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Wound healing effect of *Pistacia lentiscus* L. seed oil: confirmation of its uses in Mediterranean traditional medicine

[Efecto curativo de heridas del aceite de semilla de *Pistacia lentiscus* L.: confirmación de sus usos en medicina tradicional mediterránea]

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Abstract: In order to evaluate the potential of this formulation (*P. lentiscus* L. oil-based ointment) to heal wounds, experimental wounds were done on guinea pigs and efficiency was comparatively assessed against a reference ointment, Cicaderma®. Wound contraction was performed on days 5, 10 and 15. Tissue sections were also evaluated histopathological on days 7, 14 and 21. Results showed that for all days (5, 10 and 15), the highest wound contraction values were attained for the *P. lentiscus* oil-based ointment treated group with wound contraction values of 19.38, 55.8 and 77.11%, respectively, as compared to the reference drug Cicaderma® where contractions were 7.97%, 49.53% and 71.44%, respectively. Vehicle and negative control groups however showed no statistically significant wound healing activity on the excision wound model. These experimental studies revealed that the *P. lentiscus* oil-based ointment displays remarkable wound healing activity, in accordance with its use in traditional medicine.

Keywords: *Pistacia lentiscus* L.; Seed oil; Wound contraction; Histological evaluation

Resumen: Con el fin de evaluar el potencial de esta formulación (ungüento a base de aceite de P. lentiscus L.) para curar heridas, se realizaron heridas experimentales en cobayos y se evaluó comparativamente su eficacia respecto de un ungüento de referencia, Cicaderma®. La contracción de la herida se realizó los días 5, 10 y 15. Las secciones de tejido también se evaluaron histopatológicamente los días 7, 14 y 21. Los resultados mostraron que para todos los días (5, 10 y 15), se obtuvieron los valores más altos de contracción de la herida para el grupo tratado con ungüento a base de aceite de P. lentiscus con valores de contracción de la herida de 19.38, 55.8 y 77.11%, respectivamente, en comparación con el medicamento de referencia Cicaderma® en donde las contracciones fueron 7.97%, 49.53% y 71.44%, respectivamente. Sin embargo, los grupos de control de vehículo y negativo no mostraron actividad de curación de heridas estadísticamente significativa en el modelo de herida por escisión. Estos estudios experimentales revelaron que la pomada a base de aceite de P. lentiscus muestra una notable actividad de curación de heridas, de acuerdo con su uso en la medicina tradicional.

Palabras clave: *Pistacia lentiscus* L.; Aceite de semilla; Contracción de herida; Evaluación histológica

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INTRODUCTION

For thousands of years, plant-derived substances have been a part of the evolution of human healthcare and natural products have been used since ancient times for the treatment of many diseases.

Among the most complicated health problems, wound healing is considered as an important field of research in current biomedical sciences. Herbal medicines have been used to accelerate wound healing since early times. In recent times, scientists have been able to use scientific methods to demonstrate efficacy of many of these plants and to get a better understanding of mechanisms of their actions on wound reparation. The reputation of herbal medicines may be related to the perception that herbs cause minimal adverse effects.

In this context, several studies have reported the potential of some medicinal plants to heal wounds on the basis of their use in folk medicine (Biswas & Mukherjee, 2003; Sharma *et al*., 2013).

Some of these plants have been already screened scientifically to evaluate their wound healing activity, but the potential of most of them remains unexplored.

Pistacia lentiscus L. seed oil is a natural product known for its use to heal wounds and burns in the folk medicine of rural Italian, Tunisian and Algerian people. Several studies highlighted the nutritional value of this oil. Mezni *et al.* (2014a) and Trabelsi *et al.* (2012) demonstrated that lentisk oil is rich in unsaturated fatty acids (more than 70%) and that it is an important source of tocopherols and carotenoids. In the same context Mezni *et al.* (2018) showed that *P. lentiscus* seed oil contains a high amount of phenols. Numerous biological properties of this no wood forest product were studied. Mezni *et et al.* (2014b & 2016) demonstrated the antimicrobial and anti-proliferative effect of this oil.

