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ORIGINAL ARTICLE

Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress

Renan O. Silva[a], Francisca Beatriz M. Sousa[a], Samara R.B. Damasceno[a], Nathalia S. Carvalho[a], Valdelânia G. Silva[a], Francisco Rodrigo M.A. Oliveira[b], Damião P. Sousa[c], Karoline S. Aragão[d], André L.R. Barbosa[a], Rivelilson M. Freitas[b], Jand Venes R. Medeiros[a]*

[a]*Biotechnology and Biodiversity Center Research (BIOTEC), Federal University of Piauí, Av. São Sebastião, 2819, CEP: 64202-020, Parnaíba, PI, Brazil*
[b]*Postgraduate Program in Pharmaceutical Sciences, Federal University of Piauí, Av. N S Fátima, CEP 64049-550, Teresina, PI, Brazil*
[c]*Department of Physiology, Federal University of Paraíba, CP5009, CEP 58051-970, João Pessoa, PB, Brazil*
[d]*Department of Physiology and Pharmacology, Federal University of Ceará, Rua CR Nunes de Melo 1315, CEP 60430-270, Fortaleza, CE, Brazil*

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*Correspondence and reprints:
jandvenes@ufpi.edu.br

ABSTRACT

Studies have shown that diterpenes have anti-inflammatory and redox-protective pharmacological activities. The present study aimed to investigate the anti-inflammatory properties of phytol, a diterpene alcohol, in a mouse model of acute inflammation, and phytol effect on leukocyte recruitment, cytokines levels, and oxidative stress. The anti-inflammatory activities of phytol were assessed by measuring paw edema induced by different inflammatory agents (e.g., λ-carrageenan, compound 48/80, histamine, serotonin, bradykinin, and prostaglandin E_2 [PGE_2]), myeloperoxidase (MPO) activity, peritonitis model and cytokine levels. Further, oxidative stress was evaluated by determining glutathione (GSH) levels and malondialdehyde (MDA) concentration. The results showed that phytol (7.5, 25, 50, and 75 mg/kg) significantly reduced carrageenan-induced paw edema, in a dose-dependent manner. In addition, phytol (75 mg/kg) inhibited compound 48/80-, histamine-, serotonin-, bradykinin- and PGE_2-induced paw edema. It also inhibited the recruitment of total leukocytes and neutrophils; decreased MPO activity, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels, and MDA concentration; and increased GSH levels during carrageenan-induced acute inflammation. These results suggest that phytol attenuates the inflammatory response by inhibiting neutrophil migration that is partly caused by reduction in IL-1β and TNF-α levels and oxidative stress.

INTRODUCTION

Inflammation is a complex biological response of vascularized tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. It is well established that this process involves the local formation of kinins and cytokines that promote vascular endothelial cell activation, followed by leukocyte migration into the inflamed site [2]. Another important component of he inflammatory response is oxidative stress leading to the generation of molecules, such as hydrogen peroxide, superoxide anion, and peroxynitrite, which are produced in response to stimuli and can exacerbate this process [3].

The clinical signs and symptoms of inflammation include edema, fever, erythema, pain, and cell migration (primarily neutrophil migration) into the site of injury [4]. The drugs used to treat these symptoms, such as nonsteroidal anti-inflammatory drugs (NSA-

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IDs), are not only associated with major adverse effects, such as gastrointestinal ulcers, bleeding, and renal disorders, but also have low therapeutic efficacy [5]. Thus, the search for new products with therapeutic potential for the treatment for inflammation has increased in recent years [6].

Many studies have been conducted as a part of the search for new therapeutic options for inflammation, and classes of secondary metabolites from natural sources, such as lactones [7], alkaloids [8], and terpenoids [9], have attracted the attention of many researchers because of their pharmacological activities.

Phytol (*Figure* 1) is an acyclic diterpene alcohol found in the essential oils of some aromatic plants, such as *Cleome serrata* [10] and *Lantana radula* [11]. Various therapeutic activities of phytol have been reported in previous studies, including its activity against mycobacteria [12], and anticonvulsant [13], antispasmodic [14], and anticancer activities [15].

Some studies have demonstrated promising anti-inflammatory pharmacological activities of diterpenes [16,17], but few have focused on phytol. The aim of this study was to investigate the anti-inflammatory properties of phytol, a diterpene alcohol, in mouse models of acute inflammation. Furthermore, the study investigated the roles of leukocyte recruitment, cytokines, and oxidative stress in phytol-induced effects.

