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Gabapentin, a Synthetic Analogue of Gamma Aminobutyric Acid, Reverses Systemic Acute Inflammation and Oxidative Stress in Mice

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Abstract—The aim of this study was to investigate the potential anti-inflammatory and anti-oxidant effects of gabapentin (GBP) in mice. The anti-inflammatory and anti-oxidant effects were evaluated using various mediators that induce paw edema, peritonitis model, myeloperoxidase (MPO) activity, proinflammatory cytokine levels, glutathione (GSH) consumption, and malondialdehyde (MDA) production in mice. Pretreatment of mice with GBP (1 mg/kg) significantly reduced carrageenan or dextran-induced paw edema ($P<0.05$) when compared to vehicle group. Adding to this, GBP (1 mg/kg) significantly inhibited paw edema induced by histamine, serotonin, bradikinin, 48/80 compound, and prostaglandin E_2. In the carrageenan-induced peritonitis model, GBP significantly decreased total and differential leukocyte counts and reduced the levels of MPO activity in the plantar tissue and IL-1β and TNF-α concentrations in the peritoneal exudate. The same dose of GBP also decreased the MDA concentration and increased the levels of GSH into the peritoneal fluid. In summary, our results demonstrated that GBP exhibited anti-inflammatory activity in mice by reducing the action of inflammatory mediators, neutrophil migration and proinflammatory cytokine levels, and anti-oxidant properties by decreasing the concentration of MDA and increasing the GSH content. These observations raise the possibility that GBP could be used to improve tissue resistance to damage during inflammatory conditions.

KEY WORDS: gabapentin; anti-inflammatory effect; anti-oxidant action.

INTRODUCTION

Gabapentin (GBP) is an anti-convulsant drug structurally related to γ-aminobutyric acid. Evidence obtained in a number of experimental models of neuropathic pain and inflammatory hyperalgesia [1, 2] shows that GBP has an effective anti-nociceptive or anti-hyperalgesic action, in addition to being an anti-convulsant. In humans, GBP has become increasingly popular as a treatment for chronic neuropathic pain. Clinical studies have shown that GBP is an effective analgesic in different types of neuropathic pain syndromes, such as diabetic neuropathy [3], postherpetic neuralgia [4], and trigeminal neuralgia [5].

The literature data show that the GBP can also reverse the gastric inflammatory damage induced by indomethacin and ethanol and diminish the acute inflammatory process induced by carrageenan into the mice paw [6]. Paw edema induced by carrageenan can

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be mediated by several mediators and intense neutrophil influx [7, 8]. However, the action of GBP against the inflammatory condition established by vascular inflammatory mediators and by the release of cytokines, free radicals, and neutrophil migration is still largely unknown.

The inflammatory process involves a complex cascade of biochemical and cellular events that occur in response to cellular injury [9], which is characterized by neutrophil migration occurring in locally produced inflammatory mediators, including TNF-α and IL-1β, which activate neutrophils and promote their migration to the inflammatory site [10, 11], as well as a variety of chemical mediators such as histamines, serotonin, bradykinins, and prostaglandins [12–14]. Those mediators cause increased vascular permeability and promote extravasation of low levels of protein and neutrophils [15].

Knowing that the GBP can reduce some conditions of the inflammatory response, the aim of this study is to test whether GBP is able to inhibit the paw edema induced by several inflammatory mediators, production of free-radical scavengers, neutrophil infiltration, and release of proinflammatory cytokines.

METHODS

Animals

Male Swiss mice (25–35 g) were sourced by the Central Animal Facility of the Federal University of Piauí. The animals were housed at 25±2 °C under a 12:12-h light/dark cycle, and food and water were supplied *ad libitum*. Experiments were conducted in accordance with current established principles for the care and use of research animals (National Institutes of Health [NIH] guidelines) and were approved by the ethics committees in research of Faculdade Integral Diferencial—FACID, number of protocol: 002/13.

Drugs and Reagents

The following drugs and reagents were used: carrageenan, dextran sulfate, histamin, serotonin, prostaglandin E_2 (PGE_2), 48/80, aminoguanidine, L-arginine, and indomethacin (Sigma Aldrich, St Louis, MO, USA). These drugs were dissolved in sterile saline (0.9 % NaCl).

Experimental Protocol

Carrageenan-Induced Paw Edema

The animals were randomly divided into six groups (n=5), and edema was induced by the injection of 50 μL of a suspension of carrageenan (CG; 500 μg/paw) administered with a subplantar injection into the right paw (group I). The mice were pretreated intraperitoneally (i.p.) with either 0.9 % NaCl (group II, untreated control); 10 mg/kg indomethacin (group III, reference control); or 0.1, 0.5, or 1 mg/kg of GBP, i.p, respectively, 1 h before the carrageenan injection. Paw volume was measured immediately before (V_0) and at 1, 2, 3, and 4 h after carrageenan treatment (Vt) with a plethysmometer (PANLAB, LE7500). The effect of pretreatment was calculated as the percentage of inhibition of edema relative to the paw volume of the saline-treated controls by using the following formula [16]:

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

where V_0 is the basal volume and V_t is the final volume measured at the indicated times

Paw edema Induced by Different Inflammatory Agents

To induce paw edema with different inflammatory agents, the animals received injections of dextran (DXT; 500 μg/paw), serotonin (5-HT; 1 %w/v), histamine (HIST; 100 μg/paw), bradykinin (BK; 6.0 nmol paw), PGE_2 (3 nmol/paw), and 48/80 (12 μg/paw) into the right hind paw. One group received 50 μL of 0.9 % sterile saline and served as an untreated control group. GBP (1 mg/kg) or indomethacin (INDO; 10 mg/kg, reference control) was given i.p. 30 min before intraplantar injections of phlogistic agents. For paw edema induced by dextran, serotonin, histamine, bradykinin, PGE2, and 48/80, the paw volume was measured using plethysmometer (PANLAB, LE7500) before the injection of inflammatory agents (time zero). Hence, the paw volume was measured for 30, 60, 90, and 120 min after the injection of those inflammatory agents, except for dextran or histamin, which was measured for 30 min and 1, 2, 3, and 4 h using the same plethysmometer.

Measurement of Myeloperoxidase Activity in Mice Paw

Neutrophil infiltration in the mouse paw was measured through myeloperoxidase (MPO) activity evaluation.

