

## Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article

doi: 10.12980/jclm.4.2016J6-93

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## A comparison of the effects of honey, fish oil and their combination on wound healing in rat

Nader Tanideh<sup>1,2</sup>, Ehsan Abdordideh<sup>3</sup>, Seyedeh Leili Asadi Yousefabad<sup>4</sup>, Sajad Daneshi<sup>4\*</sup>, Omid Koohi Hosseinabadi<sup>5</sup>, Soleiman Mohamadi Samani<sup>6</sup><sup>1</sup>Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran<sup>2</sup>Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran<sup>3</sup>Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran<sup>4</sup>Young Researchers and Elite Club, Yasooj Branch, Islamic Azad University, Yasooj, Iran<sup>5</sup>Laboratory Animals Center, Shiraz University of Medical Sciences, Shiraz, Iran<sup>6</sup>Center for Nanotechnology in Drug Delivery, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

## ARTICLE INFO

## Article history:

Received 16 May 2016

Received in revised form 1 Jun 2016

Accepted 13 Jul 2016

Available online 1 Aug 2016

## Keywords:

Honey

Fish oil

Wound healing

Histopathological

Biomechanical

## ABSTRACT

**Objective:** To evaluate and compare the effects of honey and fish oil on wound healing in rat.**Methods:** A total of 80 adult female Sprague-Dawley rats were randomly divided into four groups as negative control group, Groups II, III and IV. The rats in the four groups were treated with honey, fish oil and honey + fish oil, respectively. Rats were anesthetized, hair was removed from the back, 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. The data were analyzed using One-way ANOVA.**Results:** Wound area in group IV was lower than that in other groups. Promotion of wound contraction and epithelialization in group IV was better than that in other groups. Biomechanical parameters in group IV was significantly more than other rats.**Conclusions:** A combination of honey and fish oil on wounds can enhance healing better than honey and fish oil separately.

## 1. Introduction

Wound healing is an important health problem by re-establishing the physical integrity of tissues in the outside and inside of the body's structures with several stages like inflammation, angiogenesis, proliferation, and remodeling, which involves complex relationships between cells and different factors. A variety of agents have been used topically to treat wounds for many years such as *Aloe vera*[1], glycerol[2], zinc[3], tocopherol[4], ascorbic acid[5], *Lantana (Lantana camara)*[6], honey[7,8] and fish oil[9].

Honey with a long tradition of use in wound healing for ulcers, burns and acute or chronic wounds has many records of use for

\*Corresponding author: Sajad Daneshi, Young Researchers and Elite Club, Yasooj Branch, Islamic Azad University, P.O. Box 75917-34715, Moalem St., Shahed 22 Avenue, Yasooj, Iran.

Tel: +98 713 2352757

Fax: +98 713 2286940

E-mail: sa.daneshi@yahoo.com

Foundation Project: Supported by Shiraz University of Medical Sciences (Grant No. 1274).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

treating wounds in ancient scripts from Greece, Egypt and India. Also the Koran praises the virtues of honey[10]. Different studies have mentioned the effect of honey and its higher efficacy compared with new wound healing materials[11,12]. Several studies in animal models demonstrated that honey reduced healing time, decreased scarring and improved healing process[13].

Fish oil is the main source of omega-3 which composes of long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids that have biomedical importance. Fish oil, due to the presence of an antioxidant agent - vitamin E, confers important protection against lipid peroxidation. Fish oil is rich in arachidonic acid that acts as inflammatory mediator, stimulates vessel dilation, induces platelet aggregation, and accelerates wound healing[14]. Omega-3 is an important component promoting cell integrity, development, maintenance and function; reducing arterial stiffness and cardiovascular mortality[9], and also has significant healing effects[15]. Omega-3 fatty acids inhibited arachidonic acid synthesis and incorporation into phospholipids, decreased platelet production of thromboxane A, a potent vasoconstrictor and inducer of platelet

aggregation, significantly reduced the severity of dermatitis and increased production by platelets of thromboxane A. The health benefits of EPA and DHA have been related primarily to their anti-inflammatory properties[16,17]. EPA is used for synthesis of prostaglandin I, a potent vasodilator and inhibitor of platelet aggregation[18].

