ANTI-INFLAMMATORY ACTIVITY OF SEED OILS OF *OPUNTIA FICUS-INDICA* L. AND *PUNICA GRANATUM* L. FROM MOROCCO

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ABSTRACT

*Opuntia ficus-indica* L. and *Punica granatum* L. seeds are widely used in Moroccan traditional medicine. The aim of this study is to evaluate the anti-inflammatory activity of the seed oils from this two plants to enhance its use in folk medicine. The seed oils was extracted in a soxhlet apparatus and the acute toxicity was evaluated by oral administration to swiss female mice according to the OECD guidelines 423 and The anti-inflammatory activity was evaluated in carrageenan and experimental trauma-induced inflammatory hind paw oedema in wistar rats. Our results showed that in the acute toxicity studies of *Opuntia ficus-indica* L. and *Punica granatum* L. seed oils did not produce mortality or changes in general behaviour of the test animals on the other hand the seed oils of the two plants had significant anti-inflammatory activity comparable to the control and reference drug used in both models. Owing to the anti-inflammatory properties of pomegranate and prickly pear seed oils. The studies can be further extended to exploit them for their possible application for the treating inflammation and pain in Morocco as well as their use in product application like pharmaceuticals, nutraceuticals and cosmeceuticals in Morocco.

INTRODUCTION

Opuntia ficus-indica L. known as prickly pear is a cactus plant member of the Cactaceae family[1] grows everywhere in Morocco, originated from Mexico, was introduced into North Africa in the 16th century.[2] Many species of Opuntia produce edible fruit and very fragrant.[3] Cactus pear is mainly consumed as fresh fruit and grows wild in arid and semiarid regions, where the production of more succulent food plants is severely limited. Low water exigency and a high water use efficiency ratio favor the extension of cactus production, as underlined by the Food and Agriculture Organization.[4] In traditional medicine, prickly pear fruits have been used for their hypoglycemic and hypolipidemic actions. Some authors have attributed these beneficial effects to high contents of fibers in these fruits.[5] Opuntia ficus-indica L. is used in other regions as laxative, anti-inflammatory, or against diarrhoea.[6] It is also antacid, astringent, antidermatosic, emollient, antiseptic, vulnerary, analgesic, expectorant, antiviral, sedative, antioxidant.[7]

On the other hand the pomegranate or Punica granatum L., belongs to the Punicaceae family is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States.[8] Fruits, peels and roots of pomegranate have been commonly used in herbal remedies by local healers in many countries. Pomegranate peels have been used in traditional medicine for treating diarrhea and dysentery.[9-10] In Yemen and other countries of the Arabian Peninsula, dried peels have been traditionally used for treating diarrhea, stomachache and for healing wounds. In this regard, astringency is a known pharmacological property of tannins.[11-12]

The current study was carried out to assess the safety and to evaluate the anti-inflammatory activity of P. granatum L. and O. f-indica L. seed oils in suitable experimental animal models.

MATERIAL AND METHODS

Plant Material

The fruits of Opuntia ficus-indica L. from Aït Baamrane region and Punica granatum L. from Ain Taoujdate (Meknès region) where cultivated in the experimental field of the National Institute for Agricultural Research (INRA) were collected respectively, in September and October 2012.
Extraction of the Oil
The fruits were peeled then seeds were isolated by pressing the whole edible pulp. The powdered sample (20 g) was placed into a thimble in a soxhlet apparatus and was extracted with petroleum ether until exhaust. The extract was concentrated under reduced pressure at 40-60°C using a rotary vacuum evaporator. Oil obtained from the seed was stored in a refrigerator at - 4°C and kept in air tight container protected from light until used.

Animals
Adult swiss mice (25–30 g), 8-10 weeks of age and adult wistar rats weighing 150 to 200g, were obtained from the animal centre of Mohammed V Souissi University, Medicine and Pharmacy Faculty, Rabat, Morocco. The animals were maintained under standard conditions at 23 ± 1°C and relative humidity 60 – 70 % and 12h-dark/12h-light cycle. They were housed in groups of six animals in standard cages containing a supply of pellet diet and ad libitum water. The animals submitted to oral administration of the seeds oil or drugs were fasting for 18 hrs before the experiment (water was available). All studies were conducted between 9 a.m. and 1 p.m. The experiment was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509.[13,14,15,16]

Acute Toxicity
The acute toxicity studies were carried out based on OECD guidelines 423. Twelve healthy young adult female Swiss mice, nulliparous, non-pregnant divided into 4 groups, were designed for study of acute toxicity via the oral route. Each group of 3 mice received, respectively, a single oral dose of 5000 mg/kg body weight of Opuntia ficus-indica L. and Punica granatum L. seeds oil. Female mice were chosen because of their greater sensitivity to treatment.[17] Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the mice were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes.