Briefly, 50–100-mg hind paw tissue was homogenized in 1-mL potassium buffer with 0.5 % hexadecyltrimethylammonium bromide for each 50-mg tissue. The homogenate was centrifuged at 40,000*g* for 7 min at 4 °C. MPO activity in the resuspended pellet was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride and 1 % hydrogen peroxide. The results were reported as the MPO units/milligram of tissue. A unit of MPO (UMPO) activity was defined as converting 1 μmol hydrogen peroxide to water in 1 min at 22 °C.

Peritonitis Assay

Mice were pretreated with oral administration of 250 μL sterile saline or indomethacin 10 mg/kg or GBP 1 mg/kg. One hour later, the animals were injected i.p. with 250 μL of the carrageenan (500 μg/cavity). The mice were killed by cervical dislocation under anesthesia 4 h later, and the peritoneal cavity was washed with 1.5 mL heparinized phosphate-buffered saline (PBS) to count peritoneal cells. Total cell counts were performed in a Neubauer chamber, and differential cell (neutrophils) counts (total of 100 cells) were carried out on cytocentrifuge slides stained with hematoxylin and eosin. The results were presented as the number of total leukocyte cells or neutrophils per milliliter of peritoneal exudate.

Cytokine Measurements

The levels of IL-1β and TNF-α were evaluated using sandwich ELISA. Briefly, microliter plates were coated overnight at 4 °C with antibody against mice IL-1β or TNF-α (2 μg/mL). Blocking of nonspecific binding sites was accomplished by incubating the plates with PBS containing 2 % bovine serum albumin (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-IL-1β and anti-TNF-α (diluted 1:1000 with assay buffer 1 % BSA) was added to the wells. After a 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 the 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 picogram per milligram of protein and reported as mean ± SD.

Measurement of Malondialdehyde

The malondialdehyde (MDA) concentration was measured using the method described previously with modifications [17].

Measurement of Levels of Glutathione

The glutathione (GSH) levels in the fragments of intestinal tissue were determined according to the method described previously with modification [18].

Statistical Analysis

Results are expressed as mean ± SEM from at least five animals per group. Statistical analysis was performed using analysis of variance followed by the Newman-Keuls *post hoc* test, when appropriate. Statistical significance was set at $p<0.05$.

RESULTS

Effect of Gabapentin on Carrageenan-Induced Paw edema in Mice

Table 1 shows that the administration of carrageenan into the plantar surface (500 μg per paw) induced severe paw edema within 1 h of injection and was maintained until 4 h after injection. Indomethacin (10 mg/kg) administration significantly decreased paw edema throughout the experimental period (*$p<0.05$), with maximal inhibition of 100 %. Similarly, GBP (1 mg/kg, i.p.) inhibited edema formation in all times. At 2, 3, or 4 h, compared with the carrageenan group, the animals pretreated with 1 mg/kg of GBP showed 87.2, 77.8, and 69.4 % reduction in paw edema, respectively. GBP prevented carrageenan-induced paw edema (500 μg per paw/50 μl) with maximal inhibitory effect at dose of 1 mg/kg (2 h, 0.01±0.007 mL; 3 h, 0.022±0.001 mL; 4 h, 0.025±0.007 mL). Therefore, this dose was selected for studying the possible mechanisms of action involved in GBP-mediated decrease in inflammatory response.

Effect of Gabapentin on Paw Edema Inflammation Induced by Different Inflammatory Agents

The injection of GBP (1 mg/kg, i.p.) significantly reduced the edema induced by all the phlogistic agents during the times tested, mainly in the first 30 min, the peak time of edemas tested (Fig. 1). The group treated with GBP (0.003±0.0008) reverted the paw edema induced by dextran

Table 1. Effect of Gabapentin on Carrageenan-Induced Paw Edema in Mice

Treatment	Dose (mg/kg)	Paw edema in milliliters (time after inflammatory stimuli administration)			
		1 h	2 h	3 h	4 h
Control (Cg)[1]	–	0.067±0.002	0.078±0.006	0.101±0.010	0.081±0.001
Saline	–	0.016±0.006*	0.013±0.004*	0.005±0.005*	0±0.0*
Indomethacin (INDO)	10	0.006±0.004*	0±0.0*	0.061±0.007	0.011±0.007*
GBP	0.1	0.065±0.005 (3.7 %)	0.067±0.007 (14.5 %)	0.061±0.007 (39.4 %)*	0.077±0.006 (5.1 %)
	0.5	0.061±0.012 (8.6 %)	0.066±0.010 (14.9 %)	0.067±0.006 (50 %)*	0.075±0.009 (8.16 %)
	1	0.03±0.004 (55.6 %)*	0.01±0.007 (87.2 %)*	0.022±0.001 (77.8 %)*	0.025±0.007 (69.4 %)*

Values of paw edema are expressed as mean±SEM (n=5). The % inhibition of paw edema is indicated in parentheses
*p<0.05 compared with control (one-way analysis of variance followed by the Newman-Keuls *post hoc* test)

(0.0312±0.0030; Fig. 1a). GBP also significantly inhibited the increase in paw volume of animals treated with serotonin (0.048±0.015; Fig. 1b), histamine (0.103±0.025; Fig. 1c), bradykinin (0.058±0.005; Fig. 1d), PGE2 (0.033±0.014; Fig. 1e), and 48/80 (0.079±0.013; Fig. 1f). On the other hand, the saline injected into the paw did not induce any effect. The values given are means ± SEM (n=5).

Effect of Gabapentin on Carrageenan-Induced Myeloperoxidase Activity in Paw Tissue

We can observe in Fig. 2 that the carrageenan subplantar injection elevated the concentration of MPO in the plantar tissue (17.73±3.85 UMPO/mg of tissue) when this group is compared to the saline group (0.84±0.19 UMPO/mg of tissue). On the other hand, pretreatment with GBP (1 mg/kg) reduced the action of this tissue enzyme (1.10±0.33 UMPO/mg of tissue; Fig. 2).