MATERIALS AND METHODS

Drugs and chemicals

λ-Carrageenan, compound 48/80, serotonin, histamine, bradykinin, prostaglandin E2 (PGE2), indomethacin, dimethyl sulfoxide (DMSO), and phytol (97% purity) were purchased from Sigma Chemical (St. Louis, MO, USA). Heparin and morphine were provided by Merck (Brazil). All drugs were dissolved in sterile 0.9% (w/v) NaCl (saline). The phytol was dissolved in 2% DMSO. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Animals

Male Swiss mice weighing 25–30 g were randomly housed in appropriate cages at 23 ± 2 °C under a 12/12-h light/dark cycle with free access to food (Purina®, Brazil) and water. Experimental protocols were approved by the Ethics Committee in Research of the Federal University of Piauí (protocol No. 0066/10), and handling procedures were in accordance with the *Guide for Care and Use of Laboratory Animals* (National Institute of Health, Bethesda, MD, USA).

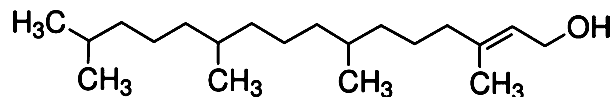

Figure 1 Chemical structure of phytol.

Effect of phytol on carrageenan-induced paw edema

Initially, the animals were randomly divided into seven groups (n = 5), and edema acute hind paw edema was produced by injecting of 50 μL of a suspension of carrageenan (500 μg/paw, prepared in 0.9% sterile saline) into the right hind paw (group I). In the other groups, mice were pretreated intraperitoneally (i.p.) with carrageenan + 2% DMSO (group II) or only 2% DMSO (group III); indomethacin 10 mg/kg (group IV, standard drug) or phytol 7.5, 25, 50, or 75 mg/kg (groups V, VI, VII and VIII, respectively). Right paw volume was measured by the dislocation of the water column of a plethysmometer (Panlab, Barcelona, Spain) before (V_0; time zero) at 1, 2, 3, and 4 h after carrageenan treatment (V_t) as previously described [18]. The effect of pretreatment was calculated as percent inhibition of edema relative to the paw volume of the DMSO-treated controls using the following formula:

$$\% \text{ inhibition of edma} = \frac{(V_t - V_0)\text{Control} - (V_t - V_0)\text{Treated}}{(V_t - V_0)\text{Control}} \times 100$$

Effect of phytol on paw edema induced by different agents

To induce edema with different agents, the animals received 50-μL injections of compound 48/80 (12 μg/paw), serotonin (5-HT; 1% w/v), histamine (Hist; 100 μg/paw) bradykinin (BK; 6.0 nmol/paw), or PGE_2 (3 nmol/paw) into the right hind paw, as previously described [4,19]. The contralateral paw received 50 μL of 2.0% DMSO and served as control untreated. Phytol (75 mg/kg) or indomethacin (10 mg/kg, reference control) was injected i.p. Thirty minutes before intraplantar injections of phlogistic agents. Right paw volume was measured by the dislocation of the water column of a plethysmometer (Panlab, Barcelona, Spain) before (V_0; time zero) at 30, 60, 90, and 120 min after (V_t) phlogistic agents administration.

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Myeloperoxidase (MPO) activity

Briefly, 50–100 mg of paw tissue was homogenized in potassium buffer containing 0.5% hexadecyltrimethylammonium bromide (HTAB). The homogenate was centrifuged at 4500 ***g*** for 15 min at 4 °C. The pellet was resuspended, and MPO activity was assayed by measuring the change in absorbance at 450 nm using o-dianisidinedihydrochloride and 1% hydrogen peroxide. Myeloperoxidase activity was reported as units/mg of tissue. A unit of MPO activity was defined as that converting 1 μmol of hydrogen peroxide to water in 1 min at 22 °C.

Evaluation of neutrophil migration

Mice were injected intraperitoneally with 2.0% DMSO, phytol 75 mg/kg, or indomethacin 10 mg/kg. Thirty minutes later, the animals were injected with carrageenan (500 μg/cavity; 250 μl). After 4 h, mice were sacrificed and peritoneal cavity was washed with 1.5 mL of heparinized phosphate-buffered saline (PBS). The volumes recovered were similar in all experimental groups and were equivalent to ~95% of the injected volume. Total cell counts were performed in a Neubauer chamber, and differential cell counts (100 cells total) were carried out on cytocentrifuge slides stained with hematoxylin and eosin. The results are presented as the number of neutrophils per milliliter of peritoneal exudate. Aliquots of the peritoneal exudates were stored at −70 °C for later analysis of cytokine content, glutathione (GSH) levels, and malondialdehyde (MDA) concentration.