Only a few scientific researchers have been interested in studying the healing effects of this oil. Djerrou *et al.* (2010) demonstrated the efficiency of virgin fatty oil of *Pistacia lentiscus* for healing burn wounds. Maameri *et al.* (2012) compared potential wound healing of *P. lentiscus* seed oil with that of honey. All of these authors mentioned an increase in wound contraction but no histological evaluation was conducted to confirm the efficiency of *P. lentiscus* seed oil in healing the wounds.

This work evaluates, for the first time, both the clinical and histopathological aspects of cutaneous application of *P. lentiscus* oil-based ointment on healing surgically-created wounds in guinea pigs.

MATERIALS AND METHODS

Plant material and ointment formulation

The fruits of *Pistacia lentiscus* (Lentisk-mastic tree) were collected from the Feija area located in the northwest of Tunisia (N 36°28’, E 8°19’).

The plant was identified by Dr. A. Khaldi from I.N.R.G.R.E.F-Tunisia. The certified specimens (VS1-PL2009) were deposited at the Herbarium run by I.N.R.G.R.E.F.

The plant name corresponds to “The Plant List” (www.theplantlist.org).

The oil was extracted using cold pressure. The fruits were ground. The unguent obtained was mixed and placed in a hydraulic press. The floating oil was removed and used to formulate a 30% (v/v) oil-based ointment using soft white Vaseline as a vehicle.

Chemicals and reagent

Ketamin chlorhydrate, Xylocaïn hydrate, vaseline and Cicaderma® were acquired from Central Pharmacy (Tunisia). Neutral buffered formalin was purchased from Phytoderm Laboratory (Tunisia). Paraffin and hematoxylin–eosin were acquired from Sigma-Aldrich (France).

Animals

Male guinea pigs (250-300 g) were purchased from the Experimental Commodities and Animal Care Service (Pasteur Institute, Tunisia).

The animals were left for 2 weeks at room condition for acclimatization. They were kept on a standard pellet diet with water *ad libitum* throughout the experiment. A minimum of eight animals were used in each group. Throughout the experiments, animals were handled according to the suggested international ethical guidelines for the care of laboratory animals (Authorization issued by Pasteur Institute ethical committee).

Wound healing studies

Excision wound model

An excision wound was inflicted on the shaved dorsal back skin of the guinea pigs according to methods described by Saha *et al*. (1997) under total anesthesia with Ketamin chlorhydrate (1.5 mg/kg body weight intramuscularly) and then a local anesthesia using Xylocaïn hydrate (2%) on the excision area. Full skin thickness was excised from the marked area to get a wound measuring about 225 mm^2. The animals' wounds were treated topically according to group (8 animals in each group). Group 1 was untreated and considered a negative control group. Animals in Group 2 were treated with a simple ointment base (placebo group). Group 3 was treated with a well used commercial wound healing herbal ointment (Cicaderma®) and Group 4 was treated with the oil-based ointment prepared from *P. lentiscus* fruits.

Treatment was applied topically once a day, starting from the wound induction until complete healing. Ointment was applied in enough quantity to cover all wounds.

Planimetry

Planimetry was performed on days 0, 5, 10 and 15. Contractions, which contribute to wound closure, were studied by tracing the raw wound on transparent paper with a special marking pen. Wound area was determined using a digital planimeter. The percentage of wound contraction was calculated using the following formula: [% wound contraction = (healed area/total area) x 100] and used for statistical analysis.

Histological evaluation

Skin specimens from the wound of each animal were collected on days 7, 14 and 21. The specimens were fixed in 10% neutral buffered formalin, then processed and blocked with paraffin. Five-micrometer cross sections were cut and stained with hematoxylin–eosin. The tissues were qualitatively assessed under a light microscope at 400× magnification and evaluated for the following histological criteria: the extent of re-epithelialization, maturation and organization of the epidermal squamous cells, thickness of the granular cell layer and degree of granulation tissue formation.

Statistical analysis

Data were processed using the GLM procedure (General Linear Models) of the SAS (9.0) program. An analysis of variance was done of the studied parameters. Significant correlations were retained ($p < 0.05$). All values represent the mean of five replications.

