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Histological assessment of topical mixture of *Thymus vulgaris* honey and *Nigella sativa L* seed powder on skin wound healing in sheep

Deffa Ouafa[1, 2,*], Daikh Badis[1, 3]

[1]*Department of Biology of Organisms, University of Batna 2, Batna, Algeria*

[2]*Laboratory of Biology and Environment, Faculty of Nature and Life Sciences, University of Mentouri Brothers, Constantine, Algeria. 3. Biotechnology's Laboratory of the Bioactive Molecules and the Cellular Physiopathology, University of Batna2, Batna, Algeria*

[3]*Biotechnology's Laboratory of the Bioactive Molecules and the Cellular Physiopathology, University of Batna2, Batna, Algeria*

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Abstract

Honey and *Nigella sativa L* has been known for millennia of years for its therapeutic healing properties. This present histopathological study aims to evaluate the healing activity of Algerian bee honey derived from the nectar of *Thymus vulgaris* mixed with *N. sativa* seed powder on a sheep model. Induction of wounds with longitudinal incisions of full thickness, was carried out in the dorsal part of 09 healthy sheep belonging to the animal facility of the veterinary department of the University of Batna 1. After inductions of the wounds, the animals were distributed to the randomly in 03 batches of animals of 03 sheep for each with appropriate topical care for each batch: the batch of animals treated with *Thymus vulgaris* honey mixed with *N. sativa* seed powder (HNS), the Madicassol batch and the Vaseline. The topical application of the various treatments was carried out once a day for 02 successive weeks. Histopathological evaluation was performed by measuring scar tissue thickness. In the batch of animals treated with HNS mixture, the thickness of the scar tissues revealed a significant decrease compared to the Madicassol and Vaseline batches. No keloid or detectable in the wounds of the mixture HNS lot. The results of this experimental study suggested that the topical use of local honey from the nectar of *T.vulgaris* mixed with *N. sativa* seed powder significantly reduced the thickness of wound healing in sheep.

* **Corresponding Author:** Deffa Ouafa ✉ o.deffa@univ-batna2.dz

Introduction

A wound is defined as a disturbance of the normal structure and function of the epidermis which corresponds to the first defensive barrier against external aggressions (Rodrigues *et al.*, 2019). Various mechanisms that can cause wounds, such as wounds, burns.... Wound healing is a complex process involving numerous physiological and molecular mediating reactions in order to restore the cellular integrity of damaged tissue (Sabine *et al.*, 2014). Several empirical stages are the subject of this restoration, initiated by a fleeting stage of inflammation followed by more or less long stages, called remodeling and restoration (li *et al.*, 2007).

Several treatments and preventive modalities, such as laser therapy, intralesional agents, cryotherapy, radiation therapy, pressotherapy, occlusive dressings, topical scarring agents, can be used in the prevention and treatment of abnormal scarring. (Niessen, *et al.*, 1999; Alster *et al.*, 2003). Currently, topical agents have been proposed as an excellent avenue for non-invasive therapy and prevention of scars showing keloids (Niessen *et al.*, 1999; O'Shaughnessy *et al.*, 2009). Honey has been known for millennia of years for its therapeutic healing properties. Still used for a long time in the 20th century, it was gradually abandoned after the 2nd World War in favor of more innovative products such as Asiaticosides. Equally ranked, in recent years, honey has shown a remarkable resurgence due to its antibacterial activity, and its potential to promote wound healing through many extremely complex processes.

It promotes fibroplasia, as well as stimulates the formation of a granulation model. It in fact decreases collagenase which considerably impairs healing (Uwatosin *et al.*, 2000). The seeds of *Nigella sativa* (black seeds) is also recognized as another natural remedy. It's an annual flowering plant, belongs to the family of Ranunculaceae, used in alternative medicine to cure numerous diseases. The consumption of its seeds have been reported to be antioxidative ,analgesic, antibacterial, anti-inflammatory, and wound healing processing (Forouzanfar *et al.*, 2014).

