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Preliminary study to assess cicatrizing activity of honey and *Pistacia lentiscus* fatty oil mixture on experimental burns in rabbits

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Abstract: The present study was undertaken to assess cicatrizing activity of a mixture of honey and *Pistacia lentiscus* fatty oil (PLFO) on dermal burn wounds. It was carried out on 8 male adult New Zealand rabbits. After anesthesia, 4 equal burns were realized on the back of each animal (2 dorsal and 2 lumbar). The wounds were treated, immediately after burning and repeated once daily until 22nd day of experiment, by 0.5 g of honey, 0.5 ml of PLFO or 0.5 g of mixture Honey + PLFO (v/v), the last wound was treated by 0.5 g of Cicatryl[®] as a reference drug. The healing process was evaluated by calculating the percentage of wound contraction at days 2, 6, 10, 14, 18 and 22. The results showed that both of honey, PLFO and the mixture honey + PLFO promote significantly ($P < 0.05$) the wound contraction when compared to the standard drug at the different time intervals. In addition, PLFO showed better contraction than honey during the inflammatory and proliferative phases. The mixture showed a percentage of wound contraction better than that of honey but lower than that of PLFO used separately during the inflammatory phase, this difference became non significant at day 10 ($P > 0.05$) and marked a significant reduction at the 14th day ($P < 0.05$). After that the differences were not statically significant ($P > 0.05$). In conclusion, the current study suggests that PLFO may ameliorate the healing properties of honey when mixed to it during the inflammatory phase of cicatrizing process in rabbit model.

Keywords: Honey; *Pistacia lentiscus*; fatty oil; burns; wound healing; rabbits.

Introduction

Honey has been used for its medicinal properties in many cultures, since the ancient times. According to Al-Mamary et al. (2002), Honey has been reported to be effective in gastrointestinal disorders, in healing of wounds and burns, to provide gastric protection against acute and chronic gastric lesions and as an anti-microbial agent.

Honey is primarily made of water and carbohydrates. It also contains trace amounts of several minerals and vitamins. You can find niacin, calcium, copper, riboflavin, iron, magnesi-

um, potassium and zinc in honey. Honey also contains a blend of flavonoids and phenolic acids (Sampath Kumar et al. 2010).

Pistacia lentiscus L. (Anacardiaceae) is a dense bush with a strong characteristic aroma and green leaves, which grows in many Mediterranean countries (Zrira et al. 2003). The essential oil and gum from this plant have been widely used as food and beverage flavoring additives and traditional medicines in the Mediterranean region since ancient times without any reported toxicity in humans (Loutrari et al. 2006). *P. lentiscus* fatty oil is edible oil extracted from fruits of this plant. In Algeria this oil is

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used by people in traditional medicine as an anti-diarrheal, as a component of cattle feed (Trabelsi et al. 2012), it is recommended for diabetics, for the treatment of pain of stomach, and in case of circumcision (Hmimsa. 2004) and back pain (Bellakhdar. 1997), it is largely used in the treatment of respiratory disorders and dermal burns in Algerian folk medicine (Djerrou et al. 2011). Scientifically tested, the oil showed a real healing activity on experimental burns in the rabbit model, by decreasing the inflammatory phase, promoting wound contraction and reducing the epithelialization period (Djerrou et al. 2010).

The present study was undertaken to evaluate the efficacy of honey and *pistacia lentiscus* fatty oil mixture in the management of dermal burn wounds in the rabbit model.

Material and methods

Drugs

Pistacia lentiscus fatty oil was provided by an herbalist who has collected *P. lentiscus* berries in November 2010 in El Milia region of northern Algeria and has extracted oil by a traditional method. The oil was kept cool and protected from light until use. Honey was provided by a beekeeper located in El Milia. Cicatryl® was purchased from a private pharmacy, it contains as ingredients: Purified water, Cety alcohol, Petrolatum, isopropyl myristate, Sorbitol, Glyceryl stearate, Hydroxypropyl starch phosphate, PEG 75-stearate, Chlohexidinedigluconate, Tocophery acetate, Ceteth 20, Steareth-20, Polyacrylamide, C13-14 isoparaffin, Laureth 7, Methyl paraben, Propyl paraben, imidazolidinyl urea.

Animals

The trial included eight male New Zealand rabbits, weighing 2.49 ± 0.1 kg at the beginning of the experiment, from a private farm located in Ain M'lila North of Algeria. The animals were kept in individual cages in a standard environment, with a temperature of $22 \pm 2^\circ\text{C}$ and a cycle of 12h light / dark. Food and water were provided *ad libitum*.

Experimental protocol

The study was conducted according to the technique described by HamdiPacha et al. (2002). The rabbits were sedated (5 mg Diazepam®, IM), and the back of each animal was shaved by an electric clipper. Then the 4 zones to be burned were anesthetized locally (Lidocain 3%, SC). After that, 4 circular burns (figure 1) were realized on the back of each rabbit; 2 dorsal (left and right) and 2 lumbar (left and right) using a metallic cylinder (22 mm diameter) previously immersed in built water for 3mn and immediately placed on the skin for 15 seconds. The treatments were applied immediately after burnings, and repeated once daily until day 22. The first wound was treated with 0.5 ml of *pistacia lentiscus* fatty oil, on the second wound was applied 0.5 g of honey, a 0.5 g of mixture honey + 0.5 ml of *P. lentiscus* fatty oil (v/v) was used to treat the third wound, and the last wound was treated with 0.5 g of Cicatryl® as a standard drug.



Figure 1: Circular burn at the first day of experiment.

