Anti-inflammatory and immunomodulatory properties of *Pistacia lentiscus* extracts

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ABSTRACT

This study aimed to evaluate the anti-inflammatory activity of alcoholic and aqueous extracts of *Pistacia lentiscus* leaves. Croton oil-induced ear edema in mice, acetic acid-induced vascular permeability in mice and carrageenan induced-pleurisy in rats were conducted as acute inflammation models. Chemotaxis and elastase activity of human neutrophils were assayed *in vitro*. Results showed that local treatment with 2 mg/ear of alcoholic extract decreased significantly the ear edema (65%), while the aqueous extract exerted a lower inhibitory effect (51%). Moreover, the oral treatment with 200 mg/kg of alcoholic extract inhibited the vascular permeability by 46%, whereas the aqueous extract caused only 28% of inhibition. Furthermore, both extracts reduced significant the carrageenan induced-pleurisy. Indeed, at 400 mg/kg, the extracts inhibited the neutrophil migration by 29% and 38%, respectively, and reduced the number of the PMNS migrated into the pleural exudates by 49% and 43%, respectively. At 100 µg/mL, the methanolic and aqueous extracts inhibited neutrophil chemataxis by 81% and 71%, respectively, and reduced significantly the elastase activity with maximum values of 82% and 90%, respectively. These findings provide valuable evidence for the potential anti-inflammatory of *Pistacia lentiscus* leaves, suggesting that this plant can be exploited as a natural source of anti-inflammatory agents.

INTRODUCTION

Inflammation is frequently associated with vasodilation and increased vascular permeability, plasma exudation and leukocyte migration to the site of injury. The activation of the biochemical cascade of inflammation causes the release of inflammatory mediators such as cytokines, histamines, kinins, complement factors, clotting factors, nitric oxide, and proteases (Grover *et al.*, 2011). During the early phase (1 - 2h) of the inflammatory response, histamine and serotonin are released from mast cells, starting a cascade of events that produce other mediators such as leukotrienes, prostaglandins and platelet-activating factor (PAF). These factors cause vascular changes leading to plasma exudation and contribute to the establishment of the acute inflammatory response. During the late phase of the inflammatory process (4 - 12h), the chemoattractant factors induce the recruitment of leukocytes towards the inflammatory site (Cuzzocrea *et al.*, 1998). These cells are triggered to produce inflammatory mediators, which collectively contribute to tissue degradation by activation of several distinct host degradative pathways.

Polymorphonuclear neutrophils (PMNs) are the principal immunity cells which migrate to inflammatory sites, where they release of high quantity of elastase, which plays a key role in bacteria killing and host defense. However, the excessive migration of PMNs into the inflammatory site and the excessive elastase release, which can escape the regulatory systems, are associated with irreversible tissue damage. Elastase is involved in impairing host defense, injuring bronchial epithelial cells, and destroying components of extracellular matrix in lung and contributes to several inflammatory disorders such as acute respiratory distress syndrome and lung emphysema (Houghton, 2015).

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Many medicines of plant origin have been used to treat inflammatory disorders since long time without any adverse effects, and extensive researches prove that many plants represent a source of new compounds with anti-inflammatory activity. *Pistacia lentiscus* L. (*P. lentiscus*) belongs to Anacardiaceae family is an evergreen Mediterranean small tree or shrub (AL-Saghir and Porter, 2012).

It is a traditional natural remedy that has been used by ancient Mediterranean civilizations. The extract from different parts of this plant shows various activities like antiatherogenic, antioxidant, antimicrobial, antitumor, antiarthritic, antigout, anticancer, hypotensive and antifungal activities (Mohd *et al.*, 2014). Phytochemical studies of *P. lentiscus* extracts revealed the presence of essential oils and phenolic compounds (Bozorgi *et al.*, 2013).

Biological activities of essential oils from *P. lentiscus* fruits are largely studied. However, there are very few studies comparing the anti-inflammatory activity of the extracts from *P. lentiscus* leaves. Hence this study was conducted for the evaluation of the anti-inflammatory activity of *P. lentiscus* leaf extracts using *in vivo* and *in vitro* models of inflammation.

