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Dermal Wound Healing Effect of *Pistacia Lentiscus* Fruit's Fatty Oil.

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ABSTRACT
Several natural products have been shown to accelerate wound healing process. The present study was undertaken to evaluate the effect of *Pistacia lentiscus* fruits fatty oil on cutaneous wound healing in rat, and to compare this effect to that of saponifiable and unsaponifiable oily fractions. Full-thickness excision wounds were made on the back of anesthetised rats. The fruit's oil and the two fractions were assessed together with a conventional drug, i.e. Madecassol®. Preparations were topically applied on the area of excised wounded once a day and assessed for a period of 26 days. During this period, wound area was measured and photographically documented. Wound contraction, expressed as percentage, was significantly ($P<0.05$) enhanced in the presence of *Pistacia lentiscus* oil, unsaponifiable oily fraction and Madecassol® treatments compared to the control, untreated animals. Furthermore, wound healing potentially effect was more pronounced in case of the oily unsaponifiable fraction-treated group compared to the others groups. Results clearly substantiate the healing potential effect on wound of a topic application of the *Pistacia lentiscus* fruits fatty oil and its unsaponifiable fraction.
KEYS WORDS : *Pistacia lentiscus*, dermal wound, oil, healing activity

INTRODUCTION

Wound healing is a complex process that involves a series of biochemical and cellular reactions, beginning with homeostasis, repithelialisation, granulation tissue formation and remodelling of the extracellular matrix (1-4). Searches for safer and effective wound healing agents from medicinal plants have become more important areas of active research. According to traditional medicine, wounds have been treated topically with various medicinal herbs or their extracts since time immemorial. Many plants have been shown to possess therapeutic potential as promoters of wound healing. *Aloe vera* (5), *Centella asiatica* (6), *Pterocarpus angolensis* (7), *Channa striatus-cetrimide* (8), *Datura alba* (9), *Terminalia chebula* (10), *Cinnamomum zeylanicum* (11), *Pterocarpus santalinus* (12), *Phellinus gilvus* (13), *Napoleona imperialis, Ocimum gratissimum and Ageratum conyzoides* (14), *Butea monosperma* (15), *Cassia fistula* (16), *Plagiochasma appendiculatum* (17), *Embelia ribes* (18), *Sphaeranthus indicus* (19), *Tragia involucrata* (20), *Tephrosia purpurea* (21).

Pistacia lentiscus is a small tree very widespread in the whole of Mediterranean basin to the South-West of black Sea. Its use as a medicinal herb is known since antiquity (22). The mastic obtained by incising the trunk contains 30% of resin, an essential oil (2%) and a bitter principle. The leaf contains flavonoïds, an essential oil and tannins. The fruit is a small drupe, from which is expressed a fatty oil (23).

Pistacia lentiscus is well known medicinal plant in the Mediterranean region. All parts of the plants posses medicinal uses. The uses of its roots, leaves, fruits and

mastic have been reported in many traditional pharmacopoeias. However, medicinal virtues of the fatty fruit's oil are particularly known in North Africa, in the eastern region of Algeria to Tunisia. The fruit oil is used internally for respiratory allergies, externally to treat sore throats, and locally applied for wounds and burns (23,24).

The aerial parts of *Pistacia lentiscus* L. has traditionally been used in the treatmant of hypertension and possesses stimulant and diuretic properties (25-27). The leaves of the plant were found to have antimicrobial, antioxidant, hepatoprotective activities (28-32). The resin obtained from mastic *Pistacia lentiscus* is known as antimicrobial, antioxidant, antiatherogenic, anti-ulcer, antiproliferatif agent [33-39]. Pharmacological evaluation of this species has also revealed an essential oil with an anti-inflammatory, antifungal, antibacterial and antioxidant activities (40-44).

Despite its wide use in North Africa traditional medicine as a well known wound and burn healing traditional remedy, a little has been done on pharmacological evaluation of the fatty oil of *Pistacia lentiscus* fruits. This present study is the first report dealing with the in vivo experimental evaluation of the wound healing effect of *Pistacia lentiscus* fruit's oil.