Today, several therapeutic techniques and remedies for skin healing have been considered by a number of research teams. Indeed, several publications have been established, but few studies have compared their modes of action with each other (Salman *et al.*, 2008; Santuzzi *et al.*, 2011). With this in mind, we have carried out an experimental study, the aim of which is to evaluate the healing activity of local honey from the nectar of *Thymus vulgaris* combined with *N. sativa* seed powder on experimentally induced skin wounds in sheep.

Material and methods

Ethics approval

This study was approved by the Biological Animal Ethics Committee of the University of Batna-2, Algeria (approval n°28/DBO/FSNV/UB2/2020).

Period and place of study

The study was carried out in the spring of 2020, on healthy sheep raised in the animal facility of the veterinary department of the University of Batna 1, Algeria. During the experiment, all surgical maneuvers were performed under rigorous hygienic conditions. For the histopathological study, the preparation of the various sections was carried out within the histopathology laboratory of the University of Batna 1. The reading of the various histopathological sections was made blind by a medical specialist belonging to the University Hospital of Batna.

Experimental animals

In this study we used 09 adult and clinically healthy male sheep. The weight of these animals varied from 20 to 25 kg. All these animals come from the animal facility of the veterinary department of the University of Batna 1.

An ordinary diet (straw and barley) was provided to the animals. One month before induction of the various wounds, extensive vermification was performed in all experimental subjects using ivermectin (CEVAMECTIN) by subcutaneous injection in the neck at a dose of 0.2 ml/ kg.

T.vulgaris honey and N.sativa L. seeds preparation steps

T. vulgaris honey from bees raised in the mountainous region of Arris (35° 33′ 0″ N, 6° 10′ 12″ E) of Batna (Algeria) was harvested in late spring. This honey was used as it is and without any particular preparation except for impurities, which were removed using a Whatman 0.5 mm filter. The sample its PH is 3.74 was placed in a glass bottle at temperature 25 °C. The seeds of *N. sativa* L. were *acquired* from an herbalist in BATNA (Algeria). The sample was cleaned with water, then dried at room temperature in a ventilated place, to better preserve the sensitive molecules. And finally the seeds of *N. sativa L* are ground using a manual mortar and sieved using 0.3 mm standard test to obtain a medium fine powder.

Induction of wounds

Before carrying out the various skin wounds, the animals underwent a tranquilization treatment using acepromazine intramuscularly at a dose of 0.1 mg/kg. Then, a broad shave was made in the dorsal region of the animals. The skin was then disinfected with 10% Betadine. On the shaved and disinfected dorsal surface, 02 longitudinal incisions of thick wounds (7 cm in length) were created for each animal. Before the actual wound induction, local anesthesia (lidocaine hydrochloride 02%) was performed according to the technique recommended by Alishahi *et al.* (2014). Indeed, anesthesia was performed subcutaneously at a dose of 1ml/1cm3. Then, all the wounds were sutured using a non-absorbable thread (STERIM*, T-30mm-4/8). Subsequently, the animals were grouped into 03 batches of 03 sheep for each. Hygienic asepsis measures were widely applied and respected with daily changing of the litter in the animal boxes.

Experimental grouping of animals

Before the application of the different topicals, the animals were separated blind into 03 lots of 03 sheep for each. The application of the different topicals (mixture of *T.vulgaris* honey and *N.sativa* powder (H N S), madicassol and vaseline) was carried out once a day for 02 successive weeks. The different topicals were applied directly to the surface of the wound at a dose of 1g. Concerning the mixture, mix of 1:1 ratio of honey and *N. sativa L* powder. The composition was freshly prepared and given.

- Lot I: the animals were treated with mixture (H N S)
- Lot II: Animals treated with madicassol.
- Lot III: corresponds to control sheep, treated with vaseline.

