Pistacia lentiscus Resin Regulates Intestinal Damage and Inflammation in Trinitrobenzene Sulfonic Acid–Induced Colitis

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ABSTRACT   Mastic (Pistacia lentiscus) of the Anacardiaceae family has exhibited anti-inflammatory and antioxidant properties in patients with Crohn's disease. This study was based on the hypothesis that mastic inhibits intestinal damage in inflammatory bowel disease, regulating inflammation and oxidative stress in intestinal epithelium. Four different dosages of P. lentiscus powder in the form of powder were administered orally to trinitrobenzene sulfonic acid–induced colitic rats. Eighty-four male Wistar rats were randomly assigned to seven groups: A, control; B, colitic; C–F, colitic rats daily supplemented with P. lentiscus powder at (C) 50 mg/kg, (D) 100 mg/kg, (E) 200 mg/kg, and (F) 300 mg/kg of body weight; and G, colitic rats treated daily with cortisone (25 μg/kg of body weight). Colonic damage was assessed microscopically. The cytokines tumor necrosis factor-α, intercellular adhesion molecule-1 (ICAM-1), interleukin (IL)-6, IL-8, and IL-10 and malonaldehyde were measured in colonic specimens. Results were expressed as mean ± SE values. Histological amelioration of colitis ($P \leq .001$) and significant differences in colonic indices occurred after 3 days of treatment. Daily administration of 100 mg of P. lentiscus powder/kg of body weight decreased all inflammatory cytokines ($P \leq .05$), whereas 50 mg of P. lentiscus powder/kg of body weight and cortisone treatment reduced only ICAM-1 ($P \leq .05$ and $P \leq .01$, respectively). Malonaldehyde was significantly suppressed in all treated groups ($P \leq .01$). IL-10 remained unchanged. Cytokines and malonaldehyde remained unaltered after 6 days of treatment. Thus P. lentiscus powder could possibly have a therapeutic role in Crohn's disease, regulating oxidant/antioxidant balance and modulating inflammation.

KEY WORDS: • Crohn's disease • immunomodulation • oxidative stress • Pistacia lentiscus • rats

INTRODUCTION

THE INFLAMMATORY BOWEL DISEASES (IBDs) refer to ulcerative colitis and Crohn's disease (CD). Incidence rates of three to 14 cases per 100,000 people are reported in the western world,[1] but pathogenesis remains unknown; genetic, environmental, and immunologic factors seem to be responsible for the suffering in IBD, resulting in dysregulation of the immune system.[2] Cytokines, a group of polypeptides produced by immune cells, contribute to the inflammatory response.[3] Failure to control leukocyte recruitment enhances immune cell activation, which leads to further chemotaxis. Several inflammatory cytokines have been implicated in IBD; tumor necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1), interleukin

(IL)-6, and IL-8 are elevated in colonic tissue and peripheral blood of IBD patients.[3] Imbalance between oxidant and antioxidant factors is also observed. In the presence of inflammation, reactive oxygen species (ROS) inhibit antioxidant actions, increasing lipid peroxidation. As a result, oxidative stress occurs.[4] Corticosteroids, antibiotics, and immunosuppressants are used to standardize symptoms.[2]

Low compliance to treatment and the potential side effects raise the need for using natural products in IBD treatment. Chios mastic is the resin excreted from the trunk of Pistacia lentiscus, an evergreen shrub of the Anacardiaceae family. Chios mastic, exported from the island of Chios, Greece, to all over the world, is the basis for the production of a great variety of mastic products, such as bakery products, sweets, jams, ice creams, chocolates, chewing gums, candies, beverages, tea, coffee, dairy products, pasta, sauces, liquors, ouzo, and wine. In certain areas of Greece, mostly around the Aegean Sea, mastic is often used as a flavoring for Easter sweets. Modern Greek chefs have proved that this spice with its unique aromatic, wood- and

pine-like, exotic taste can go along with almost everything. In Lebanon and Syria they make a sort of traditional mastic-flavored cheese. For Arabs, mastic is considered as a great luxury for flavoring food, sweets, or milk. As a spoon sweet, mastic is served in a particularly traditional way, inside a glass of water, a version known as *ypovryhio* (submarine). Its health benefits have been known since antiquity. Earlier studies showed that mastic possesses anti-ulcer activities.[5–7] Antibacterial, antioxidant, and anticancer properties of the resin have ben found. A recent study showed that mastic administration (0.75 mg/day) reduces *Helicobacter pylori* colonization in stomach of infected mice ($P \le .01$).[8] In human subjects, mastic consumption (5 g/day) resulted in decreases of serum cardiologic and hepatic biochemical parameters ($P \le .05$).[9] *In vitro* protection against low-density lipoprotein oxidation ranged between 75.3% and 99.9%.[10] When isolated blood peripheral mononuclear cells were exposed to oxidized low-density lipoprotein, mastic managed to prevent cytotoxicity.[11] Recently it was shown that intraperitoneal infusion of a mastic extract suppressed colorectal tumor growth in mice.[12] Polyphenols and triterpenes are considered to be the bioactive compounds.[8,10] Data about resin's effects on IBD are still limited. A study on patients with CD showed that mastic intake (2.2 g/day) improves the disease activity index ($P \le .05$), because of the regulation of inflammatory and antioxidant status in peripheral blood ($P \le .05$).[13] In the same study, mastic's effects on isolated circulating mononuclear cells confirmed its potential properties ($P \le .05$).[14]

The aim of the present study was to investigate the effect of *P. lentiscus* resin in an animal model that mimics CD.