Cytokine measurements

The levels tumor necrosis factor (TNF)-α and interleukin (IL)-1β were evaluated using sandwich ELISA as described previously [20]. Briefly, microliter plates were coated overnight at 4 °C with antibody against mice TNF-α or IL-1β (2 μg/mL). Blocking of nonspecific binding sites was accomplished by incubating plates with PBS containing 2% BSA for 90 min at 37 °C. After blocking the plates, the test samples and each standard at various dilutions were added in duplicate and incubated at 4 °C for 24 h. The plates were washed three times with buffer. After washing the plates, 50 μL of biotinylated sheep polyclonal anti-TNF-α and anti-IL-1β (diluted 1 : 1000 with assay buffer 1% BSA) was added to the wells. After further incubation at room temperature for 1 h, the plates were washed, and 50 μL of streptavidin-HRP diluted 1 : 5000 was added to all wells. The reagent o-phenylenediamine dihydrochloride (50 μL) was added 15 min later, and the plates were incubated in the dark at 37 °C for 15–20 min. After color development, the reaction was stopped with the addition of sulfuric acid (1 M), and absorbance was measured at 490 nm. The results are expressed as pg/mg protein and reported as mean ± SD.

Glutathione (GSH) levels

The concentration of glutathione in the peritoneal exudates was estimated according to the method previously described [21]. Aliquots (600 μL) of the peritoneal exudates were centrifuged at 3000 rpm for 15 min at 4 °C. Next, 400 μL of each supernatant was mixed with 800 μL of Tris buffer (0.4 M, pH 8.9) and 20 μL of 0.01 M 5,5-dithio-bis (2-nitrobenzoic acid). Subsequently, the samples were stirred for 3 min and read on a spectrophotometer at 412 nm. Glutathione concentration was determined via a reduced GSH standard curve, which was generated in parallel. Glutathione levels are expressed as μg/ml of exudates.

Malondialdehyde (MDA) concentration

The MDA concentration in the peritoneal exudates from each group was measured according to the method previously described [22]. Briefly, aliquots (500 μL) peritoneal exudates were centrifuged at 3000 ***g*** for 15 min at 4 °C, then 250 μL each supernatant was added to 1.5 mL of 1% phosphoric acid (H_3PO_4) and 0.5 mL of 0.6% thiobarbituric acid (aqueous solution). Then, this mixture was stirred and heated in a boiling water bath for 45 min. The mixture was then cooled immediately in an ice water bath followed by the addition of 4 mL of n-butanol. This mixture was shaken, and the butanol layer was separated by centrifugation at 1200 × ***g*** for 10 min. Optical density was determined to be 535 and 520 nm, and the optical density difference between the two determinations was calculated as the tert-butyl alcohol value. Malondialdehyde concentrations are expressed as mmol/ml of exudates.

Statistical analysis

Results are expressed as mean ± SEM from at least five animals per group, and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Newman–Keuls post hoc test, when appropriate. Statistical significance was set at $P < 0.05$.

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RESULTS

Phytol reduced carrageenan-induced paw edema

As shown in *Table* I, carrageenan administration was effective in inducing time-dependent paw edema, which peaked after 4 h (0.054 ± 0.004 mL). Phytol (25, 50, and 75 mg/kg) significantly inhibited ($P < 0.05$) the development of carrageenan-induced paw edema in a dose-dependent manner, at all time points. The maximum inhibitory effect—51.8% inhibition—was achieved 4 h after the administration of 75 mg/kg phytol (0.026 ± 0.002 mL). The reference drug indomethacin (10 mg/kg) also significantly decreased ($P < 0.05$) paw edema throughout the experimental period, with the maximum inhibition being 59.3% 4 h after treatment with carrageenan (*Table* I). Because the 75 mg/kg phytol dose produced the maximum inhibitory effect against carrageenan-induced paw edema, it was used to study the possible mechanisms involved in the anti-inflammatory activity of phytol.