RESULTS

Wound contraction

The wound healing potential of a formulation of *P. lentiscus* seed oil based on traditional medicine was investigated. The experimental results of wound contraction are summarized in Figure No. 1.

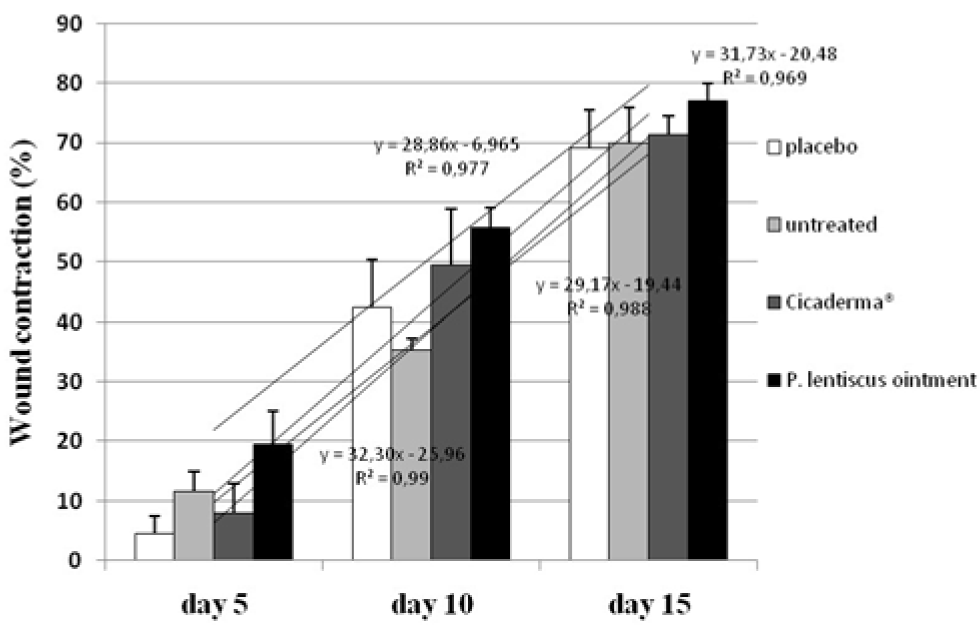

Figure No. 1

Percentage of wound contraction in treated groups (with Cicaderma®or *P. lentiscus* ointment) and control groups (untreated and placebo) at days 5, 10 and 15

The results obtained showed that both the reference drug Cicaderma® and the *Pistacia lentiscus* oil-based ointment were found to have wound healing potential, while the vehicle and negative control groups showed no statistically significant wound healing activity on the excision wound model. Wound contraction started from day 5 in all groups. However, the wound healing rate was faster in the reference drug Cicaderma® and *Pistacia lentiscus* oil-based ointments groups ($p < 0.007$).

As shown in Figure No. 1, the highest wound contraction values were reached for the *P. lentiscus* oil-based ointment treated group on days 5, 10 and 15 with wound contraction values of 19.38%, 55.80% and 77.11%, respectively.

However, wound contractions were 7.97%, 49.53% and 71.44% for the reference drug Cicaderma® treated group, significantly comparable to the *Pistacia lentiscus* oil-based ointment on days 10 and 15.

The negative control and placebo groups showed the same rate of wound healing with contraction values increasing from 11.55% to 69.89% and from 4.47% to 69.08%, respectively (from day 5 to day 15).

Histological evaluation

Seven days after the wound was inflicted, the treated groups showed an enhanced healing process compared to that of the untreated groups. The best results were obtained with the *P. lentiscus* oil-based ointment and the Cicaderma® treated animals.

On day 7, both animals topically treated with the *P. lentiscus* ointment and the reference drug showed high cellular infiltration. The control wounds had a large presence of crust, highly infiltrated granulation tissue with inflammatory cells (Figure No. 2a). These features indicate an initial organization of the granulation tissue. In the treated wounds, no crust was observed and granulation tissue was more developed with minor inflammatory infiltration and an abundant extracellular matrix.