MATERIALS AND METHODS

Materials

Trinitrobenzene sulfonic acid (TNBS), phosphate-buffered saline (PBS), butylated hydroxytoluene, and Bradford reagent were all purchased from Sigma-Aldrich Co. (Steinheim, Germany). Ketamine and pentobarbital (Dolethal®) were from Vétoquinol Co. (Lure, France). Xylazine was supplied from Pfizer Co. (Karlsruhe, Germany). *P. lentiscus* resin in powder form consisting of 60% resin and 40% inulin was kindly donated by the Chios Mastic Growers' Association (Chios, Greece). The malonaldehyde (MDA) reagent pack was purchased from OxisResearch (Portland, OR, USA). The enzyme-linked immunosorbent assay kits for the quantification of TNF-α, ICAM, IL-6, IL-8, and IL-10 were all from R&D Systems (Abington, United Kingdom). All solvents were of high-performance liquid chromatography grade and were all from Merck Co. (Darmstadt, Germany).

Collection and preparation of mastic

P. lentiscus resin was collected in 2009 in the Mastiha villages, Chios, where the *P. lentiscus* tree is exclusively grown. The *P. lentiscus* tree or lentisk is an evergreen shrub, 2–3 m high, that develops very slowly and becomes fully grown after 40–50 years, reaching up to the height of 5 m at

its mature age. Its average annual yield per tree is 150–180 g of resin, although there are certain rare cases of trees yielding up to 2,000 g and others that only give 10 g. Certain soil and weather conditions favor the *P. lentiscus* tree's cultivation only in Southern Chios. Collection of resin is done according to standard collection methods. In brief, the collection method includes the *kentos*, the term for the carving of small scars on the lentisk's bark, which is the most crucial stage in *P. lentiscus* resin production. It begins in July and goes on throughout August, while sometimes there may be more carvings up until the end of September. With the help of a small sharp iron tool with grooved ends, called *kentitiri*, small cuts on the tree's trunk and main branches are made, beginning from the lowest part of the trunk and going up to the branches. The first gathering is done after August 15. *P. lentiscus* resin starts solidifying within 15–20 days from the first carving. *P. lentiscus* resin is then put into wooden boxes and stored in cool places where it is diligently cleaned. Production of mastic-related products occurs within a 6-month period of time. *P. lentiscus* powder applied in this experimental protocol was produced simply by crushing the resin and by addition of inulin (6:4 resin:inulin) in order to prevent coagulation. *P. lentiscus* powder was finally administered to the animals dispersed in water.

Experimental animals

Adult male Wistar rats were supplied by the Greek Pasteur Institute (Athens, Greece) and were housed in stainless cages at constant environmental conditions (temperature of 22°C and humidity of 50%) with a 24-hour day–night cycle. An acclimatization period lasting 2 weeks preceded the experiments. All animals were fed with isocaloric diet of standard macro- and micronutrient content (Vergerio Mangimi srl, Cadoneghe Padova, Italy). Access to food and water was *ad libitum*. The study was approved by the Animal Care Committee and was in agreement with the European Union Act and Greek Law 160, A-64, May 1991.

TNBS model of colitis

On day 0, body weight was recorded, and rats were lightly anesthetized with an intramuscular administration of a ketamine–xylazine solution (80 and 16 mg/kg of body weight, respectively). Colitis was then induced by intracolonical instillation of 25 mg of TNBS dissolved in 0.25 mL of 50% ethanol. All animals were held in the head-down position for about 1 minute to avoid leakage. A total of 84 animals weighing 261.0 ($\pm$4.1) g were randomly assigned to two parallel experiments of seven groups each (six rats per group in each experiment) as follows: Group A, control, untreated, healthy rats; Group B, TNBS-induced colitic animals; Group C, colitic rats daily supplemented with 50 mg of *P. lentiscus* powder/kg of body weight; Group D, colitic rats daily supplemented with 100 mg of *P. lentiscus* powder/kg of body weight; Group E, colitic rats daily supplemented with 200 mg of *P. lentiscus* powder/kg of body weight; Group F, colitic rats daily supplemented with 300 mg of *P. lentiscus* powder/kg of body weight; and Group G, colitic

rats daily treated with subcutaneously infused cortisone (25 μg/kg of body weight).

The first experiment lasted 4 days: the first day for colitis induction and the next 3 days for *P. lentiscus* powder treatment. The second experiment lasted 7 days; the first day for colitis induction and the next 6 days for *P. lentiscus* powder treatment. *P. lentiscus* powder dispersed in 5 mL of water was administered orally via gastric intubation. At the end of the 3-day ($n=42$) and 6-day ($n=42$) treatments, all animals were anesthetized with ketamine–xylazine (100 and 20 mg/kg of body weight, respectively) and were euthanized with Dolethal infused directly in the heart (0.6 mg/kg of body weight). After colon removal (approximately 5 cm starting from rectum), each specimen was longitudinally separated into two parts for (1) histological assessment and (2) measurements of cytokine concentrations and oxidative stress.

