Anti-inflammatory activity of acetonic extract of *Pistacia lentiscus fruits*

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Abstract : ***Pistacia lentiscus* L. (*P. lentiscus*) has traditionally been used as a stimulant, for its diuretic properties, and to treat hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice. In the present study, the anti-inflammatory effect of acetonic extract of P. lentiscus fruits was evaluated. The quantitative estimation of total phenolic compounds showed that the acetonic extract is rich in polyphenols (250.6 ± 14 µg GAE/mg of extract) and flavonoïds (20.6 ± 5.8µg QE/mg of extract). The anti-inflammatory activity of *P. lentiscus* was evaluated *in vivo* using the ear edema model induced by Croton oil and the air pouch model induced by Lambda carrageenan. Three treatments were used. The simultaneously use *of* the extract (3mg of extract/ear) with the irritant agent, the topic use of 3mg of extract/ear and oral administration of 300mg of extract/kg 1hour before the induction of the inflammation. Results obtained showed that *P. lentiscus* extract inhibited significantly the ear edema by the percentages of 68%, 72% and 80%, respectively. These inhibitions were statistically similar to the effect of indomethacin used as a standard anti-inflammatory agent. On the other hand, an inhibitory activity of leukocytes migration was observed in the murine air pouch exudate. The treatment by acetonic extract (1mg/pouch) decreased significantly the number of leucocytes in the air-pouch (34%). This inhibition was statistically similar to the effect of indomethacin. Finally, we can conclude that the acetonic extract of *P. lentiscus* fruits have a considerable anti-inflammatory activity, which support the use of this plant specially their fruits in folkloric medicine.**

Keywords: Inflammation, anti-inflammatory, *Pistacia lentiscus*, polyphenols, flavonoids

Introduction

Medicinal plants have received great interest in biomedical research. They are considered to be an important source of therapeutic compounds and the therapeutic benefit of many medicinal plants is often attributed to their anti-inflammatory and antioxidant properties (Shahidi, et al., 1992; Tunón et al., 1995). The preservative effect of many plant species and herbs suggests the presence of bioactive compounds such as flavonoids, phenolic acids, and phenolic diterpenes (Shahidi et al., 1992; Dutra et al., 2008). *Pistacia lentiscus* (*P. lentiscus*) Linn. (Family - Anacardiaceae), commonly known as mastic tree or mastagi, one of the many evergreen bushes found in the eastern Mediterranean region has a long tradition in folk medicine dating from the times of the ancient. *P. lentiscus* has traditionally been used as a stimulant, for its diuretic properties, and to treat hypertension, coughs, sore throats, eczema, kidney stones and jaundice (Palevitch and Yaniv, 2000). *P. lenticus* gum had a great effect in healing of mucosa, and its current use is limited to treating stomach aches, heartburn and respiratory problems (Ali-Shtayeh et al., 2000; Lev and Amar, 2002). Also, anti-*Helicobacter pylori* activity has been reported (Serafino et al., 2001). The extract of the different parts of the plant shows various activities like anti-atherogenic, anti-inflammatory, antioxidant, antimicrobial, hypotensive, anticancer, anti-arthritis and anti-gout and in treatment of wound, antiasthmatic and anthelmitic activity (Ansari and Siddiqui, 2012). Polyphenols from the leaves are gallic acid and galloylderivatives (Abdewahab et al., 2007), flavonol glycoside and anthocyanins (Romani et al., 2002). Traces amount of myrcetine derivative and catechin are also present (Kıvcak and Akay, 2005). Some reports

available in the literature, studying the antioxidant properties of extract from this plant (Baratto et al., 2003), as well as the total flavonoids content. A little additional research exists on *P. lenticus* anti-inflammatory effects. Therefore, the present study was designed to investigate and evaluate the pharmacological basis for the use of *P. lentiscus* fruits extract in folklore medicine for the treatment of inflammatory disorders. Thus, the anti-inflammatory activity of acetonic extracts of *P. lentiscus* fruits were evaluated on two models of acute inflammation, ear edema and air pouch.

Materials and Method

Chemicals and all reagents were purchased from fluka and Sigma (Germany) and were of analytical grade. The plant material, *P. lentiscus* fruits were collected in November 2010 from Skikda, Algeria. The plant material was identified and voucher specimen was deposited at the laboratory of botany in the University of Sétif, Algeria. The fruits were cleaned and frozen at -32 ° C until use. Swiss albino mice weighing 30–40 g were purchased from the Pasteur Institute of Algiers, Algeria. All animals were divided into different groups each consisting of 7 animals, and were allowed to acclimatize to the animal room conditions for 1 week and had free access to food and water *ad libitum*. Animals were fasted overnight prior the experiments.