The main objective of this study was the clinical assessment of the effect of honey and fish oil on wound healing process in rats.

## 2. Materials and methods

### 2.1. Animals

A total of 80 adult female Sprague-Dawley rats, weighing ( $200 \pm 20$ ) g were randomly divided into four groups of twenty 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 fish oil, while Group IV with honey and fish oil. Animals were maintained under controlled conditions of temperature ( $23 \pm 1$ ) °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).

### 2.2. Experimental procedure

Firstly, all rats were checked to make sure they were clinically healthy. Animals in each group were gently held, and anesthetized through intramuscular injection of a combination of 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 was made on the back of their necks with a sterile surgical blade. 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 in Groups II and III was completely performed with honey (0.5–1.0 mL) and fish oil (0.5–1.0 mL) every day, respectively. In Group IV, wound was dressed daily at morning and afternoon with honey (0.5–1.0 mL) and fish oil (0.5–1.0 mL), respectively. And after recovery, the rats were kept in their individual cages ( $25 \times 25 \times 40$  cm<sup>3</sup>) under suitable ventilation until the end of the study (21 days).

### 2.3. 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, *i.p.*, Reza-Daru Pars Co. Iran). Then sections ( $1 \times 2$  cm<sup>2</sup>) from each rat were taken for histopathological study. Sections ( $1 \times 2$  cm<sup>2</sup>) 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 hematoxylin-eosin (H&E) and observed under a light microscope. Pathologic changes of the wound were assessed and reported.

### 2.4. Biomechanical study

The method used has been described previously by Oryan and Moshiri[19]. After operation, five rats on days 14 and 21 were euthanized with thiopental sodium (100 mg/kg, *i.p.*). 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 (5 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. Samples were then attached to tensiometer holders (Tensimetry®, Co. Zovic, Germany). The following parameters were measured: yield strength (yield point) (kg), ultimate strength (kg) and stiffness (kg.cm<sup>2</sup>).

### 2.5. 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 were analyzed using One-way ANOVA. A value of  $P < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Visual observation of wound areas

The wound photos and visual observations on different days showed that wound area decreased in a time-dependent manner in the four groups. Wound area in the negative control group was larger than those in other groups on all days of experiment. In those which were given fish oil, wound area was larger than that in positive control group on days 14 and 21. Also wound area in rats which were taken fish oil and honey was smaller than that in positive control group on days 14 and 21 and it showed significant decrease in wound area ( $P < 0.05$ ) (Figure 1). Improvement of wound in treatment groups on different days was illustrated in Figure 2.