Histological analysis

Colon samples fixed in 10% buffered formalin were embedded in paraffin blocks. Tissue sections 4 μm thick were stained with hematoxylin–eosin and were examined by two pathologists blinded as to treatment by light microscopy. Criteria of histological score were based on previously described protocols.[15] In particular, the following parameters were assessed: (0) architectural damage, 0.0=no abnormality, 0.1=mild abnormality, 0.2=moderate diffuse or multifocal abnormalities, and 0.3=severe diffuse or multifocal abnormalities; (1) chronic inflammatory infiltrate, 1.0=no increase, 1.1=mild but unequivocal increase, 1.2=moderate increase, and 1.3=marked increase; (2) eosinophils and neutrophils, 2.0=no increase, 2.1=mild but unequivocal increase, 2.2=moderate increase, and 2.3=marked increase; (3) neutrophils in epithelium, 3.0=none, 3.1=<5% crypts involved, 3.2=<50% crypts involved, and 3.3=>50% crypts involved; (4) crypt destruction, 4.0=none, 4.1=probable local excess of neutrophils in part of the crypt, 4.2=probable marked attenuation, and 4.3=unequivocal crypt destruction; and (5) erosion or ulceration, 5.0=none, 5.1=recovering epithelium + adjacent inflammation; 5.2=probable erosion focally stripped, 5.3=unequivocal erosion, and 5.4=ulcer or granulation tissue.

Sample preparation

All colonic specimens were first weighed and homogenized in PBS using an Ultra-Turrax (IKA-Labortechnik, Staufen, Germany) blender. Prior to homogenization 0.5 M butylated hydroxytoluene in acetonitrile was added (10 μL/mL of tissue homogenate) to avoid further oxidation of the samples. To each individual tissue specimen (weighing 0.2 g) 1 mL of PBS (20 mM, pH 7.4) was added. Samples were centrifuged at 3,000 g for 15 minutes at 4°C, aliquoted, and kept at −80°C. During the same day of homogenization, total protein content was measured following the assay of Bradford.[16]

Tissue cytokine assays

Tissue concentrations of TNF-α, ICAM-1, IL-6, IL-8, and IL-10 were measured. Assessment was based on quantita-tive enzyme-linked immunosorbent assay kits according to the instructions of the manufacturer (R&D Systems). Results were expressed as pg/mg of total protein.

Tissue MDA measurement

To quantify MDA content a commercial kit was applied following the instructions of the manufacturer (OxisResearch). Measurement was based on the reaction of a chromogenic reagent, *N*-methyl-2-phenylindole, with MDA at 45°C. The stable chromophore product exhibits maximal absorbance at 586 nm. Results were expressed as pmol/mg of total protein.

Statistical analysis

Power calculation with anticipated power (π) over 90% and SE 10% revealed that each animal group should consist of six rats. All results were expressed as mean±SE values. Data were processed with SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). When appropriate, differences between the groups were analyzed for significance by the nonparametric Kruskal–Wallis test. Bonferroni's *post hoc* test was applied for multiple comparisons (more than two groups compared). The statistical significance level was set at $P \le .05$.

RESULTS

Histological evaluation

As shown in Table 1, microscopy assessment of the colon revealed significant differences between healthy controls

TABLE 1. COLONIC DAMAGE SCORE AFTER COLITIS INDUCTION, FOLLOWED BY 3 DAYS OF TREATMENT WITH *P. LENTISCUS* POWDER

Group	Histological grade at 3 days	P value
Group A: control, untreated	0.0±0.0	
Group B: TNBS-induced colitic animals	4.7±0.5*	.00
Group C: colitic rats, 50 mg of *P. lentiscus* powder/kg of BW/day	1.4±0.3**	.00
Group D: colitic rats, 100 mg of *P. lentiscus* powder/kg of BW/day	1.3±0.1**	.00
Group E: colitic rats, 200 mg of *P. lentiscus* powder/kg of BW/day	2.5±0.6**	.001
Group F: colitic rats, 300 mg of *P. lentiscus* powder/kg of BW/day	1.7±0.2**	.00
Group G: colitic rats, treated with cortisone	2.1±0.0**	.00

Histological grades are expressed as mean±SE values.
The Kruskal–Wallis nonparametric test showed statistically significant differences among ranks: *significant differences between untreated and trinitrobenzene sulfonic acid (TNBS)-treated; **statistically significant differences between TNBS-treated and *P. lentiscus*– or cortisone-treated animals. Bonferroni's analysis of variance was applied with the statistical significance level set at $P \le .05$.
BW, body weight.

(Fig. 1A) and TNBS-treated animals (Fig. 1B) at the end of the 3-day period. Cortisone decreased histological damage (Fig. 1C). *P. lentiscus* powder administration diminished the elevated histological damage score at all doses tested (characteristic picture in Fig. 1D), leading to remission of the disease. No significant changes were observed when *P. lentiscus* powder was administered for 6 days after colitis induction.

Immunocytochemistry

The severity of colonic inflammation was further evaluated by measurement of several cytokines. As shown in Figure 2, the lack of microscopic evidence of colitis in Group A (healthy controls) agreed with low concentrations of tissue cytokines. Significantly elevated concentrations of TNF-α (332 ± 36 vs. 114 ± 14 pg/mg of protein, $P = .001$), IL-6 ($2,398 \pm 396$ vs. 898 ± 67 pg/mg, $P = .028$), IL-8 (161 ± 20 vs. 41 ± 2 pg/mg, $P = .011$), and ICAM-1 ($20,030 \pm 2,312$ vs. $6,541 \pm 494$ pg/mg, $P = .00$) were observed in Group B of chemically induced colitis compared with Group A. No significant differences were observed in IL-10 content (464 ± 123 vs. 267 ± 28 pg/mg, $P = 1.0$), 3 days after induction of colitis.