MATERIAL AND METHODS

Plant materials and fatty oil expression

Pistacia lentiscus L. (Anacardiaceae) was selected as a potential wound healing popular remedy based on ethnopharmacological information, provided by the local communities. Fresh ripe fruits (1 kg) of *Pistacia lentiscus* L were collected in mid November 2006 from the locality of Mila (50 miles North West of Constantine, Algeria). Plant was properly identified and voucher specimen (n° PL1106) was deposited in pharmacognosy and botany laboratory of the faculty of medicine, Mentouri University of Constantine (Algeria). The freshly harvested fruits of *Pistacia lentiscus* were extracted according to traditional manner, by cold expression using a traditional screw press to deliver, after filtration, clear green yellowish oil (225 g).

Saponification of oil

An oily fraction (50 g) was added to 200 ml of alkaline alcoholic solution (KOH 0.5N) and heated for 1 hour in a reflux set. After cooling, alcohol was removed under reduced pressure using a rotavapor. 100 ml of distilled water was then added to the remaining residue and the aqueous solution was exhaustively extracted with freshly distilled diethyl ether (3x150 ml) to remove completely the organic fraction. The latter was dried over anhydrous sodium sulphate Na_2SO_4 before total evaporation of the organic solvent to deliver 1.54 g of unsaponifiable fraction, denote thereafter (UNSAP). The remaining aqueous fraction was acidified to neutral pH and extracted with distilled diethyl ether (3x150 ml) to remove completely the free fatty acid containing saponifiable fraction (18.87 g) , referred to thereafter as (SAP).

Animals

Adult male albino Wistar rats weighting 300 ± 20 g were used. Animals were fed on a commercial pellet diet and water ad libitum. All rats were acclimatized to laboratory hygienic conditions 10 days before starting the experiment.

The animals were divided in six groups of five (05) animals each: group I: Control (untreated group, CT), group II: Madecassol-treated group (MAD), group III: Pistacia fruit's oil-treated rats (OIL), group IV: Saponifiable oily fraction-treated rats (SAP), group V: Unsaponifiable oily fraction-treated rats (UNSAP) and group VI: Paraffin oil-treated rats (PRF as Vehicle).

The study was carried out following the guidelines of the principals of Laboratory animal Care "Guide for the Care and Use of Laboratory Animals" (DHHS, 1985)

Excision wound model

Twenty four (24) hours before the beginning of the wound healing experiment, the dorsal skin of rats were (was) shaved. After 24 hours, all animals were anesthetized by intramuscular injection of Ketamin chlohydrate (15 mg/kg). A predetermined dorsal area (0.59 in x 0.39 in**)** was first sterilized (70% alcoholic solution) and was then inflicted by cutting away a full thickness of skin. The wounds were left undressed to the open environment and the animals were kept individually in separates cages.

Wounds treatment

Animal were treated with a daily topic administration of 15 mg/kg from each of *Pistacia lentiscus* oil (OIL), saponifiable (SAP), unsaponifiable fraction (UNSAP), Madecassol® (MAD) as reference healing drug (45-47) and paraffin oil as vehicle (16,19). The used Pistacia based phytopreparations (OIL, SAP, UNSAP) were dissolved in paraffin oil (10 %, v/v) before testing. 50 µl of each tested products was applied slowly, using an insulin syringe, from the central point extending outside the wound area to ensure inclusion of the edges.

Measurement of wound area

Throughout the experimentation period (26 days), measures of wound area were made on days 0, 2, 6, 10, 14, 18, 22 and finally 26 days using a electronic

digital calliper (Fukuoka Japan); precision 0,001 inch) and tracing wound margin using a transparent paper (20). Percentage wound contraction was determined using the following formula (21):

P.W.C. = [Healed area / Original wound area] x 100.

Wound areas were photographed documented using a professional AGFA780c camera equipped with macro zoom.

Chemicals

Paraffin liquid was purchased from Grifrer Barbezat, Pharmacien, Decines (Rhône), Madecasol® (powder 2%) from Ind. Farmaceutica, Nova Argentia, Ketamine hydrochloride injection from Bioniche, Canada. All chemicals were of analytical grade.

