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ORIGINAL ARTICLE

Basic Study

Anti-inflammatory intestinal activity of *Combretum duarteanum* Cambess. in trinitrobenzene sulfonic acid colitis model

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Abstract

AIM

To evaluate the anti-inflammatory intestinal effect of the ethanolic extract (EtOHE) and hexane phase (HexP) obtained from the leaves of *Combretum duarteanum* (*Cd*).

METHODS

Inflammatory bowel disease was induced using trinitrobenzenesulfonic acid in acute and relapsed ulcerative colitis in rat models. Damage scores, and biochemical, histological and immunohistochemical parameters were evaluated.

RESULTS

Both *Cd*-EtOHE and *Cd*-HexP caused significant reductions in macroscopic lesion scores and ulcerative lesion areas. The vegetable samples inhibited myeloperoxidase increase, as well as pro-inflammatory cytokines TNF-α and IL-1β. Anti-inflammatory cytokine IL-10 also increased in animals treated with the tested plant samples. The anti-inflammatory intestinal effect is related to decreased expression of cyclooxygenase-2, proliferating cell nuclear antigen, and an increase in superoxide dismutase.

CONCLUSION

The data indicate anti-inflammatory intestinal activity. The effects may also involve participation of the antioxidant system and principal cytokines relating to inflammatory bowel disease.

Key words: *Combretum duarteanum*; Medicinal plants; Combretaceae; Inflammatory bowel disease; Anti-inflammatory intestinal activity

Core tip: Inflammatory bowel diseases are chronic inflammatory disorders that include Crohn's disease and ulcerative colitis (UC). Genetic, immunologic and environmental factors are postulated as possible etiologic agents. Their conventional treatment is centered in reducing inflammation and abnormal symptom relief. A variety of herbal medicines have been demonstrated to produce promising results in the treatment of those diseases. *Combretum duarteanum* is a species popularly used in folk medicine to treat inflammation. Thus, the present study was designed to evaluate the intestinal anti-inflammatory effect in an UC rat model, contributing to the safe use and collaborating with the scientific knowledge of natural products.

de Morais Lima GR, Machado FDF, Périco LL, de Faria FM, Luiz-Ferreira A, Souza Brito ARM, Pellizzon CH, Hiruma-Lima CA, Tavares JF, Barbosa Filho JM, Batista LM. Anti-inflammatory intestinal activity of *Combretum duarteanum* Cambess. in trinitrobenzene sulfonic acid colitis model. *World J Gastroenterol* 2017; 23(8): 1353-1366 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i8/1353.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i8.1353

INTRODUCTION

The inflammatory bowel diseases (IBDs) are chronic disorders of the gastrointestinal (GI) tract characterized by alternating periods of remission and relapse[1]. These diseases represent a large group of inflammatory disorders, the most common being Crohn's disease (CD) and ulcerative colitis (UC)[2,3].

CD can affect any part of the GI tract and has the classic symptoms of fatigue, prolonged diarrhea (with or without bleeding), abdominal pain, weight loss, and fever[4,5]. UC is a type of chronic inflammation restricted to the colon; however, the entire large intestine may be affected[6]. Affected patients show symptoms such as rectal bleeding, frequent bowel movements, tenesmus, rectal mucus discharge, and abdominal pain[5].

The etiology of IBDs is still not fully understood; however, it is believed that environmental, genetic and immunologic factors have important roles in their occurrence and progression[4,7].

Emerging models in the study of IBD pathogenesis suggest three key disease development factors: (1) breaking the intestinal barrier function; (2) lamina immune cell exposure to luminal contents; and (3) exacerbation of immune response. However, the factors responsible for initiation and perpetuation of the cycle leading to exacerbation of the disease are still unclear[8,9].

A combination of genetic and environmental factors may foment changes in the intestinal mucosal barrier function; this allows translocation of luminal antigens (commensal bacteria or microbial products) into the intestinal wall, and consequent immune cell activation and excessive production of cytokines, causing the acute phase of inflammation. If the acute inflammatory process is not resolved by anti-inflammatory mechanisms and suppression of pro-inflammatory cytokines, chronic intestinal inflammation develops. This can lead to tissue destruction and complications of the disease[10].

Conventional treatments are aimed at reducing inflammation and consequent abnormal symptom relief. Patients with UC are treated with amino-salicylates, corticosteroids, and immunomodulatory drugs[11].

Natural products have become the most attractive source of new drugs for the treatment and prevention of diseases and their use is constantly expanding worldwide. A variety of herbal medicines have been shown to produce promising results in the treatment of peptic ulcer and IBD[11-14].

Combretum duarteanum (*C. duarteanum, Cd*) Cambess., the species selected for this study, is popularly known as "mufumbo, cipiúba, cipaúba". This

shrubby species is exclusive to South America with registrations in Bolivia, Paraguay, and Brazil. It occurs in the northern and northeastern regions of Brazil, being associated with the "caatinga" biome[15,16].

In folk medicine, *C. duarteanum* is used to treat pain, inflammation and GI tract disorders, which justifies its selection, using ethno-pharmacological indication as the criterion of choice. Phytochemical studies suggest the presence of flavonoids and triterpenes, whose pharmacological effects have been attributed[16-18].

C. duarteanum has presented *in vitro* and *in vivo* anti-inflammatory, anti-nociceptive, and antioxidant capacities[15]. Quintans *et al*[17] demonstrated orofacial nociceptive activity as promoted by the hexane phase and Fridelin terpenes, isolated from the species studied.

de Morais Lima *et al*[16,19] demonstrated gastro-protective and antiulcer activity in *C. duarteanum* in different models of acute ulcer induction (acidified ethanol, ethanol, nonsteroidal anti-inflammatory drugs (commonly known as NSAIDs), stress, pylorus ligature, acetic acid) in animals. Previous studies demonstrated low toxicity and no change of liver enzymes in animals treated with the tested plant sample for 15 d in acid acetic-induced gastric ulcer model[19].

Given the need for new IBD therapies, this study aimed to evaluate the intestinal anti-inflammatory activity promoted by the species *C. duarteanum*, validating its popular use and contributing to the search for new therapies for diseases that affect the GI tract.

MATERIALS AND METHODS

Reagents

The drugs and reagents were prepared immediately before use. The following drugs were used: trinitro-benzenesulfonic acid (TNBS) (Sigma-Aldrich), ketamine 5% (Vetanarcol), xilazine 2% (Dorcipec), ethanol (Merck®), Tween 80 (Merck®), sodium chloride (Sigma-Aldrich). TNF-α, IL-1β and IL-10 in enzyme-linked immunosorbent assay (ELISA) kits were provided by R&D Systems Inc.

Plant material

Plant samples used in intestinal anti-inflammatory activity research experiments in rats were obtained from the leaves of *C. duarteanum* Cambess., collected at Serra Branca City, Paraíba State, Brazil, in 2010. The species was identified by Dr. Maria Fatima Agra and a voucher specimen (No. 6767) was deposited in the Herbarium Prof. Lauro Pires Xavier (JPB) at the Universidade Federal da Paraíba.

The ethanolic extract (*Cd*-EtOHE) and the hexane phase (*Cd*-HexP) obtained from the leaves of *C. duarteanum* Cambess. were provided by Dr. Josean Fechine Tavares and his group, all of PgPNSB/UFPB.

The dried leaves (5 kg) were powdered, extracted with ethanol, stirred, and macerated at room temperature for approximately 48 h, with the procedure being repeated three times. The solvent was fully evaporated under reduced pressure, and the extract (yield of 200 g) was concentrated. The *Cd*-EtOHE was subjected to liquid-liquid partition with the following solvents: hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc), obtaining their respective phases. This step was repeated to secure the required quantity for the study.

Pharmacological assays

Investigation of *Cd*-EtOHE and *Cd*-HexP effects on acute phase intestinal inflammation (TNBS)-induced in rats: The intestinal anti-inflammatory activity of *Cd*-EtOHE and *Cd*-HexP was assessed in rats using the Morris *et al*[20] method. Male Wistar rats (n = 5-8) fasted for 24 h were divided into four groups: non-colitic, colitic, *Cd*-EtOHE and *Cd*-HexP. The animals were anesthetized for rectal administration of TNBS (2,4,6-trinitro-benzene sulfonic acid) - 10 mg solubilized in 0.25 mL of 50% v/v ethanol. The induction of inflammation was performed with the aid of a probe (2-mm diameter), which was inserted about 8 cm into the rectum of the animal. After TNBS administration, animals were maintained upside down for 15 min to enable total absorption of the administered inducing agent. The non-colitic group underwent the same procedures but they did not receive TNBS.

