Original Communication

Reduction of paw edema and liver oxidative stress in carrageenan-induced acute inflammation by *Lobaria pulmonaria* and *Parmelia caperata*, lichen species, in mice

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Abstract: Paw edema volume reduction is a useful marker in determining the anti-inflammatory effect of drugs and plant extracts in carrageenan-induced acute inflammation. In this study, the anti-inflammatory effect of *Lobaria pulmonaria* (LP) and *Parmelia caperata* (PC), two lichen species, was examined in carrageenan-induced mouse paw edema test. Compared to the controls in carrageenan-induced inflammation (n = 5/group), our results showed that pretreatment by single oral doses with PC extract (50–500 mg/kg) gives better results than LP extract (50–500 mg/kg) in terms of anti-edematous activity, as after 4 h of carrageenan subplantar injection, paw edema formation was inhibited at 82–99% by PC while at 35–49% by LP. The higher anti-inflammatory effect of PC, at all doses, was also observed on the time-course of carrageenan-induced paw edema, displaying profile closely similar to that obtained with diclofenac (25 mg/kg), an anti-inflammatory drug reference (all $p < 0.001$). Both LP and PC, at all doses, significantly ameliorated liver catalase (CAT) activity (all $p < 0.05$). However, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity and glutathione (GSH) levels were found increased in liver of PC- compared to LP-carrageenan-injected mice. Our findings demonstrated on one hand higher preventive effects of PC compared to LP in a mouse carrageenan-induced inflammatory model and suggested, on the other hand, that anti-inflammatory effects elicited by the two lichens were closely associated with the amelioration in the endogenous antioxidant status of liver.

Keywords: Anti-inflammatory effect, Antioxidant effect, Anti-edematous effect, Carrageenan, Lichen

Introduction

Lichens are complex organisms that encompass more than 25000 species [1], resulting from the symbiosis between an autotrophic organism including a green alga and/or a cyanobacterium, and mycobionte, i.e., a heterotrophic fungal species that is mainly an ascomycete, providing to the photobiont partner, among other, shelter [2]. In the environment, the presence of these composite organisms, especially fruticose and foliose lichens, indicates an "excellent" air quality level [3, 4]. Lichens adapt to the most hostile environmental conditions such as extreme temperatures, and thus they can grow in earth-arctic tundra, icebergs, deserts and rocky coast. Adaptive functions of lichens are mainly attributed to their contents of specific secondary metabolites (e.g., salizinic acid, evernic acid, caperatic acid, norstictic acid etc.), constituting 85% of the total metabolites. Lichen secondary metabolites are not found in higher plants or free-living fungi, except for a small minority (e.g., parietin and lecanoric acid), which are common to other fungi or plants [5, 6]. Lichen secondary metabolites make up more than 30% of the dry mass of the thalli, which usually constitutes the most visually prominent parts of the lichen, and are produced by the fungi partner in the form of crystals deposited on the hyphae surface [8, 9]. Nowadays, approximately 1050 different secondary metabolites have been identified, including phenolics that comprise more than 800 compounds

such as dibenzofurans, depsides, depsidones, depsones, quinones and pulvinic acid derivatives [8, 10, 11].

In addition to their numerous utilizations in human food items, animal feeds, dyestuff industry and perfumery, lichens are also largely employed as drugs [12]. In this respect, lichens could constitute a good alternative for synthetic drugs such as anti-inflammatory agents, which have, especially for long-term use, serious undesirable side effects, including gastric ulceration. Research has demonstrated that lichen secondary compounds possess diverse biological activities including anti-inflammatory, antioxidant, antipyretic, analgesic, antiviral, antibiotic, antifungal, antiherbivore, antitumor, and anticancer activities [13–15].

In this study, *Lobaria pulmonaria* (LP) and *Parmelia caperata* (PC), epiphytic foliose lichens commonly employed in folk medicine, were studied. Traditional healers have been using LP in the treatment of respiratory and pulmonary diseases (e.g., tuberculosis, asthma, coughs, spitting blood), eczema, arthritis, diarrhea, and heavy menstrual flow. Research on animal models and *in vitro* studies conducted on *L. pulmonaria* from different regions in the word (e.g., Germany and Turkey), employing various extraction procedures, have emphasized several health beneficial effects, including anti-inflammatory and antioxidant effects [16–20]. Concerning PC, it has been reported that this lichen is one of the ingredients of Chinese, Russia and northern Mexico medicine [21, 22]. Research has demonstrated that PC contains a large array of bioactive secondary metabolites (e.g., usnic acid, protocetraric acid and caperatic acid) that possess potent antioxidant power, antimicrobial capacity, and anticancer activity against melanoma and colon carcinoma [23].