MATERIAL AND METHODES

Chemicals

Croton oil, indomethacin, λ-carrageenan, heparin lithium salt, dextran, Hanks' balanced salt solution with Ca^{+2} and Mg^{+2} ($HBSS_2$) or without Ca^{+2} and Mg^{+2}($HBSS_1$), Gallic acid, quercetin, fMet-Leu-Phe (fMLP), Cytochalasine B (CB), Histopaque®-1077, HEPES, elastase substrate: MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide, Ovalbumin and Wright stain were supplied by Sigma (Germany). All other reagents were of analytical grade and were supplied by Sigma and Fluka (Germany).

Animals

Swiss albino mice of either sex weighting 25-30 g and Wistar albino male rats weighing 210-270 g were used *in vivo* in this study. These animals were procured from the Pasteur Institute of Algiers, Algeria. All animals were kept to acclimatize under the laboratory conditions for one week and were provided with standard rodent diet and water *ad libitum*. Animals were randomly selected for different experimental groups (6-7 animal/group) and fasted overnight prior the experiments. All procedures were performed in accordance with European Union Guidelines for Animals Experimentation (2007/526/EC).

Plant material

Leaves of *P. lentiscus* were collected from Skikda in eastern Algeria, during autumn 2010. The plant was identified and authenticated taxonomically by Pr. H. Laouer, Univesity of Sétif 1, Algeria. A voucher specimen (No. P. L. 2010-1) was preserved in the local Herbarium of Botany, University of Sétif 1 for future reference. The leaves were air-dried at room temperature, reduced to powder and then stored in a paper bags at room temperature away from moisture until use.

Extraction

Methanolic extract (Met E) was obtained by maceration of 30 g of the powdered leaves of the plant with 80% methanolic for 24 h under continuous shaking at room temperature. After filtration, the filtrate was concentrated under reduced pressure at 40°C. The residue was lyophilized to give a powder (yield: 16.41%).

Aqueous extract (Aq E) of *P. lentiscus* leaves was prepared by boiling 25g of the powdered leave plant material in 250 mL of distilled water for 20 min, followed by filtration and centrifugation for 10min. The supernatant obtained was lyophilized to give a powder (yield: 25.7%). Both extracts were stored at -32 °C until use.

Determination of total phenolic and flavonoid contents

Total phenolic content in *P. lentiscus* extracts was determined using the Folin-Ciocalteu reagent according to Slinkard and Singleton (1977). Gallic acid was used as a standard and the results were expressed as μg of Gallic acid equivalent (GAE)/mg of extract. Flavonoids amount was further estimated by the aluminium trichloride method (Bahorun *et al.*, 1996) using quercetin as standard. Results were expressed as μg of quercetin equivalent (QE)/mg of extract.

Croton oil-induced ear edema in mice

The effect of *P. lentiscus* leaf extracts on Croton-oil induced ear edema was evaluated according to Manga *et al.* (2004). Briefly, cutaneous inflammation was induced in the inner surface of the right ear of mice (6 mice/group) by application of 15 μl of acetone containing 80 μg of Croton oil as irritant. Treated animals received topically 2 mg/ear of extract or 0.5 mg/ear of indomethacin, used as reference drug. The control animals received only the irritant agent. The thickness of ears was measured before and 6 h after induction of inflammation using a dial calipers. The edema was expressed as an increase in the ear thickness due to Croton oil application.

Acetic acid-induced vascular permeability in mice

The effect of *P. lentiscus* leaf extracts on vascular permeability was evaluated according to the method of Kou *et al.* (2006) with slight modifications. The treated mice (7 mice/group) received orally 0.2 mL of either 200 mg/kg of the Met E, Aq E or 50 mg/kg indomethacin. The control group received the same volume (0.2 mL) of vehicle orally. One hour later, 10 mg/kg of 1% (w/v) Evan's blue was injected intravenously through the tail vain followed by intra-peritoneal injection of 10 mL/kg of 0.7% acetic acid. Thirty minutes later, the mice were sacrificed by cervical dislocation and the peritoneal exudates were collected after being washed with 3 mL of normal saline, and centrifuged at 2000 rpm for 10 min. The absorbance of the supernatant was read at 610 nm. The dye content of the exudates, which refers to the rate of

vascular permeability, was calculated according to the standard curve of Evans Blue.