Statistical analysis

Results are expressed as mean ± SD. of at five animals in each group. The results were analyzed statistically using Student's *t*-test to identify the differences between groups. The data were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Healing was assessed by morphological evaluation of the wound at regular time intervals for a period of 26 days. Contraction of wound for treated and untreated animal groups were measured and results were expressed as percentage wound contraction (P.W.C.) and shown in table 1 and figure 1.

At day 2 after wounding, no significant wound healing effect was observed for treated and untreated groups.

Values of PWC, observed at 6, 10, 14 and 18, clearly indicates that evolution of healing process is significantly ($P<0.05$) potentiated in case of UNSAP-treated group respectively (36.0, 53.5, 63.0, and 86.0 %) and at a lesser extend for OIL-(15.7, 30.4, 64.0 and 92.0 %) and MAD-(16.0, 19.5, 58.0 and 88.5) treated groups, when compared to control CT- (16.5, 14.5, 38.5 and 65.0 %) and paraffin oil PAR-(15.0, 20.0, 35.2, 74.6) groups.

Its worth noting, that at time interval of 6 to 10 days after wounding, the values of PWC no significantly varied in case of CONT- (from 16.5 to 14.5 %), MAD-(from 16.0 to 19.5%), SAP-(from 20.6 to 16.0%) and PAR-(15.0 to 20.0%) treated groups. However for the same period (6 to 10 days), PWC values was significantly upward for UNSAP-group (from 36.0 to 53.5%, $p<0.01$) and OIL-treated group (from 15.7 to 30.4%, $p<0.05$). Furthermore, daily visually observations indicates the presence of signs of infection around wounds area of CONT-, MAD-, SAP and PAR-treated rats, and absence of such infections for UNSAP- and OIL-treated groups.

Finally, from 22 to 26 days, PWC values evolution in different groups become no statistically different. Time for wound closure for different groups (treated and untreated rats) are comparable and all showed effective wound healing process (96% - 99.8 %) after 26 days treatment

Results of area measurements, expressed as PWC, showed in table 1 and figure 1 clearly indicate a wound healing potentiating effect in *Pistacia lentiscus* fruit's oil (OIL) and its unsaponifiable fractions (UNSAP) treated rats. However, this effect seems not clearly established in case of oily saponifiable (SAP) fraction.

Healing potentiating effect of UNSAP-treated animals is distinct and more pronounced, from 6 to 10 days after wounding, contrasting to the rest of groups where the healing process was distinctly slowed, as confirmed by visually observation of wound animals. Inspection of wound area confirmed the presence of signs of infection in most animals, except those treated with fruit's oil and oily unsaponifiable fraction. Probably this observation is due to the presence of potential anti-microbial effect of oily unsaponifiable fraction of the *Pistacia lentiscus* fruits. This may justify the light observed advantage of the oily unsaponifiable fraction with regard to the Madecassol as reference healing agent. Antimicrobial effect is reported from leaves, mastic gum and essential oil of *Pistacia lentiscus* (30,34,42,43). No report of study in literature is concerned by antimicrobial effect of the fatty oil or its unsaponifiable fraction.

Morphological evaluation indicates that healing potentiating effect of oily unsaponifiable fractions was specifically observed in period from 6 to 18 days after wounding.

According to previous studies, this period (6 to 18 days) might represent the proliferative phase of the healing process (3,4,48). This step might be affected by phytochemicals from the UNSAP-fraction. Preliminary phytochemical investigations of the latter fraction has revealed the well-know unsaponifiable containing compound, such as tocopherols and phytosterols. Many biological and pharmacological activities have been associated with such compounds. In particularly, phytosterols have been previously found to exhibit anti-inflammatory and anti-oxydant activities (49,50) and tocopherols (e.g. vitamine E) are major anti-oxydant agents in cellular membranes and plasmatic lipoproteins and have hypolipimiant and immunostimulant activities (51,52). Its worth asking whether such compounds are implicated in the wound

Table 1: The percentage wound contraction (P.W.C.) in control (CT) and [lentisc oil (OIL), constituents (UNSAP and SAP), Madecasol (MAD), Paraffin oil (PRF)- treated rats at different days (during wounding period).