LP is an indicator of the forest antiquity, and thus it is found in the ancient ones [24]. Moreover, PC is also sensitive to pollution, and thus, it has been used as a bioindicator of air quality [3]. In Algeria, a country in North Africa, LP and PC can be found in the forest of Jijel, which is known by its biodiversity, encompassing more than 130 medicinal plants and 60 lichen species [25]. Traditional medicine constitutes a deeply rooted culture in inhabitants of Jijel. The purpose of this study is to study the anti-inflammatory and antioxidant effects of LP and PC from Jijel, using carrageenan-induced paw edema in mice, which is considered as the best characterized acute inflammatory model [17, 26].

Materials and methods

Preparation of the lichen extract

L. pulmonaria (L.) Hoffm. and *P. caperata* (L.) Ach. were collected in March 2015 from Taza National parc of Jijel, located in the Northeast of Algeria. Botanical identification of these species was done based on a guide to lichens by Tievant [3] (Voucher number 10.5.2015. Laboratoire de Biotechnologie, Environnement et Santé. Jijel). Dried thalli were ground rapidly for 15 sec, using an electric spice grinder WSG30 (Waring, USA) and then 30 g of the powder were macerated, at ambient temperature, using 200 mL of 80% aqueous methanol. The mixture was then filtered through a Whatmann filter paper. Extraction of the residue was repeated using the same conditions. The two filtrates were combined, then concentrated under reduced pressure in a rotary evaporator (Evaporator E100, Heidolph-Instruments, Germany) at 40 °C to a final volume of approximately 4–6 ml, and finally lyophilized and stored at –18 °C until oral treatment of mice.

Animals

A total of 55 male mice, 9 weeks old and ranging in weight from 30 to 45 g at the time of reception from the breeder (Pasteur institute, Algiers, Algeria). Prior to experimental testing, they were housed in groups of five in standard cages containing a supply of food pellets and water available *ad libitum*. Experiments were performed after a 2-week period of acclimatization. All animal procedures were carried out in accordance with the relevant European Union regulations (Directive 2010/63/EU).

Carrageenan-induced hind paw edema in mice

Carrageenan-induced paw edema is a commonly employed test for determining the acute phase of inflammation. To assess the anti-edematous effect of lichen extract, the method described by Winter [26] was used. Prior to subplantar injection of λ-carrageenan (0.1 ml, 1%), 5 mice were randomly assigned to each of the 8 experimental groups receiving by gavage 50, 150, 250 or 500 mg/kg of LP or PC extract and to the two control groups, receiving *per os* either only distilled water (negative controls) or the anti-inflammatory reference drug diclofenac (25 mg/kg) (positive controls). Moreover, in this study, a group of non-injected mice (n = 5) was allocated, which receiving only distilled water.

For each mouse, the initial volume (V0) of the paw was measured, immediately before carrageenan injection, with a digital vernier caliper (Stainless Steel Electronic Caliper, China). Forty-five minutes after gavage, mice were then injected, into the subplantar region of the right hind paw, by 0.1 ml of carrageenan 1%, dissolved in sterile saline solution (NaCl, 0.9%), in order to induce acute paw edema. The paw volumes (V) in carrageenan-injected mice were

further measured at each hour (h). On 4 h (V4), percent inhibition of edema was calculated according to the following formula:

$$\%\, inhibition = \frac{(V4 - V0)\, negative\ controls - (V4 - V0)\, tested\ mice}{(V4 - V0) negative\ control} \times 100$$