Phytol reduced paw edema induced by different phlogistic agents

As shown in *Figure* 2, the injection of compound 48/80 (0.108± 0.011 mL; *Figure 2a*), Hist (0.082 ± 0.003 mL; *Figure 2b*), 5-HT (0.084 ± 0.008 mL; *Figure 2c*), BK (0.070 ± 0.009 mL; *Figure 2d*), or PGE_2 (0.069 ± 0.003 mL; *Figure 2e*) induced intense paw edema. Pretreatment with phytol (75 mg/kg) significantly inhibited ($P < 0.05$) paw edema induced by compound 48/80 (0.038 ± 0.008 mL; 64.8% inhibition), Hist (0.030 ± 0.010 mL; 64.4% inhibition), 5-HT (0.028 ± 0.005 mL; 64.4% inhibition), BK (0.018 ± 0.06 mL; 74.3% inhibition), or PGE_2 (0.030 ± 0.007 mL; 56.5% inhibition). Indomethacin (10 mg/kg) also significantly reduced the paw edema induced by different phlogistic agents.

Phytol reduced carrageenan-induced MPO activity

Figure 3 shows that compared with the group treated with carrageenan alone (9.85 ± 0.96 U/mg of tissue), the groups pretreated with phytol (75 mg/kg) should significantly reduced ($P < 0.05$) MPO activity (3.21 ± 0.82 U/mg of tissue). Furthermore, compared with treatment with carrageenan alone, treatment with the reference drug, indomethacin, significantly reduced ($P < 0.05$) MPO activity (4.37 ± 1.45 U/mg of tissue).

Phytol inhibited cell migration in the carrageenan-induced peritonitis model

Carrageenan administration induced a significant increase in the total leukocyte ($4.83 \pm 0.99 \times 10^3$ cells/mL; *Figure 4a*) and neutrophil recruitment ($3.55 \pm 0.32 \times 10^3$ cells/mL; *Figure 4b*) to the peritoneal cavity. Compared with treatment with carrageenan alone, pretreatment with phytol (75 mg/kg) significantly reduced ($P < 0.05$) the leukocyte recruitment ($2.39 \pm 0.62 \times 10^3$ cells/mL) and neutrophil migration ($0.42 \pm 0.45 \times 10^3$ cells/mL) to the peritoneal cavity. Indomethacin (10 mg/kg) reduced leukocyte ($1.98 \pm 0.32 \times 10^3$ cells/mL) and neutrophil counts ($0.58 \pm 0.11 \times 10^3$ cells/mL) in the peritoneal cavity (*Figure* 4).

Phytol decreased carrageenan-induced TNF-α and IL-1β production

Carrageenan significantly increased ($P < 0.05$) TNF-α ($208.7 \pm 39.2 \times 10^3$ pg/mL; *Figure 5a*) and IL-1β

Table I Effect of phytol on carrageenan-induced paw edema.

Treatment	Dose (mg/kg)	Paw edema (mL)			
		1 h	2 h	3 h	4 h
DMSO		0.006 ± 0.004	0.004 ± 0.002	0.004 ± 0.002	0.002 ± 0.002
Carrageenan		0.026 ± 0.002	0.037 ± 0.002	0.046 ± 0.003	0.054 ± 0.004
Carrageenan + DMSO		0.025 ± 0.004	0.040 ± 0.002	0.046 ± 0.004	0.052 ± 0.005
Indomethacin	10	0.012 ± 0.002* (53.8)	0.016 ± 0.002* (56.7)	0.018 ± 0.002* (60.8)	0.022 ± 0.003* (59.3)
Phytol	7.5	0.023 ± 0.003	0.033 ± 0.003	0.040 ± 0.003	0.041 ± 0.004
	25	0.015 ± 0.003* (42.3)	0.020 ± 0.003* (45.9)	0.030 ± 0.005 (34.7)	0.034 ± 0.002 (37.0)
	50	0.017 ± 0.003 (34.6)	0.018 ± 0.002* (52.6)	0.026 ± 0.003* (43.4)	0.030 ± 0.003* (44.4)
	75	0.016 ± 0.002* (38.5)	0.016 ± 0.002* (56.8)	0.022 ± 0.003* (52.2)	0.026 ± 0.002* (51.8)

Values of paw edema are expressed in mean ± SEM of 5–6 animals per group.

% Inhibition of paw edema is indicated in parenthesis.

*$P < 0.05$ vs. control group.