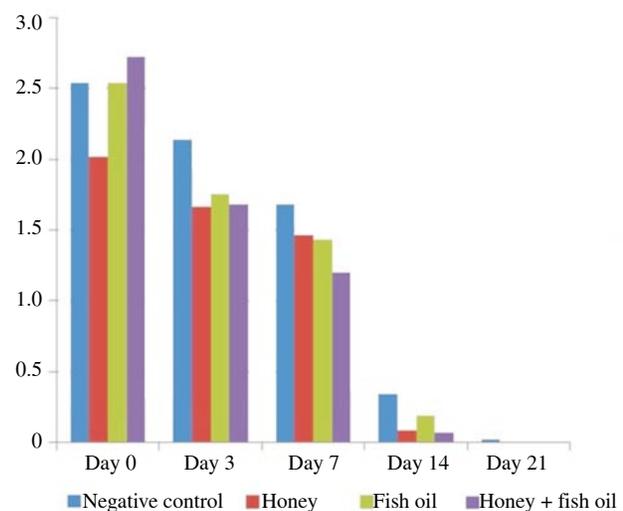
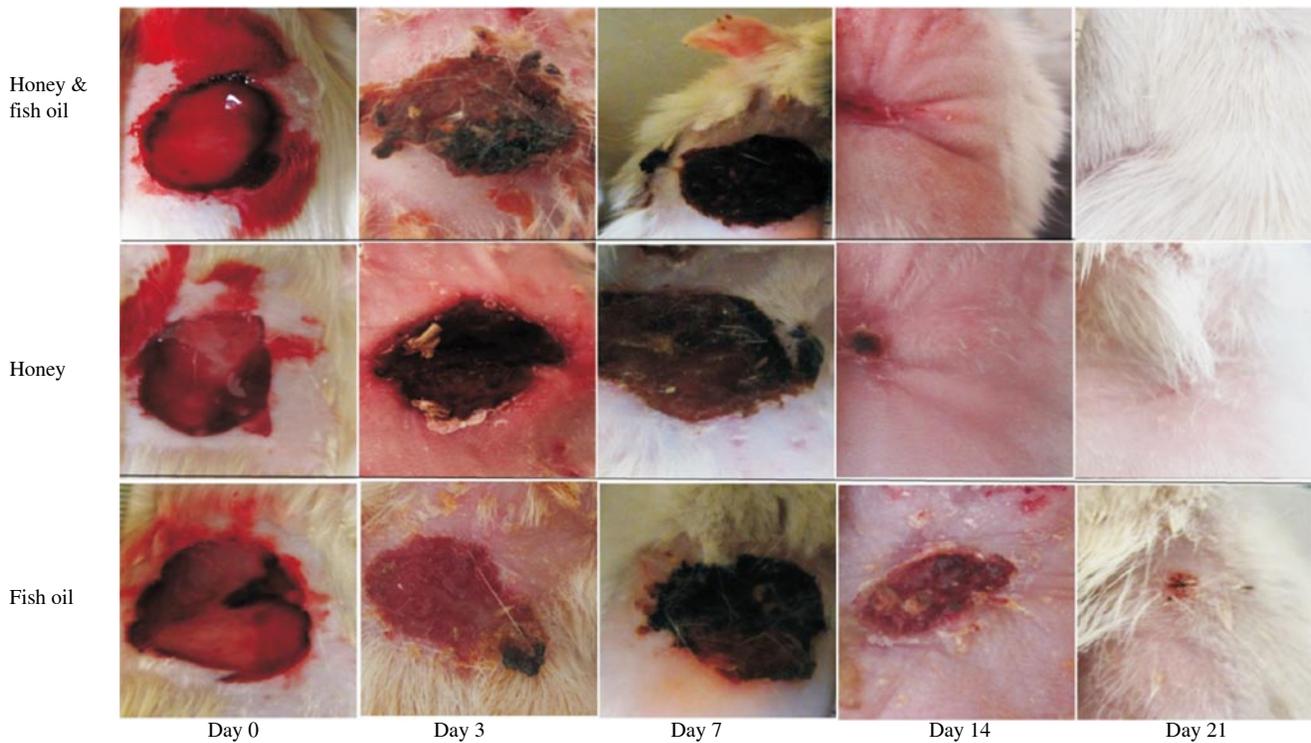


Figure 1. Wound area for all groups on different days.