Histopathological study

Skin biopsies using a punch biopsy (4mm BP-40F) from the different incision wounds were performed on the 7th, 14th, 30th day after wound induction. The various samples underwent a classic histological treatment of preparation, first fixed by formalin buffered at 10%, then passage through a series of alcohol baths in the automaton. All the samples were embedded in a paraffin solution. The preparation of different sections was carried out by transverse sectioning of the skin with a microtome 4 µm thick. Then, the tissue sections were stained with hematoxylin-eosin and examined by a doctor specializing in histopathology belonging to the University Hospital of Batna. For microscopic measurements of the thickness of the epidermis we used the same technique established by Won *et al.* (2014). The thickness is then defined as the depth from the lower edge of the basement membrane of the epidermis to the lower part of the dense collagen mesh. In order to carry out the different measurements, we used the Image J software.

Statistical analysis

A global descriptive statistical study was carried out and a calculation of the mean and standard deviations (SD) of different thicknesses was carried out by SPSS 23.0 (2015) software. The comparison between the different means relating to the measurements of the thickness of the epidermis was carried out using an ANOVA. The multiple comparison between the different batches was carried out using the Tukey test. To determine the level of significance of the differences, we retained the values of $p < 0.05$ and $p < 0.01$.

Results and discussion

The results

Clinical follow-up

Animals tolerated wound induction well, and no symptoms of aggression or hypersensitivity were detected. The application of the different treatments was done in an easy way.

Histopathological study

Variations in the thickness of the epidermis

Histopathological evaluation revealed variations in the thickness of the newly formed epidermis (Table 1). Significant differences were recorded in healing progression in the three study groups. 01 weeks after surgery, the re-epithelialization is incomplete and the bed of a certain number of wounds will still seem filled with scabs, especially observed at the level of the wounds of the vaseline group. Inflammatory cells were also isolated from the breasts of the various wounds, especially from the control wounds.

Regarding the variations in thickness, we recorded a priori significant differences in the wounds of the batch of animals treated with the mixture (H N S) in comparison with those of the Madicassol and Vaseline batches: 191.95 ± 5.13 against: 196.8 ± 4.16 and 196.8±4.16 respectively (Fig.1). 02 weeks post-surgery, re-epithelialization is completed in all of the wounds, and a modeled synthesis of dense collagen fibers is observed especially in the wounds treated with the mixture (H N S), which gives the wounds a moderate appearance of fibrosis (Fig. 2a).

Table 1. Variations in the thickness of the epidermis of the different study groups expressed in µm.

Day of measurement	Group	Mean ± SD	N	95 % Confidence Interval		F	Significance P-value p < 0,05(*) p < 0,01(**) p < 0,001(***)
				Lower limit	Upper limit		
Day 7	HNS mixture	191,95±5,13***	6	186,6	202,0	12,18	0,001 (***)
	Madicassol	196,8±4,16	6	190,0	201,0		
	Vaseline	196,8±4,16	6	200,0	208,0		
Day 14	HNS mixture	68,25±7,34***	6	89,24	108,0	29.51	0,001 (***)
	Madicassol	82,36±04,07***	6	78,90	90,0		
	Vaseline	97,16±7,54***	6	89,24	108,0		
Day 30	HNS mixture	40,35±2,77***	6	36,8	44,3	391.64	0,001 (***)
	Madicassol	57,58±01,90***	6	54,58	59,57		
	Vaseline	84,76±3,42***	6	80,20	88,36		

At equal rank, our histopathological measurements of the thickness of the epidermis revealed a well-marked regression trend within the wounds treated with the mixture (H N S) : 191.95 ± 5.13 at 02 weeks, against 68.25 ± 7, 34 to 04 weeks (Fig.2). 01 month after wound induction, we recorded a significant difference within the batch of animals treated with the mixture (H N S). The group of animals treated with mixture (H N S) showed perfect healing, with a normal appearance of the epidermis. Fibrosis is reduced in all wounds, especially in wounds treated with the mixture (H N S) (Fig.2d). However, the vaseline group wounds showed poor healing, with more pronounced keloids (Fig.2f).