Each group of rats was pretreated with vehicle (12% Tween 80), *Cd*-EtOHE (31.25, 62.5, 125, 250 mg/kg) or *Cd*-HexP (31.25, 62.5, 125, 250 mg/kg), at 48, 24 and 1 h prior to administration of TNBS/50% ethanol, and at 24 h after colitis induction. At 48 h after inducing inflammation, the animals were euthanized and colonic segments were removed, opened, washed, and photographed for quantification of ulcerative lesion area (ULA) and macroscopic score evaluation of the intestinal inflammatory process. Analysis of the extent of intestinal injury was performed according to the scale described previously by Bell *et al*[21]. General parameters such as diarrhea and the colon weight/length ratio were also evaluated. The most effective doses obtained in this model were used in the chronic model with relapse of UC in rats.

Investigation of *Cd*-EtOHE and *Cd*-HexP effects in the chronic phase with intestinal inflammation relapse induced by TNBS in rats: Male Wistar rats (n = 7-9) were divided into non-colitic, colitic, *Cd*-EtOHE and *Cd*-Hexp groups. After 24 h of fasting, induction of intestinal inflammation was performed with TNBS (10 mg/0.25 mL ethanol 50% v/v, rectally)[20]. At 24 h after initial induction, the animal groups were treated orally with 12% Tween 80 solution (non-colitic and colitic), *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg). On day 14 after the first induction, the second administration

(relapse) was performed with TNBS (10 mg/0.25 mL ethanol 50% v/v, rectally) to mimic recurrent relapses in IBDs in humans.

General parameters such as diarrhea, water and food intake, and body weight were recorded daily throughout the treatment period. At day 21 all animals were euthanized, the colon removed, opened, and washed for macroscopic lesion analysis and evaluation of the intestinal inflammatory process[21]. Collection of material for biochemical and histological analysis was also performed. The samples were stored at -80 ℃ for evaluation of myeloperoxidase (MPO) and cytokines involved in intestinal inflammation.

Histological analysis

Colonic segments intended for light microscopy were collected. For this, the tissues were fixed in Alfac solution for 24 h at room temperature. Afterwards, the pieces were kept in 80% alcohol until the block assembly time. The pieces were dehydrated and embedded in paraplast forming blocks, and then cut to 10-mm thickness for mounting on slides. These were stained with hematoxylin and eosin for morphological analysis[22].

Quantification of MPO activity

Colon segments, stored at -80 ℃ were used with dosages of MPO and pro-inflammatory and anti-inflammatory cytokines. The samples were homogenized in hexadecyltrimethylammonium bromide buffer (HTAB) (0.5% in 50 mmol/L sodium phosphate buffer, pH 6.0) that acts as a detergent, lysing granules of neutrophils containing MPO, which is released. The sample was centrifuged for 10 min at 4 ℃. The homogenate was subjected to a three-fold freezing and thawing process to facilitate the rupturing of cell structures and the consequent release of the enzyme. On ELISA plates were placed 50 μL of supernatant from each sample and 150 μL of reaction buffer[23].

The results were expressed as MPO units per gram of tissue, where 1 U of MPO activity is defined as that degrading 1 μmol of hydrogen peroxide per min at 25 ℃.

Assessment of the involvement of pro-inflammatory (TNF-α and IL-1β) and anti-inflammatory cytokines (IL-10)

TNF-α, IL-1β and IL-10 levels were determined from colonic specimens, frozen in -80 ℃, and collected in the UC relapse model. For this, we used PBS buffer pH 7.4 (1:5) to homogenize the samples. The homogenate tubes were centrifuged at 12000 rpm for 10 min. The supernatants were frozen at -80 ℃ until assay. Subsequently, the samples were shaken in water bath at 37 ℃ for 20 min and then centrifuged at 10000 rpm for 5 min at 4 ℃. The supernatant was collected and the cytokines TNF-α, IL-1β and IL-10 were quantitated using ELISA assay kits (DuoSet®; R & D Systems Inc.). The concentrations of the cytokines in relation to the amount of total protein was quantified by bicinchoninic acid method[24].

Immunohistochemical analysis cyclooxygenase-2, proliferating cell nuclear antigen, and superoxide dismutase expression

Histological samples were incubated with anti-cyclo-oxygenase-2 (COX-2) secondary antibody (marker for assessing anti-inflammatory effect), anti-proliferating cell nuclear antigen (PCNA) (cell division marker to assess potential for regeneration), and anti-superoxide dismutase (SOD) (marker to evaluate the antioxidant effect). The positively stained cells were counted for the various immunohistochemical reactions in a fixed number of fields by means of an image analyzer (Q-Win Standard Version 3.1.0; Leica) coupled to the Leica DM microscope. They were photographed and analyzed by AVSoft program Bioview Spectra and Seeker 4.0.

Animal care and use statement

The experimental protocols were approved by the Committee for Ethics in Animal Experimentation (CEPA/LTF/UFPB) under number 1112/10. Male Wistar albino rats (180-250 g) from the "Prof. Thomas George Vivarium" of LTF/UFPB were fed a certified Presence® diet, with free access to water under fixed conditions of illumination (12/12 h light/dark cycle), humidity (60% ± 1.0%), and temperature (21.5 ± 1.0 ℃). Fasting was used prior to all assays since standard drugs were administered orally (by gavage) or by intra-rectal route, using a 12% solution of Tween 80 (10 mL/kg) as the vehicle. The animals were kept in cages with raised, wide-mesh floors to prevent coprophagy.

Statistical analysis

Results with parameter values (inflammatory bowel lesion area and weight/length ratio) were subjected to analysis of variance (ANOVA) followed by Dunnett's or Tukey test, and expressed as mean ± SD of the average. In quantitation assays of antioxidant enzymes, cytokines and MPO values obtained were presented as mean ± standard error of mean (SEM).

For nonparametric values (score of intestinal inflammation), the Kruskal-Wallis test (ANOVA, Dunn's post-test) was used. The results were expressed as median (minimum-maximum). Data were analyzed using the software GraphPad Prism 6.0, and the significance level was set at $P < 0.05$.

RESULTS

Investigation of Cd-EtOHE and Cd-HexP effect on induced acute phase intestinal inflammation (TNBS) in rats

A significant reduction in the intestinal ULA for rats

Table 1 Effects of oral administration of *Cd*-EtOHE or *Cd*-HexP in acute phase of intestinal inflammation in trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Dose (mg/kg)	ULA (mm^2)	Inhibition (%)	Lesion score	Weight/length (mg/cm)	Diarrhea (%)
Non-colitics	-	-	100		110 ± 7.7	0
Colitics	-	107 ± 38	-	6.0 (5-7)	148 ± 17[f]	100
Cd-EtOHE	31.25	79 ± 31	26	5.0 (3-7)	152 ± 19[f]	100
	62.5	46 ± 12[b]	57	4.0 (1-5)[a]	146 ± 19[f]	57
	125	19 ± 8[c]	82	3.0 (2-5)[b]	134 ± 5[d]	14[b]
	250	76 ± 23	29	6.0 (4-7)	152 ± 14[f]	86
Non-colitics	-	-	-	-	102 ± 14	0
Colitics	-	101 ± 45	-	7.0 (5-8)	154 ± 27[f]	87
Cd-HexP	31.25	52 ± 18[b]	49	5.0 (1-6)[a]	142 ± 20[e]	87
	62.5	21 ± 7[c]	79	5.0 (4-5)[b]	129 ± 20[a]	29[a]
	125	95 ± 33	7	6.0 (5-6)	154 ± 10[f]	71
	250	80 ± 12	22	5.0 (4-7)	155 ± 16[f]	86

Results expressed as mean ± SD or median (minimum-maximum) of the parameters analyzed (n = 5-8). For the parametric data, mean ± SD was used, with ANOVA and *a posteriori* Dunnett's test. For the non-parametric data, median (minimum-maximum) was used, with Kruskal-Wallis test and *a posteriori* Dunn. [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ *vs* colitic group; [d]$P < 0.05$, [e]$P < 0.01$ and [f]$P < 0.001$ *vs* non-colitic group. *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

treated with *Cd*-EtOHE at doses of 62.5 and 125 mg/kg (46 ± 12, $P < 0.01$ and 19 ± 8, $P < 0.001$ respectively) was observed compared to the colitic group (107 ± 38). In the experimental evaluation of the effect of *Cd*-HexP, a significant reduction was observed at doses of 31.25 and 62.5 mg/kg (52 ± 18, $P < 0.01$ and 21 ± 7, $P < 0.001$ respectively), when compared to the colitic animals (101 ± 45) (Table 1).

For the lesion score, *Cd*-EtOHE at doses of 62.5 and 125 mg/kg significantly reduced the amounts of lesion to 4.0 (1-5) ($P < 0.05$) and 3.0 (2-5) ($P < 0.01$) respectively, compared to the colitic control of 6 (5-7). *Cd*-HexP 31.25 and 62.5 mg/kg significantly reduced lesion to 5.0 (1-6) ($P < 0.05$) and 5.0 (4-5) ($P < 0.01$) respectively, compared to the colitic group of 7 (5-8) (Table 1).