**Figure 2.** Photographs of rats show various phases of wound healing in treatment groups.

### 3.2. Histopathological evaluations

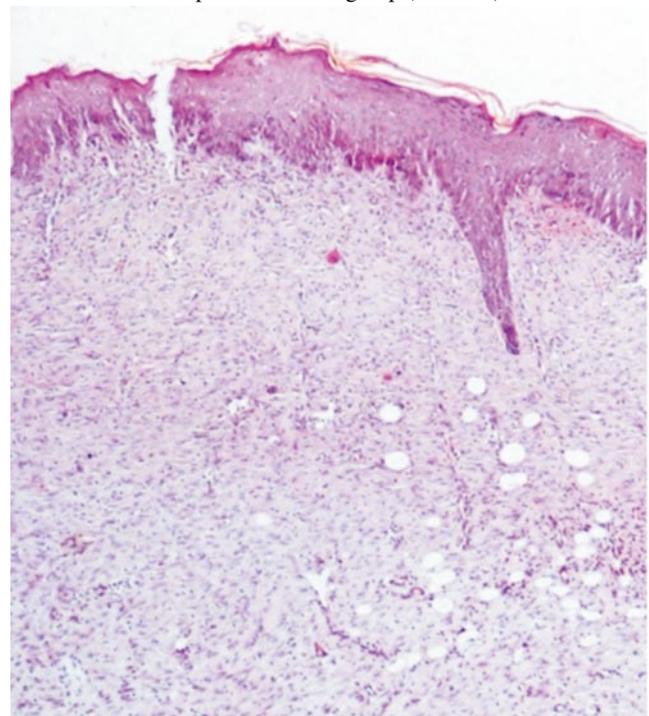
On day 3, the number of lymphocytes and macrophages in the negative control group was less than that in other groups. Epithelialization in the negative control group was not observed but granulation tissue, epithelialization, angiogenesis and collagen formation were detected in other groups especially in the group treated with fish oil and honey.

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

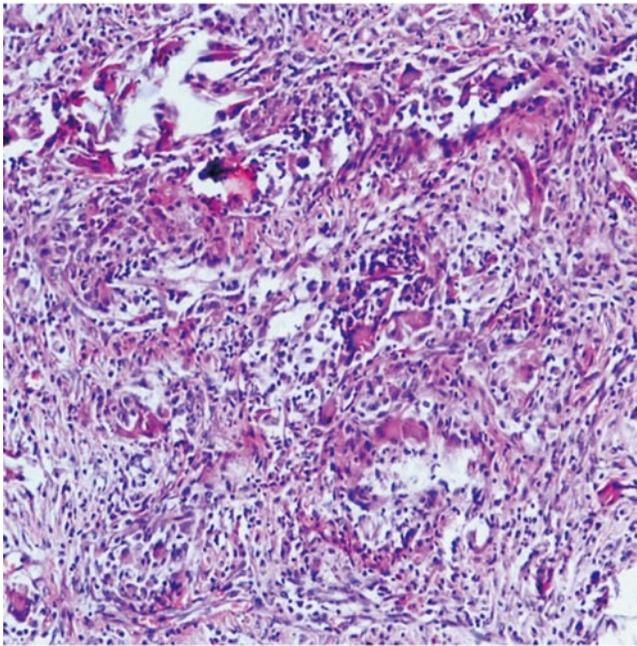
On day 21, in the treatment groups epithelialization had completed, especially in those received honey and fish oil. The number of inflammatory cells was the least and hair follicles were seen in the closer of surgical line. Furthermore, collagenous fibers were observed regular and tight. These observations in negative control group were not as well as those in the treatment groups. Tissue repair in treatment groups was significantly maturer than that in negative control group ( $P < 0.05$ ). The wound healing status of the treated animals 21 days after operation are shown in Figures 3–5.

### 3.3. Biomechanical study

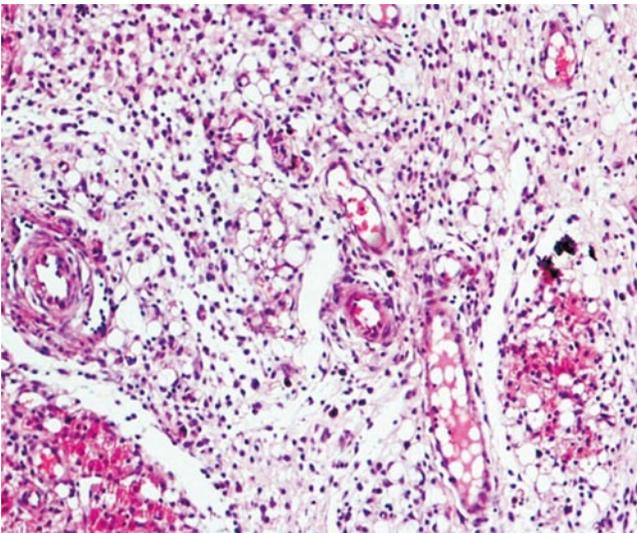
The stiffness, yield point and ultimate strength on day 21 in animals treated with fish oil and honey were significantly higher than those in the negative control group ( $P < 0.05$ ), which showed better biomechanical properties of the treated tissues. Also were more effective than positive control group ( $P < 0.05$ ).