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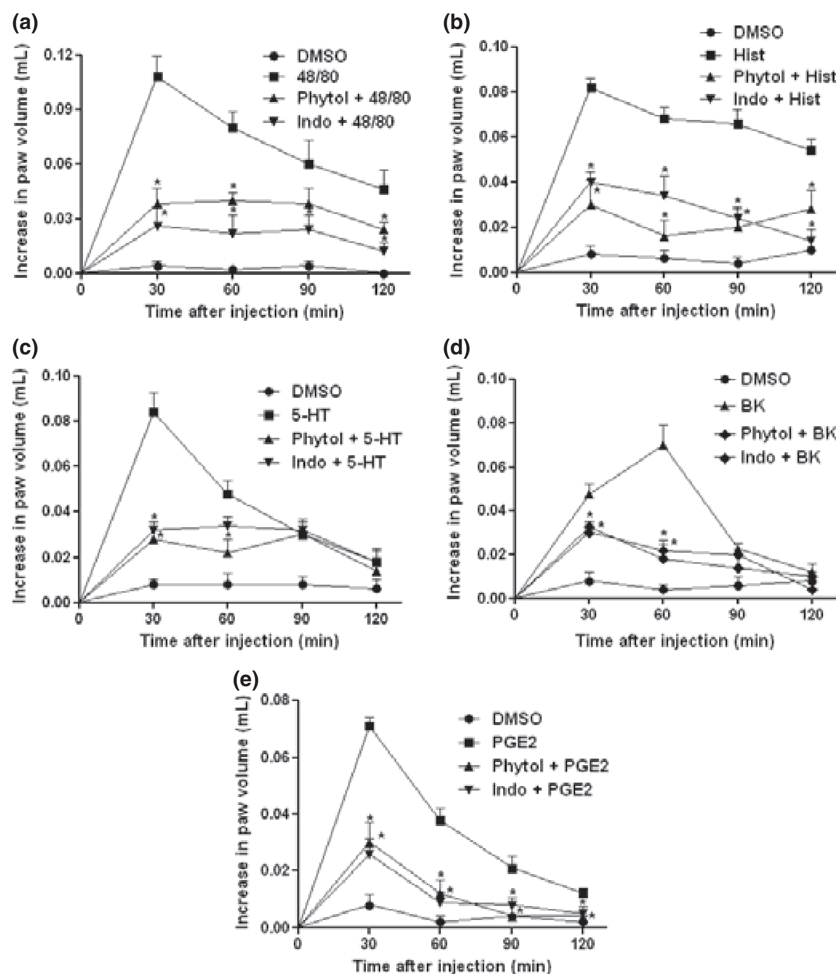

Figure 2 Effect of phytol on paw edema induced by different agents. Animals were pretreated with 2% DMSO, phytol (75 mg/kg, i.p.), or indomethacin (Indo; 10 mg/kg, i.p.). Edema was induced 30 min later by compound 48/80 (panel a), histamine (Hist; panel b), serotonin (5-HT; panel c), bradykinin (BK; panel d), and prostaglandin E2 (PGE_2; panel e). Each column represents the mean ± SEM of 5–6 animals per group. *$P < 0.05$ vs. phlogistic agent.

(1073.0 ± 180.1 × 10^3 pg/mL; *Figure* 5*b*) production in peritoneal exudates 4 h after stimulus injection, compared with the control treatment (100.7 ± 15.5 × 10^3 pg/mL and 176.9 ± 99.5 pg/mL, for TNF-α and IL-1β, respectively). However, pretreatment with phytol (75 mg/kg) significantly decreased ($P < 0.05$) TNF-α (106.8 ± 32.81 pg/mL; *Figure* 5*a*) and IL-1β (607.1 ± 147.6 pg/mL;
Figure 5*b*) levels. Similarly, indomethacin produced significant inhibitory effects on both the parameters analyzed.

Phytol inhibited the carrageenan-induced increase in GSH levels

Figure 6 shows that carrageenan treatment significantly increased ($P < 0.05$) GSH levels (59.73 ± 8.19 μg/mL) in peritoneal exudates, 4 h after stimulus injection, compared with the control treatment(127.60 ± 20.12 μg/mL). Compared with treatment with carrageenan alone, pretreatment with phytol (75 mg/kg) significantly reduced ($P < 0.05$) GSH levels (94.05 ± 17.17 μg/mL).

Phytol reduced the carrageenan-induced increase in MDA concentration

Malondialdehyde concentration increased significantly (25.17 ± 4.47 nmol/mL) in peritoneal exudates 4 h after carrageenan injection ($P < 0.05$), compared with the control treatment (14.12 ± 1.71 nmol/mL) (*Figure* 7). However, compared with treatment with carrageenan alone, pretreatment with phytol (75 mg/kg) significantly decreased ($P < 0.05$) MDA concentration (10.73 ± 1.75 nmol/mL).