A significant increase in weight/length for the colitic group (148 ± 17, $P < 0.001$, 154 ± 27, $P < 0.001$) was also observed when compared to the non-colitic group (110 ± 8, 102 ± 14 respectively). Treatment with different doses of *Cd*-EtOHE (31.25, 62.5, 125 and 250 mg/kg) did not reduce the weight/length ratio (152 ± 19, 146 ± 19, 134 ± 5 and 152 ± 14 respectively) for the parameter compared to the colitic group (148 ± 17). However, treatment with *Cd*-HexP at a dose of 62.5 mg/kg significantly reduced the ratio to 129 ± 20 ($P < 0.05$), compared to their respective colitic group (154 ± 27) (Table 1).

The administration of TNBS resulted in a diarrhea rate of 100% in the colitic animals. *Cd*-EtOHE at a dose of 125 mg/kg significantly reduced the diarrhea involvement to 14%. For *Cd*-HexP, treatment at dose of 62.5 mg/kg significantly reduced diarrhea to 29% ($P < 0.05$) when compared to their respective colitic control (87%) (Table 1).

Intestines of colitic, non-colitic and treated rats with different tested doses of *Cd*-EtOHE or *Cd*-HexP in the model can be seen in Figures 1 and 2 respectively.

Investigation of Cd-EtOHE and Cd-HexP effects in chronic phase intestinal inflammation with induced relapse using TNBS in rats

A significant reduction in macroscopic damage scores was observed for both *Cd*-EtOHE (125 mg/kg) and *Cd*-HexP (62.5 mg/kg), to 1.0 (1-4) ($P < 0.05$) and 1.0 (1-4) ($P < 0.01$) respectively, compared to the colitic control 4 (3-6). Moreover, the tested plant sample reduced the onset of diarrhea by 56% when compared to colitic animals (94%) (see Table 2).

The weight/length ratio significantly increased in the colitic, *Cd*-EtOHE and *Cd*-HexP groups (143 ± 14, $P < 0.001$; 132 ± 11, $P < 0.001$; 122 ± 12, $P < 0.01$, respectively) compared to the non-colitic group (97 ± 9). However, *Cd*-HexP 62.5 mg/kg caused a significant reduction (122 ± 12, $P < 0.01$), compared to the colitic group (143 ± 14). These results are shown in Table 2, and can be seen best in Figure 3.

A significant decrease in water (28 ± 3, $P < 0.01$) and food (19 ± 2, $P < 0.01$) intake was observed in the colitic group, compared to the non-colitic animals (31 ± 4 and 22 ± 2 respectively). Only treatment with *Cd*-HexP increased water (31 ± 2, $P < 0.05$) and food (22 ± 3, $P < 0.01$) intake, compared to colitic animals (28 ± 3, $P < 0.01$ and 19 ± 2, $P < 0.01$ respectively) (Table 3).

As an additional parameter to the data described above, we evaluated the effect of repeated administrations of *Cd*-EtOHE (125 mg/kg) and *Cd*-HexP (62.5 mg/kg) on the body weights of animals affected with intestinal inflammation. At the end of the experiment, there was a significant reduction in mean body weight for the colitic group (215 ± 21, $P < 0.001$) when compared to the non-colitic group (261 ± 35). However, when the treatments were performed with *Cd*-HexP (62.5 mg/kg), a significant increase in mean body weight (239 ± 17, $P < 0$ 05) was observed compared to the colitic animals (215 ± 21) (Table 4).

A significant increase was observed in spleen weight

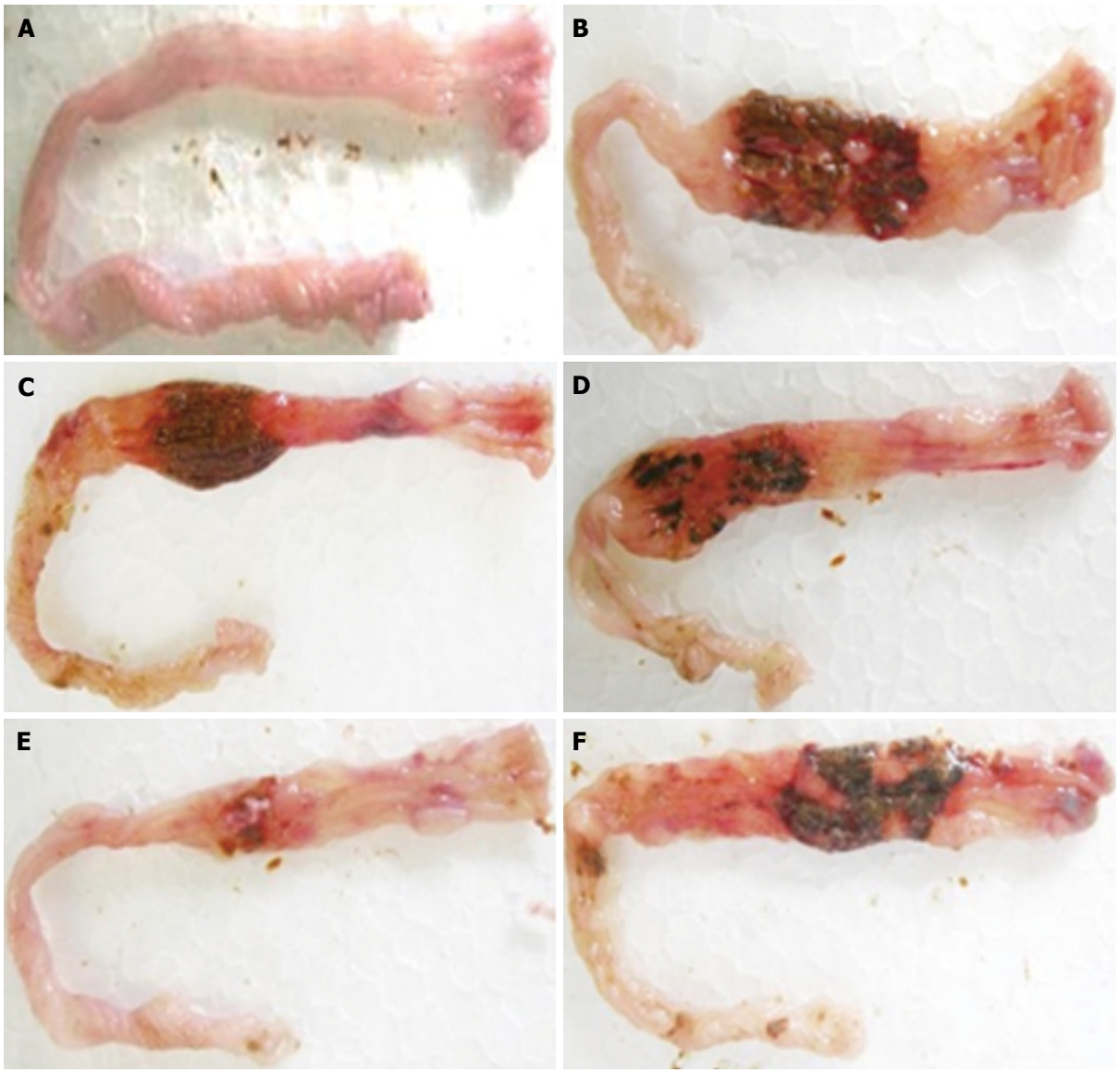

Figure 1 Representative macroscopic images of rat colonic mucosa in non-colitics (A), colitics (B), *Cd*-EtOHE at 31.25 mg/kg (C), *Cd*-EtOHE at 62.50 mg/kg (D), *Cd*-EtOHE at 125 mg/kg (E), and *Cd*-EtOHE at 250 mg/kg (F). *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract.

in the colitic group (2.8 ± 0.5, $P < 0.05$) compared to the non-colitic group (2.1 ± 0.2). Analyzing the organs of animals treated with *Cd*-EtOHE or *Cd*-HexP, a significant increase in spleen weight for animals treated with *Cd*-EtOHE as compared to the non-colitic group was demonstrated (Table 5). In other evaluations (heart, liver and kidneys) no significant changes, compared to the non-colitic group, were observed.

Histological analysis

Histological examination of the colon of non-colitic rats showed normal histological structure, highlighting the structure of the mucosal straight intestinal glands with large numbers of goblet cells and lamina propria classical (or normal). The animals belonging to the colitic group had transmural inflammation, necrosis of the mucosa with disruption of glands, and loss of goblet and epithelial cells. The presence of granulation, highlighting neutrophilic and lymphocytic infiltration, was also observed.

