the healing processes and appears to have an important property that makes it ideal as a dressing for skin wounds. Base on our finding, the presence of inflammatory cells on days 3 and 7 and their absence on days 14 and 21 in treated lesions in comparison to untreated ones that contained numerous inflammatory cells even up to the end of the experiment proves the antibacterial activity of honey. The present findings are in concordance with previous investigations as Vandamme et al. (2013), Hampton et al. (2011), and Tan et al. (2009). Additionally, this investigation revealed that honey accelerates wound healing due to its components like the vitamins bioten, nicotinic acid, folic acid, pantothenic acid, pyridoxin, and thiamine which in agreement with previous reports (Nisbet et al. 2010; Jull et al. 2013; Vandamme et al. 2013). Also, colostrum contains growth factors; vitamins like A, E, and C; minerals; and amino acids that make it useful for wound dressing. Our findings are in agreement with Torre et al. (2006) that used bovine colostrum to heal abdominal skin wound in dog; reported colostrum promotes the cellular growth and stimulates dog fibroblasts. So, colostrum helps wound to be closed due to its insulin-like growth factor 1 (IGF-1) that stimulates proliferation and epithelialization (Ginjala and Pakkanen 1998).

In this study, tensile strength of wound treated with honey and colostrum was highest especially on day 21 due to the potency of honey and colostrum to stimulate collagen formation (Thapa 2005; Takayama et al. 2001) that is the most important factor in tensile strength of wound. This finding is similar with Alizadeh et al. (2011) that mentioned that honey promoted wound contraction, closure time, and tensile strength. Also, our results are in accordance with the findings of Sazegar et al. (2011).

Conclusions

The present study demonstrated that local use of a combination of honey and colostrum on wounds can enhance healing with regards to their tensile strength property. Their anti-inflammatory property decreases exudates and reduces scar. They also stimulate the growth of granulation tissue,

neoangiogenesis, and epithelialization so that healing is accelerated. And also, they are easy to use, provides good pain relief, nonimmunogenic, and protect wound from infection.

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Compliance with ethiccal standard

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed and approved by the Animal Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran and administered by the National Animal Ethics Advisory Committee.

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