The TNBS-induced increase of pro-inflammatory cytokines was prevented in the *P. lentiscus* powder–supplemented groups, largely in Group D. In particular, the increase in TNF-α, a key molecule in colonic inflammation, was statistically suppressed by *P. lentiscus* powder in Group D compared with colitic Group B (143 ± 27 vs. 332 ± 36 pg/mg, $P = .008$) (Fig. 2). Likewise, in Group D, concentrations of IL-6 and IL-8 were statistically reduced compared with Group B (935 ± 137 vs. $2,398 \pm 396$ pg/mg [$P = .036$] and 41 ± 5 vs. 161 ± 20 pg/mg [$P = .010$], respectively) (Fig. 2). When colonic ICAM-1 was quantified, a statistically significant decrease occurred in both Groups C and D (Fig. 2): $11,612 \pm 622$ pg/mg ($P = .027$) and $10,132 \pm 1,821$ pg/mg ($P = .005$), respectively, versus $20,030 \pm 2,312$ pg/mg. Treatment with cortisone (Group G) failed to regulate cytokine content other than ICAM-1 ($101,800 \pm 2,158$ vs. $20,030 \pm 2,312$ pg/mg, $P = .005$) (Fig. 2). No significant changes were observed in IL-10 with *P. lentiscus* powder treatment at all doses examined.

At the end of the 6-day period, significantly elevated concentrations of ICAM-1 between healthy and colitic rats ($3,220 \pm 739$ vs. $6,659 \pm 500$ pg/mg, $P = .026$) were detected, whereas all other cytokines (TNF-α, IL-6, and IL-8) re-
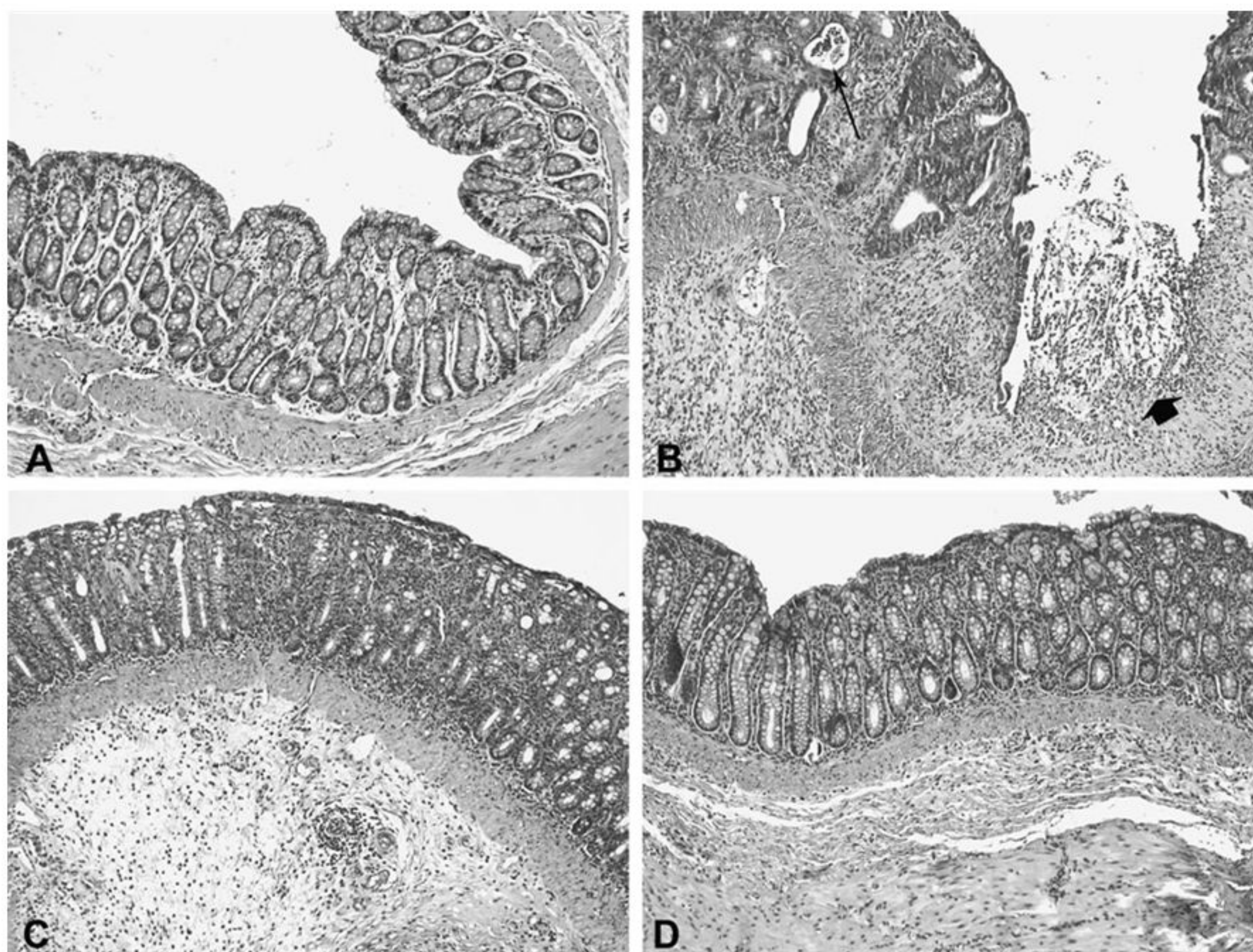