**Figure 3.** Wound treated with honey and fish oil 21 days after operation. Epithelialization had completed and the third level of epithelialization is seen (H&E, 400 $\times$ ).



**Figure 4.** Wound treated with honey and fish oil 21 days after operation. The fibroblast and collagen fibers with angiogenesis are present (H&E, 400 $\times$ ).



**Figure 5.** Wound treated with honey and fish oil 21 days after operation. Well-vascularized tissue and inflammatory cells can be seen (H&E, 400 $\times$ ).

#### 4. Discussion

Honey and fish oil are studied by many researchers and were used in ancient medicine for wound healing. But today they are transformed from an ancient remedy to a modern therapy.

In this study, macroscopic evaluation and visual observation 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 fish oil was observed. This finding was in agreement with Gogus and Smith who stated that fish oil dressings can reduce wound area and accelerate the healing process[15]. Fish oil comprises a lot of growth factors which can reduce inflammatory cells[14], promote wound healing and are

important for healthy cell growth[20]. Also fish oil is a mediator of angiogenesis that helps in the healing of blood vessels[21]. Our results are in accordance with findings of Baie and Sheikh[22] that reported fish oil is very impressive at increasing healing of wounds when it is used topically to the injured area.

Falih *et al.*[23] and Sazegar *et al.*[24] reported a considerable increase in the collagen fibers in the wound healing process in rats treated by honey, which confirms our results. The number of fibroblasts during healing process increased. In the current study, on the first day post operation there were a large number of lymphocytes and tissue macrophages in wound area but in the process of healing their number was 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 the operation day to the last day and closed wound became more and more and also collagen fibers became more regulated, increased cross-linking and the fibers length and improved fiber thickness. The collagen level was significantly higher in rats treated by the mix of honey and fish oil on days 14 and 21. Honey contains a large number of biologically active peptides, including melittin, apamin and adolapin that increase membrane permeability, thereby leading to production of growth factors like vascular endothelial growth factor and transforming growth factor  $\beta$ 1 and also collagen formation[25]. Alizadeh *et al.*[26] reported that tissues treated by honey revealed relative epithelial proliferation and improved angiogenesis, which confirms our findings. Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Fish oil is rich in glycine and proline, so fish oil stimulates the repair and regeneration of collagen[27]. Gercek *et al.*[28] reported that fish oil accelerates collagen formation. Arnold and Barbul[29] stated that fish oil improves the systemic immune function and thus reducing infectious complications due to its rich omega-3 fatty acids contents. Fish oil enhances epithelization and neovascularization in wound healing[30].

Visual observations showed that wound area in rats taken a combination of honey and fish oil was significantly decreased especially on 21 days after operation. The evidences for the effectiveness of honey and fish oil in wound healing are in numerous published reports 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[31,32], which provide a unsuitable place to prevent the growth of micro-organisms so that it helps to wound healing.

Histological evaluation, especially on group four revealed the inflammatory phase during the first three days after incision. Blood cells and a fibrin network filled the incisional space, creating a scaffold for migrating fibroblasts and the number was increased 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 more apparent in group four than those in other groups. Honey dressing as compared to fish oil dressing is very effective in wound healing with faster coagulation, increasing angiogenesis, high anti-inflammatory activity, antimicrobial activity (due to its low water activity of 0.6)[33], antioxidant activity[34], increment in collagen production, quicker fibroblasts growth, high osmotic gradient[10], better epithelization, contraction and remodeling. Our results are in agreement with Allen *et al.*[35] who reported that honey is suitable for wound dressing and useful in treating infection of wounds. Also current study is similar with the study of Oryan and Zaker[36] which mentioned that honey accelerates the healing processes and appears to have an important property that makes it ideal as a dressing for skin wounds. Based 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 of Vandamme *et al.*[37], Hampton *et al.*[38] and Tan *et al.*[11]. Additionally, this investigation revealed that honey accelerates wound healing due to its components like the vitamins, nicotinic acid, folic acid, pantothenic acid, pyridoxine, and thiamine, which is in agreement with previous reports of Nisbet *et al.*[39], Alizadeh *et al.*[26], Jull *et al.*[40] and Vandamme *et al.*[37].