Hepatic oxidative status

Preparation of liver cytosolic fractions

Mice were anesthetized and sacrificed by cervical dislocation. Cytosol fractions were extracted as described by Iqbal et al. [27]. Briefly, livers were immediately removed and rinsed with ice-cooled physiological water. Hepatic tissues were cut into small pieces, and were directly immersed in three volumes of phosphate buffer (0.1 M at pH 7.4 with 1.17% KCl), homogenizing by an electric tissue homogenizer. The liver homogenate was firstly centrifuged at 800 rpm for 15 minutes at 4 °C, to eliminate nuclear debris, membrane fragments and unexploded cells, and secondly, at 9600 rpm for 45 minutes at 4 °C. The obtained supernatant, which corresponds to the cytosolic fraction, was used to assess catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH). Protein estimation was performed by Bradford [28], using bovine serum albumin (BSA) as standard. The cytosolic fractions were stored at −80 °C until use.

Superoxyde dismutase (SOD) activity

Cytosolic SOD activity was evaluated by the method of Beauchamp and Fridovich [29]. The assay mixture contained 2 ml of the reactive medium (sodium cyanide 10^{-2} M, nitro blue tetrazolium chloride (NBT) solution 1.76×10^{-4} M, ethylenediaminetetraacetic acid (EDTA) 66 mM, methionine 10^{-2} M, riboflavin 2 μM, pH 7.8) and 5 μl of the cytosol fraction. This mixture was exposed to the light of a 15 W lamp for 10 minutes to induce the photoreaction of riboflavin and O_2. Reduction of NBT by superoxide anions to blue formazan was assessed spectrophotometrically at 560 nm (model DU 800 ultra violet/visible spectrophotometer, Beckman Instruments, Fullerton, CA, USA). Enzymatic activity was calculated in terms of IU/mg of proteins.

Catalase (CAT) activity

CAT activity was assessed in the cytosolic fractions by measuring the rate of hydrolysis of H_2O_2 according to the method described by Aebi [30]. Using spectrophotometric analyses, the resulting decrease in absorbance at 240 nm was monitored for 2 min at 25 °C. CAT activity was expressed as μmol H_2O_2 decomposed min^{-1} mg^{-1} protein by using 0.04 mM^{-1} cm^{-1} for extinction coefficient.

Glutathione (GSH) assay

GSH level was evaluated using the method of Weckbecker and Cory [31]. Briefly, 0.8 ml of the cytosolic fraction was mixed with 0.2 ml of sulfosalicylic acid (0.25%). Then, the mixture was shaken, incubated,in an ice-bath, for 15 minutes and centrifuged at 1000 rpm for 5 min. Afterwards, 0.5 ml of the supernatant was mixed with 1 ml of Tris-EDTA buffer (EDTA 0.02 M, pH = 9). Finally, 0.025 ml of 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB, 0.01%) was added to the mixture. Hepatic GSH activity was assessed spectrophotometrically and expressed as nmol/mg proteins by using the absorbance coefficient for thiol (R-SH) at 412 nm.

Glutathione peroxidase (GPx) activity

GPx activity was evaluated using the method of Floke and Gungler [32]. Briefly, 200 μl of the cytosolic fractions were mixed with 0.4 ml of phosphate buffer (0.4 M, pH 7.0), 0.2 ml EDTA (0.8 mM), 0.1 ml GSH (4 mM) and 0.1 ml H_2O_2 (30 mM). The mixture was incubated for 10 min at room temperature. Afterwards, 0.5 ml of trichloroacetic acid (5%) was added and the mixture was centrifuged for 10 min at 1500 rpm. Finally, 0.5 ml of DTNB (0.04%) was added and the absorbance was measured spectrophotometrically at 420 nm. The GPx activity was expressed as nmol of oxidized glutathione min^{-1} mg^{-1} protein.

Statistical analyses

Normality of distribution was verified by normality plots, and was measured by conducting Shaphiro-Wilk test. Repeated measures ANOVA was used to analyze paw edema in mice considering oral treatment of the lichen as between-subject fixed factor, and paw volume as the repeated measure. One-way ANOVA was employed to analyze oxidative stress variables (GPx, CAT, SOD and GSH). The significant effects of the treatment were further assessed using Tukey's HSD test when ANOVA results suggested significant differences. Data were reported as mean ± sd. For all statistical analyses, the level of significance was set at $p < 0.05$ (2-sided). All statistical analyses were performed using SPSS 21.0 (SPSS. Armonk, NY: IBM Corp.).