Carrageenan induced-pleurisy in rats

The effect of *P. Lentiscus* extracts on carrageenan induced-pleurisy in rats was conducted according to Cuzzocrea *et al.* (2000). Treated rats (7 rats/group) were administered orally 2 mL (400 mg/kg) of alcoholic or aqueous extract of *P. lentiscus* leaves, one hour before the intra-pleural injection of 0.2 mL of λ-carrageenan (1%). Rats of the positive control group were treated orally with 2 mL of saline solution. Animals were lightly anaesthetized with chloroform and submitted to a skin incision at the level of the left sixth intercostal space. The underlying muscle was dissected and saline solution (0.2 mL) containing 1% λ - carrageenan (0.2 mL) was injected into the pleural cavity. Rats of negative control group were injected in their pleural cavity 0.2 mL of sterile 0.9% NaCl instead of the λ-carrageenan solution and were not treated with any other substance. The skin incision was closed with a suture and the animals were allowed to recover. Four hours after the injection of λ -carrageenan, rats were killed and their chests were carefully opened, the pleural cavity is subsequently washed with 2 mL of heparinized saline solution. The exudate and washing solution were removed by aspiration and the total volume was measured. Any exudate, which was contaminated with blood, was discarded. The amount of exudates was calculated by subtracting the volume injected (2 mL) from the total volume recovered. The leukocytes in the exudate were suspended in PBS and counted with an optical microscope after vital Trypan blue staining.

Human neutrophil isolation and cell viability

Human isolated neutrophils as a cellular model were used to evaluate the *ex vivo* immunomodulatory effect of *P. lentiscus* extracts. These cells were isolated from freshly heparinized (5U/mL) blood of healthy volunteers as previously described (Selloum *et al.*, 2003). Briefly, PMN isolation was carried out by centrifugation on a Ficoll-Hipaque® gradient density after a dextran-enhanced sedimentation. The remaining red blood cells were eliminated by hypotonic lysis. PMNs were preserved in $HBSS_1$ and kept on ice until use. This method routinely yielded cells that were $\geq$ 95% viable neutrophils as assessed by trypan blue exclusion test. The Trypan blue exclusion test is widely used to measure plasma membrane integrity. Therefore, it was used to assess the effects of the extracts on cell viability. Cells were incubated for 30 min with different concentrations (20 - 200 µg/mL) of *P. lentiscus* leaf extracts, and then they were incubated with trypan blue (0.4%) for 10 min and the resulting percentage of blue cells, indicating a capture of the colorant due to plasma membrane rupture, were counted. Normal cell viability was considered to be between 90% - 95%.

Neutrophil chemotaxis assay

In order to evaluate the immunomodulatory effect of *P. lentiscus* alcoholic and aqueous extracts, neutrophil chemotaxis

was assayed in 48-microwell chemotaxis chamber (Neuroprobe, Inc., USA) using a 5µm pore size polycarbonate filter according to Falk *et al.* (1980). PMNs (1.5×10^6 cells/mL) suspended in $HBSS_2$ supplemented with 0.25% ovalbumine were pre-incubated for 10 min at 37°C with various concentrations (20-100 µg/mL) of *P. lentiscus* extracts, then placed in the upper wells of the chemotaxis chamber.

Cells were then allowed to migrate towards fMLP (10^{-7} M) present in the lower wells for 90 min at 37°C in a humidified atmosphere containing CO_2 5%. After incubation, the filter was removed, fixed in absolute methanolic and stained with Wright's stain. Positive control (absence of extracts) and negative control (only medium in the lower wells) are conducted in parallel with experimental samples. Neutrophils that had migrated trough the filter were counted under light microscopy at $\times$ 400 magnification in five random fields for each of three replicate wells. A mean value was obtained for each well. The average number of cell migration in the negative control wells was subtracted as background from the number migrating in test wells, to yield the net number of neutrophils migrating per field. Each experiment was repeated at least three times.