Discussion

The skin of domestic animals, is the largest organ of the body, it is characterized by large variations depending on the region of the body it covers (Heath *et al.* 2008). The skin is essential in various physiological processes such as hydration, protection against chemical attacks and pathogens, vitamin D metabolism, thermal homeostasis. Skin damage that can put the life of the animal in danger. Healing is the result of tissue repair, it corresponds to an extraordinary mechanism comprising complex

empirical steps involving an extremely organized cellular machinery (Rodrigues *et al.*, 2019; Veith *et al.*, 2020). A failure of one or other of these stages is most often accompanied by poor healing with the appearance of keloids (Won *et al.*, 2014).

Currently, and in order to reduce the economic and health burdens associated with the various tissue lesions, which are accompanied by ineffective "bad" healing, recent results are encouraging researchers towards other alternatives and experimental studies oriented towards the realization of new, more effective therapies (Chicharro-Alcántara *et al.*, 2018). At the cutaneous level, the different treatments are classified as “conventional” or “natural”. Conventional therapy leads to the formation of scars regardless of possible aesthetic and functional alterations (Chicharro-Alcántara *et al.*, 2018). However, nat.ural so-called alternative wound therapy is a resurgent field in biomedical research.

It aims to restore the skin to its original function, restoring damaged skin cells and tissues without leaving scars (Boyce *et al.*, 2018). For this reason there are several therapeutic agents or therapies which were potentially combined with honey to enhance antimicrobial activity and skin healing (McLoone *et al.*, 2020). Indeed, a lot of results have been published but widely diversified (Gentile *et al.*, 2020).

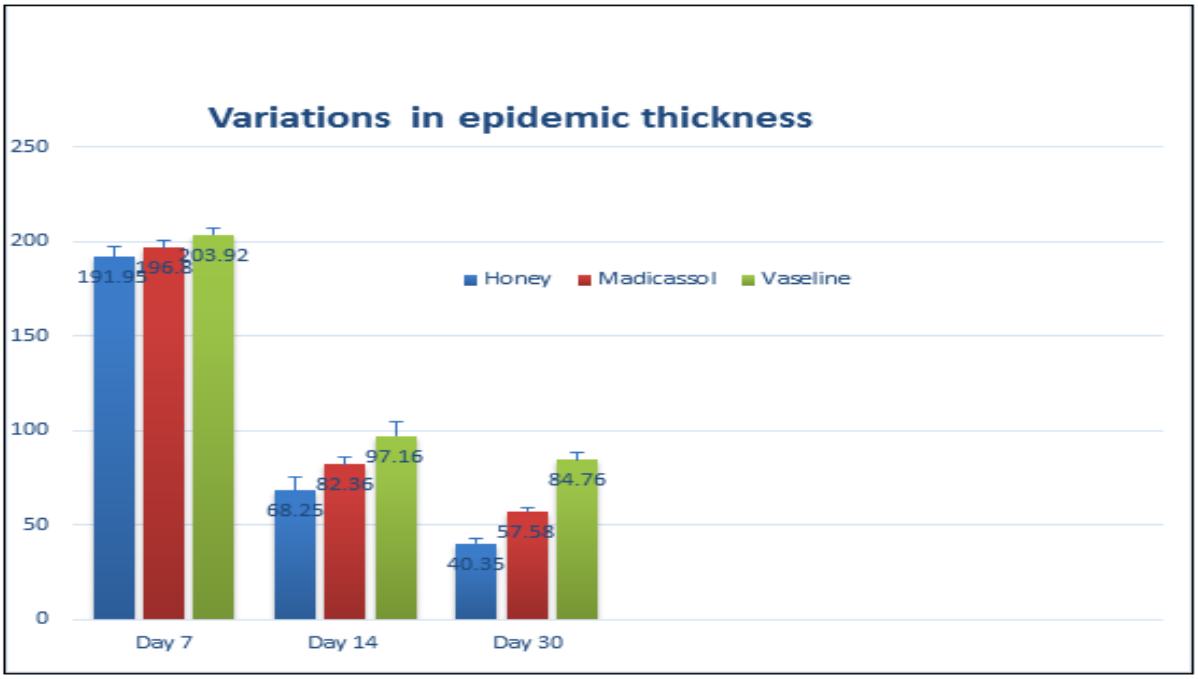

Fig. 1. Variation in the thickness of the epidermis in different groups of animals.