The acetonic extract from *P.lentiscus* fruits was prepared by maceration of 150 g of crushed fruits with 100 ml of acidified acetone/eau (7:3 V/V) at 4°C for 24 h with frequent agitation. After filtration, the filtrate was concentrated under reduced preressure at 40°C. The residue was lyophilized using a lyophilizator (PHYWE chrisa) to give a dark purple powder.

Total polyphenol contents were estimated by Follin-Ciocalteu method (Li et al. 2007). Practically, 0.5µl of Folin (10%) was added to 100 µl of extract (0-0.5 mg ml) or gallic acid (used as standard), after 4 min, 400 µl of sodium carbonate Na_2CO_3 solution (7.5%) are added to the reaction medium. After 2 h of incubation in darkness at room temperature, the absorbance was measured at 765nm. Results were expressed as µg of Gallic acid equivalent/mg of extract (µg AGE/mg of extract).

The flavonoid contents were estimated by $AlCl_3$ method (Bahorun et al., 1996). One ml of the extract (0-0.5 mg/ml) was added to 1 ml of 2% methanolic $AlCl_3$. After 10 min of incubation, the absorbance was read at 430 nm. Results were expressed as µg of quercetin equivalent/mg of extract (µg QE/mg of extract).

The anti-inflammatory effect of acetonic extract from *P.lentiscus* fruits was studied using two models of acute inflammation, Croton oil-induced ear edema and ear pouch in mice.

Croton oil-induced ear edema mice was conducted in three ways:

1. Simultaneously use of the treatment with the irritant agent: croton oil-induced ear edema was performed according to the method of Manga et al. (2004). Cutaneous inflammation was induced to the inner surface of the right ear of mice (7 mice each group) by application of 15 µL of acetone containing 80 µg of Croton oil as irritant. Treated animals received topically 3 mg/ear of acetonic extract of *P. lentiscus* fuits. Indomethacin as reference drug was applied topically (0.5 mg/ear). The control group received topically Croton oil alone. The thickness of ears was measured before and 6 h after induction of inflammation using a digital micrometer. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges, and the thickness was recorded in micrometers. To minimize technique variations, a single investigator performed the measurements throughout each experiment. The edema was expressed as an increase in the ear thickness due to Croton oil application. The inhibition of the inflammation was calculated using the following equation:

Inhibition = ($\Delta T - \Delta E / \Delta T$) x 100, were ΔT: edema size of the control and ΔE: edema size of the treated group by the extract.

2. Topical pretreatment 1 hour before the induction of inflammation: A volume of 15 µl of acetone-water solution (1:1) containing 3 mg of extract or 0.5 mg of indomethacin were applied topically on the inner surface of the right ear of mice. One hour after application of the treatment, 15 µl l of acetone-water solution (1:1) containing 80 mg of Croton oil was applied locally on the inner surface of the right ear of each mouse. The control mice received only 15 µl of the solution of Croton oil. The ear thickness was measured before the treatment and then 4h and 6h after the induction of inflammation.

3. Oral pretreatment 1 hour before the induction of inflammation: Three groups of mice were respectively received orally 0.2 ml of saline solution (control group), 300 mg/kg of acetonic extract of *P. lentiscus* fruits and 50

mg/kg of indomethacin. One hour after, 15µl of acetone-water solution (1:1) containing 80 mg of Croton oil was applied locally on the inner surface of the right ear of each mouse. The ear thickness was measured before treatment and 4 h and 6 h after the induction of inflammation.

The air pouches were raised on the dorsum by subcutaneous injection of 3 ml of sterile air, as previously described (Colville-Nash and Lawrence, 2003). After 4 days, the pouches were re-inflated with 1,5 ml of sterile air. On day 7, inflammation was induced by injection of 0.1ml of Lambda carrageenan suspension 1% (w/v, in saline) into the air pouch under light chloroform anesthesia (Gambero, 2003). One hour after, 0.1 ml of the extract (1mg/pouch) or 0.1 ml of Indomethacin (0.15 mg/pouch) was injected. The control group received only 0.1 ml of Lambda carrageenan suspension. Four hours after the treatment, the mice were sacrificed by cervical dislocation. The pouches were flushed by 0.1 ml of PBS, pH=7.4, and vigorously massage for 30 sec. The pouches were opened with a small incision and the exudates were collected. The leukocytes in the fluid were counted using hemocytometer coulter (MINDRAY Auto Hematology Analyser).