FIG. 1. Colonic damage after colitis induction, following by 3 days of treatment with *P. lentiscus* powder. Histological sections, hematoxylin–eosin staining, ×100 original magnification. (**A**) Healthy untreated group with normal mucosa. (**B**) TNBS model of colitis with severe crypt distortion, crypt abscess (arrow), inflammation, and ulceration (arrowhead). (**C**) Animals treated with cortisone characterized by elevated mucosal inflammatory cells and some crypt distortion still present. (**D**) Animals daily treated with 100 mg of *P. lentiscus* powder/kg of BW characterized by sparse mucosal inflammatory cells and mild fibrosis.
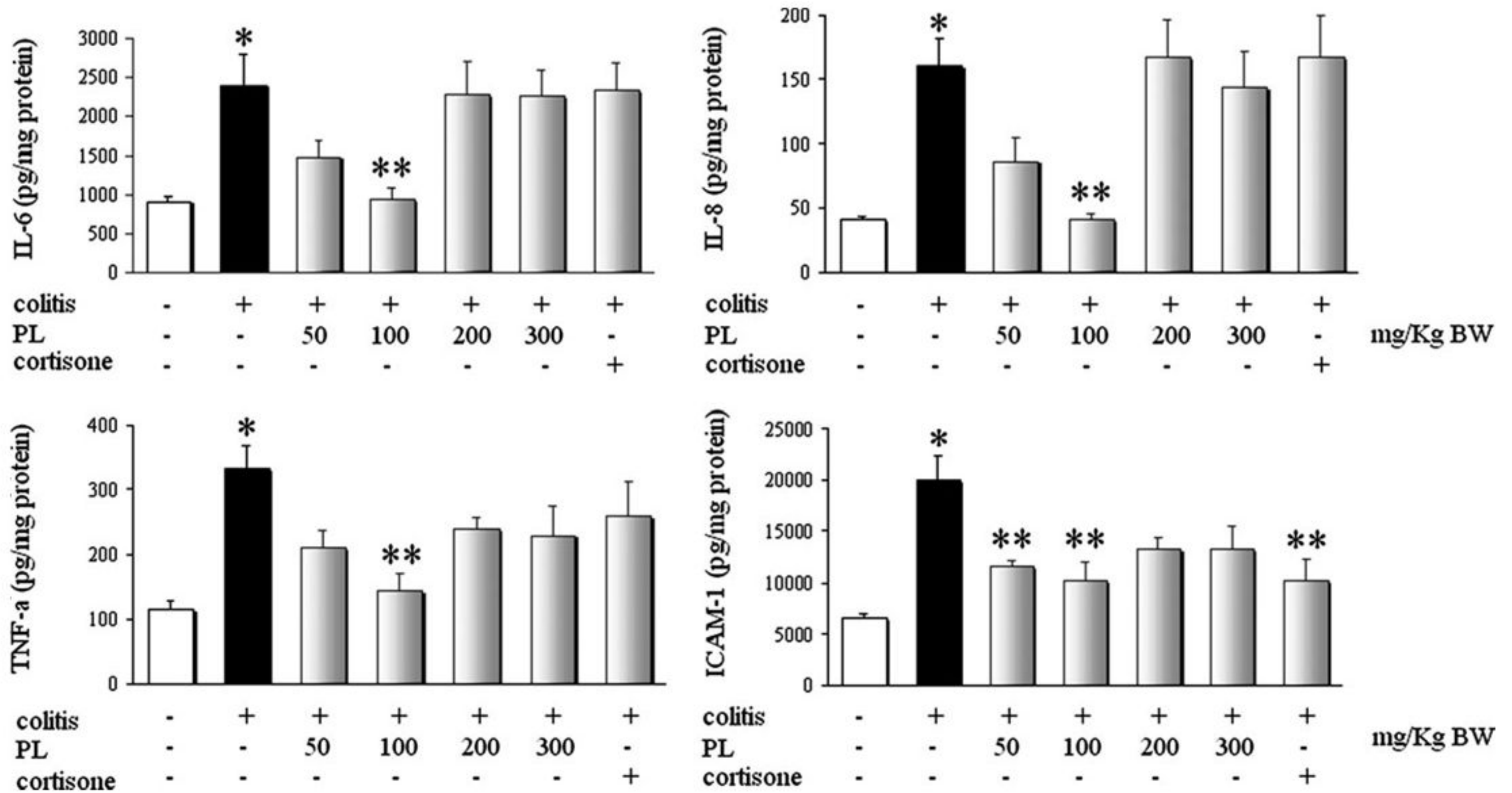
FIG. 2. Inflammatory cytokine changes in the colon after colitis induction, followed by 3 days of treatment with *P. lentiscus* (PL) powder. Data are mean ± SE values expressed as pg/mg of total protein. The statistical significance level was set at $P \leq .05$: *statistically significant differences between untreated and TNBS-treated; **statistically significant differences between TNBS-treated and PL powder– or cortisone-treated animals. ICAM-1, intercellular adhesion molecule-1; IL, interleukin; TNF-α, tumor necrosis factor-α.

mained unaltered, implying that the evolution of TNBS-induced colitis began to attenuate 6 days after challenge.

Colonic MDA concentration

To assess the potential role of ROS in the intestinal damage, the MDA content was measured. Colonic MDA was statistically increased in TNBS-treated compared with noncolitic rats (1,433 ± 100 vs. 389 ± 53 pmol/mg of protein, $P = .00$) killed at the end of the 3-day period (Fig. 3). The concentration of MDA was significantly reduced in *P. lentiscus* powder–treated colitic animals of all Groups C, D, E, and F, as well as in cortisone-treated colitic animals, compared with Group B rats with TNBS-induced colitis: 751 ± 235 pmol/mg ($P = .013$), 409 ± 36 pmol/mg ($P = .000$), 550 ± 127 pmol/mg ($P = .000$), 453 ± 104 pmol/mg ($P = .000$), and 553 ± 137 pmol/mg ($P = .001$), respectively, versus 1,433 ± 100 pmol/mg.