Results

Lichen effects on paw edema

The time-course of the acute phase of inflammation induced by carrageenan injected in the right hind paw of

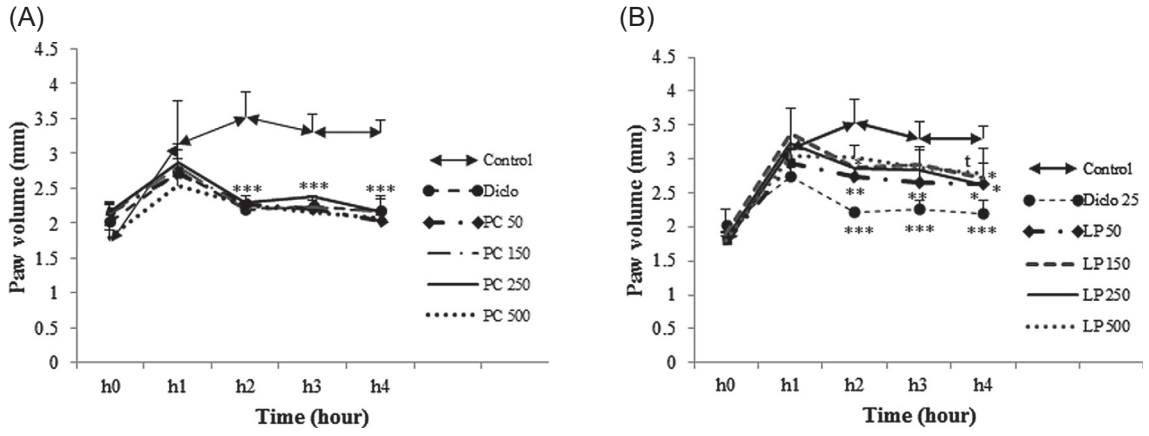

Figure 1. Effects of oral pretreatment with *Parmelia caperata* (PC) (A) or *Lobaria pulmonaria* (LP) (B), at the doses 50, 150, 250 and 500 mg/kg, and diclofenac (25 mg/kg), an anti-inflammatory reference drug, compared to distilled water (vehicle) on the time-course of the volume of carrageenan-induced paw edema in mice. PC 50, PC 150, PC 250, PC 500 stand for mice groups pretreated with *Parmelia caperata* (PC) at the doses 50, 150, 250 or 500 mg/kg, respectively. LP 50, LP 150, LP 250, LP 500 stand for mice groups pretreated with *Lobaria pulmonaria* (LP) at the doses 50, 150, 250 or 500 mg/kg, respectively. All assays were performed in quintuplicate (n = 5). The data are reported as the mean ± sd. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ significantly different from negative control mice. The "t" indicates tendency i.e., $0.05 < p < 0.1$. All p-values are 2-tailed following repeated measures ANOVA and Tukey's HSD *post hoc* test.

mice showed that paw edema volume reached the plateau starting 3 h post-induction in the negative controls (Figure 1).

The paw volumes in diclofenac-pretreated orally mice were significantly reduced compared to those in controls starting 2 h after intraplantar injection (all $p < 0.001$) (Figure 1). Similarly to the anti-inflammatory reference drug, PC extract, at all doses, induced a significant reduction of paw volumes compared to negative controls (all $p < 0.001$) (Figure 1). However, less significant reducing effects were observed for LP extract compared to negative controls (Figure 1 and Table 1). At 4 hours, the inhibition (%) of edema ranged from 81–99% for the PC extract compared to 35–48% for the LP extract (Table 1).

Table 1. Edema inhibition (%) after 4 hours of carrageenan injection in the right hind paw in mice orally pretreated, 1 h prior to carrageenan injection, with *Parmelia caperata* (PC), or *Lobaria pulmonaria* (LP) extracts, at single doses 50, 150, 250 and 500 mg/kg, and diclofenac (25 mg/kg), an anti-inflammatory reference drug, compared to distilled water (vehicle).