Anti-Inflammatory Activity
In present study anti-inflammatory activity was determined in wistar rats according to the method that used chemical stimuli (winter test) and mechanical stimuli (Riesterer and Jaques test) induced paw oedema in rats.
Carrageenan-Induced Rat Paw Oedema
Using six animals in each group. The animals were injected carrageenan (0.05 ml of 1% w/v of fresh carrageenan suspension in 0.9% saline) in the left hind foot under the plantar aponeurosis.\textsuperscript{18}

The test groups of rats were given orally 200 and 300 mg/kg of seeds oil of the two plants one hour before the carrageenan injection. Indomethacin (10 mg/kg, p.o.) was administered orally as reference drug while distilled water (5ml/kg, p.o.) was used as negative control. The inflammation was quantitated in terms of ml i.e. replacement of water by oedema using a plethysmometer Digitals 7500 immediately before carrageenan injection and then 1h 30, 3h and 6 hour after carrageenan injection. Mean differences of treated groups were compared with the mean differences of the control group.

Percent inhibition of the oedema was calculated as

\[
\text{\% of inhibition} = \frac{\text{v Left}_\text{control} - \text{v Left}_\text{treated}}{\text{v Left}_\text{control}} \times 100.
\]

\(v\) Left means volume of oedema on the left hind paw and \(v\) Right mean volume of oedema on the right hind paw.

Experimental Trauma-Induced Rat Paw Oedema
This assay was determined as described by Riesterer and Jacques test. Both plant extracts were given in the doses of 200, and 300 mg/kg. Control group received 5 mL/kg of distilled water and the standard group received the reference drug (Indomethacin 20 mg/kg). All the dose administered orally. One hour after oral administration of different substances dropping a weight of 50 g onto the dorsum of the left hind paw of all animals. The left hind paw is not treated; it is taken as a witness.\textsuperscript{19}

The anti-inflammatory activity was estimated volumetrically by measuring the mean increase in hind paw volume of rat with the help of plethysmometer digitals 7500 at 1 h 30 min, 3 h and 6 h after induction of inflammation.\textsuperscript{20} The percentage increase in paw oedema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula.
% of inhibition = mean [v Left _v Right] control - [v Left _v Right] treated / [v Left _v Right] control × 100.

v Left means volume of oedema on the left hind paw and v Right mean volume of oedema on the right hind paw.

**Statistical Analysis**
The results were expressed as mean ± SEM. The data were subjected to one-way ANOVA followed by student’s t-test. A value of p < 0.05 was considered significant.

**RESULTS**

**Oral Acute Toxicity Study**
Toxicity, safety, and efficacy data for any herbal preparation in suitable animal models as per regulatory norms can greatly help in predicting toxicity and providing guidelines for selecting a safe dose in humans. In this study Prickly pear and pomegranate seed oils extract did not exhibit any mortality up to the dose level of 5000mg/kg. So, the extract is safe for long term administration.

**Anti Inflammatory Activity**
From the results obtained the *Opuntia ficus-indica* L. and *Punica granatum* L. seed oils showed a significant P<0.05 anti-inflammatory activity comparable to the control and reference drug used in both models.

**Carrageenan-Induced Rat Paw Oedema**
We have tested the anti-inflammatory activity of the seed oils of *Opuntia ficus-indica* L. and *Punica granatum* L. in rats. We administered *per os* either vehicle (control group), seed oils of the two plants (200 mg/kg or 300 mg/kg) or Indometacin (10 mg/kg) 30 min before an oedema was induced in the rat-paw by subcutaneous injection of carrageenin.

The effect of *Opuntia ficus-indica* L. and *Punica granatum* L. seed oils on carrageenan-induced inflammation is shown in Tables 1 and 2.

The seeds oil of *Opuntia ficus-indica* L. at the dose level of 200 and 300 mg/kg decreased the oedema significantly (p<0.05) at 1hr30, 3 and 6 hours after administration of the seeds oil when compared to the control group. The effect was compared to the activity (p<0.05) produced by standard indomethacin at 1h 30, 3 and 6 hours after administration.
Pretreatment by *Punica granatum* L. significantly reduced the carrageenan-induced oedema in a dose dependent way from the dose 200–300 mg/kg, p.o. 1h30 after carrageenan injection, to reach a maximal inhibition at this time with the dose 200 mg/kg (35.11 %) and after 1h30 with the dose 300 mg/kg (51.36 %) afterwards, this effect progressively decreased.