No significant changes were observed between healthy and colitic or between colitic and *P. lentiscus* powder– or cortisone-treated animals killed at the end of a 6-day period, indicative of the attenuation in chemically induced colitis.

DISCUSSION

In the present study data show that oral ingestion of *P. lentiscus* powder decreased histological damage in TNBS-induced colitic rats after a 3-day treatment. Overall, the

histology results suggest evidence of severe pathological events in the TNBS-induced colitic animals. Evidence of histological improvement was found in all of the *P. lentiscus* powder–treated groups and in the cortisone-treated group. This improvement was in all components of the total score, and the mean total scores were significantly reduced. This
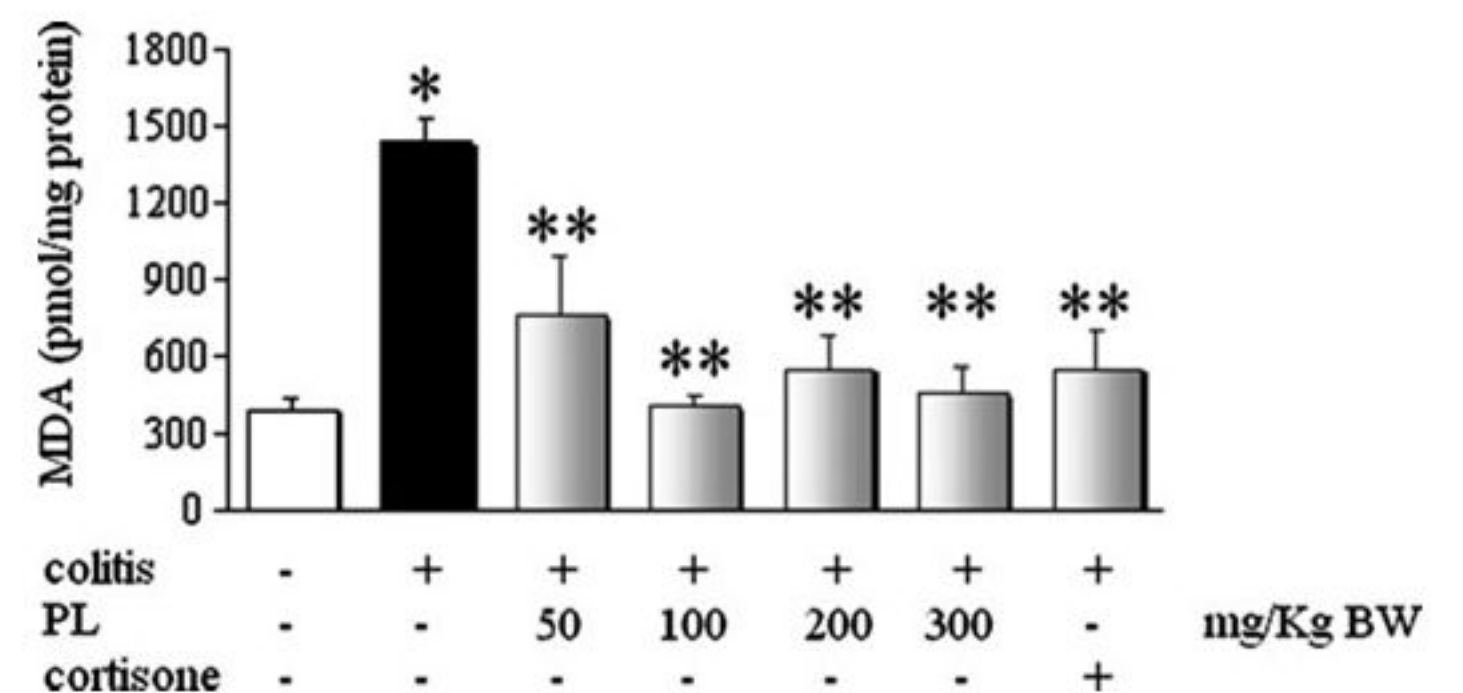

FIG. 3. Malonaldehyde (MDA) changes in the colon after colitis induction, followed by 3 days of treatment with PL powder treatment. Data are mean ± SE values expressed as pmol/mg of total protein. The statistical significance level was set at $P \leq .05$: *statistically significant differences between untreated and TNBS-treated; **statistically significant differences between TNBS-treated and PL powder– or cortisone-treated animals.

finding was associated with decreases in pro-inflammatory cytokines and oxidative stress. In pilot preliminary experiments, animals, either control or colitic, were administered water by gastric intubation in order to determine the effect of water used to disperse *P. lentiscus* powder. No effect assessed by histological damage score or tissue TNF-α level was reported in vehicle (water)-administered animals (data not shown). This is the first study demonstrating the benefit of *P. lentiscus* resin in an animal model of severe inflammation and IBD. Previous pilot studies[13,14] have demonstrated the reduced severity of CD in patients with no more than moderate disease activity index supplemented with mastic. Taken together, our data support the proposed use of *P. lentiscus* resin in the treatment of inflammatory diseases.