All results were expressed as mean ± SEM. The statistical significant of the results as analyzed by the Student t-test with a value of <0.05 are considered significant.

Results

Results showed that the the acetonic extract of *P. lentiscus* fruits is rich in polyphenols (250.6 ± 14 µg GAE/mg of extract) and flavonoïds (20.6 ± 5.8µg QE/mg of extract).

In air edema model, the mice in the control group that received the solution of Croton oil alone developed after 4 h and 6 h an ear edema characterized by increased thickness of 81 ± 9 µm and 95 ± 6 µm, respectively. Mice treated group simultaneously with the irritant agent by local application of 3 mg/ear of the acetonic extract of *P. lentiscus* fruits induced highly significant reduction ($p < 0.001$) of inflammation compared to control mice. The thickness of the edema 4h and 6h after the induction of inflammation was 26 ± 2 µm after 4 and 23 ± 3 µm after 6h (Figure 1), which correspond to an inhibition of 68% and 76%, respectively. These values are statistically very close to those obtained with Indomethacin. Indeed, mice treated by Indomethacin showed a highly significant reduction ($p < 0.001$) in the inflammation. Edema thickness of the 4h and 6h after the induction of inflammation were 17 ± 6 µm and 15 ± 4 µm, respectively, which corresponds to an inhibition of 79% and 84%, respectively.

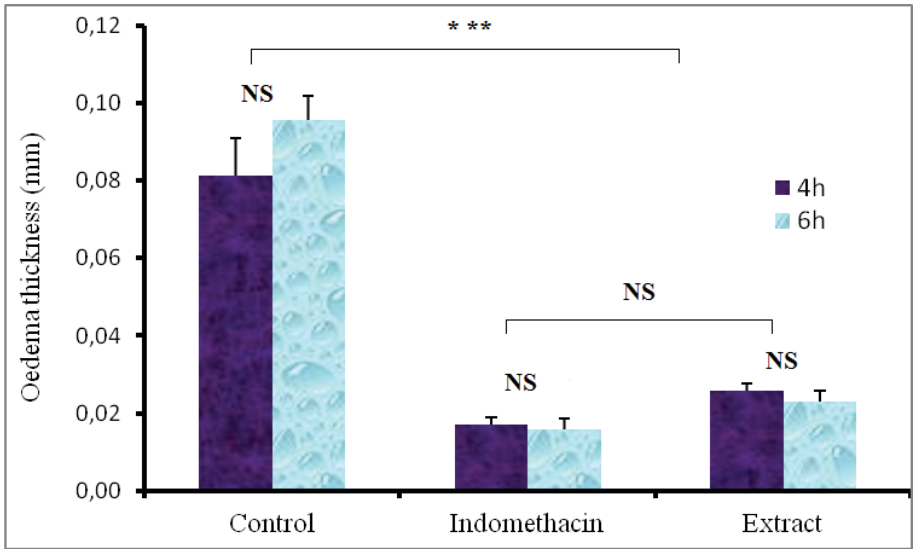

Figure 1. Effect of the acetonic extract of *P.lentiscus* fruits applied topically and simultaneously with the irritant agent on ear edema in mice. The edema was induced by topical application of 80 µg of Croton oil on the inner surface of the right ear of mice. Groups of mice were treated locally and simultaneously with croton oil by 3mg/ear of the extract or 0.5mg/ear of Indomethacin. The control group received the solution of Croton oil alone. Edema is expressed as the mean thickness increase of ears after 4h and 6 h of Croton oil application. Values are expressed as means ± SEM (n = 7). ***P < 0.001. NS: not significant versus the control.

A highly significant reduction (p <0.001) in ear edema is observed in mice locally treated by 3 mg/ear extract 1 hour before the induction of inflammation compared to control mice. The thickness of the edema was 23 ± 3 µm after 4 h and 21 ± 5 µm after 6 h, which correspond to an inhibition of 72% and 76%, respectively. These rates are statistically similar to those obtained with Indomethacin (Figure 2). In fact, a highly significant reduction (p <0.001) in ear edema is observed in mice treated locally by 0.5 mg/ear of Indomethacin 1 h before the induction of inflammation. The thickness of the edema was 11 ± 5 µm after 4 h and 10 ± 4 µm after 6, which correspond to an inhibition of 86% and 89%, respectively.