Determination of elastase activity

Neutrophil elastase activity assay was conducted to study the immunomodulatory effect of *P. lentiscus* extracts. Briefly, the elasase activity was determined in cell released supernatant, according to the method of Bieth *et al.* (1980). Elastase was produced by the incubation of PMNs suspension (5×10^6 cells/mL) for 20 min at 37°C in the presence of fMLP/CB (10^{-5} M). Thereafter, elastase rich supernatant aliquots were treated with different concentrations of *P. lentiscus* extract (20-100 µg/mL) or $HBSS_1$ (Control). On a microtitration plate well, 75µl of the treated and untreated supernatant were mixed with 75µl of the elastase synthetic substrate, *N*MeO-Suc-(Ala)$_2$-Pro-Val-*p*-nitroanilide (5×10^4 M), which was dissolved in a HEPES medium (0.1 M; pH 7.4). After incubation for 40 min at 37°C, the formation of the *p*-Nitroanilide product was quantified at 405nm using microplate reader (Stat Fax 2100, USA). Results were expressed as the percentage of elastase activity inhibition compared with the control considered as 100% of activity.

STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) and *t*-test Student. The differences between the control and samples were considered statistically significant at $p \leq 0.05$.

RESULTS

Total phenolic and flavonoid contents

The quantitative estimation of total phenolic and flavonoid in the *P. lentiscus* leaf extracts showed that methanolic extract is richer in polyphenols and flavonoids than aqueous extract (Table 1).

Table 1: Total phenolic and flavonoid contents in *P. lentiscus* leaf extracts.

Extract	Polyphenols µg of GAE/mg of extract	Flavonoids µg of QE/mg of extract
Met E	268 ± 0.7	15 ± 0.06
Aq E	227 ± 0.3	11 ± 0.1

Data are expressed as mean ± SEM (n=3).

Effect of *P. lentiscus* leaf extracts on Croton-oil induced ear edema

Six hours after the topical application of the irritating agent, the mice in the control group developed an edema at their right ears with a thickness of 112 ± 22 µm. The local treatment of mice by 2 mg of alcohol extract of *P. lentiscus* leaves decreased significantly (p < 0.001) the inflammation compared to the control group mice. The ear thickness was 39 ± 9 µm, which corresponds to 65% of inhibition. This inhibition is greater than that obtained with indomethacin, which exhibited an inhibition of 54%. The effect exerted by the aqueous extract (51%) is very close to that of indomethacin (Fig. 1).
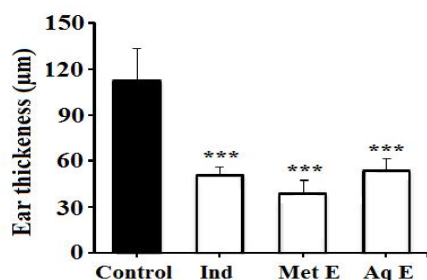

Fig. 1: Effect of *P. lentiscus* extracts on Croton oil induced-ear edema in mice. Mice were treated with 2 mg/ear of methanolic extract (**Met E**), aqueous extract (**Aq E**) or 0.5 mg/ear of indomethacin (**Ind**). Control group received sterile saline solution only. Edema is expressed as mean thickness of ears before and 6 h after Croton oil application. Values are expressed as means ± SEM (n = 6). ***: *p*<0.001 vs control.
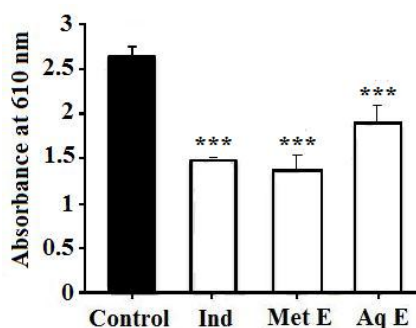

Fig. 2: Effect of *P. lentiscus* extracts on vascular permeability induced by acetic acid in mice. Animals were pre-treated orally with 200 mg/kg of methanolic extract (**Met E**), aqueous extract (**Aq E**) or 50 mg/Kg of indomethacin (**Ind**). Control group received only saline solution. Values are expressed as means ± SEM (n=7). ***P* < 0.001 vs control.

Effect of *P. lentiscus* extracts on acetic acid-induced vascular permeability

Results showed that treatment with alcohol or aqueous extract of *P. lentiscus* leaves or indomethacin, one hour before the induction of inflammation with acetic acid, inhibited significantly (*p<0.001*) the vascular permeability. Indeed, treatment by 200

mg/kg of methanolic extract reduced the vascular permeability by (46%). This effect is better than that obtained with 50 mg/kg of indomethacin (44%). In contrast, aqueous extract exerted only 28% of inhibition (Fig. 2).