The wounds margins were traced on a transparent plastic sheet, then the wounds diameters were measured by vernier calipers which were used to calculate the average wound areas in mm^2 . The percentage of wound contraction were measured on days 2, 6, 10, 14, 18 and 22 using the following formula (Srivastava et al. 2008):

Percentage of wound contraction = $(\text{Initial wound size} - \text{Specific day wound size}) \times 100 / \text{initial wound size}$.

All experimental procedures were adopted in accordance with the direction of the faculty of sciences of nature and life, Mentouri Constantine University, Algeria.

Statistical analysis

Data were expressed as mean of 8 replicates \pm 2. The results were analyzed statistically using One-way ANOVA to identify the differences between the groups of treatments. The data were considered significant at $p < 0.05$.

Results and discussion

The rabbits were survived during all the experimental period. Their mean weights were not changed significantly ($P > 0.05$) when compared with initial mean weight (2.49 ± 0.10 kg). The weights obtained after burning were 2.54 ± 0.09 kg at day 7, 2.63 ± 0.13 kg at day 14 and 2.60 ± 0.23 kg at the 22th day of experiment.

The results obtained about the evolutions of wounds, treated with the different products, were given in table 1, as percentages of wound contraction calculated each 4 days until 22th day after burning. Generally, the percentage of wound contraction in wounds treated with *Pistacia lentiscus* fatty oil alone (PLFO), honey alone or mixture (Honey + PLFO) was higher significantly ($p < 0.05$) than that of wounds treated in the standard drug from the 2nd day to the 22nd day. From D2 to D14, the wound contraction was already better in PLFO group than honey group. The mixture (PLFO + honey) has marked a contraction better than honey alone but lower significantly ($p < 0.05$) than PLFO when used alone. In the 6th day, the mixture was already better than honey but the difference with PLFO was non significant ($P > 0.05$). At day 10, the mixture has not ameliorated the percentage of wound contraction when compared to honey ($P > 0.05$), noting that this percentage was inferior significantly ($p < 0.05$) than that obtained in wounds treated in oil (PLFO). In the 14th day after burning, the contraction in mixture (PLFO + Honey) was lower significantly ($p < 0.05$) than that obtained with honey or oil used separately. At day 18 and 22, any significant difference

($P > 0.05$) was noted between the mixture of PLFO and honey and the two products used separately, in addition the results showed a similar percentage of wound contraction in case of PLFO or honey used separately.

These results obtained in the present study confirmed other previous studies which have tested PLFO or honey separately. If honey was well studied since a long time, few reports are available about PLFO. Our team has confirmed scientifically the healing properties of PLFO; this vegetable oil has showed a real healing activity on experimental burns in the rabbit model, by decreasing the inflammatory phase, promoting wound contraction and reducing the period of epithelialization (Djerrou et al. 2010).

According literature, honey produces rapid tissue regeneration and suppresses inflammation, edema and exudation. Its high viscosity provides a protective barrier to prevent wounds from becoming infected, effectively sealing the wound (Molan 1999). It has been reviewed by Mwipatayi et al. (2004), that honey provides a moist healing dressing that prevents bacterial growth even if the wound is heavily infected. A moist wound environment is known to protect the wound, reduce infection rates, reduce pain, debride necrotic tissue, and promote granulation tissue formation (Subrahmanyam 1998). The pH of honey is low and ranges from 3.2 to 4.5 with the most predominant one being gluconic acid (Abdullah Adil Ansari and Clemencia Alexander 2009). The acid pH does contribute to the ideal environment for fibroblastic activity, migration, proliferation and organisation of collagen, which results in stimulation of wound healing (Mwipatayi et al. 2004).

In the present study, the mixture of PLFO and honey (v/v) has ameliorated the effect of honey during the 6th first days after burning; this period corresponds to the inflammatory phase (Park and Barbul. 2004). At the 10th day, the difference between mixture and honey became non significant statistically but it showed a significant reduction of wound contraction in mixture at the 14th day of experiment; this period corresponds to the second half of proliferative phase and remodeling (Park and Barbul. 2004).

Table 1: Percentage of wound contraction in the different group of treatments at different time intervals.

Treatments	Wound contraction (%)*					
	D 2	D 6	D 10	D 14	D 18	D 22
Honey	16,15±1,32	20,59±2,63	52.28±1.16	73.81±1.14	94.51±1.36	98.44±0.86
PLFO	24,15±0,58	33,07±1,26	46.13±3.12	79.25±1.04	94.74±1.18	98.83±0.37
Honey + PLFO	19,18±1,95,	31,89±1,35	46.11±2.07	70.80±1.77	94.91±1.39	98.22±0.8
Cicatryl®	7,70±1,17	18,41±1,75	24.8±1.42	58±1.61	80.68±0.69	95.79±0.79
Statistical data						
PLFO Vs Honey	S	S	S	S	NS	NS
PLFO VsCicatryl®	S	S	S	S	S	S
Honey VsCicatryl®	S	S	S	S	S	S
(Honey+PLFO) Vs PLFO	S	NS	S	S	NS	NS
(Honey+PLFO)Vs Honey	S	S	NS	S	NS	NS
(Honey+PLFO)VsCicatryl®	S	S	S	S	S	S

*Results are expressed as mean \pm ² of 8 replicates. PLFO= *Pistacia lentiscus* fatty oil, S=significant ($P < 0.05$), NS=non significant ($P > 0.05$).

Conclusion

We can conclude that PLFO and honey have promoted wound contraction in the different stage of cicatrizing process. However, PLFO showed better wound contraction during the inflammatory and proliferative phases. The mixture of these two products (v/v) has ameliorated the effect of honey during the inflammatory phase, but the difference became non significant at the 10th ($P > 0.05$) and marked a significant reduction at the 14th day ($P < 0.05$). After that the differences were not statically significant ($P > 0.05$). In view of these results, we suggest that the mixture of honey and PLFO may be justified during the inflammatory phase of cicatrizing process.

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