Effect of Gabapentin on Carrageenan-Induced Cytokine Production in Peritonitis

Figure 3 shows that intraperitoneal administration of carrageenan was found to induce a marked increase in IL-1β concentrations in the peritoneal exudates (1.125±37.40 pg/mL). The level of IL-1β in the peritoneal cavity of control animals (saline group) was 127.0±19.04 pg/mL. Compared with the carrageenan group, the animals pretreated with GBP (1 mg/kg, i.p) showed significantly decreased IL-1β peritoneal concentration (599.3±113.1 pg/mL; Fig. 3a). Furthermore, GBP treatment decreased the levels of TNF-α (90.12±14.22 pg/mL) compared to the peritoneal carrageenan group (564.9±52.32 pg/mL; Fig. 3b).

Anti-Inflammatory Effect of Gabapentin on Carrageenan-Induced Peritonitis in Mice

Figure 4 shows that the carrageenan group promoted an increase in cell migration (leukocytes) into the peritoneal cavity ($16{,}675\times10^3\pm2{,}252\times10^3$ cells/mL). However, GBP group showed significantly reduced peritoneal leukocyte count ($1.760\times10^3\pm123.9\times10^3$ cells/mL; Fig. 4a). Furthermore, the same dose of GBP significantly reduced neutrophil migration into the peritoneal cavity ($784.02\times10^3\pm55.02\times10^3$ cells/mL) compared with that in the carrageenan group ($11{,}410\times10^3\pm2{,}392\times10^3$ cells/mL; Fig. 4b). This result was consistent with the fact that neutrophils are the most abundant cells in primary inflammatory exudates.

Effect of Gabapentin on MDA Levels in the Peritoneal Exudates of Mice

Figure 5 shows that the injection of carrageenan (41.83±1.788) significantly increased the levels of MDA compared to the group that received only intraperitoneal saline (22.88±3.075). However, the group pretreated with GBP 1 mg/kg (24.55±1.192) had significantly reduced MDA levels compared to the untreated group (Fig. 5).

Effect of Gabapentin on Glutathione Levels in Peritoneal Exudate of Mice

Figure 6 shows that treatment with carrageenan (41.98±4.515) increases the consumption of GSH compared to the saline group (155.0±10.02). It was also observed that the GBP (127.6±8.197) group significantly increased GSH levels compared to the untreated group.

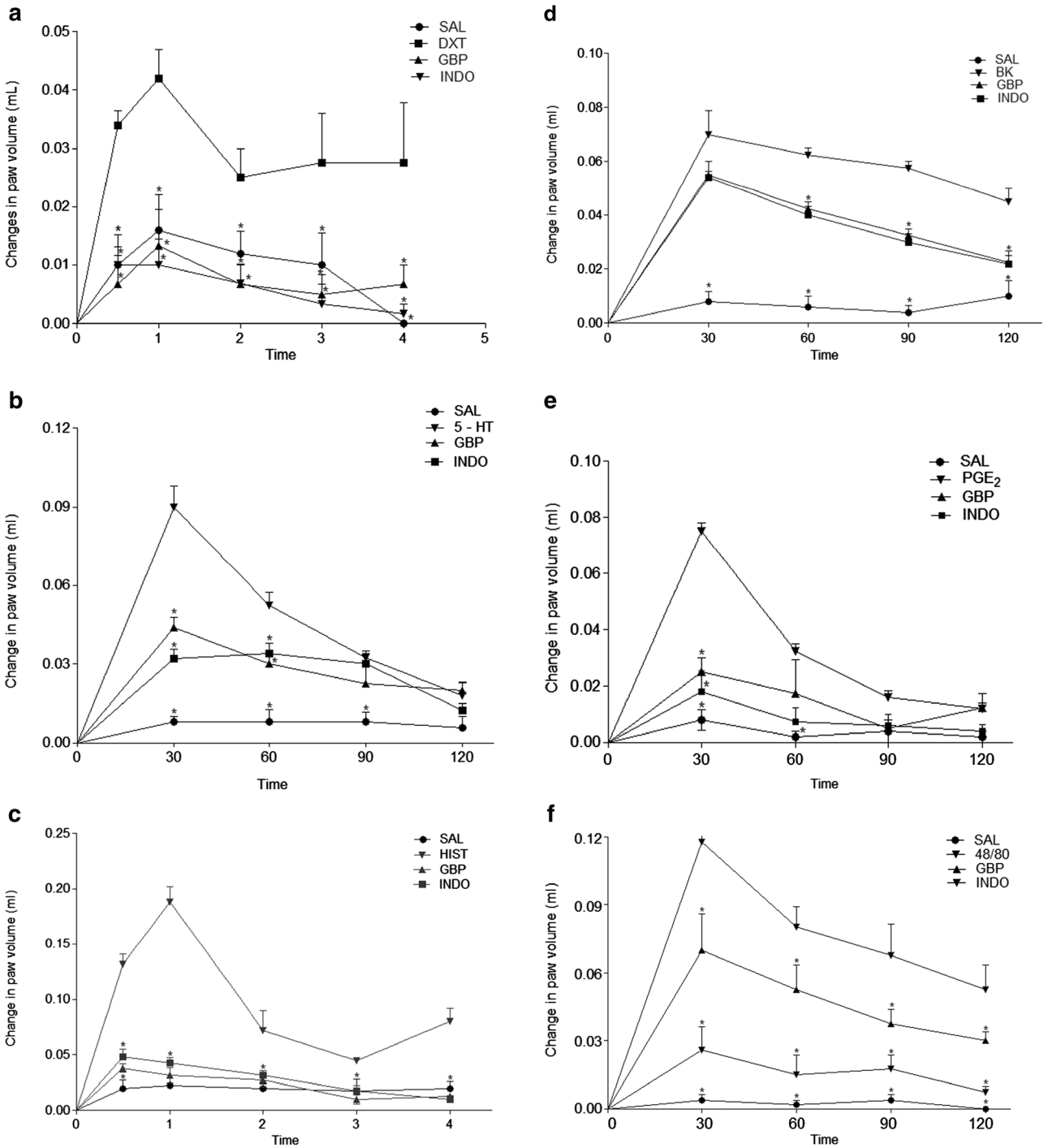

Fig. 1. Effects of gabapentin on paw inflammation induced by different inflammatory agents. Edema was induced by **a** dextran (DXT; 500 μg/paw), **b** serotonin (5-HT; 1 %*w*/*v*), **c** histamine (HIST; 100 μg/paw), **d** bradykinin (BK; 6.0 nmol/paw), **e** PGE_2 (3 nmol/paw), and **f** 48/80 (12 μg/paw). Animals were pretreated with GBP (1 mg/kg i.p.), saline (SAL; control), or indomethacin (INDO; 10 mg/kg, i.p.). Each *point* represents the mean ± SEM of five animals. *$p<0.05$ significantly different to the control group. Statistical analysis was performed by the Newman-Keuls *post hoc* test.