Treatment with *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) maintained some areas of the mucosal structure and epithelium intact, reducing the reducing the Inflammatories cells in lamina propria as compared to the colitic group, suggesting the re-epithelialization of the animals treated with the vegetable samples (Figure 4).

Quantification of MPO activity

According to the results obtained, there was a significant increase in MPO to 40.270 ± 3.077 ($P < 0.001$) in the colitic group when compared to non-colitics (10.120 ± 1.672). When compared to the colitic controls (40.270 ± 3.077), treatments with *Cd*-EtOHE (15.187 ± 1.158, $P < 0.001$) or *Cd*-HexP (17.620 ± 2.395, $P < 0.001$) significantly reduced MPO (Figure 5).

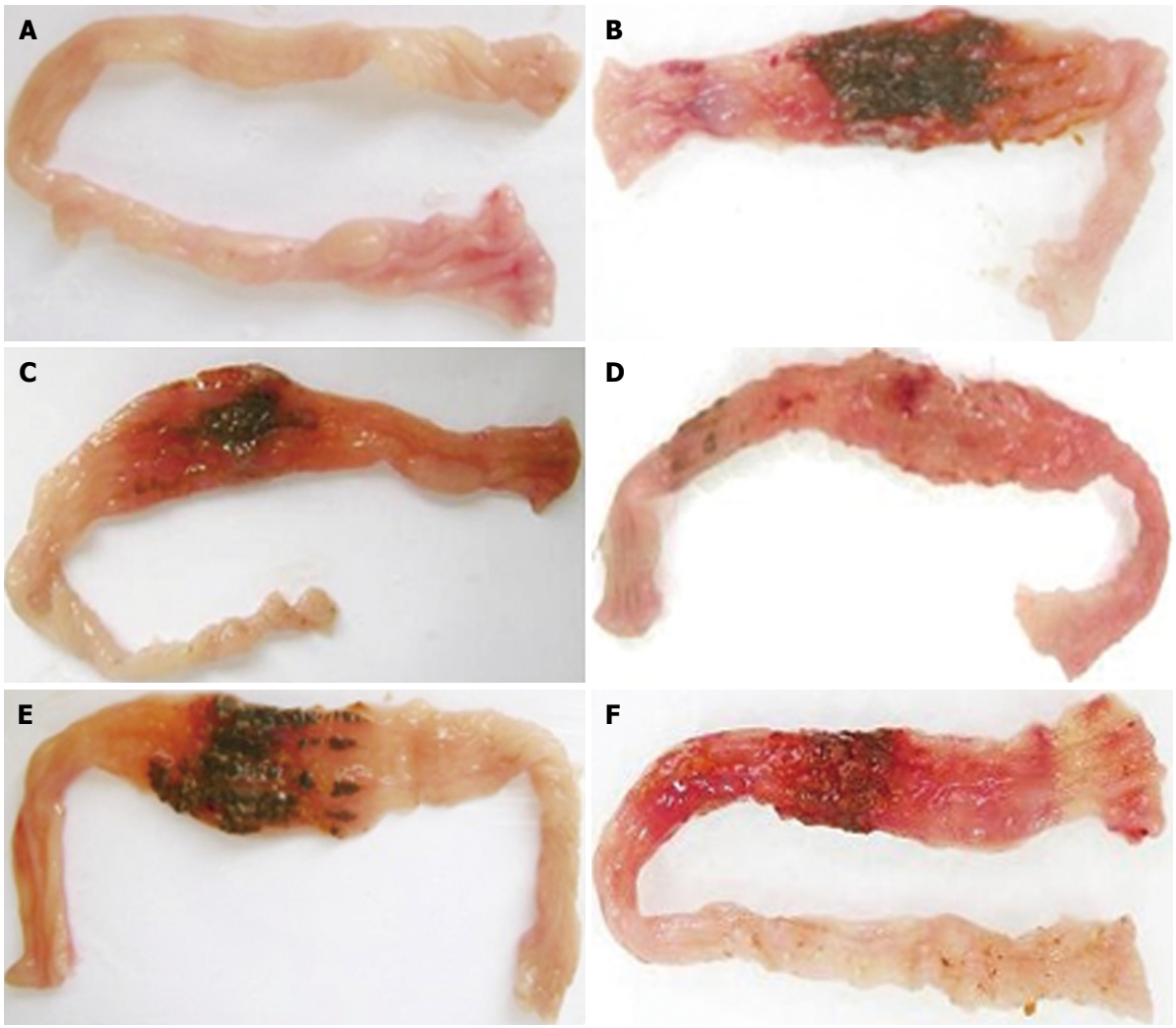

Figure 2 Representative macroscopic images of rat colonic mucosa in non-colitics (A), colitics (B), *Cd*-HexP at 31.25 mg/kg (C), *Cd*-HexP at 62.50 mg/kg (D), *Cd*-HexP at 125 mg/kg (E), and *Cd*-HexP at 250 mg/kg (F). *Cd*: *Combretum duarteanum*; HexP: Hexane phase.

Assessment of the involvement of pro-inflammatory (TNF-α and IL-1β) and anti-inflammatory cytokines (IL-10)

The results showed a significant increase in TNF-α levels in colitic animals (2.0 ± 0.2, $P < 0.01$) compared to non-colitics (1.3 ± 0.1). However, the treatment of *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) resulted in TNF-α level reduction (1.2 ± 0.2 and 1.4 ± 0.1, $P < 0.01$) respectively, compared to the colitic group (2.0 ± 0.2) (Figure 5).

IL-1β levels increased in colitic animals (63 ± 6, $P < 0.001$) when compared to the non-colitic group (32 ± 4.0). Treatment with *Cd*-EtOHE significantly reduced to 41 ± 5 ($P < 0.01$) IL-1-β, compared to the colitic group (63 ± 6.2). *Cd*-HexP did not cause significant change when compared to the negative control (Figure 5).

The results showed significant reduction of IL-10 in intestinal tissues, comparing the colitic group 3.2 ± 0.3 ($P < 0.05$) to the non-colitic group (5.1 ± 0.8). Treatment with *Cd*-EtOHE or *Cd*-HexP caused significant increases (5.0 ± 0.4, $P < 0.05$, 5.8 ± 0.3, $P < 0.01$, respectively) when compared to the colitic group (3 2 ± 0.3) (Figure 5).

Immunohistochemical analysis (COX-2, PCNA and SOD expression)

In analyzing the results of COX-2 expression shown in Table 6 and Figure 6, we observed a significant increase in COX-2 expression in the colitic group animals to 505 (100-1450) ($P < 0.05$) when compared the non-colitic group of 230 (110-700). However, treatment with *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) significantly reduced the expression of COX-2 to 90 (10-2590) ($P < 0.001$) and 205 (20-790) ($P < 0.001$) respectively, compared to the colitic group of 505 (100-1450).

A significant increase in PCNA expression was observed in the animals of the colitic group of 5380 (850-15960) ($P < 0.001$) when compared to the non-colitic group of 1425 (60-7890). However, treatment with *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) significantly reduced PCNA expression to 1.630 (90-14790) ($P < 0.001$) and 1.570 (250-9500) ($P <$

Table 2 Effects of oral administration of *Cd*-EtOHE or *Cd*-HexP in chronic phase with relapse of intestinal inflammation in trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Dose (mg/kg)	Lesion score	Weight/length (mg/cm)	Diarrhea (%)
Non-colitics	-		97 ± 9	0
Colitics	-	4.0 (3-6)	143 ± 14[f]	94
Cd-EtOHE	125	1.0 (1-4)[a]	132 ± 11[f]	56[a]
Cd-HexP	62.5	1.0 (1-4)[b]	122 ± 12[b,e]	56[a]

Results expressed as mean ± SD or median (minimum-maximum) of the parameters analyzed (n = 7-9). For the parametric data, mean ± SD was used, with ANOVA and a posteriori Dunnett's test. For non-parametric data, median (minimum-maximum) was used, with Kruskal-Wallis test and a posteriori Dunn. [a]P < 0.05, [b]P < 0.01 *vs* colitic group; [e]P < 0.01 and [f]P < 0.001 *vs* non-colitic group. *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

Table 3 Effect of oral administration of *Cd*-EtOHE or *Cd*-HexP for 21 d on water and food consumption in trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Dose (mg/kg)	Water intake (mL)	Food intake (g)
Non-colitics	-	31 ± 4	22 ± 2
Colitics	-	28 ± 3[e]	19 ± 2[e]
Cd-EtOHE	125	30 ± 4	20 ± 3[d]
Cd-HexP	62.5	31 ± 2[a]	22 ± 3[b]

Values are expressed as mean ± SD (n = 7-9). One-way ANOVA, followed by Dunnett's test, [a]P < 0.05 and [b]P < 0.01 *vs* colitic group; [d]P < 0.05 and [e]P < 0.01 *vs* non-colitic group. *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

0.001) respectively, compared to 5.380 of the colitic group (850-15960) (P < 0.001) (Table 6 and Figure 6).