Fish oil plays an important role to improve wound healing due to its properties such as increasing monocyte survival[20], collagen production[41], inflammatory effect[42], effects on leucocyte functions like chemotactic response, phagocytosis, and cytokine production[14], prostaglandin E2 reduction[43], inhibiting platelet aggregation and increasing blood flow[44], increasing cell proliferation (re-epithelialization)[45], improving the secretion of growth hormone, accelerating epithelization and angiogenesis, containing vitamin A that improves wound healing, encourages skin growth and skin strength, and possessing vitamin E that act as an antioxidant.

In this study, tensile strength of wound treated with honey and fish oil was highest especially on day 21 due to the potency of honey and fish oil to stimulate collagen formation which is the most important factor in tensile strength of wound. This finding is similar with that of Alizadeh *et al.*[26] who mentioned that honey promoted wound contraction, closure time and tensile strength. Also our results are in accordance with the findings of Sazegar *et al.*[24].

In conclusion, the present study demonstrated that local use of a combination of honey and fish oil 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. Also they are easy to use, non immunogenic, provides good pain relief and can protect wound from infection.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

The authors would like to thank Shiraz University of Medical Sciences for financial support as this paper is part of Grant No. 1274 of the university.

### References

- [1] Ghasemi S, Emami M, Maleki M, Fathi B. Histopathologic evaluation of curative impact of *Aloe vera* L. fresh gel on healing of experimental infected full-thickness open wounds induced with *Staphylococcus aureus* in dogs. *Iran J Vet Surg* 2009; **4**(1): 103-14.
- [2] Stout EI, McKessor A. Glycerin-based hydrogel for infection control. *Adv Wound Care (New Rochelle)* 2012; **1**(1): 48-51.
- [3] Voicu G, Oprea O, Vasile BS, Andronescu E. Antibacterial activity of zinc oxide-gentamicin hybrid material. *Dig J Nanomater Biostruct* 2013; **8**(3): 1191-203.
- [4] Cheng PG, Phan CW, Sabaratnam V, Abdullah N, Abdulla MA, Kuppasamy UR. Polysaccharides-rich extract of *Ganoderma lucidum* (MA Curtis: Fr.) P. Karst accelerates wound healing in streptozotocin-induced diabetic rats. *Evid Based Complement Alternat Med* 2013; **2013**(2013): 671252.
- [5] Kamer E, Recai Unalp H, Gundogan O, Diniz G, Ortac R, Olukman M, et al. Effect of ascorbic acid on incisional wound healing in streptozotocin-induced diabetic rats. *Wounds* 2010; **22**(2): 27-31.
- [6] Reddy NM. *Lantana camara* Linn. chemical constituents and medicinal properties: a review. *Scholars Acad J Pharm* 2013; **2**: 445-8.
- [7] Cooke J, Dryden M, Patton T, Brennan J, Barrett J. The antimicrobial activity of prototype modified honeys that generate reactive oxygen species (ROS) hydrogen peroxide. *BMC Res Notes* 2015; **8**(1): 20.
- [8] Jenkins R, Burton N, Cooper R. Proteomic and genomic analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) exposed to manuka honey *in vitro* demonstrated down-regulation of virulence markers. *J Antimicrob Chemother* 2014; **69**: 603-15.
- [9] Alexander JW, Supp DM. Role of arginine and omega-3 fatty acids in wound healing and infection. *Adv Wound Care (New Rochelle)* 2014; **3**(11): 682-90.
- [10] Mohammed H, Salama MA, El Lenjawi B, Hassan I. Using honey in post-excision malignant melanoma ulcers. *Diabet Foot J Middle East* 2015; **1**(1): 6-9.
- [11] Tan MK, Hasan Adli DS, Tumiran MA, Abdulla MA, Yusoff KM. The efficacy of Gelam honey dressing towards excisional wound healing. *Evid Based Complement Alternat Med* 2012; **2012**(2012): 805932.
- [12] Yaghoobi R, Kazerouni A, Kazerouni O. Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-