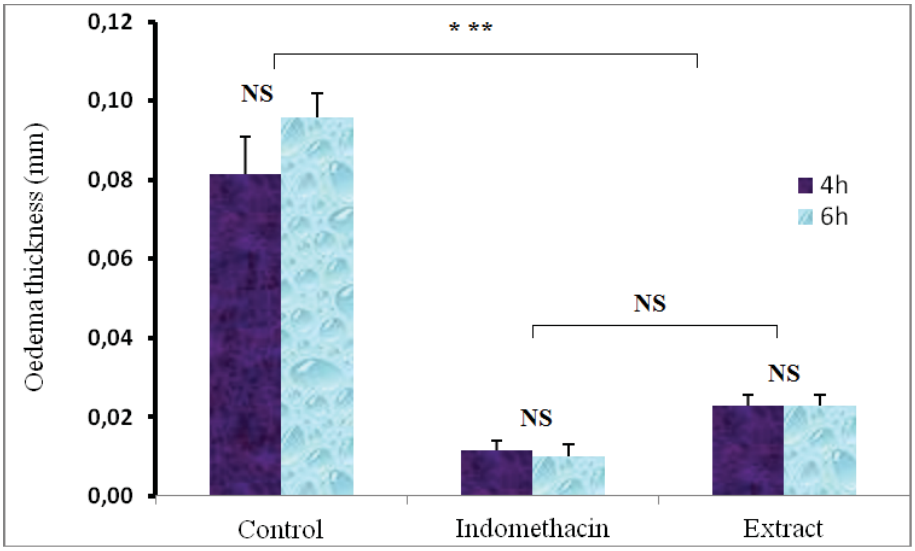

Figure 2. Effect of the acetonic extract of *P. Lentiscus* fruits applied topically 1 hour before induction of the ear edema in mice. The edema was induced by topical application of 80µg of Croton oil on the inner surface of the right ear of mice. Groups of mice were treated locally by 3 mg/ear of the extract or 0.5 mg/ear of Indomethacin 1 hour before the application of the irritant. The control group received the solution of Croton oil alone. Edema is expressed as mean thickness increase of ears after 4h and 6 h of the irritant agent application. Values are expressed as means ± SEM (n = 7). ***P < 0.001. NS: not significant versus the control.

In the oral pretreatment 1 hour before the induction of inflammation, treated mice by 300 mg/kg of acetonic extract *of P. Lentiscus* fruits induced a highly significant reduction (p <0.001) of ear edema compared with mice in the control group. The size of the edema was 16 ± 3 µm after 4 h and 14 ± 2 µm after 6h, which correspond to an inhibition of 80% and 82%. These rates are statistically similar to those obtained with Indomethacin (Figure 3). Indeed, treated mice with 50 mg/kg of Indomethacin 1 hour before the induction of inflammation showed a significant reduction (p <0.001) compared with the control group. The edema 4 h after induction of inflammation was 14 ± 4 µm and 13 ± 3 µm after 6 h, corresponding to an inhibition of 82% and 86%, respectively.