Groups	Paw volume before inflammation (mm)	Paw volume after 4 h (mm)	Inhibition (%)
Control	1.76 ± 0.14	3.30 ± 0.18	–
Diclofenac	2.03 ± 0.23	2.19 ± 0.21***	89.61
PC			
Dose 50 mg/kg	2.05 ± 0.10	2.16 ± 0.09***	92.46
Dose 150 mg/kg	2.14 ± 0.14	2.16 ± 0.18***	98.70
Dose 250 mg/kg	2.16 ± 0.16	2.17 ± 0.05***	99.35
Dose 500 mg/kg	1.80 ± 0.17	2.08 ± 0.02***	81.81
LP			
Dose 50 mg/kg	1.81 ± 0.11	2.63 ± 0.29*	46.75
Dose 150 mg/kg	1.89 ± 0.04	2.72 ± 0.05*	46.10
Dose 250 mg/kg	1.84 ± 0.07	2.63 ± 0.25*	48.70
Dose 500 mg/kg	1.80 ± 0.06	2.79 ± 0.37^{t}	35.71

All assays were performed in quintuplicate (n = 5). The data are reported as the mean ± sd. *$p < 0.05$ and ***$p < 0.001$ significantly different from negative control mice. The "t" indicates tendency i.e., $0.05 < p < 0.1$. All p-values are 2-tailed following repeated measures ANOVA and Tukey's HSD *post hoc* test.

Lichen effects on SOD activity

Pretreatment with PC at the doses 50, 250 and 500 mg/kg significantly increased liver SOD activity compared to negative controls (all $p < 0.01$). However, at the dose 150 mg/kg, this lichen had only a tendency to increase SOD activity ($p = 0.08$). Compared to non-injected mice, only pretreatment with PC at the highest dose (500 mg/kg), restored SOD activity (p = n.s).

As for LP, only pretreatment with this lichen at the highest dose (500 mg/kg) significantly increased liver SOD activity compared to negative controls ($p < 0.001$). At the dose of 250 mg/kg, LP had a tendency to increase the activity of SOD compared to negative controls ($p = 0.09$). However, compared to non-injected mice, a significant reduced SOD activity was seen in all groups (all $p < 0.01$), except for 500 mg/kg-pretreated mice (p = n.s) (Figure 2A).

Lichen effects on CAT activity

As the reference drug diclofenac, pretreatment with either PC or LP, at all doses (50–500 mg/kg), significantly