Day	CT	PRF	MAD	OIL	UNSAP	SAP
0	0	0	0	0	0	0
2	03.0 ± 6.5	05.0 ± 5.5	00.0 ± 12.1	08.5 ± 3.5	05.5 ± 8.5	05.5 ± 8.5
6	16.5 ± 10	15.0 ± 10.1	16.0 ± 7.1	15.7 ± 7.5	36.0 ± 2.3 * # ■■	20.6 ± 10.0
10	14.5 ± 7.3	20.0 ± 2.5	19.5 ± 6.5	30.4 ± 8.5 * □	53.5 ± 7.5 ** ## ■□□	16.0 ± 8.1
14	38.5 ± 9.5	35.2 ± 2.0	58.0 ± 4.5 * ##	64.0 ± 8.4 ** ##	63.0 ± 8.5 ** ##	58.5 ± 8.5 ##
18	65.0 ± 9.4	74.6 ± 3.3	88.5 ± 0.6 ** #	92.0 ± 5.5 ** ##	86.0 ± 2.5 * #	71.0 ± 7.5
22	85.0 ± 7.2	96.3 ± 1.5	97.0 ± 4.5	98.0 ± 4.5	97.0 ± 0.7	89.1 ± 7.0
26	96.0 ± 4.1	99.7 ± 0.1	99.5 ± 0.5	99.8 ± 0.2	99.8 ± 0.2	97.2 ± 0.2

Values are expressed in mean ± S.D. for five animals in each group.

P< 0.05: as compared with control (CT) using student's t-test

** p < 0.05, ** p< 0 .01: as compared with control (CT), # p< 0.05, ## p< 0.01: compared to PRF-treated group,*

■ p< 0.05 ■■ p<0.01: as compared to OIL and MAD treated groups, □ p< 0.05; □□ p< 0.01: compared to day 6.

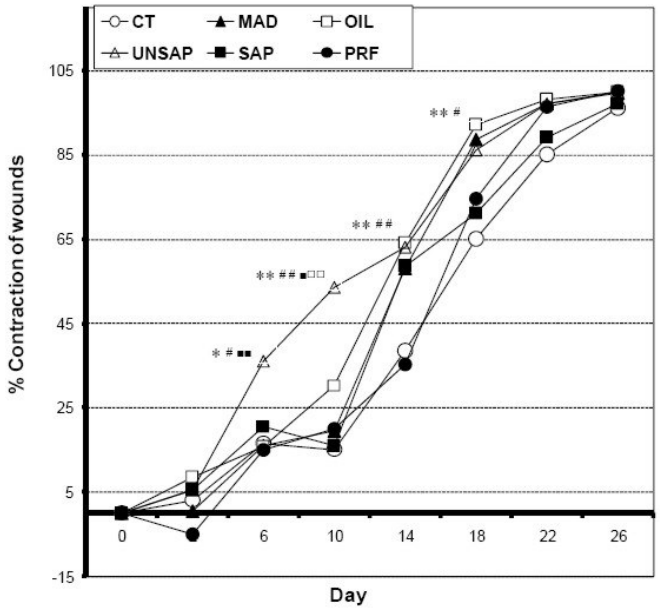

Figure 1. The percentage wound contraction (P.W.C.) in control (CT) and treated rats at different days. Values are expressed in mean ± S.D. for five animals in each group. P < 0.05; as compared with control (CT) using student's t-test

** p < 0.05, ** p< 0 .01: As compared with control (CT).*

 # p < 0.05; ## p< 0.01: compared to PRF-treated group.

■ p< 0.05; ■■ p<0.01: compared to OIL and MAD treated groups.

□□ p< 0.01: compared to day 6.

healing process observed particularly for the UNSAP-fraction treated rat.

CONCLUSION

It may therefore be concluded that under present working condition *Pistacia lentiscus* fruit's oil and particularly its unsaponifiable fraction have been determined as active healing agent. This potentiating effect is probably associated with UNSAP fraction containing compounds. Studies are underway to establish nature of the bioactive photochemicals associated with the observed antimicrobial and healing effects and the mechanism involved.

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