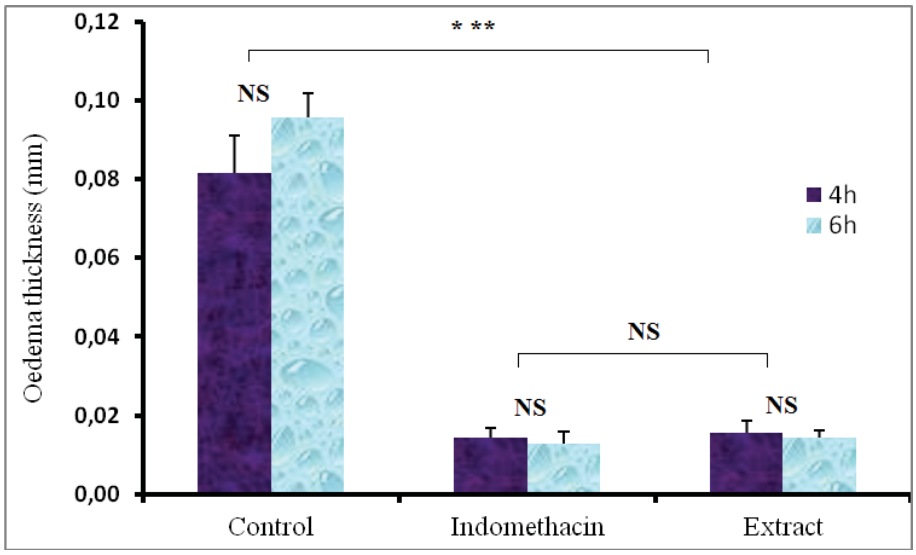

Figure 3. Effect of the acetonic extract of *P. Lentiscus* fruits applied orally 1 hour before induction of the ear edema in mice. The edema was induced by topical application of 80µg of Croton oil on the inner surface of the right ear of mice. Groups of mice are treated by oral administration of the extract (300 mg/kg) or Indomethacin (50mg/kg) 1 hour before the application of the irritant. The control group received the solution of Croton oil alone. Edema is expressed as mean thickness increase of ears after 4 h and 6 h of the irritant agent application. Values are expressed as means ± SEM (n = 7). ***P < 0.001. NS: not significant versus the control.

In air Pouch model, the mice of the control group developed after 4 h an inflammation with infiltration of leukocyte by 7.91 x 10^6 cells/ml of exudate. Treatment by 1 mg/pouch of acetonic extract of *P. Lentiscus* fruits induced a significant (P <0.01) reduction in the number of infiltrating leukocytes (5.24 x 106 cells/ml of exudates) compared to control mice. This value corresponds to an inhibition of 34%, which is similar to that of Indomethacin. Indeed, the number of leukocytes in mice treated by Indomethacin was 5.36 x 106 cells/ml of exudates after 4 h of induction of inflammation, which corresponds to an inhibition of 32% (Figure 4).

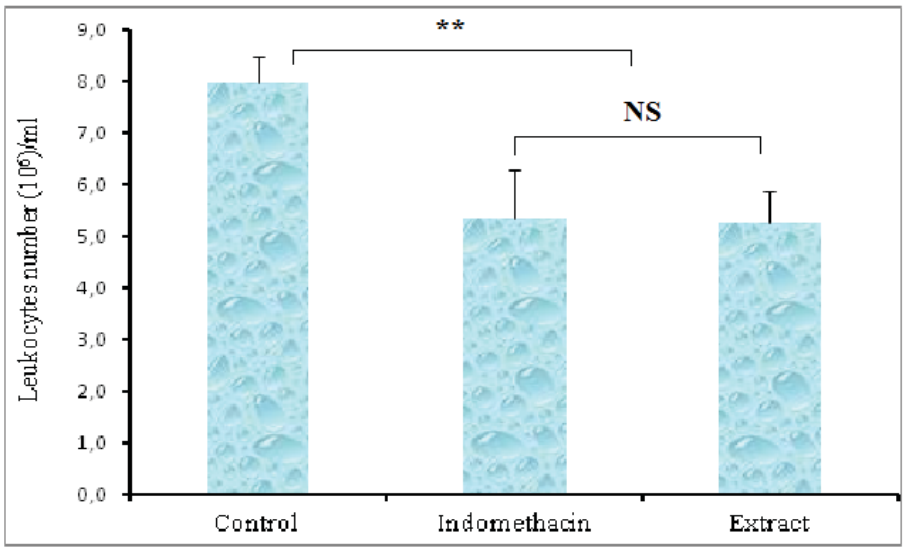

Figure 4. Effect of the acetonic extract of *P. Lentiscus* fruits on the number of infiltrating leukocytes in the exudates. The pouch inflammation was induced by carrageenan (1%). One hour after the induction of inflammation, mice were treated by injection of 1mg/Pouch of the extract or 0.15 mg/Pouch of Indomethacin. The control group of mice received only the solution of carrageenan. The comparison is made with respect to the control group. Values represent the mean ± SEM (n = 7). : ** P <0.01, NS: not significant (Student's t test).

Discussion

Acute inflammation is characterized by classical symptoms, such as heat, redness, swelling and pain. Edema is therefore a good measure of inflammation and is useful for the quantification inflammation induced by phlogistic agents such as croton oil. The Croton oil induced ear edema model has certain advantages for natural product testing and has a good predictive value for screening anti-inflammatory agents that could be useful in the treatment inflammatory disorders (Tunón et al., 1995). The effect of the irritant agent, croton oil, is due to the active 12-O-tetradecanoyl phorbol acetate (TPA) it contains. TPA induces an inflammatory response characterized by high production of pro-inflammatory mediators, increased vascular permeability, edema and neutrophil infiltration (Delaporte et al., 2004). These changes are triggered by protein kinase C, which promotes an increase in the activity of phospholipase A_2 (PLA_2) (Oskarsson et al., 1999). PLA_2 catalyzes the hydrolysis of membrane phospholipids archidonic acid, which is involved in the synthesis of eicosanoïdes, prostaglandins and leukotrienes, which constitute the first step in the inflammatory response (Serhan, 2009).