In this research, we proceeded with a comparative experimental study, in order to evaluate the healing activity of the mixture of local bee honey from the nectar of *T.vulgaris* and the seeds of *N. sativa L* powder on the thickness of the scars induced experimentally in a sheep model.

After 01 week of healing, the current results revealed a significant difference recorded in the batch of animals treated with mixture (H N S). At this stage of the evolution of healing, our comparative study showed that the thickness of the epidermis presents the highest values, then it tends to regress around the 04th week of healing. According to some authors, confirm in other similar studies that it is the inflammatory phase of the scarring process. These, could be due according to Pizzicannella *et al.* (2019) to the growth factors released locally at the level of the lesion bed by inflammatory cells which stimulate the surrounding cells. 02 consecutive weeks of healing leading to a remarkable regression in the thickness of the epidermis, especially in the batch of

animals treated with the mixture (H N S). No doubt this is the phase of remodeling and maturation. Takzare *et al.* (2017) suggested in a similar study that a hydrated topical like honey is hypertrophic of scar tissue by increasing collagen synthesis. Or furthermore, it is believed that the nature of honey, provides a hydrated model, quickly strewn with fibroblasts, and it promotes extracellular matrix components. (Abuharfeil *et al.*,1999; Daikh *et al.*,2021). Several similar studies have demonstrated the beneficial effects of topical application of *N. sativa L* oil in the treatment of skin-related diseases such as psoriasis, vitiligo and eczema. (Ghorbanibirgani *et al.*, 2014 Yousefi *et al.*, 2013 Jawad *et al.*, 2014). While the importance of honey as a better vehicle for medicinal plants has long been proven. (Alam *et al.*, 2014). Recent research has revealed the effects of combining honey with other therapies or agents with the aim of finding more efficacious treatments (McLoone *et al.*, 2020).