The results of this analysis demonstrated a significant decrease in the expression of SOD in colitic controls to 165 (10-1000) (P < 0.001) when compared to the non-colitic group of 400 (80-1115). The treatment of the animals with *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) increased the expression of SOD to 400 (50-1480) (P < 0.05) and 435 (20-1650) (P < 0.001) respectively, compared to the colitic control (Table 6 and Figure 6).

DISCUSSION

A promising area for research, many plant species and their chemical constituents exert therapeutic actions. This has led to the development of new, effective and safe drugs for the treatment of various pathological processes. There is also an interest in targeted therapy for diseases derived from oxidative stress, such as IBD[11,25].

IBDs are progressive and destructive chronic disorders of the GI tract, the most common being CD or UC. There is evidence that the pathogenesis of IBD is related to a dysfunctional interaction between the bacteria of the intestinal microflora and mucosal

Table 4 Effect of oral administration of *Cd*-EtOHE or *Cd*-HexP for 21 d on body weight on trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Initial weight (g)	Final weight (g)	Average increase (g)
Non-colitics	202 ± 32	261 ± 35	51 ± 10
Colitics	189 ± 19	215 ± 21[f]	35 ± 12[d]
Cd-EtOHE	178 ± 13	230 ± 7	43 ± 6
Cd-HexP	184 ± 14	239 ± 17[a]	48 ± 10[a]

Values are expressed as mean ± SD (n = 7-9). One-way ANOVA, followed by Dunnett's test, [a]P < 0.05 *vs* colitic group; [d]P < 0.05, [f]P < 0.001, *vs* non-colitic group. *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

Table 5 Effect of oral administration of *Cd*-EtOHE or *Cd*-HexP for 21 d on weight organs in trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Heart	Liver	Kidneys	Spleen
Non-colitics	4.0 ± 0.2	41 ± 1.5	8.6 ± 0.6	2.1 ± 0.2
Colitics	4.0 ± 0.5	43 ± 5.3	8.6 ± 1.0	2.8 ± 0.5[d]
Cd-EtOHE	4.0 ± 0.3	43 ± 3.1	8.6 ± 0.2	2.8 ± 0.4[e]
Cd-HexP	4.0 ± 0.3	44 ± 1.2	8.4 ± 0.5	2.4 ± 0.2

Values are expressed as mean ± SD (n = 7-9). One-way ANOVA, followed by Dunnett's test, [d]P < 0.05 and [e]P < 0.01 *vs* non-colitic group. *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

immune system[26,27].

CD and UC are immunologically different diseases. CD is characterized by an exaggerated cellular Th1 response (CD4+) and Th17, characterized by high levels of INF-γ/IL-17 and IL-12/IL-23. UC is characterized by a heightened Th2 response, and excessive IL-5 and IL-13[28-30].

TNBS is a hapten, administered by enema in rats in combination with 50% ethanol to break the mucus barrier and facilitate penetration of the hapten into the intestinal epithelium. TNBS reacts with autologous proteins and stimulates the development of hypersensitivity, leading to the activation of antigen-specific T cells. The immune response induced by the hapten causes severe ulceration of the mucosal and epithelial barrier, characterized by trans-mural infiltration of mononuclear cells[20].

Preventive treatment with *Cd*-EtOHE (62.5 to 125 mg/kg) or *Cd*-HexP (31.25 and 62.5 mg/kg) caused a significant reduction in the severity and extent of injury as reflected in the macroscopic lesion score. The macroscopic/microscopic damage scores and colon weight/length ratio can be considered as sensitive and reliable markers to estimate the severity of the disease, and thus the anti-inflammatory effect promoted by the test drug[31].

A low incidence of diarrhea in animals treated with *Cd*-EtOHE and *Cd*-HexP was also observed. Diarrhea is a major symptom of disease in both animals and humans and indicates loss of the absorptive capacity of the colon, which is impaired in intestinal infla-

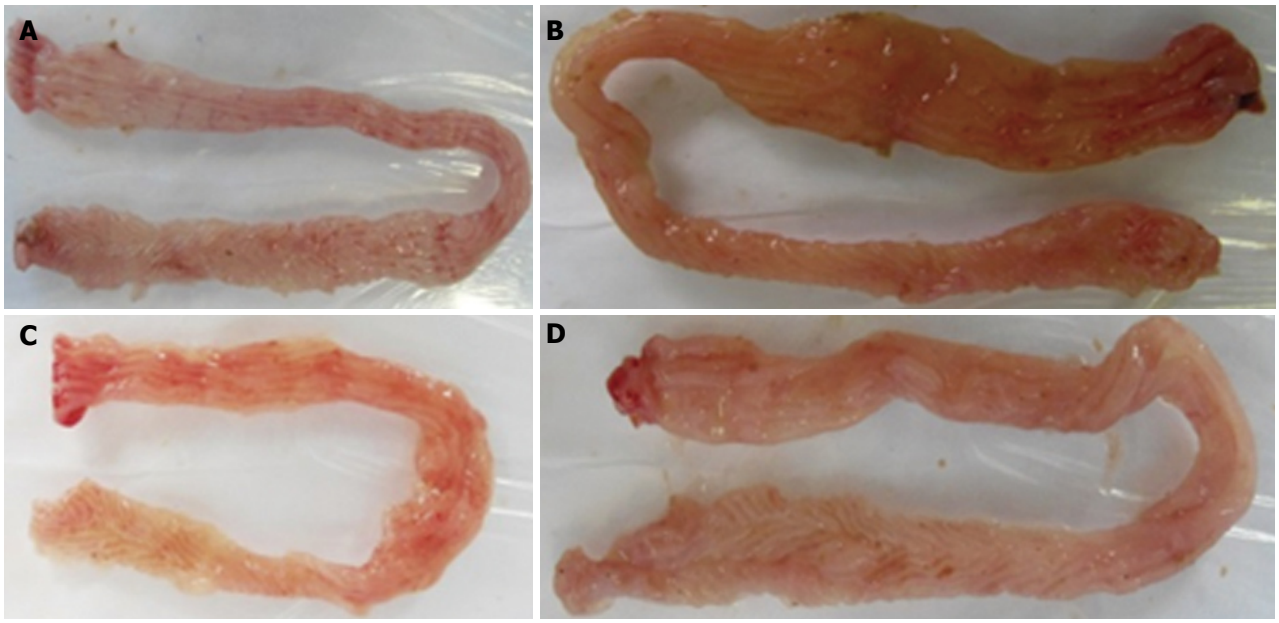

Figure 3 Representative macroscopic images of rat colonic mucosa in non-colitics (A), colitics (B), *Cd*-EtOHE at 125 mg/kg (C), and *Cd*-HexP at 62.5 mg/kg (D). *Cd*: *Combretum duarteanum*; HexP: Hexane phase; EtOHE: Ethanolic extract.

