Safety and efficacy of *Pistacia lentiscus* L. fruit’s fatty oil for the treatment of dermal burns: A synthesis report

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Article History: Received 16th September 2013, Revised 8th December 2013, Accepted 10th December 2013.

Abstract: The aim of this study was to assess the effect of *Pistacia lentiscus* fatty oil (PLFO) on healing process and its safety to the skin and mucous membranes in the rabbit model. The cicatrizing activity was evaluated in 6 NZW rabbits; 4 equal burns were realized on the back of each animal. The wounds treated with oil were compared to these treated in Vaseline, Madecassol® and untreated wounds. PLFO has shown promising healing properties by reducing the inflammatory phase, stimulation of wound contraction and reducing the epithelization period compared to the different controls. Regarding possible adverse effects of PLFO, some toxicological tests were undertaken according to OECD Testing Guidelines. The results showed that this oil is only slightly irritating to the eye and to the skin, either intact or abraded after a single dose. In the repeated dermal toxicity test, PLFO has shown a reversible slight erythema after the second week of application. One third of rabbits had presented a reversible local sensitization with skin thickening. Regarding repeated toxicity via rectal route, PLFO was well tolerated. No anatomical or functional disturbance was observed in biochemical analysis, pathological examinations and histology. The study concludes that *Pistacia lentiscus* fatty oil is effective for healing burns. It is tolerable in the short term, but may cause skin sensitization after prolonged use.

Keywords: *Pistacia lentiscus*; fatty oil; burns; wound healing; irritation; toxicity.

Introduction

Skin wound healing is a complex and dynamic process involving a sequence of integrated events including inflammation, granulation tissue formation, matrix deposition and remodeling (Sehn et al. 2009; Varedi & Englander, 2006). The search for natural compounds which can stimulate tissue repair has gained importance in the recent years aiming the development of non-toxic formulations for wound treatment (Sehn et al. 2009).

*Pistacia lentiscus* fatty oil (PLFO) is a fixed oil extracted from *Pistacia lentiscus* fruits (black berries). Medicinal virtues of this fatty oil are particularly known in North Africa, in the eastern region of Algeria to Tunisia. The people of these regions have used this fruit’s oil externally to treat sore throats, locally to remedy burns and wounds and internally for respiratory allergies (Boukef and Souissi 1982). It is also recommended for varicose veins and circumcision.

This study aims to assess the effect of PLFO on healing process and its safety to the skin and mucous membranes in the rabbit model.

Materials and methods

The New Zealand white rabbit is the animal model used in the different biological stages of this study including:

The oil of *Pistacia lentiscus* L was extracted from its fruits in a traditional press in different steps. After the filtration process, the fatty oil obtained was stored in vials away from light until use (Figure 1).

PLFO effect on experimental burn wound’s using the technique described by Hamdi Pacha et al. (2002). Four equal burns were realized on the back of each animal (Figure 2). The first
wasn’t treated; the others were covered, once daily until complete healing by Vaseline, Madecassol® or PLFO. The percentage of wound contraction was assessed (Srivastava and Durgaprasad, 2008) and the different healing times were noted (Djerrou et al. 2010).

The Eye irritation/corrosion test, in accordance with OECD guideline No. 405 (OECD, 2002). The study was conducted on 3 New Zealand rabbits (Djerrou et al. 2013).

Acute toxicity: Dermal irritation/corrosion, according to OECD guideline No. 404 (OECD, 2002). The study was conducted on 6 New Zealand rabbits (Djerrou et al. 2013).

Repeated dermal toxicity, at the dose of 1ml/kg (Limit test) for 28 days, in accordance with OECD guideline No. 410 (OECD, 1981). The study was conducted on 18 New Zealand rabbits; 6 for the control group and 12 for the tested group. 6 animals from the tested group were considered as satellite group (Djerrou et al. 2013).

Repeated toxicity via rectal route, at the dose of 1ml/Kg for six weeks (O.J. 1984). The study was carried out on 12 New Zealand rabbits. After the acclimatization period, the animals were randomly divided on two groups. The rabbits of tested group (PLFO group) were treated once daily, 5-day per week, at the dose of 1 ml/Kg body weight, via rectal route for 6 consecutive weeks and were compared with untreated animals (CRL group) (Djerrou et al. 2011).

The results obtained from these previous tests (toxicity studies) were discussed in light of OECD guidelines and other guidelines and studies described elsewhere (CPMP, 2001; Draize et al., 1944; EPA, 1998; MHLW, 2002). All the steps of these studies were approved by faculty of sciences, University of Constantine, Algeria.

Figure 1: Extraction of *Pistacia lentiscus* fruit’s fatty oil.
Statistical analysis

Data obtained from body weight measurements, hematological analysis were expressed as mean ± SD and were evaluated by one –way ANOVA. Non parametric variables were expressed as median ± IQR and were evaluated with Mann-Whitney U test. The level of significance was set at 5%.

Figure 2: Localization of the 4 wounds on the rabbit’s back.