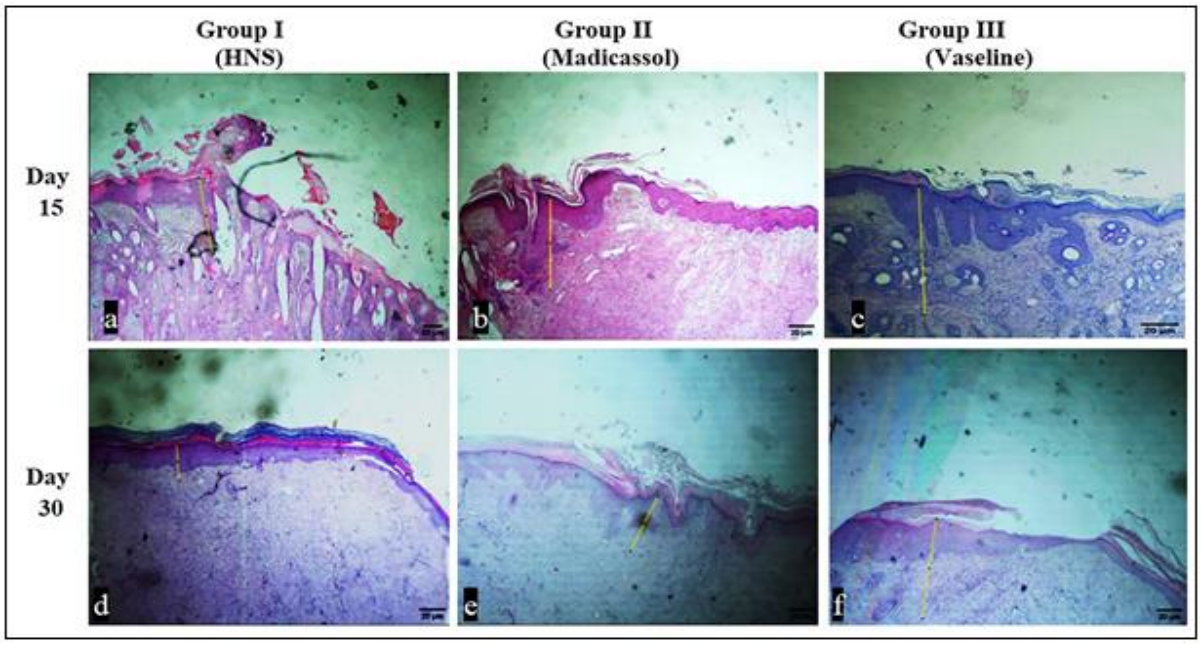

Fig. 2. Photomicrograph of various histological aspects (cross section) from skin biopsies taken on day 14 (a-c) and day30 (d-f) after surgery (magnification is 40×). Yellow arrows (measured epidermis contours). On the 15th day post-surgery, the thickness of the epidermis from the wounds of the Vaseline groups (c) is much thicker than the HNS mixture and madicassol groups (a and b). However, the dermis of HNS mixture -treated wounds (a) showed intense fibroplasia and significant angiogenesis. 01 month after surgery, healing is complete in all wounds. Variations in epidermis thickness showed the thinnest values encountered in the HNS mixture group wounds (d), with the dermis containing collagen fibers seemed better organized. Poor healing observed in the Vaseline and madicassol groups (e and f). Desquamated epidermis, and sometimes pronounced fibrosis in the dermis of wounds of the vaseline group (f).

In vitro studies have evaluated the combination of honey with N. sativa L oil to assess the anti-rheumatic and analgesic properties of this mixture (Khabbach *et al.*, 2012). After one month after induction of the wounds, healing of all the wounds with moderate fibrosis or even absent in the wounds treated with mixture (HNS) (Fig. 2d). Concerning the thickness of the epidermis, our study showed an almost total regression of the latter in the wounds of the mixture (HNS) group. In this study, honey in its hydrated and the seeds of *N. sativa* L powder form is believed to potentially improve skin wound healing in sheep. Knowing that this powdered form of *N. sativa* with honey has never been tested in research on skin lesion .Several studies have been published and confirm the usefulness of the use of hydrated occlusive topicals when managing the cutaneous palate (Won *et al.*, 2014). These preparations could lead to an increase in

skin hydration responsible for moderate angiogenesis, reduced hyperemia and maintain excessive fibroplasia responsible for the appearance of keloids (Niessen, *et al.*, 1999; O'Shaughnessy *et al.*, 2009). Other studies have also shown that hydration stabilizes the gap between the keratinocytes and the fibroblast, which can impair healing by the appearance of keloids (O'Shaughnessy *et al.*, 2009; Limandjaja*et al.*, 2020). On the other hand, the qualitative and quantitative nature of the mixture allows a combination between these biomolecules and contributes in synergy to the antimicrobial, antioxidant and anti-inflammatory activities. All of its concomitant activities characterizing the mixture are mainly correlated with its chemical composition, the pH of the honey, and the bioactive molecules such as thymoquinone and flavonoids which act individually or in synergy as natural antioxidants. This synergistic effect was investigated in similar research by AL-Waili *et al.* (2012).

Conclusion

Our results of the present study should be accepted as preliminary, and in all cases and despite the use of honey from the nectar of *T. vulgaris* and the seeds of *Nigella sativa* L powder, when managing skin wounds in sheep has shown positive results, this technique is still considered complementary to modern conventional treatments.

Authors' Contributions

DEFFA OUAFA

Realization of the experiment, handwritten writing.

DAIKH BADIS

Design of the study, Analysis and interpretation of data.