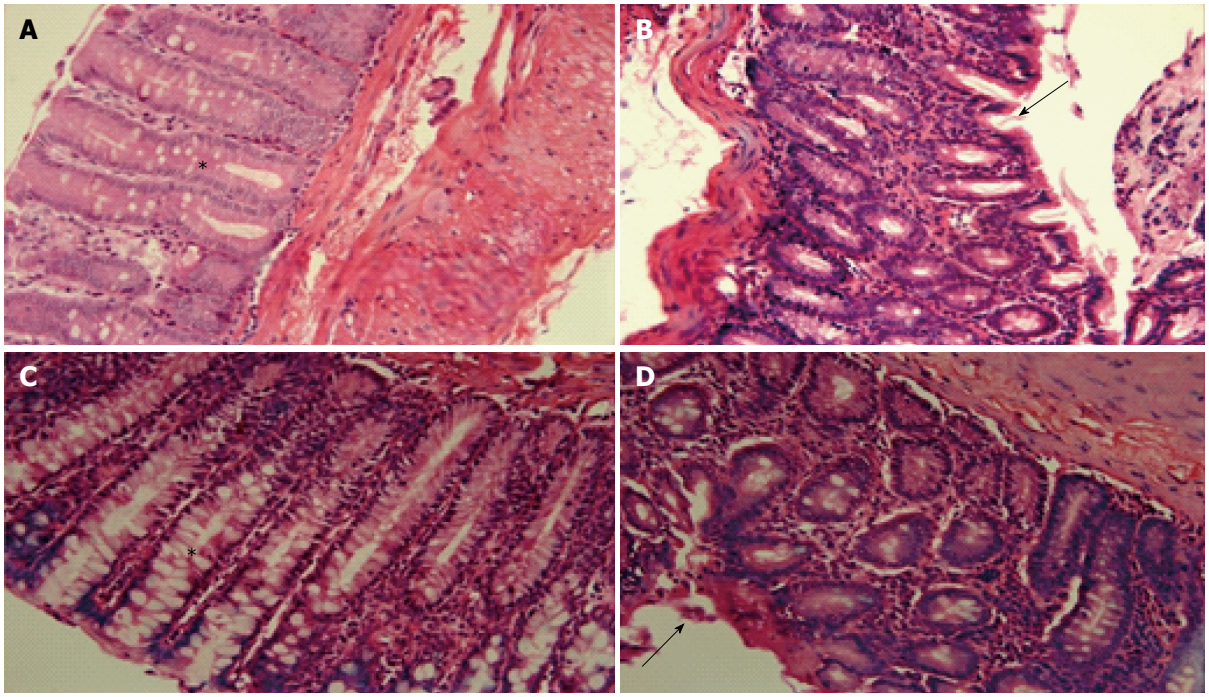

Figure 4 Representative histological appearance of rat colonic mucosa in the non-colitic group (A), colitic group (B), *Cd*-EtOHE (C), and *Cd*-HexP (D). Aumento magnification × 40 (*goblet cells; → ulceration region; 50 μm). Colonic tissue sections were stained with hematoxylin and eosin, and observed under light microscope (magnification × 40). *Cd*: *Combretum duarteanum*; HexP: Hexane phase; EtOHE: Ethanolic extract.

mmation[32]. The results suggest that treatment with the plant samples restored the intestinal absorptive capacity.

Cd-HexP at 62.5 mg/kg significantly reduced the weight/length ratio. This effect is possibly related to the scavenging capacity of free radicals caused by treatment at this dose[33]. These results demonstrate, for the first time, anti-inflammatory intestinal activity for a species belonging to the genus *Combretum*.

The more effective doses of *Cd*-EtOHE (125 mg/kg) or *Cd*-HexP (62.5 mg/kg) were selected to investigate their effects in the chronic phase with relapse in TNBS-induced UC in rats. This model mimics the disease in humans and can be used to evaluate new treatments potentially applicable in IBD[31].

Cd-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg

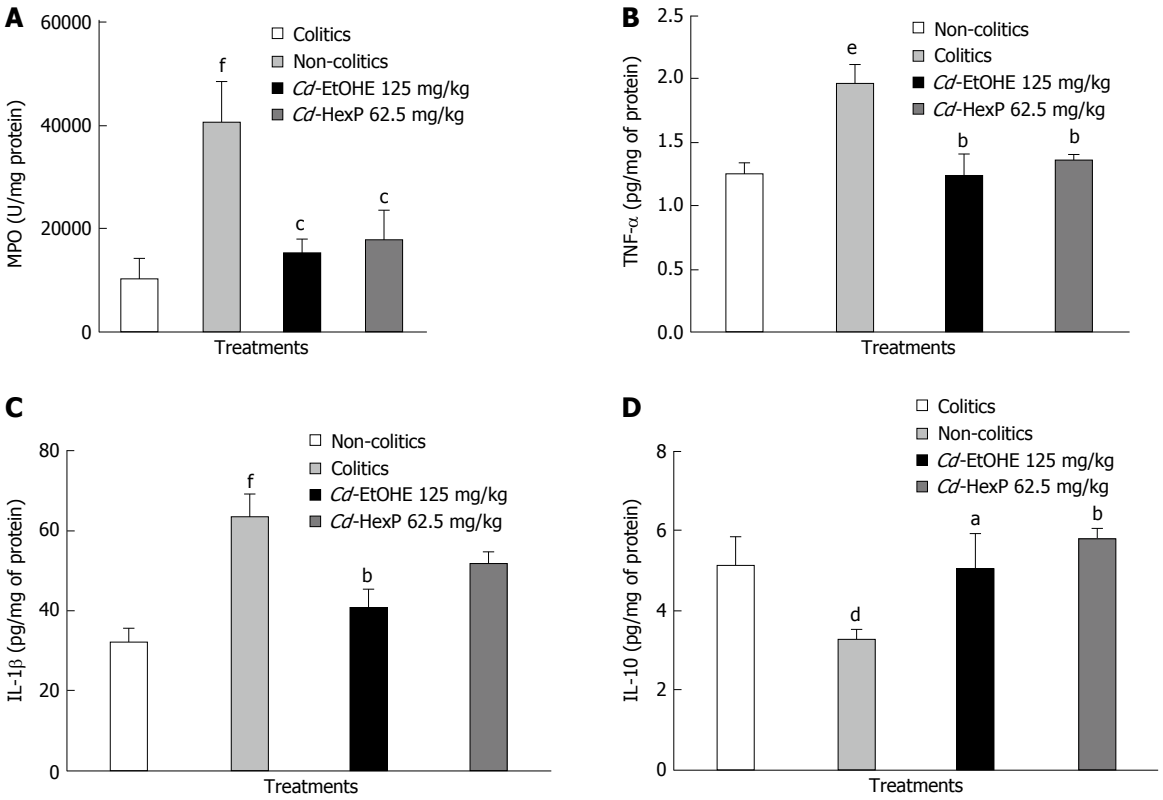

Figure 5 Effect of acute administration of *Cd*-EtOHE at 125 mg/kg and *Cd*-HexP at 62.5 mg/kg on myeloperoxidase activity (MPO, U/mg protein) (A), tumor necrosis factor-alpha (TNF-α, pg/mg protein) (B), interleukin-1β (IL-1β, pg/mg protein) (C), interleukin-10 (IL-10, pg/mg protein) (D) in trinitrobenzene sulfonic acid-induced colitis model with relapse in rats (TNBS, 10 mg/animal). Data are expressed as the mean ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ *vs* colitic group. $^{d}P < 0.05$, $^{e}P < 0.01$ and $^{f}P < 0.001$ *vs* non-colitic group. *Cd*: *Combretum duarteanum*; HexP: Hexane phase; EtOHE: Ethanolic extract.

Table 6 Effect of oral administration of *Cd*-EtOHE or *Cd*-HexP for 21 d on the expression of cyclooxygenase-2, proliferating cell nuclear antigen and superoxide dismutase in trinitrobenzenesulfonic acid-induced ulcerative colitis in rats

Group	Dose (mg/kg)	COX-2 (μm^2)	PCNA (μm^2)	SOD (μm^2)
Non-colitics	-	230 (110-700)	1425 (60-7890)	400 (80-1.115)
Colitics	-	505 (100-1.450)^{d}	5380 (850-15.960)^{f}	165 (10-1.000)^{f}
Cd-EtOHE	125	90 (10-2.590)^{c}	1630 (90-14.790)^{c}	400 (50-1.480)^{c}
Cd-HexP	62.5	205 (20-790)^{c}	1570 (250-9.500)^{c}	435 (20-1.650)^{c}

Results are expressed as median (minimum-maximum) of the analyzed parameters (n = 7-9). For nonparametric data, median (minimum-maximum) was used, with Kruskal-Wallis and Dunn's test *a posteriori*. $^{c}P < 0.001$ *vs* colitic group; $^{d}P < 0.05$ and $^{f}P < 0.001$ *vs* non-colitic group. COX-2: Cyclooxygenase-2; PCNA: Proliferating cell nuclear antigen; SOD: Superoxide dismutase; *Cd*: *Combretum duarteanum*; EtOHE: Ethanolic extract; HexP: Hexane phase.

significantly decreased signs of disease, such as macroscopic lesion (lesion area and score), weight/length ratio and diarrhea, showing the anti-inflammatory effect at 21 d of treatment.

Intestinal inflammation in the TNBS-induced model promoted the loss of 8% to 10% of body weight notably at 1 wk after induction, this is related to reduction in food intake due to abdominal pain and diarrhea during the active phase of the disease[34]. Treatment with *Cd*-HexP at 62.5 mg/kg reversed low water and food intake (and body weight loss) caused by disease.

The spleen recycles and acts as a reserve of red blood cells. The organ is the center of reticulo-endothelial system activity, and is an essential part of the immune system[35]. A significant increase in spleen weight of colitic animals when compared to non-colitic animals was demonstrated. UC and CD are mentioned in lists of factors that can cause increased spleen size, which can occur through lymphoid cell accumulation in immune functions[35,36].