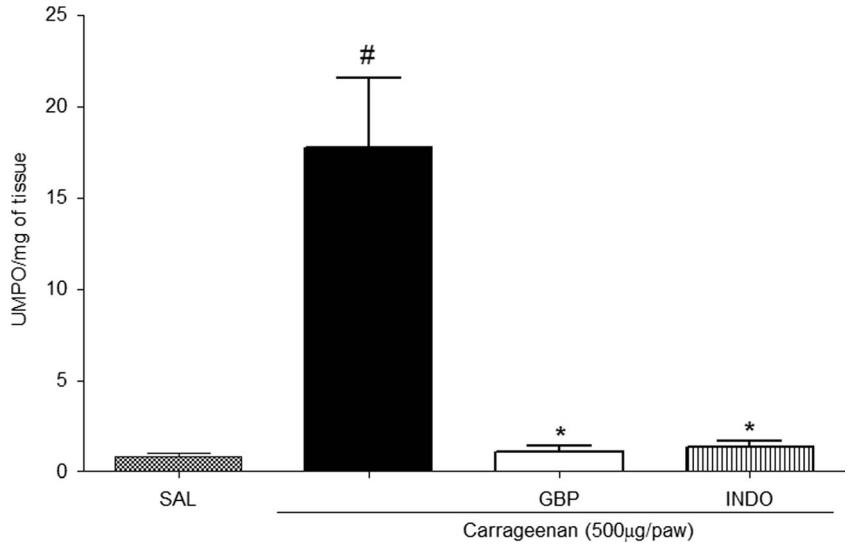

Fig. 2. Effect of gabapentin on carrageenan-induced myeloperoxidase activity in paw tissue. Saline or carrageenan (500 μg per paw) was injected into the plantar surface of mice. One hour before this injection, animals had been treated with indomethacin (INDO; 10 mg/kg, i.p.) or gabapentin (GBP; 1 mg/kg, i.p.). Myeloperoxidase (MPO) activity was detected in the paw tissue after 4 h. The results are expressed as the mean ± SEM MPO units (UMPO)/milligram of tissue. *$p<0.05$ compared with carrageenan group; $^{\#}p<0.05$ compared with saline group. Statistical analysis was performed using analysis of variance followed by the Newman-Keuls *post hoc* test.

DISCUSSION

The pharmacological approaches of GBP have drawn attention of researchers from basic and clinical areas. GBP, a drug used to improve neurological disorders such as epilepsy and seizures [19, 20], reduces the inflammatory hyperalgesia induced by acid-acetic and formalin tests in mice [1, 21–25]. Another study revealed that this substance inhibited the acute inflammatory responses that occur in the indomethacin-induced gastropathy inflammation in rats or carrageenan-induced paw edema [6]. However, the mechanisms of this latter effect of GBP were not elucidated.

In this manuscript, we report new insights into the functions and possible mechanisms of GBP, including its anti-inflammatory ability demonstrated by decreasing the paw edema induced by carrageenan, dextran, and 48/80 and induced by several mediators, such as histamine, serotonin, PGE_2, and bradikinin, the levels of proinflammatory cytokines (TNF-α and IL-1β), the neutrophil infiltration, and its anti-oxidant ability demonstrated by increased GSH levels and decreased MDA concentration.

Our results demonstrate that GBP was able to reduce paw edema induced by carrageenan or dextran. Paw edema in mice induced by carrageenan is used as a tool to investigate potential anti-inflammatory agents [26]. In this acute inflammatory model, there are two phases. The first or early phase is mediated by the release of histamine and serotonin, followed by the subsequent release of bradykinin and prostaglandins [25–27]. The late or second phase is characterized by cytokine production and release of macrophages and mast cells and intense neutrophil infiltration [28, 29, 8]. On the other hand, dextran-induced paw edema promotes inflammation by increasing vascular permeability dependent on mast cell degranulation and the subsequent release of histamine and serotonin [30]. The extravasated fluid during dextran injection contains little protein and few neutrophils [31]. The results suggested that the anti-inflammatory effect of GBP seems to be mediated by the inhibition of neutrophil infiltration into the inflammatory site, as well as the inhibition of the release or activity of inflammatory mediators.

The vascular phenomenon that occurs during the acute phase of the inflammatory process is dependent on the release of several mediators that act on the vascular endothelium causing leakage of fluid and proteins into the interstitium [32]. This event can be mediated by the action of histamine, serotonin, bradikinin, PGE_2, and/or induced by 48/80 compound, which induces paw edema by mast cell degranulation with the release of histamine, serotonin, and bradikinin [33–35]. In this manuscript, GBP reduced the paw edema induced by histamine, serotonin, bradikinin, PGE_2, and 48/80 compound. Thus, we can infer that this drug affects the vascular component of edema, which