Indomethacin pretreatment induced a significant inhibitory action on carrageenan induced oedema. This effect, sizeable from the first recording, increased slightly to reach a maximal inhibition after 6 h with the dose 10 mg/kg (67.00%).

**Experimental Trauma-Induced Rat Paw Oedema**

The seed oils of *Opuntia ficus-indica* L. and *Punica granatum* L. were evaluated for experimental trauma induced paw oedema anti-inflammatory activity in experimental animal model (Table 3 and Table 4). Doses of 200 and 300 mg/kg of the two plants seeds oil were significant inhibited in different reaction time. The inhibition was observed during the different exposures.

The maximal reduction of *Opuntia ficus-indica* L. was statistically significant (p<0.05) at 6 hours (reduction by 87.52%) at dose of 200mg/kg and at a dose of 300 mg/kg by (reduction 69.10%) on the other hand the maximal reduction of *Punica granatum* L. at 6 hours was 88.36% at dose 200mg/kg and 69.37% at dose 300mg/kg. These results are comparable to the standard drug Indomethacin at dose of 20 mg/kg p.o.

**Table 1: Effect of seed oils of Opuntia ficus-indica L. and Punica granatum L. on carrageenan-induced rat paw oedema.**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg P.O.</th>
<th>Mean volume of edema (left paw-right paw) induced by carrageenan (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h 30</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.265 ± 0.007</td>
</tr>
<tr>
<td>Indometacin:</td>
<td>10</td>
<td>0.028 ± 0.001</td>
</tr>
<tr>
<td><em>O. ficus-indica</em>:</td>
<td>200</td>
<td>0.073 ± 0.002</td>
</tr>
<tr>
<td><em>O. ficus-indica</em>:</td>
<td>300</td>
<td>0.103 ± 0.003</td>
</tr>
<tr>
<td><em>P. granatum</em> :</td>
<td>200</td>
<td>0.171 ± 0.006</td>
</tr>
<tr>
<td><em>P. granatum</em> :</td>
<td>300</td>
<td>0.125 ± 0.004</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=6), p.o.: oral route, n: number of animals per group, p< 0.05 statistically significant relative to the control and reference drug (Indomethacin).
Table 2: Percentage of inhibition of inflammation of seed oils of *Opuntia ficus-indica* L. and *Punica granatum* L. using carrageenan-induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg P.O.</th>
<th>Percentage of inhibition of inflammation induced by carrageenan (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>88.83 ± 0.677</td>
</tr>
<tr>
<td>Indomethacin : 10</td>
<td></td>
<td>72.49 ± 0.961</td>
</tr>
<tr>
<td>O. ficus-indica : 200</td>
<td></td>
<td>59.42 ± 1.253</td>
</tr>
<tr>
<td>O. ficus-indica : 300</td>
<td></td>
<td>35.11 ± 2.278</td>
</tr>
<tr>
<td>P.granatum : 200</td>
<td></td>
<td>51.36 ± 1.624</td>
</tr>
<tr>
<td>P.granatum : 300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=6; these results compared with standard drug (indomethacin 10mg/kg, p.o.) were administered by the oral route.

Table 3: Effect of seed oils of *Opuntia ficus-indica* L. and *Punica granatum* L. on experimental trauma-induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg P.O.</th>
<th>Mean volume of oedema (left paw-right paw) induced by experimental trauma (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.116 ± 0.003</td>
</tr>
<tr>
<td>Indomethacin : 20</td>
<td></td>
<td>0.035 ± 0.002</td>
</tr>
<tr>
<td>O. ficus-indica : 200</td>
<td></td>
<td>0.085 ± 0.003</td>
</tr>
<tr>
<td>O. ficus-indica : 300</td>
<td></td>
<td>0.095 ± 0.003</td>
</tr>
<tr>
<td>P.granatum : 200</td>
<td></td>
<td>0.003 ± 0.085</td>
</tr>
<tr>
<td>P.granatum : 300</td>
<td></td>
<td>0.095 ± 0.003</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=6), p.o.: oral route, n: number of animals per group, p< 0.05 statistically significant relative to the control and reference drug (Indomethacin).