DISCUSSION

Natural compounds with different mechanisms of action may be used to treat inflammatory diseases and

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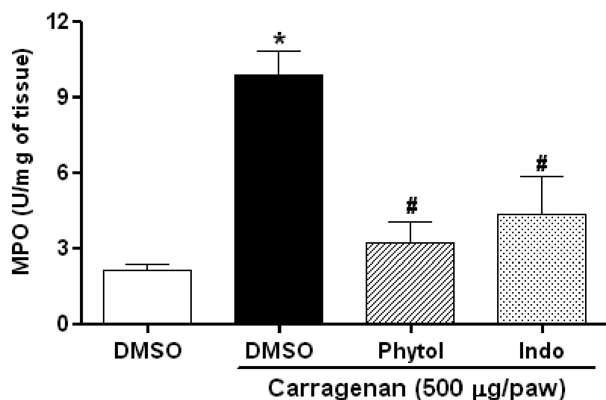

Figure 3 Effect of phytol on carrageenan-induced paw tissue myeloperoxidase (MPO) activity. Animals were pretreated with 2% DMSO, phytol (75 mg/kg, i.p.), or indomethacin (Indo; 10 mg/kg, i.p.) and injected with carrageenan (500 μg/paw) 30 min later. Myeloperoxidase activity in the paw tissue was determined 4 h later. Results are expressed as the mean ± SEM of 5–6 animals per group. *$P < 0.05$ vs. DMSO-treated group; $^{\#}P < 0.05$ vs. carrageenan group.

the essential oils, and their constituents, especially the terpenes, have been shown to possess important properties in several inflammatory processes [17]. The pharmacological results of the present study revealed that phytol, a diterpene alcohol, could reduce neutrophil migration, cytokine levels, and oxidative stress in acute inflammation models.

The carrageenan-induced paw edema model was used to evaluate the anti-inflammatory effects of phytol. This inflammation model has been well established as a standard for screening the anti-inflammatory activity of natural product-derived compounds [23]. The initial phase (1–2 h) of edema is associated with alterations in the vascular permeability induced by the action of vasoactive amines, such as histamine and serotonin released from mast cells. The delayed phase (3–4 h) involves the overproduction and secretion of BK and prostaglandins in paw tissue, which is accompanied by increased neutrophil migration and production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide, and hydroxyl radicals [24]. In this study, we found that phytol was equally effective in both phases of carrageenan-induced paw edema, suggesting that the anti-inflammatory effect observed is due, at least in part, to the inhibition of pro-inflammatory mediators, and reduction in neutrophil migration and oxidative stress.

To elucidate this mechanism, different inflammatory agents, including compound 48/80, Hist, 5-HT, BK, and PGE_2, were used in the paw edema study. Compound 48/80 induces edema by Hist and 5-HT release from mast cell degranulation, leading to osmotic edema, characterized by increased vascular permeability [25]. Our data demonstrated that phytol inhibited the paw edema induced by compound 48/80, which could be because of the stabilization of mast cell membranes and prevention of degranulation. This was confirmed by the fact that phytol inhibited paw edema induced by Hist and 5-HT.

Furthermore, phytol reduced BK- and PGE_2-induced paw edema. BK is a peptide with potent activity on vascular permeability and promotes arteriolar vasodilation and inflammatory cell chemotaxis [26]. Once released, BKs activate their receptors, resulting in the production and release of pro-inflammatory mediators derived from the arachidonic acid pathway, including

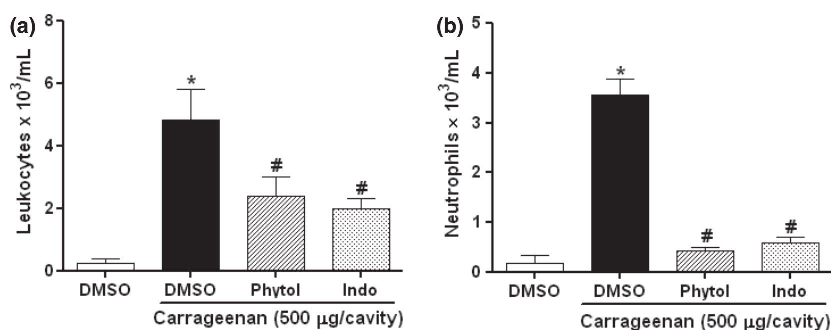

Figure 4 Effect of phytol on cell migration in carrageenan-induced peritonitis. Animals were pretreated with 2% DMSO, phytol (75 mg/kg, i.p.), or indomethacin (Indo; 10 mg/kg, i.p.) and injected with 250 μL of carrageenan (500 μg/cavity, i.p.) 30 min later. Neutrophil migration was evaluated 4 h later. Panel (a), leukocyte counts; panel (b), neutrophil counts. Each column represents the mean ± SEM of 5–6 animals per group. *$P < 0.05$ vs. DMSO-treated group; $^{\#}P < 0.05$ vs. carrageenan group.