- oxidant and anti-viral agent: a review. *Jundishapur J Nat Pharm Prod* 2013; **8**(3): 100-4.
- [13] Anyanechi CE, Saheeb BD. Honey and wound dehiscence: a study of surgical wounds in the mandibular bed. *Niger J Clin Pract* 2015; **18**(2): 251-5.
- [14] Patterson WL 3rd, Georgel PT. Breaking the cycle: the role of omega-3 polyunsaturated fatty acids in inflammation-driven cancers. *Biochem Cell Biol* 2014; **92**(5): 321-8.
- [15] Gogus U, Smith C. n-3 Omega fatty acids: a review of current knowledge. *Int J Food Sci Technol* 2010; **45**(3): 417-36.
- [16] Bazan NG, Molina MF, Gordon WC. Docosaehaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. *Annu Rev Nutr* 2011; **31**: 321-51.
- [17] Han YY, Lai SL, Ko WJ, Chou CH, Lai HS. Effects of fish oil on inflammatory modulation in arginine and fish oil: wound healing and infection 689 surgical intensive care unit patients. *Nutr Clin Pract* 2012; **27**: 91-8.
- [18] El-Sayyad HIH, Abdraboh ME, Aljebali AMA. Positive impact of fish oil on diabetic and hypercholesterolemic skin disorders. *J Nutr Health* 2015; **1**(2): 8.
- [19] Oryan A, Moshiri A. A long term study on the role of exogenous human recombinant basic fibroblast growth factor on the superficial digital flexor tendon healing in rabbits. *J Musculoskelet Neuronal Interact* 2011; **11**(2): 185-95.
- [20] Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Adv Nutr* 2012; **3**(1): 1-7.
- [21] Shingel KI, Faure MP, Azoulay L, Roberge C, Deckelbaum RJ. Solid emulsion gel as a vehicle for delivery of polyunsaturated fatty acids: implications for tissue repair, dermal angiogenesis and wound healing. *J Tissue Eng Regen Med* 2008; **2**(7): 383-93.
- [22] Baie SH, Sheikh KA. The wound healing properties of *Channa striatus*-cetrimide cream--tensile strength measurement. *J Ethnopharmacol* 2000; **71**(1): 93-100.
- [23] Falih AB, Al-Byatee AJ, Al-khazraji KI. Therapeutic use of honey for skin wounds healing in Iraqi Awassi sheep. *Kufa J Vet Med Sci* 2012; **3**(2): 17-22.
- [24] Sazegar G, Seyed Reza AH, Behravan E. The effects of supplemental zinc and honey on wound healing in rats. *Iran J Basic Med Sci* 2011; **14**(4): 391-8.
- [25] Shin JM, Jeong YJ, Cho HJ, Park KK, Chung IK, Lee IK, et al. Melittin suppresses HIF-1 $\alpha$ /VEGF expression through inhibition of ERK and mTOR/p70S6K pathway in human cervical carcinoma cells. *PLoS One* 2013; **8**(7): e69380.
- [26] Alizadeh AM, Sohanaki H, Khaniki M, Mohaghheghi MA, Ghmami G, Mosavi M. The effect of *Teucrium polium* honey on the wound healing and tensile strength in rat. *Iran J Basic Med Sci* 2011; **14**(6): 499-505.
- [27] Hananeh W, Ismail ZB, Alshehabat MA, Ali J. Review of animal models used to study effects of bee products on wound healing: findings and applications. *Bull Vet Inst Pulawy* 2015; **59**(3): 425-31.
- [28] Gercek A, Yildirim O, Konya D, Bozkurt S, Ozgen S, Kilic T, et al. Effects of parenteral fish-oil emulsion (Omegaven) on cutaneous wound healing in rats treated with dexamethasone. *JPEN J Parenter Enteral Nutr* 2007; **31**(3): 161-6.