Both authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

Abuharfeil N, Al-Oran R, Abo-Shehada M. 1999. Effect of bee honey on the proliferative activity of human B- and T-Lymphocytes and the activity of phagocytes. Food and Agricultural Immunology **11,** 169-177. https://dx.doi.org/10.1080/09540109999843.

Alishahi KM, Mofidpoor H, Alishahi MAK. 2014. Histopathological evaluation of the effect of platelet-rich fibrin on canine cutaneous incisional wound healing. World Applied Sciences Journal **31,** 676-680.

Alster TS, Tanzi EL. 2003. Cicatrices hypertrophiques et chéloïdes : étiologie et prise en charge. American Journal of Clinical Dermatology **4,** 235-243.

AL-Waili N, Al-Ghamdi A, Javed Ansari M, Al-Attal Y, Salom K. 2012 .Synergistic Effects of Honey and Propolis toward Drug Multi-Resistant *Staphylococcus Aureus, Escherichia Coli* and *Candida Albicans* Isolates in Single and Polymicrobial Cultures. International Journal of Medical Sciences **9,** 793-800. https://dx.doi.org/10.7150/ijms.4722.

Boyce ST, Lalley AL. 2018. Tissue engineering of skin and regenerative medicine for wound care. Burns & Trauma **6,** 4. https://dx.doi.org/10.1186/s41038-017-0103-y.

Chicharro-Alcántara D, Rubio-Zaragoza M, Damiá- Giménez E, Carrillo-Poveda JM, Cuervo-Serrato B, Peláez-Gorrea P, Sopena-Juncosa JJ. 2018. Platelet-rich plasma: New insights for cutaneous wound healing management. Journal of Functional Biomaterials **9,** 1-20. https://dx.doi.org/10.3390/jfb901001010.

Daikh B, Deffa O. 2021. Comparative study of the therapeutic efficacy of autologous platelet-rich plasma and honey in healing skin wounds in sheep. Veterinary World **14,** 2170-2177. https://dx.doi.org/10.14202/vetworld.2021.2170-2177.

Forouzanfar F, Bazzaz BS, Hosseinzadeh H. 2014. Black cumin (Nigella sativa) and its constituent: a review on antimicrobial effects. *Iranian* Journal of Basic Medical Sciences **17,** 929-38.

Gentile P , Calabrese C, De Angelis B, Dionisi L, Pizzicannella J, Kothari A, De Fazio D, Garcovich S. 2020. Impact of the Different Preparation Methods to Obtain Autologous Non-Activated Platelet-Rich Plasma (A-PRP) and Activated Platelet-Rich Plasma (AA-PRP) in Plastic Surgery: Wound Healing and Hair Regrowth Evaluation. International Journal of Molecular Sciences **2,** 431. https://dx.doi.org/10.3390/ijms21020431.

Ghorbanibirgani A, Khalili A, Rokhafrooz D. 2014. Comparing *Nigella sativa* oil and fish oil in treatment of vitiligo. Iranian Red Crescent Medical **16,** e4515. https://dx.doi.org/ 10.5812/ircmj.4515.

Heath M, Jaimes Na, Lemos B, Mostaghimi A, C Wang L, JDdPablo FP, Nghiem P. 2008. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. The Journal of the American Academy of Dermatology **58,** 375-381. https://dx.doi.org/10.1016/j.jaad.2007.11.020.

Jawad AH, Azhar IY, Khalil IH. 2014. Evaluation of efficacy safety and antioxidant effect of *Nigella sativa* in patients with psoriasis: a randomized clinical trial. *Clinical* and Experimental Investigations **5,** 188–193. https://dx.doi.org/ 10.5799/ahinjs.01.2014.02.0387.

Li J, Chen J, Kirsner RS. 2007. Pathophysiology of acute wound healing. Clin Dermato **25,** 9-18. https://dx.doi.org/10.1016/j.clindermatol.2006.09.007.