Carrageenan induced-pleurisy

Rats of the negative control injected with sterile saline solution did not produce any exudate in their pleural cavity. The exudate volume represents only 6% compared to the volume produced by the rats of the positive control group (Fig. 3). Further, the infiltration of PMNs into the pleural cavity of negative control rats is also very low (4 ± 1.4×10^6 PMNs/exudate), or approximately 6% compared to the number of PMNs recovered from the positive control group rats (66 ± 5 ×10^6 PMNs/exudate) (Fig. 4).
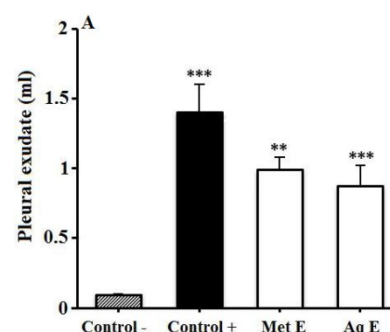

Fig. 3: Effect of *P. lentiscus* leaf extracts on the Volume of exudate in λ-carrageenan-induced pleurisy in the rats. Rats were pretreated orally with 200 mg/kg methanolic (**Met E**) or aqueous (**Aq E**) extracts. The positive control (**control +**) was pretreated with normal saline solution and then injected by λ-carrageenan, while the negative control (**control-**) without injection of λ-carrageenan, received only saline solution. TheVolume of exudate aspirated from the pleural cavity 4h after the injection of λ-carrageenan. Results are expressed as mean ± SEM (n=7). ***P* < 0.001; ***P* < 0.05 vs positive control.
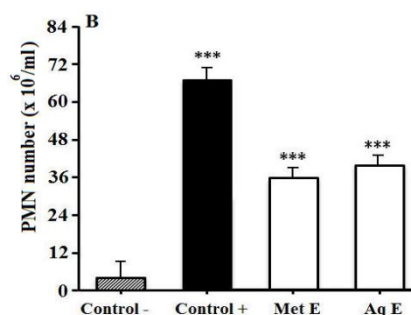

Fig. 4: Effect of *P. lentiscus* leaf extracts on the number of PMNs migrated into exudates 4h after the injection of λ-carrageenan. Rats were pretreated orally with 200 mg/kg methanolic (**Met E**) or aqueous (**Aq E**) extracts. The positive control (**control +**) was pretreated with normal saline solution and then injected by λ-carrageenan, while the negative control (**control-**) without injection of λ-carrageenan, received only saline solution. TheVolume of exudate aspirated from the pleural cavity 4h after the injection of λ-carrageenan. Results are expressed as mean ± SEM (n=7). ***: *P* < 0.001vs positive control.

Oral administration of 400 mg/kg of methanolic or aqueous extract of *P. lentiscus* decreased significantly (*p*<0.001) the development of pleurisy. Indeed, the methanolic extract inhibited the exudation by 29% compared with the rats of the

positive control group (Fig. 3), and exerted 49% of inhibition on the number of PMNs migrated into the exudates (Fig. 4). Furthermore, the aqueous extract exerted an inhibitory effect of 38% on the volume of the exudates (Fig. 3), and reduced the number of the PMNS migrated into the exudates by 43%. The number of PMNs present in the exudate, was only $37.66 \pm 3 \times 10^6$ PMNs/rat (Fig. 4).

Effect of *P. lentiscus* extracts on neutrophil chemotaxis

The chemoattractant fMLP activated strongly the chemotaxis response of isolated neutrophils. Indeed, in the presence of 10^{-7} M of fMLP the number of transmigrated PMNs (74 ± 4 cells/field) was 3 times more than that in the absence of fMLP. *P. lentiscus* extracts inhibited significantly ($p<0.05$) and in a dose-dependent manner the fMLP-stimulated neutrophils chemotaxis. At 100 µg/mL, the methanolic and aqueous extracts inhibited PMN migration by 81% and 71%, respectively (Fig. 5). The IC_{50} values were 19.8 ± 0.8 and 26.7 ± 0.7, respectively.
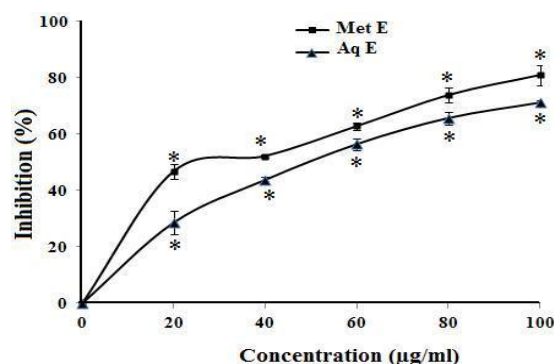