A steadily increasing number of experimental animal models have recently been developed and have contributed greatly to important advances in our current understanding of the immunological, pathological, and physiological features of chronic intestinal inflammation. Although none of these models truly represents human IBD, however, they mimic key clinical, histological, and immunological findings in IBD patients. Among the inducible models, the TNBS model, a model developed by Morris *et al.*,[17] is particularly useful for studying biochemical inflammatory pathways.[18] The TNBS model is quite similar to CD in humans with elevated levels of T-helper 1 cytokines such as TNF-α and low production of T-helper 2 cytokines such as IL-10. TNF-α is considered the key molecule, shown by the fact that when mice with TNBS-induced colitis were treated by intraperitoneal injection of antibodies to TNF-α, improvements of both the clinical and histopathologic signs of disease were found.[19] In the present study, *P. lentiscus* powder administration resulted in a TNF-α level decrease, indicating that *P. lentiscus* resin ameliorates colonic injury by preventing the induction of TNF-α. The significance of this activity is immense, and although some groups have not demonstrated increased concentrations of TNF-α in CD compared with healthy controls,[20] others have done so.[21] TNF-α has been proven critical for the remission of inflammation in patients with CD.[22] Nowadays many therapeutic strategies for CD target inhibition of the TNF-α cascade. The results of previous trials with infliximab and CDP571, which neutralize antibodies for TNF-α, are very promising.[23]

Apart from TNF-α, other cytokines play central roles in IBD. The pro-inflammatory cytokines IL-6 and IL-8 are produced in excess, whereas the level of the immunosuppressive IL-10 is reduced in inflamed tissues. Both the excess of pro-inflammatory cytokines and the relative inefficiency of counterregulatory molecules are required for maintaining, amplifying, and perpetuating chronic inflammation in IBD.[24] In addition, different cytokines induce the expression of adhesion molecules such as ICAM-1 on the endothelium, thus favoring the recruitment of new inflammatory cells.[3]

Administration of mastic reduced production of IL-6, IL-8, and ICAM-1 in the colonic tissue. The importance of IL-6 in patients with CD has been well documented. In patients

with active CD, IL-6 is overexpressed in the inflamed mucosa, and plasma content is elevated.[25] Also, IL-6 is the main cytokine factor responsible for hepatic induction of acute-phase proteins in CD. Here, we show that *P. lentiscus* resin in the form of powder reduces the colonic IL-6 level. Because IL-6 stimulates neutrophil chemotaxis and relates to the presence of necrosis and tissue destruction, *P. lentiscus* powder might significantly improve the histology of the colon by mitigating IL-6 production. Concerning the recruitment and activation of polymorphonuclear neutrophils, IL-8 is also an important cytokine.[26] Increased mucosal generation of IL-8 may attract neutrophils from the circulation into the inflammatory site and induce binding of neutrophils in the intestinal tissue, contributing to the accumulation and activation of neutrophils in the affected mucosa. A large number of studies have shown increased content of IL-8 in CD.[27] Therefore, the inhibition of IL-8 production is believed to provide a novel approach to the treatment of IBD. In contrast, the effectiveness of ICAM-1 immunoblockade in IBD management remains uncertain. A significant reduction in leukocyte recruitment in response to ICAM-1 immunoneutralization in TNBS-induced colitis in rats has been documented.[28] A study in patients with active CD receiving an anti-ICAM-1 antisense oligonucleotide demonstrated an increase in the number of patients achieving remission[29] and a reduction in steroid requirements in patients treated with drug compared with those receiving placebo.[30] Another randomized, controlled, study showed promising acute-phase and long-term benefits of this anti-ICAM-1 in patients with mild to moderate descending ulcerative colitis.[31] Here, *P. lentiscus* powder was proven able to diminish levels of both parameters, IL-8 and ICAM-1; however, it did not alter the immunosuppressant IL-10, and this may occur naturally in a highly complex cytokine network such as in IBD.

IBD is characterized by infiltration of neutrophils and mononuclear cells into colonic tissues. Neutrophils are crucial for the breakdown and remodeling of injured tissue, while they also release mediators of gastric injury. Neutrophils cause the production of free radicals into the inflamed mucosa. ROS, including hydroxyl radicals, hydrogen peroxide, and nitric oxide, induce cellular injury including peroxidation of membrane lipids, protein denaturation, and DNA damage. Several studies have shown that colonic tissues in IBD possess higher amounts of ROS compared with healthy subjects.[32] Oxidative stress is monitored in inflamed colonic tissues by measurement of lipid peroxidation and antioxidant enzyme activity. It has been shown that colonic MDA increased and superoxide dismutase decreased in both human and experimental animal studies of IBD.[33,34] Increased levels of ROS promote the production of inflammatory cytokines in the intestinal epithelium, resulting in imbalance between oxidant and antioxidant factors, and inflammation is constantly triggered (Fig. 4). To evaluate oxidative stress, MDA concentration in colonic tissues was measured. *P. lentiscus* powder diminished lipid peroxidation, indicative of a radical scavenging activity as the potential mechanism of action. This is supported by the fact
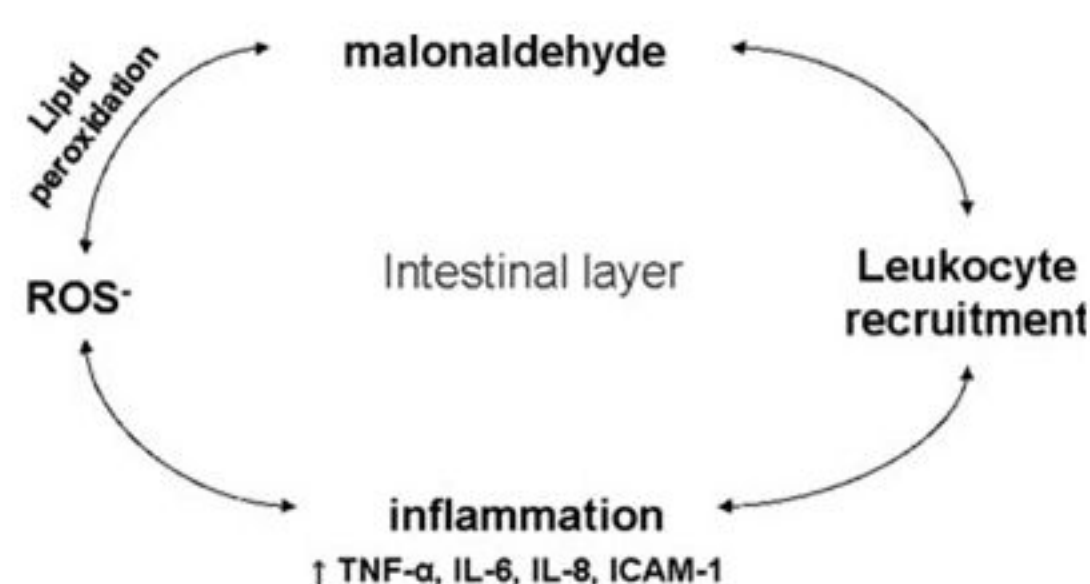
FIG. 4. Scheme for production of inflammatory cytokines in the presence of reactive oxygen species (ROS) in the intestinal layer of TNBS-induced colitic rats.