Table 4: Percentage of inhibition of inflammation of seed oils of *Opuntia ficus-indica* L. and *Punica granatum* L. using experimental trauma-induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg P.O.</th>
<th>Percentage of inhibition of inflammation induced by experimental trauma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>70.50 ± 1.828</td>
</tr>
<tr>
<td>Indomethacin : 20</td>
<td></td>
<td>15.08 ± 1.448</td>
</tr>
<tr>
<td>O. ficus-indica : 200</td>
<td></td>
<td>16.13 ± 1.799</td>
</tr>
<tr>
<td>O. ficus-indica : 300</td>
<td></td>
<td>30.30 ± 1.273</td>
</tr>
<tr>
<td>P.granatum : 200</td>
<td></td>
<td>16.47 ± 1.361</td>
</tr>
<tr>
<td>P.granatum : 300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=6 ; these results compared with standard drug (Indomethacin, 20 mg/kg, p.o.) were administered by the oral route.
DISCUSSION

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs: Redness and heat are due to increased blood flow at body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes.[21]

In control animals, the subplantar injection of carrageenan produced a local oedema that increased progressively after the injection of the phlogistic agent. The rat-paw volume was measured 1h30, 3h and 6h after injection of carrageenin. The Indometacin, at a dose of 10 mg/kg, reduced significantly paw volume by 88.83 %, 71.57% and 67.00 % at 1h30, 3 et 6 hours respectively.

At a dose of 200 mg/kg Opuntia ficus-indica L. seeds oil inhibited significantly the development of oedema at 1h30, 3 and 6 hours (reduction by 72.49 %, 63.94 % and 92.01% respectively). Similar results were obtained with the dose of 300 mg/kg. On the other hand, the Punica granatum L. (200–300 mg/kg, p.o.) inhibited, in a dose-related manner, carrageenan-induced paw oedema in rats.

Result showed that, Punica granatum L. at doses 200 and 300mg/kg showed maximum percentage inhibition of rat paw oedema with 35.11 % & 51.36 % respectively at 1h30 as compared with reference drug indomethacin (P<0.05) with 88.83 % inhibition of oedema. On the other hand the evaluation of the anti-inflammatory activities of seed oils of the two plant by experimental trauma- induced rat paw oedema at 6 hours showed significant inhibition of rat paw oedema with 87.52% and 69.10% of Opuntia ficus-indica L. and (88.36% - 69.37%) of Punica granatum L. at dose 200 and 300mg/kg respectively as compared with reference drug indomethacin (P<0.05) with 89.82% inhibition of oedema.

Carrageenan and experimental trauma- induced rat paw oedema is a suitable experimental animal model for evaluating the anti-edematous effect of natural products [22] and this is believed to have two phases i.e. early phase (upto 2 hrs) and late phase (1-6 hrs). The early phase was associated with significantly severe inflammation; whereas late phase was observed to have slow increase in volume of paw oedema.

The initial phase has been attributed to the action of mediators such as histamine, serotonin & bradykinin on vascular permeability. The late phase oedema has been shown to be a result of
over production of prostaglandins. The anti-edematogenic mechanism of action of *Opuntia ficus-indica* L. and *Punica granatum* L. may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of indomethacin by Ferreira et al. (1973).\[^{23}\] Inflammatory pain results from the release of hyperalgesic mediators e.g. prostaglandins and catecholamines which are supposed to act by regulating the sensitivity of pain receptors.\[^{24}\]

The human medical potential of Opuntia and Punica depends on its chemical compositions. The anti-inflammatory activities may be due to their content of tannins, flavanoids, alkaloids, glycosides, saponins and carbohydrates.

In previous study the anti-inflammatory Mechanisms Cold pressed pomegranate seed oil has been shown to inhibit both cyclooxygenase and lipoxygenase enzymes in vitro. Cyclooxygenase, a key enzyme in the conversion of arachidonic acid to prostaglandins (important inflammatory mediators), was inhibited by 37 percent by a CPSO extract. Lipoxygenase, which catalyzes the conversion of arachidonic acid to leukotrienes, also key mediators of inflammation, was inhibited by 75 percent by a CPSO extract.\[^{25}\]

These results which suggest that the anti-inflammatory compounds of the seed oils of *Punica granatum* L. and *Opuntia ficus-indica* L., justify the rational use of this plant to prevent the inflammatory processes.

**CONCLUSION**

We can conclude that this study demonstrates the efficacy of *Opuntia ficus-indica* L. and *Punica granatum* L. seed oils as an anti-inflammatory agent and also scientifically justifies the use of this plant as an anti-edematous agent in folk medicine, however, further studies are required to determine the constituents responsible for its anti-inflammatory activity and further authenticate its mechanism of action.

**Conflict of Interests**

The authors declare that there are no conflicts of interests in this study.

**ACKNOWLEDGMENTS**

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REFERENCES