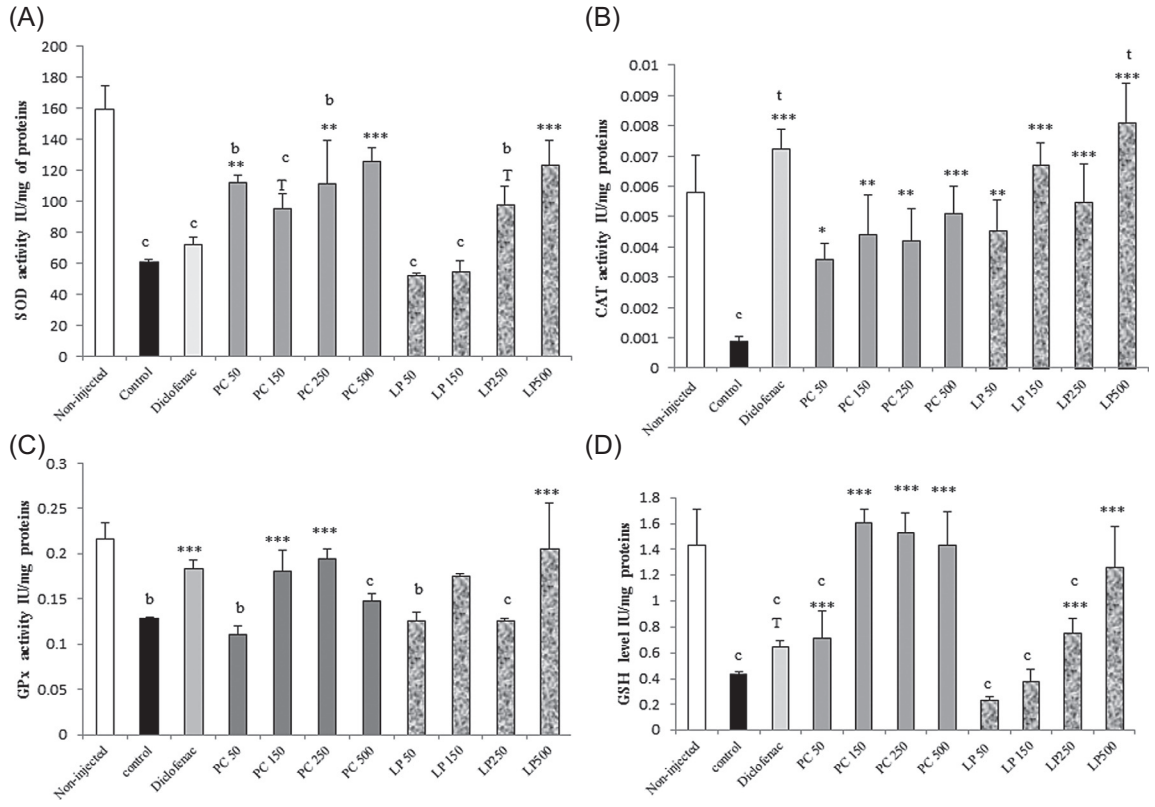

Figure 2. Effects of oral pretreatment with *Parmelia caperata* (PC) or *Lobaria pulmonaria* (LP), at the doses of 50, 150, 250 and 500 mg/kg, and diclofenac (25 mg/kg), an anti-inflammatory reference drug, compared to distilled water (vehicle) on liver oxidative stress markers in mice with carrageenan-induced paw edema. (A) Superoxide dismutase (SOD) activity is expressed in units per mg protein. (B) Catalase (CAT) activity is expressed in μmol of H_2O_2/min/mg of proteins. (C) Glutathione peroxidase (GPx) activity is expressed in nmol of GSH/min/mg of proteins. (D) Reduced glutathione (GSH) level is expressed as nmol/mg proteins. PC 50, PC 150, PC 250, PC 500 stand for mice groups pretreated with *Parmelia caperata* (PC) at the doses 50, 150, 250 or 500 mg/kg, respectively. LP 50, LP 150, LP 250, LP 500 stand for mice groups pretreated with *Lobaria pulmonaria* (LP) at the doses 50, 150, 250 or 500 mg/kg, respectively. All assays were performed in quintuplicate (n = 5). The data are reported as the mean ± sd. $^b p < 0.01$ and $^c p < 0.001$ significantly different from non-injected mice (receiving only distilled water as oral pretreatment); The "t" indicates tendency i.e., $0.05 < p < 0.1$ compared to non-injected mice; $^* p < 0.05$, $^{**} p < 0.01$ and $^{***} p < 0.001$ significantly different from negative control mice. The "T" indicates tendency i.e., $0.05 < p < 0.1$ compared to controls. All *p*-values are 2-tailed following one-way ANOVA and Tukey's HSD *post hoc* test.

increased CAT activity compared to negative controls (all $p < 0.05$). Moreover, CAT activity level in all PC- or LP-pretreated mice was not significantly different from that in non-injected mice (p = n.s), suggesting that normal levels of CAT activity were restored in these experimental groups.

Lichen effects on GPx activity

Compared to negative controls, pretreatment with PC at the intermediate doses (150 and 250 mg/kg) significantly increased GPx activity (both $p < 0.001$). Such an effect was also seen with diclofenac ($p < 0.001$) and LP at the highest dose (500 mg/kg) ($p < 0.001$). Compared to non-injected mice, GPx activity was significantly lower in experimental groups pretreated only with LP at 50 and 250 mg/kg and PC at 50 and 500 mg/kg (all $p < 0.01$), suggesting that normal levels of GPx activity were not restored in these experimental groups.

Lichen effects on GSH level

Pretreatment with PC (all doses) and with the higher doses (250–500 mg/kg) of LP significantly increased liver GSH levels compared to negative control mice (all $p < 0.001$). Compared to non-injected mice, GSH levels were not significantly different between PC-pretreated groups at the doses 150–500 mg/kg, and LP, at the highest dose (500 mg/kg) (all p = n.s), suggesting that normal levels of GSH were restored in these experimental groups.