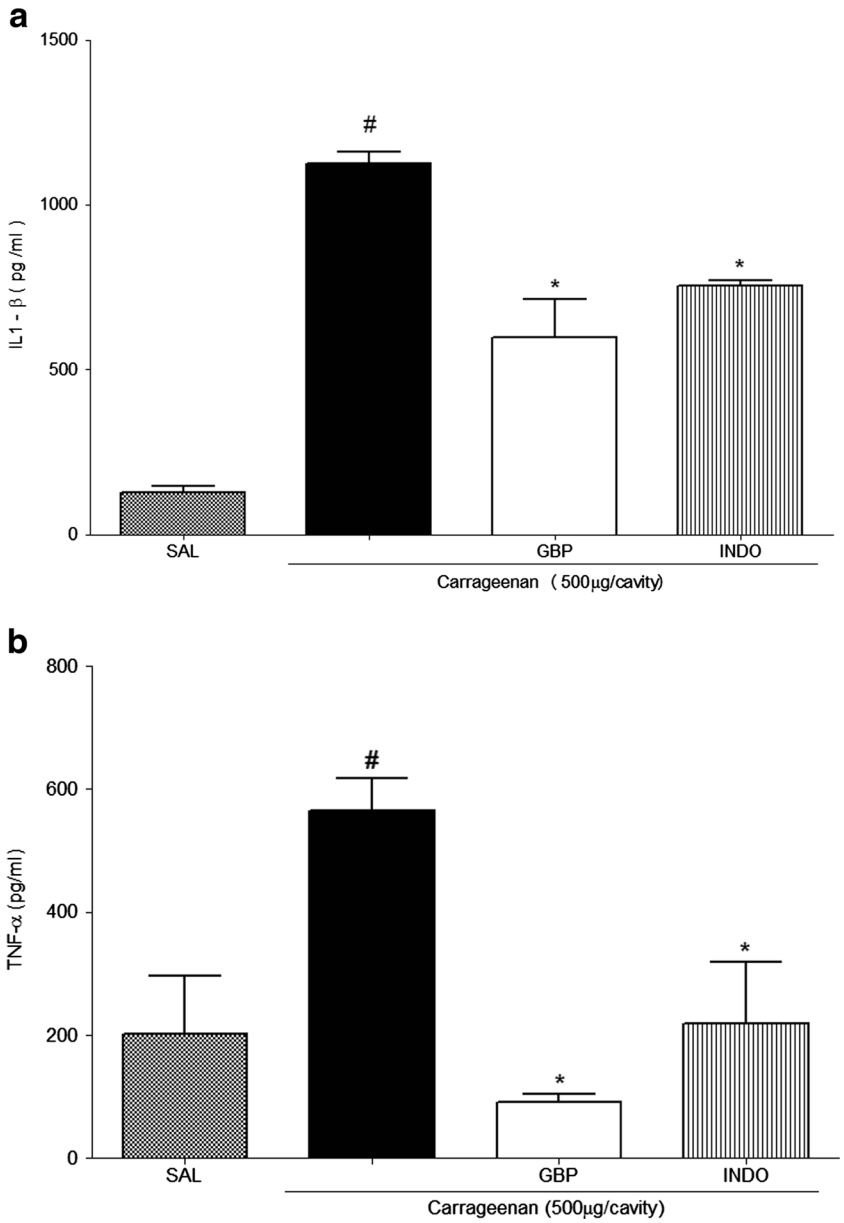

Fig. 3. Effect of gabapentin on carrageenan-induced cytokine production in peritonitis. **a** The level of interleukin (IL)-1β and **b** TNF-α. The level of IL-1β or TNF-α in the peritoneal cavity was measured 4 h after carrageenan injection. Mice were intraperitoneally administered with GBP (1 mg/kg) or indomethacin (INDO; 10 mg/kg), followed by injection of 250 μL carrageenan (500 μg per cavity, i.p.) after 1 h. Each *point* represents the mean ± SEM values obtained from five animals. $^{*}p<0.05$ compared with carrageenan group; $^{\#}p<0.05$ compared with saline group. Statistical analysis was carried out using one-way analysis of variance followed by the Newman-Keuls *post hoc* test.

appears to be mediated by the decreased action of these several inflammatory mediators.

The carrageenan-induced inflammatory response in paw tissue is known to be accompanied by intense leukocyte migration, primarily neutrophils [12]. MPO activity has been found in neutrophil azurophilic granules, which is an indicator of neutrophil accumulation [36, 37]. During the neutrophil migration, MPO can be released on the inflamed tissue, inducing damage to adjacent cells and thus contributing to the pathogenesis of inflammatory process [38]. Our results obtained showed that GBP (1 mg/kg) or indomethacin (positive control) reduced the MPO concentration in paw tissue after carrageenan injection. Thus, we can suggest that GBP-reduced inflammatory process