On day 14, cross sections showed a highly-advanced organization of granulation tissue and a greater epithelialization in the treated groups,

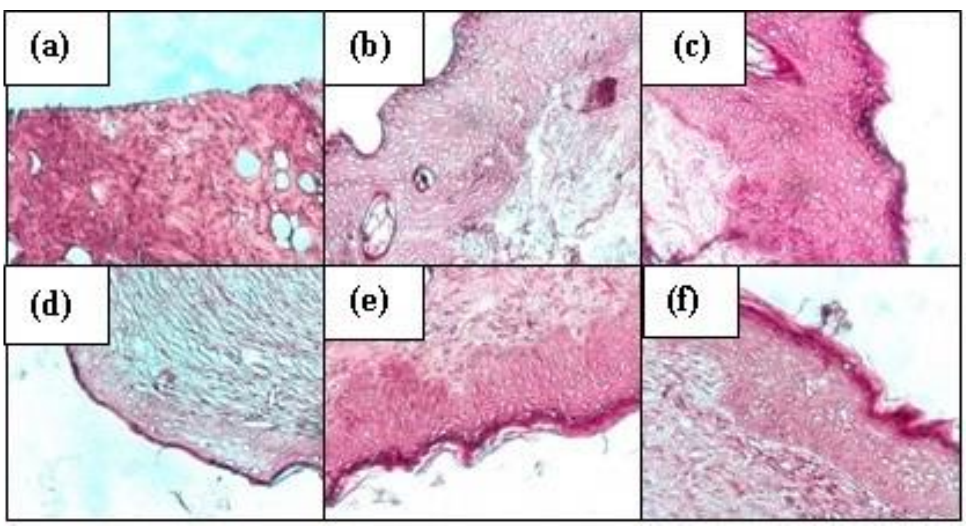

Figure No. 2
Histological evaluation of wound repair by Haematoxylin and Eosin (H&E 200x) staining of granulation tissue on different days of healing: (a) untreated group on day 7, (b) untreated group on day 14 (c) *P. lentiscus* treated wounds on day 14, (d) untreated group on day 21, (e) *P. lentiscus* oil-based ointment day 21, (f) Cicaderma® treated wounds on day 21

whether with vehicle and control (Figure No. 2b) than the Cicaderma® or *P. lentiscus* ointment groups (Figure No. 2c). The granular layer was thicker (4-5 cells) and well-formed in the two ointment-treated groups, as compared to the control and vehicle groups (2-3 cells).

On day 21, cross sections of animals treated topically with the *P. lentiscus* ointment and the reference drug showed full thickness re-epithelialization (Figure No. 2e and Figure No. 2f), in which the epidermis was thick and well organized, and the boundary between the epidermal and the dermis was clear. A remarkable improvement in the maturation and organization of epidermal layers with no debridement crust covering the epidermal surface and the presence of highly vascularized areas in the granulation tissue was observed.

For both untreated (Figure No. 2d) and vehicle groups, the epidermis was thin and disorganized. Cross sections showed immature and disorganized epidermal layers due to a delay in full-thickness re-epithelialization.

DISCUSSION

The present study was conducted to evaluate whether P. lentiscus seed oil promotes wound healing in experimentally-induced incisions in guinea pigs. The results obtained substantiate the use of this traditional medicinal oil for the treatment of wounds. These results confirm those obtained by Maameri *et al*. (2012) which demonstrated the efficiency of *P. lentiscus* seed oil in healing wounds. Although a histopathological evaluation of the wounds has not been made, planimetry findings point to accelerated healing rates and increased wound contraction.

Under the conditions of the present investigation, *P. lentiscus* seed oil-based ointment accelerated wound healing process by influencing its different aspects at various days after wounding. It is well known that wound healing is a complex process that involves four overlapping phases: hemostasis, inflammation, proliferation, and maturation.

The first phase of hemostasis begins immediately after wounding. It is the process of the wound being closed by clotting. Hemostasis starts when blood leaks out of the body (Flanagan, 1997).