Discussion

In this study, the anti-edematous effect of PC and LP (50–500 mg/kg), two lichen species, was examined in mice employing the carrageenan-induced paw edema test, a model of acute phase of inflammation. Moreover, the

preventive effect of either PC or LP on liver oxidative stress was explored in carrageenan-induced inflammation. Although results showed that both PC and LP reduced paw edema; better anti-edematous effect was found with PC extract, which exhibited, at all doses, diclofenac-like effects, an anti-inflammatory drug standard, on the time-course of carrageenan-induced paw edema (Figure 1). After 4 h of carrageenan subplantar injection, oral pretreatment of mice with LP, PC and diclofenac has inhibited 35–49%, 82–99% and 90% of paw edema formation, respectively (Table 1). This result suggests a strong anti-edematous effect of PC lichen species. Furthermore, both lichens enhanced liver endogenous antioxidant markers including CAT; however higher effects were found with PC, especially on GSH level and GPx activity (Figure 2).

Pain, redness, heat or warmness and swelling, called edema, are the four clinical signs that characterize the inflamed tissue [33, 34]. A reduction in paw edema volume is considered as a good marker in determining the anti-inflammatory activity of drugs and plant extracts [35, 36]. In this study, mouse paw edema was induced by carrageenan subplantar injection. The edema is caused primarily by the accumulation of fluid outside the blood vessels, at the inflammatory site, and also owing to the accumulation of proteins and infiltration of neutrophils, which are the first leukocytes recruited at the inflammatory site in the body [33, 34, 37–39]. Inflammation mediators including reactive oxygen species, nitric oxide, prostaglandins and cytokines have a role in the formation of this edema [40]. It has been discussed that carrageenan-induced acute inflammation is biphasic, involving the release of histamine and serotonin in the early phase (i.e., during the first hour) and prostaglandins (cyclooxygenase products) in the second phase (>1 hour) [26, 35, 41, 42].

Based on the effect of LP and PC extracts on the time-course of edema formation (Figure 1), our results suggest a significant inhibition of the edema in the second phase, which may suppose that LP and PC ingredients possess anti-inflammatory properties comparable to non-steroidal anti-inflammatory drugs (NSAIDs), through inhibition of cyclooxygenase enzymes [37, 43]. However, anti-inflammatory mechanisms of plant ingredients can also be attributed, among other, to their anti-oxidant properties e.g., by enhancing catalase levels, which may exert inhibiting effects on cyclooxygenase gene expression [44]. PC extract was better than LP extract in terms of anti-edematous effect (Figure 1 and Table 1). It has been previously revealed that LP possesses only moderate anti-inflammatory effects [17]. Pretreatment with PC provided an anti-edematous profile very close to that exhibited by diclofenac, an NSAID (Figure 1 and Table 1). Besides inflammation, previous studies have demonstrated that carrageenan induced oxidative stress disturbances including lipid peroxidation [45, 46] and a reduction of antioxidant markers such as GSH and SOD [35, 46]. Studies have demonstrated that local injection of carrageenan, into the subplantar region of the right hind paw, induced local as well as systematic oxidative stress disturbances in rodents, as revealed locally in paw tissues [35, 46], and in other body parts including the spleen [45] and the liver [45, 47, 48]. In this study, oxidative stress was assessed in the liver, the major organ responsible for the metabolism of drugs and chemicals [49]. In our animal model, carrageenan has induced liver oxidative stress, as highlighted in negative control mice by a significant decrease of either activity or level of endogenous antioxidants including GPx, CAT, SOD and GSH compared to their non-injected counterparts (Figure 2). However, oral pretreatment of mice with LP, PC or diclofenac significantly enhanced antioxidant status of carrageenan-injected mice. Compared to non-injected mice, LP and especially PC relatively restored the normal values of the studied antioxidant markers including CAT in carrageenan-injected mice (Figure 2). In addition to its antioxidative role permitting the detoxification of H_2O_2, CAT may exert a bidirectional effect on prostaglandin biosynthesis, by stimulating, at low concentrations, prostaglandin production and vice versa [44]. Our findings showed a significant decrease of CAT activity in the controls of carrageenan-induced inflammation while a significant increase of this antioxidant enzyme was found in all pretreated carrageenan-injected mice (Figure 2). From the above, it can be inferred that pro-inflammatory effect of carrageenan as well as anti-inflammatory effects of diclofenac, LP and PC might be mediated by CAT activity levels. Moreover, Tanas et al. [46] have also suggested a role of SOD in carrageenan-induced acute inflammation in a rat model. Nevertheless, although SOD activity was significantly diminished in control mice compared to their non-injected counterparts, which may highlight the role of SOD in carrageenan-induced inflammation, our results suggest a relatively limited role of this antioxidant-related enzyme in the anti-inflammatory effects of different pretreatments, as significant increased SOD activity was seen only in few pretreated groups (Figure 2).