Fig. 5: Effect of *P. lentiscus* leaf extracts on neutrophil chemotaxis. Results are expressed as the percentage of neutrophils migrating per field compared with the positive control (without treatment) set to 100%. Values represent the mean ± SEM of 3 independent experiments each performed in triplicate.*$p < 0.05$ vs control.
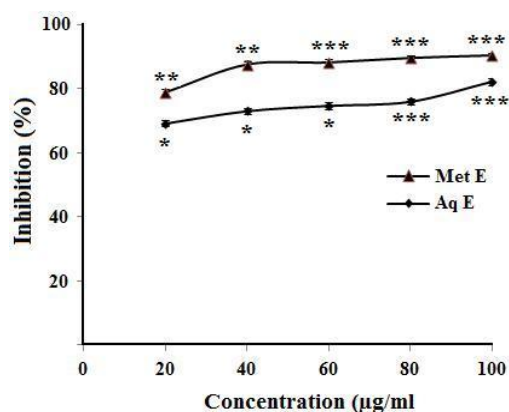

Fig. 6: Effect of *P.lentiscus* extracts on the human neutrophil elastase activity. Results are expressed as inhibitory percentage of elastase activity compared to the positive control (untreated elastase) set at 100%. Values represent the mean ± SEM of 3 independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Effect of *P. lentiscus* extracts on elastase activity

Results revealed that methanolic and aqueous extracts of *P. lentiscus* inhibited significantly ($p<0.01$) the elastase activity

(Fig. 6). This inhibition remained stable with maximum values of 82% and 90%, respectively.

DISCUSSION

Anti-inflammatory and immunomodulatory properties of *Pistacia lentiscus* leaf extracts were evaluated in the current study, using different experimental models that could provide different mechanisms underlying the anti-inflammatory activities of this plant. Ear edema induced by Croton oil is a wide used model to study the inflammatory processes, as well as identifying new anti-inflammatory compounds. In this model, edema events are triggered by protein kinase C (PKC), which leads to PLA_2 activation and then the release of arachidonic acid from membrane phospholipids. Arachidonic acid is subsequently metabolized by cyclooxygenase (COX) and lipooxygenase (LOX) to generate a variety of bioactive eicosanoids, including prostaglandins and leukotriene, which are implicated in the development of inflammatory conditions. PKC also promotes various immune mediators such as cytokines and chemokines that increase and maintain the inflammatory response (Kim *et al.* 2013). Local pre-treatment of ear mice with *P. lentiscus* alcohol and aqueous extracts reduced significantly the size of the ear edema. The presence of phenolic compounds in these extracts might be partly responsible for this effect. Indeed, *P. lentiscus* leaf extracts contain important quantities of flavonoids and gallic acid (Bozorgi *et al.*, 2013). Furthermore, Bendifallah *et al.* (2014) affirmed the richness of *P. lentiscus* leaf extracts with the condensing tannin precursors. Flavonoids such as quercetin and myricetin exert a strong inhibition on COX and LOX (Kutil *et al.*, 2014). Gallic acid and its derivatives are responsible for the inhibition of p38 mitogen-activated protein kinase (MAPK) activation and inhibition of NF-kB activation (Ahad *et al.*, 2015), which are essential for histamine release and pro-inflammatory cytokine expression such as TNF-α and IL-6. The decrease in ear swelling is also probably due to the antioxidant effects of the extracts, since reactive oxygen species produced during inflammation by phagocytic cells and during the arachidonic acid metabolism may activate phospholipase A2. Indeed, *P. lentiscus* extracts exhibit a good antioxidant effect (Remila *et al.*, 2015).