- [29] Arnold M, Barbul A. Nutrition and wound healing. *Plast Reconstr Surg* 2006; **117**(7 Suppl): 42S-58S.
- [30] Mahmoud Ali M, Radad K. Cod liver oil/honey mixture: an effective treatment of equine complicated lower leg wounds. *Vet World* 2011; **4**(7): 304-10.
- [31] Kwakman PHS, Zaat SAJ. Antibacterial components of honey. *IUBMB Life* 2012; **64**(1): 48-55.
- [32] Mahendran S, Kumarasamy D. Antimicrobial activity of some honey samples against pathogenic bacteria. *Int Lett Nat Sci* 2015; **34**: 15-20.
- [33] Dluva T. Critical review: antioxidant properties and antibiotic mechanism of honey against infectious diseases. *Int J Adv Life Sci Technol* 2015; **2**: 16-24.
- [34] Bischofberger AS, Dart CM, Perkins NR, Kelly A, Jeffcott L, Dart AJ. The effect of short- and long-term treatment with manuka honey on second intention healing of contaminated and noncontaminated wounds on the distal aspect of the forelimbs in horses. *Vet Surg* 2013; **42**: 154-60.
- [35] Allen KL, Hutchinson G, Molan PC. The potential for using honey to treat wounds infected with MRSA and VRE. In: FirstWorld Wound Healing Congress; 2000 Sep 10-13; Melbourne, Australia.
- [36] Oryan A, Zaker SR. Effects of topical application of honey on cutaneous wound healing in rabbits. *Zentralbl Veterinarmed A* 1998; **45**(3): 181-8.
- [37] Vandamme L, Heyneman A, Hoeksema H, Verbelen J, Monstrey S. Honey in modern wound care: a systematic review. *Burns* 2013; **39**(8): 1514-25.
- [38] Hampton S, Coulborn A, Tadej M, Bree-Aslan C. Using a superabsorbent dressing and antimicrobial for a venous ulcer. *Br J Nurs* 2011; **20**: S38, S40-3.
- [39] Nisbet HO, Nisbet C, Yarim M, Guler A, Ozak A. Effects of three types of honey on cutaneous wound healing. *Wounds* 2010; **22**(11): 275-83.
- [40] Jull AB, Rodgers A, Walker N. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 2008; (4): CD005083.
- [41] Hayakawa S, Yoshikawa D, Ishii H, Tanaka M, Kumagai S, Matsumoto M, et al. Association of plasma omega-3 to omega-6 polyunsaturated fatty acid ratio with complexity of coronary artery lesion. *Intern Med* 2012; **51**(9): 1009-14.
- [42] Melo RB, Guimarães SB, Silva PG, Oriá RB, Melo JU, Vasconcelos PR. Antiperoxidative properties of oil mixes of high ratio omega-9:omega-6 and low ratio omega-6:omega-3 after molar extraction in rats. *Acta Cir Bras* 2014; **29**(6): 371-5.
- [43] Niemoller TD, Bazan NG. Docosaehaenoic acid neurolipidomics. *Prostaglandins Other Lipid Mediat* 2010; **91**(3-4): 85-9.
- [44] Waite N, Lodge J, Robertson M, Badley E, Burton S, Hart K. Do fish-oil supplements affect insulin sensitivity? A pilot intervention study. *J Hum Nutr Diet* 2008; **21**(3): 288-9.
- [45] Campelo AP, Campelo MW, Brito GA, Jamacaru FV, Leitão RF, Vasconcelos PR. Oil mixes omega 9, 6 and 3, enriched with seaweed, promoted reduction of thermal burned modulating NF-kB and Ki-67. *Acta Cir Bras* 2015; **30**(6): 430-8.