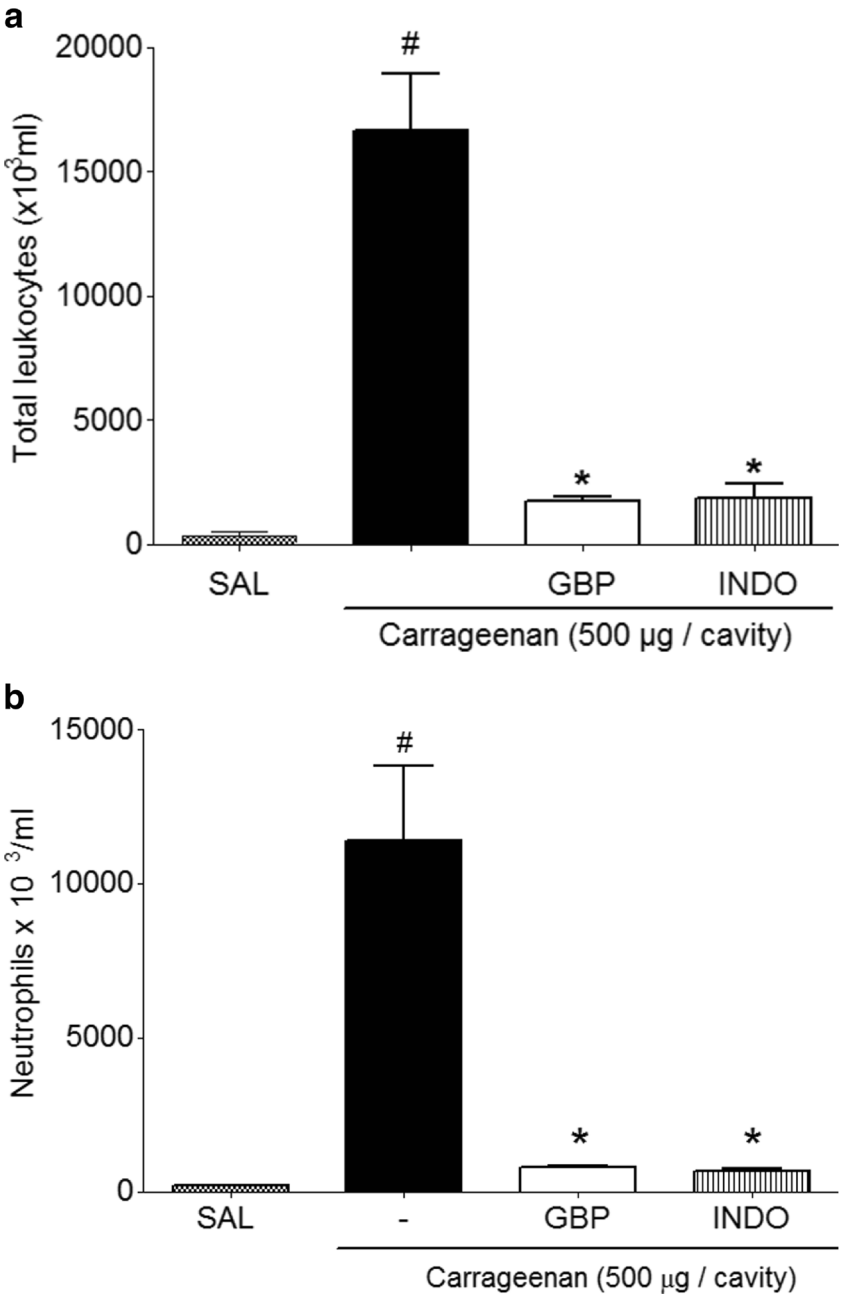

Fig. 4. Anti-inflammatory effect of gabapentin carrageenan-induced peritonitis in mice. **a** Total count of leukocytes. **b** Count of neutrophils per cavity. Mice received 250 μL saline (i.p.), indomethacin (INDO; 10 mg/kg, p.o.), or gabapentin (GBP; 1 mg/kg, p.o.), followed by injection of 500 μg carrageenan diluted in 250-μL saline solution (i.p.) after 1 h. Mice were killed 4 h later, and the peritoneal cavity was washed with 1.5 mL heparinized phosphate-buffered saline (PBS) to harvest the peritoneal cells. The values are represented as mean ± SEM. *$p<0.05$ compared to carrageenan group; $^{\#}p<0.05$ compared with saline group. Statistical analysis was performed by analysis of variance followed by the Newman-Keuls *post hoc* test.

involves the inhibition of neutrophil migration into the inflammatory site.

The carrageenan-induced peritonitis has been linked to the neutrophil infiltration, the release of neutrophil-derived mediators [27], and this phenomenon occurs through an indirect mechanism that involves the activation of resident cells and the release of proinflammatory cytokines [39]. In the

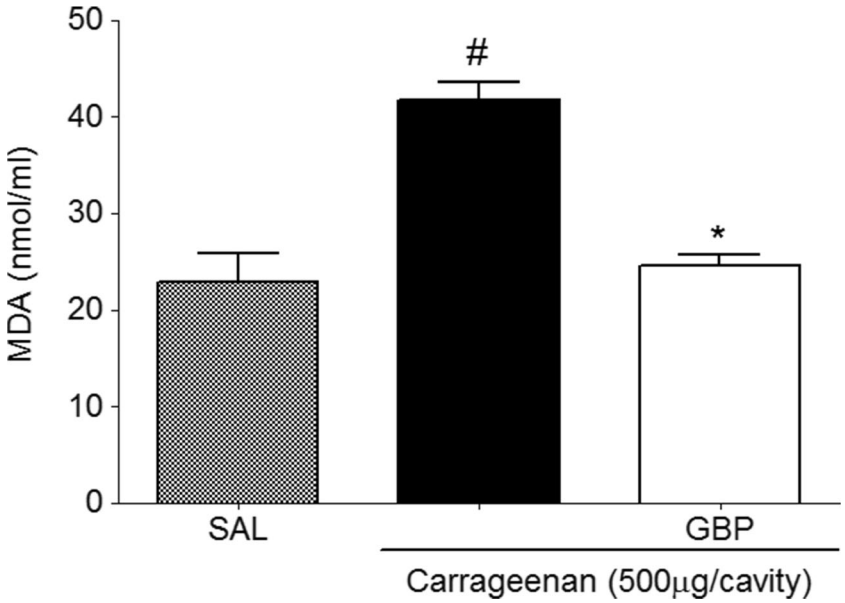

Fig. 5. Effect of gabapentin on MDA levels in the peritoneal exudate of mice. MDA levels in the peritoneal exudate were evaluated 4 h after carrageenan administration. Values are expressed as mean±EPM in nanomole per milliliter of MDA *$p<0.05$ compared to carrageenan group; $^{\#}p<0.05$ compared to saline group. Statistical analysis was performed using analysis of variance followed by the Newman-Keuls test.

present study, we showed that GBP (1 mg/kg) diminished the carrageenan-induced neutrophil migration into the peritoneal cavity. According to our findings, we can infer that this compound decreased inflammatory response by inhibiting the action and release of cytokines such as IL-1β and TNF-α.

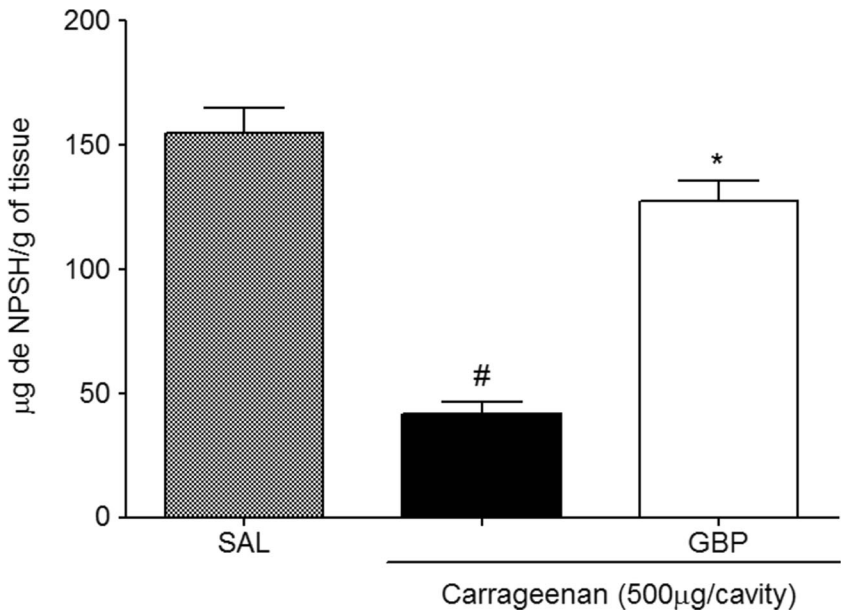

Fig. 6. Effect of gabapentin on GSH levels in peritoneal exudate of mice. The animals were killed 4 h after induction by carrageenan peritonitis. One hour before the experiment, they were pretreated with GBP (1 mg/kg). Values are expressed as mean ± EPM in microgram NPSH/gram of tissue. *$p<0.05$ compared to carrageenan group; $^{\#}p<0.05$ compared to saline group. Statistical analysis was performed using analysis of variance followed by the Newman-Keuls test.