Results and discussion

PLFO has shown promising healing properties by reducing the inflammatory phase, stimulation of wound contraction and reducing the epithelization period. The epithelization period was significantly shortened (p<0.05) in tested oil (30±3.94 days) compared to the different controls (VAS group with 34.66±3.88 days and CRL group with 37.16±3.54 days) (Figure 3). No significant difference was obtained between PLFO and MAD groups (30±3.94 and 33.5±3.78 days respectively). This healing property may be attributed to the unsaponifiable fraction of this oil dominated by tocopherols known as a powerful antioxidant. The saponifiable fraction dominated by three fatty acids (oleic, linoleic and palmitic acids) may also potentiate the effect of the first fraction. Other studies are required to understand the role of the different components in the healing process (tocopherols, sterols, phenolic components, unsaturated and saturated fatty acids).

The test of the Eye irritation / corrosion (Figure 4) showed that this oil is only slightly irritating to the eye of rabbits. The results registered in table 1 showed that individual ocular index has not exceeded 8. The mean ocular index at 1h was 4.33, noting that the ocular irritation index calculated per zone was 0 in cornea and iris. It is only the conjunctiva which has reacted with a rating of 4.33 at 1h.

Similarly, the acute toxicity test: Dermal irritation/corrosion allowed us to classify PLFO as slightly irritating to the skin; either intact or abraded (Table 2).

Figure 3: Epithelization period of the different groups.

CRL= Control (No treatment), VAS= Vaseline, MAD= Madecassol®, PLFO= Pistacia lentiscus fatty oil. Values are expressed as median ± IQR (n = 6).

*= PLFO versus CRL: significantly different at P. ≤ 0.05, @=PLFO versus VAS: significantly different at P. ≤ 0.05, &= PLFO versus MAD: Non significant at P. ≤0.05.

Figure 4: Eye of rabbit after instillation of Pistacia lentiscus fatty oil.
Table 1: Score of ocular irritation (Djerrou et al. 2013).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Sum of I.O.I</th>
<th>O.I.</th>
<th>Score / zone</th>
<th>Conj.</th>
<th>Iris</th>
<th>Cornea</th>
<th>Conj.</th>
<th>Iris</th>
<th>Cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>26</td>
<td>4.33</td>
<td>0.33</td>
<td>4.33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24h</td>
<td>2</td>
<td>0.33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

I.O.I. = individual ocular index, O.I. = ocular index, conj. = conjunctiva.

Repeated dermal toxicity test showed that this oil produced no edema, but a slight erythema was observed after the second week of application (Figure 5). This phenomenon is growing and regressing alternately until the end of the experiment. Noting that the erythematic index does not exceed the rating of 2. One third of rabbits had presented a phenomenon of local sensitization with skin thickening, which could reach 100% of the untreated skin regions. This phenomenon had been perfectly reversible and the skin has found its normal texture in a few days.

Regarding repeated toxicity via rectal route, PLFO was well tolerated (Table 3). No anatomical or functional disturbance was observed in pathological examinations and histology. The biochemical parameters showed a significant decrease in AST and ALT enzymes finding a positive impact of this oil on liver function. A significant increase in blood glucose, but still within the range of physiological norms, was recorded in suggesting a possible anti glycogenesis effect (Figure 6).

Table 3: Clinical signs and body weights of New Zealand male rabbits treated by PLFO for 6-weeks via rectal route.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Clinical signs</th>
<th>Time after administration (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of animals with abnormality (Hypo activity)</td>
<td>1</td>
</tr>
<tr>
<td>CRL group (n=6)</td>
<td>Body weight (MN ±SD)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weight gain (g/rabbit)</td>
<td>1980±140</td>
</tr>
<tr>
<td>Test group (n=6)</td>
<td>(1ml abnormality (Hypo activity))</td>
<td>0</td>
</tr>
<tr>
<td>PLFO/Kg BW</td>
<td>Number of animals with abnormality (Diarrhea)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weight gain (g/rabbit)</td>
<td>990±72.5</td>
</tr>
<tr>
<td></td>
<td>Erythema of anus (-,+;++++)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Body weight (MN ±SD)</td>
<td>1930±115</td>
</tr>
<tr>
<td></td>
<td>Number of animals with rectum lesions</td>
<td>0</td>
</tr>
</tbody>
</table>

MN: mean, SD: standard deviation, -: no erythema, +: slight erythema, ++: moderate erythema, +++: very erythema.

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Figure 7: Comparison of some biochemical parameters in the plasma of rabbits from control group (CRL) and rabbits treated rectally in Pistacia lentiscus fatty oil (PLFO). (A) Enzymatic activities of ALT and AST. (B) Glucose level. (C) Creatinine concentration. (D) Urea concentration. (E) Total protein concentration. (F) Albumin concentration. Values are expressed as means ± SD (n = 6).

* P<0.05, *** P<0.001 vs. control group.

Conclusion

The present study has confirmed a traditional use of PLFO in the management of dermal burns and ascertains its use topically at least in short term. We can conclude that Pistacia lentiscus fatty oil is effective for healing burns by reducing the inflammatory phase, stimulation of wound contraction and reducing the epithelization period. It is tolerable in the short term, but may cause skin sensitization after prolonged use.
References