Inflammation is the second stage of wound healing and begins right after the injury when the injured blood vessels leak transudate causing localized swelling. Inflammation both controls bleeding and prevents infection. The fluid engorgement allows healing and repair cells to move to the site of the wound. During the inflammatory phase, damaged cells, pathogens, and bacteria are removed from the wound area (Clark, 1998; Guo & DiPietro, 2010).

The proliferative phase (granulation stage) of wound healing is when the wound is rebuilt with new tissue made up of collagen and extracellular matrix. In the proliferative phase, the wound contracts as new tissues are built. In addition, a new network of blood vessels must be constructed so that the granulation tissue can be healthy and receive sufficient oxygen and nutrients. Myofibroblasts cause the wound to contract by gripping the wound edges and pulling them together using a mechanism similar to that of smooth muscle cells.

Maturation is the final phase and occurs once the wound has closed. It is when collagen is remodeled from type III to type I and the wound fully closes. The cells that had been used to repair the wound but which are no longer needed are removed by apoptosis, or programmed cell death (Flanagan, 2000).

The healing potential of *P. lentiscus* oil was marked by reduced inflammation and closure of the wound which are a due to rapid wound contraction and full-thickness epidermal regeneration and organization. This great potential of the studied oil could be explained by its powerful anti-microbial and antioxidant effects demonstrated in our previous studies (Mezni *et al*., 2012). During the wound healing process, inflammation plays an important role in fighting infection and inducing the proliferation phase necessary for healing. It has been reported that if a wound becomes infected, the inflammatory phase becomes chronic and normal healing is disrupted (Guo & Dipietro, 2010).

During the healing process, free radicals are generated at the site of injury and cause damage to cellular membranes, nucleotides, lipids and proteins. In this context, several antioxidants such as phenols and vitamin E have been reported to protect against oxidative damage to tissues (Pascoe *et al*., 1987). Many plant extracts and medicinal herbs have been shown to possess potent antioxidant activity. Carotenoids and tocopherols, the main antioxidant components of P. lentiscus seed oil (Dhifi *et al*., 2013) are powerful free-radical scavengers and have been shown to promote wound healing. This could explain the healing capacity observed in this investigation.

On the other hand, the wound healing property of *P. lentiscus* fixed oil may be due to its biologically active fatty acids. This oil is rich in long-chain fatty acids (16-18 C), the major one being oleic acid. Fatty acids are bioactive molecules which have been proven to modulate cellular proliferation, cell signaling and growth factor activities (Jiang *et al*., 1995; Bandyopadhyay *et al*., 1999). Previous studies have shown that poly- and monounsaturated fatty acids exert major functions on inflammatory responses, either in the form of phospholipids anchored in the cell membrane or as soluble lipoic mediators.

Mat Jais *et al*. (1998), Fredalina *et al*. (1999), Baie & Sheikh (2000) described the effects of polyunsaturated fatty acids in the wound healing process. These authors demonstrated that wounds in rats, when treated with oil containing a high concentration of polyunsaturated fatty acid, had improved dermal healing.

Cardoso *et al*. (2004) presented evidence that linolenic, linoleic and oleic acids can modulate the closure of surgically induced skin wounds. They showed that especially oleic acid, which is the major fatty acid in P. lentiscus seed oil, induced faster wound closure as compared to linoleic and linolenic acids.

Moch *et al*. (1990) have demonstrated that linoleic acid, with more than 25% of the total fatty acid content in *P. lentiscus* seed oil (Trabelsi *et al*., 2012), is a powerful pro-inflammatory mediator that causes a migration of granulocytes and macrophages as well as important changes in granulation tissue. They observed efficient topical action of linoleic acid on abdominal lesions produced experimentally in rats.

CONCLUSIONS

The present study has demonstrated that the regular topical application of Pistacia lentiscus seed oil-based ointment accelerates the wound healing process, confirming the rationale of the traditional ethnomedicinal application of this oil. This wound healing potential of *P. lentiscus* seed oil might be due to the presence of oleic and linoleic fatty acids, tocopherols and carotenoids. Although it is not known which of the constituents of *P. lentiscus* seed oil are responsible for improved wound healing, it is plausible that nonpolar compounds play a key role in its therapeutic effect.

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