MPO is an enzyme found in neutrophils and has been used as a quantitative index of neutrophil influx into inflamed intestines. The recruitment and activation of neutrophils results in a significant increase in free radical production, capable of overcoming antioxidant protections, and resulting in oxidative stress and inflammation[37,38]. *Cd*-EtOHE at 125 mg/kg and *Cd*-HexP at 62.5 mg/kg significantly decreased MPO activity when compared to colitic animals. This may

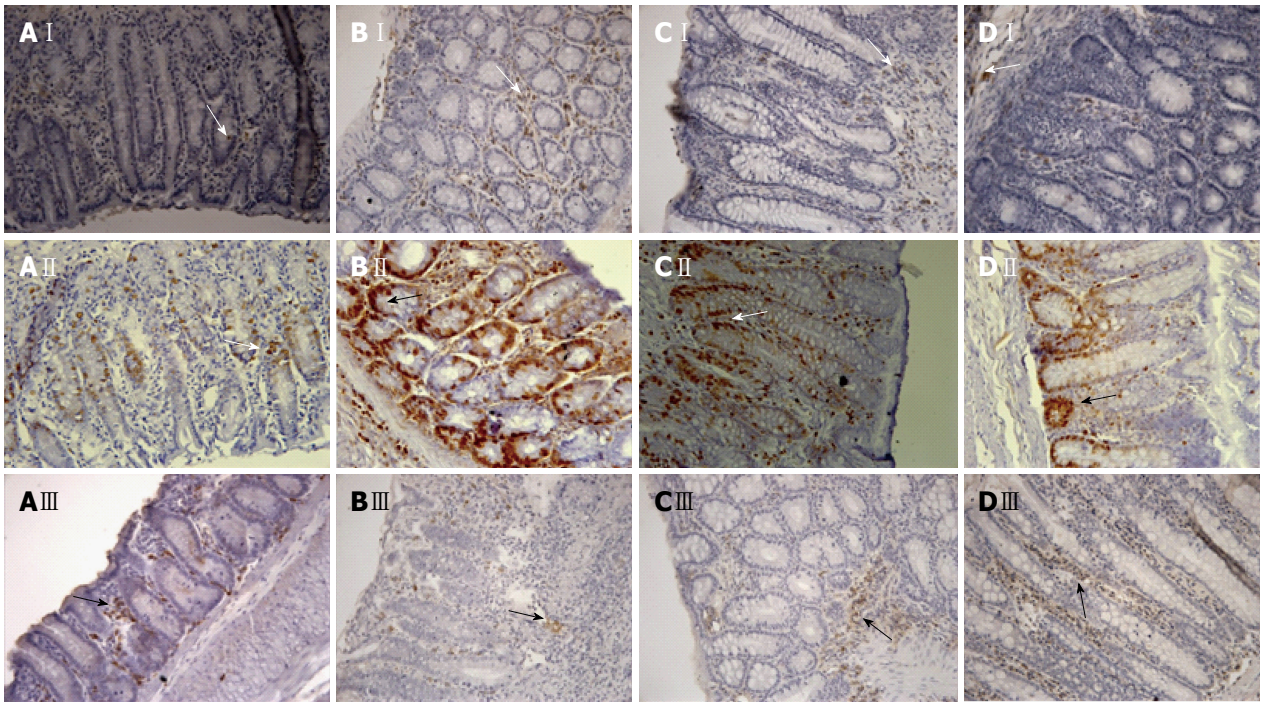

Figure 6 Photomicrograph of colonic samples from rats submitted to trinitrobenzene sulfonic acid-induced colitis model with relapse in rats after 21 d of treatment. A: Non-colitic group; B: Colitic group; C: *Cd*-EtOHE at 125 mg/kg; D: *Cd*-HexP at 62.5 mg/kg. Immunohistochemical localization of Line Ⅰ: COX-2; Line Ⅱ: PCNA; Line Ⅲ: SOD (→ COX-2, PCNA and SOD respectively). COX-2: Cyclooxygenase-2; PCNA: Proliferating cell nuclear antigen; SOD: Superoxide dismutase; *Cd*: *Combretum duarteanum*; HexP: Hexane phase; EtOHE: Ethanolic extract.

be interpreted as a manifestation of anti-inflammatory effect for *C. duarteanum* species.

The inflammation in the TNBS-induced colitis model is characterized by a Th1 pathway immune response, in which there is an increase in TNF-α, IL-1β, IL-12, IL-17, IL-18 and IL-6[39-41].

Up-regulation of the inflammatory state with increased TNF-α and IL-1β levels in colitic rats was observed, which corroborates the literature findings[42]. The treatment with *Cd*-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg was able to significantly reduce TNF-α levels when compared to the colitic controls, suggesting that TNF-α suppression is related to the anti-inflammatory effect promoted by the vegetable samples studied. *Cd*-EtOHE at 125 mg/kg was able to decrease IL-1β levels, suggesting that compounds of this plant sample may interfere with the synthesis machinery of IL-1β activation by inhibiting production[10,43,44].

IL-10 suppresses production of pro-inflammatory cytokines, such as IL-12, IL-6, IL-1 and TNF-α, in activated macrophages *in vitro,* and blocks the ability of macrophages stimulating the production of interferon by Th1 cells. IL-10 is produced in large amounts by TCD4+ regulatory cells subtype (Tregs). Tregs maintain homeostasis by suppressing the adaptive response of T cells and preventing autoimmunity[45].

A significant elevation in IL-10 levels in animals treated with *Cd*-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg, compared to the colitic group, was demonstrated. This increase is attributable to compensatory mechanisms against colonic injury, possibly playing a role in reducing mucosal inflammation and preventing it from becoming uncontrolled. IL-10 down-regulates antigen presentation, and thereafter the release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6[46,47].

Studies have shown that COX-2 is expressed predominantly in experimental colitis. Colotic humans and animals show considerable improvement in the inflammatory process when COX-2 inhibitors are used[48]. The vegetable samples tested prevented the increase in expression of this enzyme, and suggest that the intestinal anti-inflammatory effect promoted by *Cd*-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg is mediated by a reduction in COX-2 expression.

PCNA is an intra-nuclear protein, whose expression is related to cell proliferation and DNA repair. It is highly expressed during the S phase of the cell cycle[49]. Studies show that PCNA expression is up-regulated during chronic inflammation, inducing the proliferation of epithelial cells to repair the mucous tissue[50,51].

The positive expression of PCNA was increased in colitic animals in this study, while *Cd*-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg treatment significantly decreased expression of this protein. Treatment with the plant samples studied protected against intestinal epithelial cell damage induced by TNBS and the effect was mediated *via* regulation of PCNA.

SOD is a key enzyme that converts superoxide to H_2O_2, a more stable metabolite. During oxidative stress and inflammation, SOD activity is decreased in inflamed tissues as compared to non-inflamed tissues. The decreased SOD activity allows superoxide accumulation and subsequent oxidative effects in the intestinal tissue, as well as increased expression of adhesion molecules[52].

Treatment with *Cd*-EtOHE at 125 mg/kg or *Cd*-HexP at 62.5 mg/kg significantly increased the expression of this enzyme when compared to colitic animals, suggesting involvement of an antioxidant effect in the intestinal anti-inflammatory activity promoted by the vegetable samples.

In conclusion, *C. duarteanum* presents promising anti-inflammatory intestinal effects that are related to reduced levels of the pro-inflammatory cytokines (TNF-α and IL-1β) and increased anti-inflammatory cytokine (IL-10), which feature regulatory effects on the immune response, with reduction in the expression of COX-2, PCNA, and an increase in the antioxidant enzyme SOD.

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COMMENTS

Background

A variety of herbal medicines have been shown to produce promising results in the treatment of peptic ulcer and inflammatory bowel disease. *Combretum duarteanum* (*C. duarteanum*, *Cd*), the species selected for this study, is popularly known as "mufumbo, cipiúba, cipaúba". In folk medicine, it is used to treat pain, inflammation and gastrointestinal (GI) tract disorders. Given the need for new inflammatory bowel disease therapies, this study aimed to evaluate, for the first time, the intestinal anti-inflammatory activity promoted by the species *C. duarteanum*, validating its popular use and contributing to the search for new therapies for diseases that affect the GI tract.

Research frontiers

C. duarteanum has presented *in vitro* and *in vivo* anti-inflammatory, antinociceptive and antioxidant capacities. Furthermore, it has demonstrated low toxicity, gastroprotective and antiulcer activity in different models of acute ulcer induction (acidified ethanol, ethanol, nonsteroidal anti-inflammatory drugs, stress, pylorus ligature and acetic acid) in animals.

Innovations and breakthroughs

This study evaluated, for the first time, the intestinal anti-inflammatory activity promoted by the species *C. duarteanum* Cambess.

Applications

This study validated the popular use of *C. duarteanum* and contributes to the search for new therapies for diseases that affect the GI tract.

Peer-review

The authors demonstrated that *Cd*-EtOHE and *Cd*-HexP obtained from leaves of *C. duarteanum* displays anti-inflammatory effect in trinitrobenzenesulfonic acid colitis model in rats. These results are promising. The vegetal samples may have a role in antioxidant activity and mucosal healing in ulcerative colitis.