During acetic acid induced-vascular permeability model, the increase of serotonin, histamine, PAF and, leukotrienes and prostaglandins lead to a dilation of arterioles and venules thereby increase the vascular permeability. As a consequence, fluid and plasma protein are extravasated and edema is formed (Cuzzocrea *et al.*, 1998). Pre-treatment with *P. lentiscus* extracts inhibited significantly the vascular permeability in mice, which indicates that these extracts are able to control the amplification of the inflammatory response. The presence of anthocyanins, flavonoids and tannins in *P. lentiscus* leaf extracts may contribute to this anti-inflammatory effect. Indeed, it has been reported that anthocyanins reduce nuclear factor-κB (NF-κB) transactivation and decreased plasma concentrations of pro-inflammatory chemokines, cytokines, and inflammatory mediators (Karlsen *et al.*, 2007).

Flavonoids have been found to have anti-inflammatory activity in both proliferative and exudative of inflammation phases, they inhibit histamine, cytokine, prostaglandins and leukotrienes release (Park, 2008; Permender *et al.*, 2009). Furthermore, *in vitro* and *in vivo* assays indicated that hydrolysable tannins are a very effective treatment against inflammatory disorders (Ismail *et al.*, 2012).

The plasma exudation and the infiltration of PMNs into the pleural cavity exudates of rats, during the first 4 hours following the induction of pleurisy by λ-carrageenan has been exploited in this study to evaluate the anti-inflammatory effect *in vivo* of alcohol and aqueous *P. lentiscus* leaf extracts. Oral administration of these extracts significantly attenuated the development of pleurisy, by inhibiting plasma exudation as well as leukocyte recruitment to the inflated site. This result indicates that *P. lentiscus* leaf extracts are able to reduce the production of inflammatory mediators involved in the development of the acute inflammatory reaction. In fact, alcohol and aqueous *P. lentiscus* leaf extracts are rich in flavonoids and polyphenols. These compounds are good inhibitors of serotonin, histamine and leukocyte migration by blocking their adhesion to the vascular wall. It has been reported that quercetin blocks adhesion of leukocytes to the endothelial wall of the umbilical veins by inhibiting the expression of ICAM-1 (Middleton *et al.*, 2000), while Gallic acid inhibits leukocyte migration by inhibiting the adhesion molecule VCAM-1, ICAM-1 and E-selectin in vascular endothelial cells (Triantafyllou *et al.*, 2011). This inhibition is due to the inhibition of the synthesis of IL-1 and TNF- α major inducers of the expression of adhesion molecules on the vascular wall (Calixto *et al.*, 2004). In addition, cyanidin 3-O-beta-glucoside reduced the increase of mediators induced neutrophil migration, such as NO, TNF-α, IL-1ß and normalizes the levels of several acute phase proteins (Calixto *et al.*, 2004).

The modulation of immune cell activities is of active interest for the treatment of inflammation. Therefore, many immunological studies used the human isolated neutrophils as a cellular model to evaluate the *ex vivo* effect of immunomodulatory drugs on the PMNs excessive activation. In the present study, the immunomodulatory activity of *P. lentiscus* extracts was carried out by neutrophil migration and elastase release tests. The results showed that *P. lentiscus* extracts inhibited significantly the fMLP-stimulated neutrophils chemotaxis and elastase activity. Under our experimental conditions, *P. lentiscus* leaf extracts were not toxic to PMNs as assessed by trypan blue exclusion test. Therefore, the extract activities could not be due to the cytotoxicity effect. The immunomodulatory activities of medicinal plants were associated extremely to their contents of flavonoid and phenolic compounds. In this context, Selloum *et al.* (2003) reported that the natural flavonol, rutin, exerted a significant inhibitory effect on the human PMNs migration induced by the fMLP, while Souto *et al.* (2011) reported that quercetin acts as a potent chemotaxis inhibitor. Moreover, Liu *et al.* (2012) showed that flavonoids such as quercetin, myricetin and genistein could inhibit the PI3-kinase, which involved in neutrophil response signaling pathways. On the other hand, Sartor *et al.* (2002) revealed that the group glycosidic of flavones can affect the steric conformation of the elastase and lead to interactions enzyme-phenols, consequently the inhibition of its proteolytic activity. However, Piwowarski *et al.* (2011) demonstrated that the anti-elastase effect of tannin-rich plant is due to its ability to bind the enzymatic protein, which cause protein precipitation.

CONCLUSION

Pistacia lentiscus leaf extracts exerted a potent anti-inflammatory effect by inhibiting different phases of inflammation. So, this plant can constitute a promising natural source of bioactive compounds that give significant anti-inflammatory properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

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