In the present study, the thickness of the edema is measured 4h and 6 h after topical application of croton oil to assess the anti-edematous effect of the acetonic extract of *P. lentiscus* fruits. Simultaneous treatment of mice with topical application of croton oil and the acetonic extract inhibited the edema formation similarly to the pretreatment by the extract topically or orally 1 h before the induction of the inflammation. This result indicates that there is no interaction between the irritant and the anti-inflammatory agent. The ear edema thickness (68-86%) measured 4h and 6h after induction of inflammation is not statistically significant. This means that 4 hours are sufficient to induce inflammation with croton oil. These results are in agreement with those obtained by Giner-Larza et al. (2002) who reported that the ethanolic extract of the fruits of *Pistacia vera* (a species of the same genus as *P. lentiscus*) inhibited edema induced by carrageenan in mice by a rate of 63%. The anti-inflammatory effect of the acetonic extract of the fruit of *P. lentiscus* is probably attributed to lipophilic soluble substances that are able to penetrate through the skin barrier (Okoli et al., 2007) and exerted its anti-inflammatory activity. Likely candidates for these anti-inflammatory substances are flavonoids, polyphenols, which were isolated from *P. lentuscus*. Indeed, the quantitative estimation of total phenolic compounds showed that the acetonic extract is rich in polyphenols (250.6 ± 14 µg GAE/mg of extract) and flavonoïds (20.6 ± 5.8µg QE/mg of extract), respectively. Phenolic compounds are known to interact with and penetrate through lipid bilayers (Rice-Evans, 2004). The anti-inflammatory effect of the acetonic extract of the fruit of *P. lentiscus* was similar to that of Indomethacin, used as standard anti-inflammatory agent. The mechanism of action of indomethacin on inflammation is based on inhibition of the synthesis of pro-inflammatory prostaglandins (Hull et al., 2003). The anti-inflammatory effect observed is also probably due to the presence of antioxidant compounds in the extract. Abdelwahed et al. (2007) reported that that gallic acid and 1, 2, 3, 4, 6-pentagalloylglucose, two polyphenols isolated from the fruits of *P. lentiscus* fruits exhibit antioxidant activity which is an additional advantage for the anti-inflammatory activity. In fact, Ying et al. (2010) reported that the content of flavonoids in plant extracts, allows them to act at several levels of the inflammatory response.

Leukocyte recruitment in inflamed tissues is essential to the inflammatory response to cleaning the home lesion and thereby adequate tissue repair. In contrast, the excessive leukocyte migration leads to inflammatory disorders. That is why we are interested in counting the cells in a household of acute inflammation, created by carrageenan in an air pouch in mice. The mice in the control group developed after 4h an inflammation at the pouch characterized by a high number of leukocytes 7.91 x 10^6 leukocytes/ml in the exudate. Carrageenan stimulates the release of histamine and serotonin from mast cells, initiating a cascade of events that produce other mediators that contribute to the development of the acute inflammatory reaction (Cuzzocrea et al., 1998). Mice treated by acetonic extract of *P. lentuscus* showed significant reduction (34%) in the number of leukocytes. This inhibition is similar to that of

Indomethacin, used as standard anti-inflammatory agent. Secondary metabolites in fruits of *P.lentiscus* inhibit leukocyte recruitment into the cavity of the air pouch probably by inhibiting the expression of adhesion molecules on endothelial cell as reported before (Anné et al., 1994). Moreover, it reported that anthocyanins and gallic acid inhibit leukocyte migration to inflammatory sites by inhibiting the adhesion molecules ICAM-1 and VCAM-1 and E-selectin in the vascular endothelial cells, this inhibition is due to inhibition of IL-1, TNF-α and NF-κB (Takatoshi et al., 1999; Calixto et al., 2003).

Conclusions

The acetonic extract of the *P. lentiscus* applied topically or administered orally has a good anti-inflammatory effect by inhibiting the development of ear edema and the recruitment of immune cells into the inflammatory site. The anti-inflammatory properties of *P. lentiscus* may be explained in part by the activity of the polyphenols and flavonoids present in its extract. This activities assign a potential role of *P. lentiscus* extracts in human health care and support the traditional uses of this plant in the treatment of inflammatory disorders.

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