Limandjaja GC, Niessen FB, Scheper RJ, Gibbs S. 2020. The Keloid Disorder: Heterogeneity, Histopathology, Mechanisms and Models. Frontiers in Cell and Developmental Biology 8, 360.

McLoone P, Tabys D, Fyfe, L. 2020 .Honey Combination Therapies for Skin and Wound Infections: A Systematic Review of the Literature. *Clinical, Cosmetic* and Investigational *Dermatology* **13,** 875–888.

Niessen FB, Spauwen PHM, Schalkwijk JK, Moshe MD. 1999. On the Nature of Hypertrophic Scars and Keloids: A Review. Plastic *and* Reconstructive Surgery **104,** 1435–1458. https://dx.doi.org/10.1097/00006534-199910000-00031.

O'Shaughnessy KD, De La Garza M, Roy NK, Mustoe TA. 2009. Homeostasis of the epidermal barrier layé: A theory of how occlusion reduces hypertrophic scarring. Wound Repair and Regeneration **17,** 700–708. https://dx.doi.org/ 10.1177/1721727X1401200307.

Pizzicannella J, Gugliandolo A, Orsini T, Fontana A, Ventrella A, Mazzon E, Bramanti P, Diomede F, Trubiani O. 2019. Engineered extracellular vesicles from human periodontal-ligament stem cells increase VEGF/ VEGFR2 expression during bone regeneration. Frontiers in Physiology **10,** 512. https://dx.doi.org/ 10.3389/fphys.2019.00512.

Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. 2019. Wound Healing: A Cellular Perspective. Physiological Reviews **99,** 665–706. https://dx.doi.org/ 10.1152/physrev.00067.2017.

Sabine AE, Paul M, Tomic-Canic M. 2014. Wound repair and regeneration: Mechanisms, signaling, and translation. Science Translational Medicine **6,** 265sr6. https://dx.doi.org/10.1126/scitranslmed.3009337

Salman H, Kashmoola MA, Al-Waiz MM, Al-Sandooq TA. 2008. Differences between low level laser therapy and triamcinolone acetonide kenalog on healing of recurrent aphthous ulceration. Annals of the College of Medicine Mosul **34,** 35–41.

Santuzzi CH, Buss HF, Pedrosa DF, Freire MO, Nogueira BV, Gonçalves WL. 2011. Combined use of low level laser therapy and cyclooxygenase-2 selective inhibition on skin incisional wound reepithelialization in mice: a preclinical study. *An Bras Dermatol* **86,** 278–283. https://dx.doi.org/10.1590/s0365-05962011000200011.

Takzare N, Hassanzadeh G, Rouini MR, Manayi A, Hadjiakhondi A, Zolbin MM. 2017. Evaluation of the effects of local application of thyme honey in open cutaneous wound healing. Iranian Journal of Public Health **46,** 545-551.

Uwatosin OM, Olabanji JK, Oluwatosin OA, Tijani LA, Onyechi HU. 2000. A comparison of topical honey and phenytoin in the treatment of chronic leg ulcers. African Journal of Medicine and Medical Sciences **29,** 31–34.

Veith AP, Henderson K, Spencer A, Sligar AD, Bake AB. 2020. Therapeutic Strategies for Enhancing Angiogenesis in Wound Healing. Advanced Drug Delivery Reviews **146,** 97–125. https://dx.doi.org/10.1016/j.addr.2018.09.010.

Won JK, Young CN, Bum SS, Hyeon AK, Woo HH, Gum HA, Choi HC, Seo UL. 2013. The effects of topical agent (Kelo-Cote or contractubex) massage on the thickness of post-burn scar tissue formed in rats. Archives of Plastic Surgery **40,** 697-704.

Yousefi M, Barikbin B, Kamalinejad M, Abolhasani E, Ebadi A, Younespour S. 2013. Comparison of therapeutic effect of topical *Nigella* with Betamethasone and Eucerin in hand eczema. Journal of the European Academy of Dermatology and Venereology **27,** 1498–1504. https://dx.doi.org/10.1111/jdv.12033.