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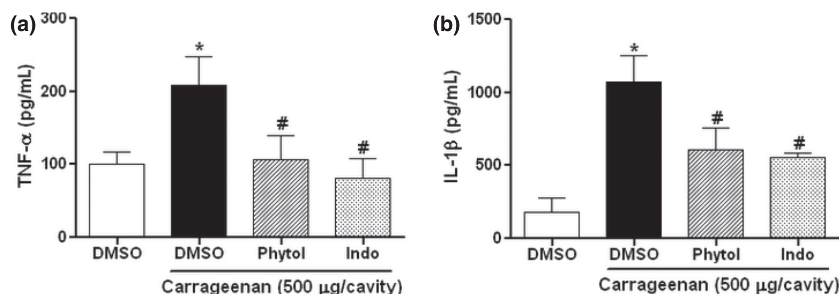

Figure 5 Effect of phytol on cytokine production in carrageenan-induced peritonitis. Animals were pretreated with 2% DMSO, phytol (75 mg/kg, i.p.), or indomethacin (Indo; 10 mg/kg, i.p.), and 250 μL of carrageenan (500 μg/cavity, i.p.) was injected 30 min later. The levels of tumor necrosis factor (TNF)-α (panel a) and interleukin (IL)-1β (panel b) in the peritoneal cavity were measured 4 h after carrageenan injection. Each point represents the mean ± SEM of 5–6 animals per group.*P < 0.05 vs. DMSO-treated group; #P < 0.05 vs. carrageenan group.

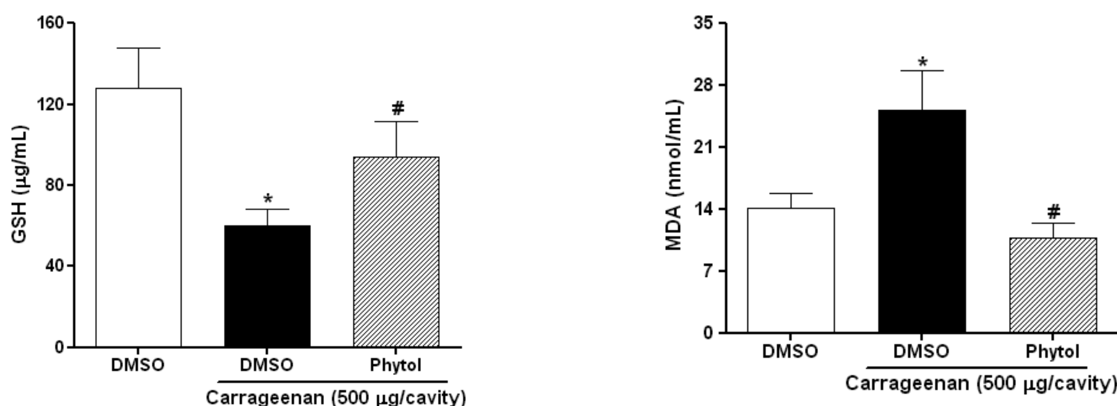

Figure 6 Effect of the phytol on glutathione (GSH) levels in carrageenan-induced peritonitis. Animals were pretreated with 2% DMSO or phytol (75 mg/kg, i.p.), and 250 μL of carrageenan (500 μg/cavity, i.p.) was injected 30 min later. Glutathione levels in the peritoneal cavity were measured 4 h after carrageenan injection. The results are expressed as the mean ± SEM of 5–6 animals per group. *P < 0.05 vs. DMSO-treated group; #P < 0.05 vs. carrageenan group.

Figure 7 Effect of phytol on malondialdehyde (MDA) concentration in carrageenan-induced peritonitis. Animals were pretreated with 2% DMSO or phytol (75 mg/kg, i.p.), and 250 μL of carrageenan (500 μg/cavity, i.p.) was injected 30 min later. Malondialdehyde concentration in the peritoneal cavity was measured 4 h after carrageenan injection. The results are expressed as the mean ± SEM of 5–6 animals per group. *P < 0.05 vs. DMSO-treated group; #P < 0.05 vs. carrageenan group.