that mastic gum has been previously shown to possess antioxidant effects on low-density lipoprotein oxidation,[10] on the cytotoxicity induced by oxidized low-density lipoprotein on mononuclear cells,[11] and *in vivo* on lipid peroxidation in patients with active CD.[13,14] Antioxidant activity, together with the anti-inflammatory efficacy of mastic, has been attributed to the polyphenols, as well as to the terpenes, that are abundant in mastic. However, regarding antiradical properties, the phenolic structure is more likely to contribute to the antioxidant effect. Previous research of our team has shown that phenolic compounds with established antiradical activity are present in mastic.[35]

Several studies have shown the potential beneficial effect of dietary treatment in the management of IBD. Because the drugs used for IBD management may produce adverse effects and are therefore hazardous when administered constantly, the use of natural nutritional products effective in IBD is encouraged. Dietary fiber, (*n*-3) polyunsaturated fatty acids, and polyphenols[36,37] have been proven to have anti-inflammatory activity in IBD. In most cases these naturally occurring constituents are well tolerated. However, (*n*-3) polyunsaturated fatty acids cause a decrease in the colonic antioxidant system and subsequent oxidative injury at the site of inflammation.[38] Concurrent administration of (*n*-3) polyunsaturated fatty acids and flavonoids blocks this phenomenon.[37] Among the dietary constituents examined for their efficiency in the management of IBD is also inulin, a prebiotic carbohydrate that resists digestion by intestinal and pancreatic enzymes in the human gastrointestinal tract and is fermented by bacteria living in the intestinal ecosystem.[39] When administered in adequate amounts, it increases saccharolytic activity within the intestine and promotes selectively the growth of bifidobacteria. The effect of the prebiotic inulin has been tested in the rat model of colitis induced by dextran sodium sulfate, which mimics ulcerative colitis in humans.[40] In rats, treatment with oral inulin reduced tissue myeloperoxidase activity, mucosal release of inflammatory mediators, and the extent of damage. From experimental models of colitis inulin seems to offer an opportunity to prevent intestinal inflammatory lesions in human ulcerative colitis; nevertheless, in the above studies it is administered prior to the development of inflammation, and this is in mega doses (*e.g.*, 400 mg/day). Controlled clinical trials of an appropriate sample size to confirm this hypothesis

are lacking. In our study, commercially available *P. lentiscus* powder contains mastic:inulin at 6:4. Still, the activity shown here could not be credited to inulin as (1) a very low dose is applied and (2) administration follows the development of severe colonic inflammation.

No statistical differences were observed in animal body weight throughout the experiments (data not shown). This was actually expected as the intervention time of the study was limited and colitis began to attenuate 6 days after TNBS challenge. Also seen was that dose-dependent interventions failed to show a dose-dependent activity of *P. lentiscus* powder. Instead, in doses above 100 mg/kg of body weight the increased β-polymer content might inhibit the activity of bioactive compounds on the epithelium.

In conclusion, the present results demonstrate that *P. lentiscus* in the form of powder exerts a beneficial effect in severe TNBS-induced colitis in rats. These outcomes regarding the anti-inflammatory efficacy of *P. lentiscus* resin in IBD are in accordance with those in patients with active mild to moderate CD,[13,14] although there are significant differences between the rat and human intestine, with respect to the mechanisms by which they utilize micronutrients; however, a possible mechanism could be the scavenging of free radicals and the regulation of key inflammatory mediators of IBD by the terpenes and phenolic compounds present in *P. lentiscus* resin. Further studies on the molecular mechanism underlying this effect and on the active *P. lentiscus* resin fraction are now in progress.

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AUTHOR DISCLOSURE STATEMENT

A.P. is an employee of ELPEN-Pharmaceuticals Co. Inc. No competing financial interests exist for A.G., A.C.K., G.A., J.K.T., and N.K.A.

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