Recent studies have shown that administration of carrageenan into the peritoneal cavity induces the release of TNF-α and IL-1β [40]. IL-1β and TNF-α are potent proinflammatory cytokines that have multiple effects, including the activation of inflammatory cells, induction of several inflammatory proteins, cytotoxicity, and neutrophil migration [41]. These cytokines have been recognized as a powerful chemotactic factor that activates the inflammatory cells, such as mature neutrophils, and induces the diapedesis to the inflammatory site [42]. GBP also inhibited the levels of IL-1β and TNF-α, and based on our results, we could infer that the anti-inflammatory action of this anti-convulsant drug might occur through the inhibition of cytokines involved in carrageenan-induced peritonitis.

Oxidative stress has been proposed to play an important role in the pathogenesis of inflammatory process and is related to promote the production of several cytokines, including proinflammatory cytokines IL-1β, IL-6, and TNF-α [43, 44] and the recruitment of neutrophils during inflammatory process. This pathological event is characterized by the overproduction of reactive oxygen resulting in tissue damage [44]. Thus, the present study also investigated the effect of GBP on two oxidative stress markers: GSH and MDA.

Our results demonstrated that GBP increased the levels of GSH and decreased the MDA concentration in mice carrageenan-induced peritonitis. GSH, an endogenous anti-oxidant, protects the cells against oxidative stress, keeping the sulfhydryl groups of proteins reduced and preventing them from reacting with free radicals [45] MDA is a product of lipoperoxidative processes that take place as a consequence of the tissue oxidative insult [46]. According to that result, we can infer that GBP decreased the tissue damage during the installation of the inflammatory process by stimulating the production and action of endogenous anti-oxidants and decreased the lipid peroxidation into the peritoneal cavity.

CONCLUSION

In summary, our results may show that GBP has an anti-inflammatory action to reduce the inflammatory response by inhibiting the action of various inflammatory mediators, neutrophil infiltration, proinflammatory cytokines, and oxidative stress. These observations raise the possibility that GBP could be used to improve tissue resistance to damage during inflammatory conditions.

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Conflict of Interest. The authors report no conflict of interest.

REFERENCES

1. Shimoyama, M., N. Shimoyama, C.E. Inturrisi, and K.J. Elliott. 1997. Gabapentin enhances the antinociceptive effects of spinal morphine in the rat tail-flick test. *Pain* 72: 375–382.
2. Hwang, J.H., and T.L. Yaksh. 1997. Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. *Reg Anesth* 22: 249–256.
3. Backonja, M., A. Beydoun, K.R. Edwards, S.L. Schwartz, V. Fonseca, M. Hes, L. La Moreaux, and E. Garofalo. 1998. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. *JAMA* b280: 1831–1836.
4. Singh, D., and D.H. Kennedy. 2003. The use of gabapentin for the treatment of postherpetic neuralgia. *Clin Ther* 25: 852–889.
5. Pöllmann, W., and W. Feneberg. 2008. Current management of pain associated with multiple sclerosis. *CNS Drugs* 22: 291–324.
6. Abdel-Salam, O.M.E., and A.A. Sleem. 2009. Study of the analgesic, anti-inflammatory, and gastric effects of gabapentin. *Drug Discov Ther* 3: 18–26.
7. Silva, V.G., R.O. Silva, S.R. Damasceno, N.S. Carvalho, R.S. Prudêncio, K.S. Aragão, M.A. Guimarães, S.A. Campos, L.M. Véras, M. Godejohann, J.R. Leite, A.L. Barbosa, and J.V. Medeiros. 2013. Anti-inflammatory and antinociceptive activity of epiisopiloturine, an imidazole alkaloid isolated from Pilocarpus microphyllus. *J Nat Prod* 76: 1071–1077.
8. Vinegar, R., J.F. Truax, J.L. Selph, P.R. Johnston, A.L. Venable, and K.K. McKenzie. 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Fed Proc* 46: 118–126.
9. Carvalho, W.A., and L. Lemônica. 1998. Mecanismos celulares e moleculares da dor inflamatória. *Rev Bras Anestesiol* 48: 137–158.
10. Greer, C.W., I. Shomera, M.E. Goldsteinb, and W. Yaphe. 1984. Analysis of carrageenan from Hypnea musciformis by using κ- and ι-carrageenases and ^{13}C-N.M.R. spectroscopy. *Carbohydr Res* 129: 189–196.
11. Aziza, M., T. Givernaud, M. Chikhaoui-khay, and L. Bennasser. 2008. Seasonal variation of the growth, chemical composition and carrageenan extracted from Hypnea musciformis (Wulfen) Lamouroux harvested along the Atlantic coast of Morocco. *Sci Res Essays* 2: 509–514.
12. Carvalho, J.C., J.R. Teixeira, P.J. Souza, J.K. Bastos, D. dos Santos Filho, and S.J. Sarti. 1996. Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinea ferrea* crude extract. *J Ethnopharmacol* 53: 175–178.
13. Hajare, S.W., S. Chandra, J. Sharma, S.K. Tandan, J. Lal, and A.G. Telang. 2001. Anti-inflammatory activity of Dalbergia sissoo leaves. *Fitoterapia* 72: 131–139.
14. Srinivasan, K., S. Muruganandan, J. Lal, S. Chandra, S.K. Tandan, and V.R. Prakash. 2001. Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. *J Ethnopharmacol* 78: 151–157.
15. Calixto, J.B., M.M. Campos, M.F. Otuki, and A.R. Santos. 2004. Anti-inflammatory compounds of plant origin. Part II. Modulation of

pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med* 70: 93–103.
16. Lanhers, M.C., J. Fleurentin, P. Dorfman, F. Mortier, and J.M. Pelt. 1991. Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta. Planta Med* 57: 225–231.
17. Sedlak, J., and R.H. Lindsay. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 24: 1992–2005.
19. Jensen, A.A., J. Mosbacher, S. Elg, K. Lingenhoehl, T. Lohmann, T.N. Johansen, B. Abrahamsen, J.P. Mattsson, A. Lehmann, B. Bettler, and H. Bräuner-Osborne. 2002. The anticonvulsant gabapentin (neurontin) does not act through gamma-aminobutyric acid-B receptors. *Mol Pharmacol* 6: 1377–1384.
20. Bennett, M.I., and K.H. Simpson. 2004. Gabapentin in the treatment of neuropathic pain. *Palliat Med* 18: 5–11.
21. Abdi, S., D.H. Lee, and J.M. Chung. 1998. The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. *Anesth Analg* 87: 1360–1366.
22. Yasuda, T., S. Miki, N. Yoshinaga, and E. Senba. 2005. Effects of amitriptyline and gabapentin on bilateral hyperalgesia observed in an animal model of unilateral axotomy. *Pain* 115: 161–170.
23. Shimoyama, N., M. Shimoyama, A.M. Davis, C.E. Inturrisi, and K.J. Elliott. 1997. Spinal gabapentin is antinociceptive in the rat formalin test. *Neurosci Lett* 222: 65–67.
24. Hunter, J.C., K.R. Gogas, L.R. Hedley, L.O. Jacobson, L. Kassotakis, J. Thompson, and D.J. Fontana. 1997. The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. *Eur J Pharmacol* 324: 153–160.
25. Vinegar, R., W. Schreiber, and R. Hugo. 1969. Biphasic development of carrageenin edema in rats. *J Pharmacol Exp Ther* 166: 96–103.
26. de Brito, T.V., R.S. Prudêncio, A.B.. Sales, F. Vieira Jr., S.J. Candeira, Á.X. Franco, K.S. Aragão, R.A. Ribeiro, Ponte de Souza MH, L.S. Chaves, A.L. Freitas, J.V. Medeiros, and A.L.R. Barbosa. 2013. Anti-inflammatory effect of a sulphated polysaccharide fraction extracted from the red algae Hypnea musciformis via the suppression of neutrophil migration by the nitric oxide signalling pathway. *J Pharm Pharmacol* 65: 724–733.
27. Dawson, J., A.D. Sedgwick, J.C.W. Edwards, and P. Lees. 1991. A comparative study of the cellular, exudative and histological responses to carrageenan, dextran and zymosan in the mouse. *Inter J Tissue Reac* 13–14: 171–185.
28. Cragg, G.M., D.J. Newman, and K.M. Snader. 1997. Natural products in drug discovery and development. *J Nat Prod* 60: 52–60.
29. De Smet, P.A. 2007. The role of plant-derived drugs and herbal medicines in healthcare. *Drugs* 54: 801–840.
30. Rowley, D.A., and E.P. Benditt. 1956. 5-hydroxytryptamine and histamine as mediators of the vascular injury produced by agents which damage mast cells in rats. *J Exp Med* 103: 399–415.
31. Kulkarni, S.K., A.K. Mehta, and J. Kunchandy. 1986. Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammagen-induced acute paw oedema in rats. *Arch Int Pharmacodyn Ther* 279: 324–334.
32. Seibert, K., Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee, and P. Isakson. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci* 91: 12013–12017.
33. Lago, J., A. Alfonso, M.R. Vieytes, and L.M. Botana. 2001. Ouabain-induced enhancement of rat mast cells response. Modulation by protein phosphorylation and intracellular pH. *Cell Signal* 13: 515–524.
34. Okazaki, T., V.S. Ilea, A. Okazaki, K. Wicher, R.E. Reisman, and C.E. Arbesman. 1976. Inhibition of antigen-induced histamine release by ouabain. *J Allergy Clin Immunol* 57: 454–462.
35. Senol, M., I.H. Ozerol, A.V. Patel, and D.P. Skoner. 2007. The effect of Na+-K+ATPase inhibition by ouabain on histamine release from human cutaneous mast cells. *Mol Cell Biochem* 294: 25–29.
36. Ajuebor, M.N., A. Singh, and J.L. Wallace. 2000. Cyclooxygenase-2-derived prostaglandin D2 is an early anti-inflammatory signal in experimental colitis. *J Physiol Gastrointest Liver Physiol* 279: 238–244.
37. Souza, G.E., F.Q. Cunha, R. Mello, and S.H. Ferreira. 1988. Neutrophil migration induced by inflammatory stimuli is reduced by macrophage depletion. *Agents Actions* 24: 377–380.
38. Klebanoff, S.J. 1999. Myeloperoxidase. *Proc. Assoc. Am. Physicians* 111: 383–389.
39. Gupta, M., U.K. Mazumdar, T. Sivakumar, M.L. Vamsi, S.S. Karki, R. Sambathkumar, and L. Manikandan. 2003. Evaluation of anti-inflammatory activity of chloroform extract of Bryonia laciniosa in experimental animal models. *Biol Pharm Bull* 26: 1342–1344.
40. Chaves, L.S., L.A. Nicolau, R.O. Silva, F.C. Barros, A.L. Freitas, K.S. Aragão, R.A. Ribeiro, M.H. Souza, A.L. Barbosa, and J.V. Medeiros. 2013. Antiinflammatory and antinociceptive effects in mice of a sulfated polysaccharide fraction extracted from the marine red algae Gracilaria caudate. *Immunopharmacol Immunotoxicol* 35: 93–100.
41. Metcalfe, D.D. 2008. Mast cells and mastocytosis. *Blood* 15: 946–956.
42. Sutbeyaz, Y., B. Yakan, H. Ozdemir, M. Karasen, F. Doner, and I. Kufrevioglu. 1996. Effect of SC-41930, a potent selective leukotriene B4 receptor antagonist, in the guinea pig model of middle ear inflammation. *Ann Otol Rhinol Laryngol* 105: 476–480.
43. Josse, C., J.R. Boelaert, M. Best-Belpomme, and J. Piette. 2001. Importance of post-transcriptional regulation of chemokine genes by oxidative stress. *Biochem J* 360: 321–333.
44. Cuzzocrea, S., D.P. Riley, A.P. Caputi, and D. Salvemini. 2001. Antioxidant therapy: a new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. *Pharmacol Rev* 53: 135–159.
45. Amirshahrokhi, K., S. Bohlooli, and M.M. Chinifroush. 2011. The effect of methylsulfonylmethane on the experimental colitis in the rat. *T Appl Pharmacol* 253: 197–202.
46. Pacher, P., J.S. Beckman, and L. Liaudet. 2007. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87: 315–424.