PGE_2 [27], as well as cytokines, and histamine and serotonin following mast cell degranulation [28]. Therefore, PGE_2 is also considered being a key pro-inflammatory mediator and causes an increase in paw volume by altering vascular permeability in synergy with the other mediators described earlier [29]. Thus, our findings suggest that the anti-inflammatory activities of phytol are due to inhibition of the synthesis, release, or action of pro-inflammatory mediators. This notion is reinforced by the inhibitory effect of phytol in the initial phase of carrageenan-induced edema, as described previously.

Cell migration within the injured tissue is an important step of the inflammatory process. Thus, to evaluate whether the anti-inflammatory effect of phytol involved the inhibition of neutrophil migration, we measured MPO activity in paw tissue. Myeloperoxidase is an enzyme abundantly found in the azurophilic granules of neutrophils and is released after their activation, within the phagosome or in the extracellular space [30]. Myeloperoxidase activity is directly proportional to neutrophil chemotaxis and infiltration into inflamed tissues [31]. Our results showed that phytol inhibited neutrophil infiltration, which was evident

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from the reduced MPO activity. This suggests that phytol can suppress neutrophil recruitment to the site of inflammation.

The carrageenan-induced peritonitis model was used to confirm the role of cell migration in the anti-inflammatory effect of phytol. Literature shows that carrageenan induces an inflammatory response in the peritoneal cavity, which is characterized by intense exudation and migration of inflammatory cells, especially neutrophils [32]. In the present study, we showed that phytol significantly reduced the migration of polymorphonuclear cells to the peritoneal cavity, as demonstrated by neutrophil counts. This inhibitory effect corroborated the data analyzing MPO activity and confirmed the importance of cell migration in the maintenance and exacerbation of the carrageenan-induced inflammatory response in the peritoneal cavity.

Next, we demonstrated that the carrageenan-induced inflammatory response in the peritoneal cavity led to a substantial increase in TNF-α and IL-1β levels; this finding was in agreement with those of previous studies [2,8,33]. These pro-inflammatory cytokines are important mediators associated with several inflammatory diseases, such as bacterial sepsis, rheumatoid arthritis, and skin inflammation [34,35]. Therefore, suppressing these mediators is believed to be an effective strategy for the treatment of various pathological conditions. In our study, pretreatment with phytol significantly reversed this significant increase, suggesting that phytol exerted an anti-inflammatory action and inhibited polymorphonuclear cell migration, probably by decreasing TNF-α and IL-1βlevels.

Oxidative stress products, primarily generated by infiltrating neutrophils, are another important aspect of the inflammatory process [36,37]. It has been shown that cytokines, including TNF-α and IL-1β, can induce the production of H_2O_2 and superoxide, leading to nuclear factor κB (NFκB) activation, which upregulates cytokine production [38]. Free radicals have been established as one of the major causes of damage from inflammation [39]. Reactive oxygen species are molecules with one or more unpaired outer shell electrons, which are generally highly unstable and extremely reactive [3].

Glutathione levels and MDA concentration were evaluated to explore the redox-protective action of phytol during acute inflammation. Under normal conditions, oxidative stress is kept under control by the endogenous antioxidant system, which includes enzymatic and nonenzymatic antioxidants, such as superoxide dismutase (SOD) and reduced GSH [40,41]. However, during inflammation, excess free radicals lead to tissue damage and activate inflammatory mediators, leading to marked downregulation of endogenous defense mechanisms [42].

In this context, MDA is the end-product of lipid peroxidation and reflects an imbalance between the oxidative and antioxidant systems. Increased MDA concentration, due to free radical-induced plasma membrane damage, has been reported in the carrageenan-induced inflammation model [43,44]. On the other hand, GSH is a free radical scavenger and has been suggested to play an important role against carrageenan-induced local inflammation, by promoting hydrogen transference and acting as a cofactor for the enzyme GSH peroxidase [43,45].

Our results confirmed that carrageenan administration caused an increase in MDA concentration, whereas GSH level decreased, indicating a role of oxidative stress in this model [46,47]. However, pretreatment with phytol reduced MDA formation and restored the depleted GSH content in peritoneal exudates. Therefore, we could infer that the redox-protective effect of phytol might be explained, at least in part, by an increase in GSH concentration. An alternative possibility is that the increase in GSH levels could be secondary to a decrease in free radical production.

In summary, our results suggest that phytol has an anti-inflammatory activity in acute inflammation models. Although there are many mechanisms through which this effect can occur, our data support the hypothesis that the inhibition of neutrophil migration is of essential importance. This effect is due, at least in part, to reduce IL-1β and TNF-α levels and oxidative stress.

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