GSH is an endogenous non-enzymatic reducer functioning as an important cellular redox buffer antioxidant [50]. This major antioxidant in aerobic cells constitutes also a determinant substrate for the enzymatic antioxidant reaction catalyzed by GPx [50]. GSH can also scavenge directly free radicals, and stabilizes nitric oxide ($^{\cdot}NO$), an important cellular messenger molecule with vasodilator effect, playing a substantial role in the development of the edema [46, 50, 51]. In addition to its antioxidant and antiradical roles [50], GSH participates in a number of critical cellular processes, including the metabolism of inflammatory

compounds such as prostaglandins and leukotrienes (lipoxygenase products) [52], which may explain both reduced and increased GSH levels in the controls of carrageenan-induced inflammation and in almost pretreated carrageenan-injected mice, respectively (Figure 2). Like SOD and CAT, GSH levels and GPx activity were significantly reduced in controls compared to non-injected mice, suggesting a close association between decreased endogenous antioxidant status and acute phase of inflammation (Figure 2). Unlike our findings, Tanas et al. [46] did not found a significant decrease of GPx in paw tissues of control rats in carrageenan-induced inflammation. However, they found that anti-inflammatory drugs including indomethacin and diclofenac induced a significant increase of GPx activity. In keeping with this last result, our findings showed that GPx activity is involved in anti-inflammatory response, as pretreatment with diclofenac, LP or PC generally increased GPx activity in carrageenan-injected mice.

In carrageenan-induced inflammation, anti-inflammatory effects could be elicited by mechanisms other than those discussed above e.g., via among other the inhibition (or attenuation) of NF-kB and p38 MAPK activation, the inhibition of inducible nitric oxide synthase (iNOS), the inhibition of synthesis, release or action of inflammatory mediators including histamine, serotonin and pro-inflammatory cytokines [38, 53, 54].

Many studies have shown that primary or secondary substances may confer anti-inflammatory effects for lichens [53, 55]. Lichen polysaccharides have exhibited interesting anti-inflammatory effects via their ability to modulate cytokine production in macrophages [56, 57]. Lichen phenolic compounds, the major group of secondary substances, have displayed inhibitory effects on the enzymes involved in the inflammatory process [55]. In this regard, it has been demonstrated that gyrophoric acid, one of the major components of LP, possesses an inhibitory effect on the 5-lipoxygenase pathway of arachidonic-acid metabolism [16, 58]. In addition, atranorin, a depside present in thalli of different species of lichen, has exhibited anti-inflammatory properties, acting as cyclooxygenase inhibitor [55]. In fact, it has been highlighted that depsides, an interesting group of secondary lichen metabolites, are inhibitors of prostaglandin biosynthesis [59]. Vijayakumar et al. [60] reported a dose-dependent anti-inflammatory effect of usnic acid, a compound of the dibenzofuran structure, present in many lichen species including PC [61]. Although results from Folin–Ciocalteu's phenol assay showed that LP possesses more phenolics (61.07 µg gallic acid equivalents (GAE)/mg dry weight) than PC (33.60 µg GAE/mg dw) (data not shown), PC exhibited higher preventive effects compared to LP in carrageenan-induced inflammation. This could be interpreted by qualitative differences in terms of individual phenolics present in the two-lichens.

Conclusion

Our results demonstrated that LP and PC extracts possess anti-inflammatory and anti-oxidant effects *in vivo*. However, PC has exhibited better effects than LP in carrageenan-induced inflammation. Thus, it could be interesting to determine the active substances in PC. Although anti-inflammatory effects of PC were closely associated with enhanced endogenous antioxidant status, further studies are necessary to explore the anti-inflammatory mechanisms for both PC species extract and its active substances.

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