REFERENCES

1 **Loftus EV**, Sandborn WJ. Epidemiology of inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 1-20 [PMID: 12122726 DOI: 10.1016/S0889-8553(01)00002-4]
2 **Hendrickson BA**, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev* 2002; **15**: 79-94 [PMID: 11781268 DOI: 10.1128/CMR.15.1.79-94.2002]
3 **Yen EF**, Pardi DS. Non-IBD colitides (eosinophilic, microscopic). *Best Pract Res Clin Gastroenterol* 2012; **26**: 611-622 [PMID: 23384806 DOI: 10.1016/j.bpg.2012.11.012]
4 **Kondamudi PK**, Malayandi R, Eaga C, Aggarwal D. Drugs as causative agents and therapeutic agents in inflammatory bowel disease. *Acta Pharm Sin B* 2013; **3**: 289-296 [DOI: 10.1016/j.apsb.2013.06.004]
5 **Wallace KL**, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 6-21 [PMID: 24415853 DOI: 10.3748/wjg.v20.i1.6]
6 **Langholz E**. Current trends in inflammatory bowel disease: the natural history. *Therap Adv Gastroenterol* 2010; **3**: 77-86 [PMID: 21180592 DOI: 10.1177/1756283X10361304]
7 **Dharmani P**, Chadee K. Biologic therapies against inflammatory bowel disease: a dysregulated immune system and the cross talk with gastrointestinal mucosa hold the key. *Curr Mol Pharmacol* 2008; **1**: 195-212 [PMID: 20021434 DOI: 10.2174/1874467210801030195]
8 **Clayburgh DR**, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 2004; **84**: 282-291 [PMID: 14767487 DOI: 10.1038/labinvest.3700050]
9 **Groschwitz KR**, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009; **124**: 3-20; quiz 21-22 [PMID: 19560575 DOI: 10.1016/j.jaci.2009.05.038]
10 **Neurath MF**. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 2014; **14**: 329-342 [PMID: 24751956 DOI: 10.1038/nri3661]
11 **Awaad AS**, El-Meligy RM, Soliman GA. Natural products in treatment of ulcerative colitis and peptic ulcer. *J Saudi Chem Soc* 2013; **17**: 101-124 [DOI: 10.1016/j.jscs.2012.03.002]
12 **Pagano E**, Capasso R, Piscitelli F, Romano B, Parisi OA, Finizio S, Lauritano A, Marzo VD, Izzo AA, Borrelli F. An Orally Active Cannabis Extract with High Content in Cannabidiol attenuates Chemically-induced Intestinal Inflammation and Hypermotility in the Mouse. *Front Pharmacol* 2016; **7**: 341 [PMID: 27757083 DOI: 10.3389/fphar.2016.00341]
13 **Borrelli F**, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, Battista G, Orlando P, Di Marzo V, Izzo AA. Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. *Br J Pharmacol* 2015; **172**: 142-158 [PMID: 25205418 DOI: 10.1111/bph.12907]
14 **Capasso R**, Orlando P, Pagano E, Aveta T, Buono L, Borrelli F, Di Marzo V, Izzo AA. Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of CB_1 receptors and TRPV1 channels. *Br J Pharmacol* 2014; **171**: 4026-4037 [PMID: 24818658 DOI: 10.1111/bph.12759]
15 **Gouveia MG**, Xavier MA, Barreto AS, Gelain DP, Santos JP, Araújo AA, Silva FA, Quintans JS, Agra MF, Cabral AG, Tavares JF, Silva MS, Quintans-Júnior LJ. Antioxidant, antinociceptive, and anti-inflammatory properties of the ethanolic extract of Combretum duarteanum in rodents. *J Med Food* 2011; **14**: 1389-1396 [PMID: 21663477 DOI: 10.1089/jmf.2010.0212]

16 **de Morais Lima GR**, de Albuquerque Montenegro C, de Sousa Falcão H, de Jesus NZ, Cabral AG, Gomes IF, Agra Mde F, Tavares JF, Batista LM. Gastroprotective activity of the ethanolic extract and hexane phase of Combretum duarteanum Cambess. (Combretaceae). *J Nat Med* 2013; **67**: 492-502 [PMID: 22983657 DOI: 10.1007/s11418-012-0706-3]

17 **Quintans JS**, Costa E V, Tavares JF, Souza TT, Araújo SS, Estevam CS, Barison A, Cabral AG, Silva MS, Serafini MR, Quintans-Júnior LJ. Phytochemical study and antinociceptive effect of the hexanic extract of leaves from Combretum duarteanum and friedelin, a triterpene isolated from the hexanic extract, in orofacial nociceptive protocols. *Rev Bras Farmacogn* 2014; **24**: 60-66 [DOI: 10.1590/0102-695X20142413347]

18 **Guimarães AG**, Oliveira GF, Melo MS, Cavalcanti SC, Antoniolli AR, Bonjardim LR, Silva FA, Santos JP, Rocha RF, Moreira JC, Araújo AA, Gelain DP, Quintans-Júnior LJ. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic Clin Pharmacol Toxicol* 2010; **107**: 949-957 [PMID: 20849525 DOI: 10.1111/j.1742-7843.2010.00609.x]

19 **de Morais Lima GR**. Atividade Gastroprotetora de Combretum duarteanum Cambess (Combretaceae) em modelos animais [Dissertação]: Universidade Federal da Paraíba, 2011; João Pessoa, Paraiba, Brazil. 133 p. Available from: URL: http://tede.biblioteca.ufpb.br/bitstream/tede/6735/1/arquivototal.pdf

20 **Morris GP**, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989; **96**: 795-803 [PMID: 2914642 DOI: 10.1016/S0016-5085(89)80079-4]

21 **Bell CJ**, Gall DG, Wallace JL. Disruption of colonic electrolyte transport in experimental colitis. *Am J Physiol* 1995; **268**: G622-G630 [PMID: 7733288]

22 **Behmer OA**, Tolosa EMC, Freitas-Neto AG. Manual de técnicas para histologia normal e patológica. São Paulo/SP: Editora da Universidade de São Paulo; 1976

23 **Krawisz JE**, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; **87**: 1344-1350 [PMID: 6092199]

24 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051 DOI: 10.1016/0003-2697(76)90527-3]

25 **Calixto JB**, Campos MM, Otuki MF, Santos AR. Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med* 2004; **70**: 93-103 [PMID: 14994184 DOI: 10.1055/s-2004-815483]

26 **Podolsky DK**. The current future understanding of inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2002; **16**: 933-943 [PMID: 12473299 DOI: 10.1053/bega.2002.0354]

27 **Swidsinski A**, Loening-Baucke V, Vaneechoutte M, Doerffel Y. Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. *Inflamm Bowel Dis* 2008; **14**: 147-161 [PMID: 18050295 DOI: 10.1002/ibd.20330]

28 **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521 [PMID: 17332878 DOI: 10.1172/JCI30587]

29 **Sheikh SZ**, Plevy SE. The role of the macrophage in sentinel responses in intestinal immunity. *Curr Opin Gastroenterol* 2010; **26**: 578-582 [PMID: 20717022 DOI: 10.1097/MOG.0b013e32833d4b71]

30 **Bouma G**, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; **3**: 521-533 [PMID: 12876555 DOI: 10.1038/nri1132]

31 **Yang XL**, Guo TK, Wang YH, Gao MT, Qin H, Wu YJ. Therapeutic effect of ginsenoside Rd in rats with TNBS-induced recurrent ulcerative colitis. *Arch Pharm Res* 2012; **35**: 1231-1239 [PMID: 22864746 DOI: 10.1007/s12272-012-0714-6]

32 **Sánchez de Medina F**, Vera B, Gálvez J, Zarzuelo A. Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. *Life Sci* 2002; **70**: 3097-3108 [PMID: 12008093 DOI: 10.1016/S0024-3205(02)01568-0]

33 **Luchini AC**, Rodrigues-Orsi P, Cestari SH, Seito LN, Witaicenis A, Pellizzon CH, Di Stasi LC. Intestinal anti-inflammatory activity of coumarin and 4-hydroxycoumarin in the trinitrobenzenesulphonic acid model of rat colitis. *Biol Pharm Bull* 2008; **31**: 1343-1350 [PMID: 18591772 DOI: 10.1248/bpb.31.1343]

34 **Campos FG**, Waitzberg DL, Teixeira MG, Mucerino DR, Habr-Gama A, Kiss DR. Inflammatory bowel diseases: principles of nutritional therapy. *Rev Hosp Clin Fac Med Sao Paulo* 2014; **57**: 187-198 [PMID: 12244339 DOI: 10.1590/S0041-87812002000400009]

35 **Cho EJ**, Shin JS, Noh YS, Cho YW, Hong SJ, Park JH, Lee JY, Lee JY, Lee KT. Anti-inflammatory effects of methanol extract of Patrinia scabiosaefolia in mice with ulcerative colitis. *J Ethnopharmacol* 2011; **136**: 428-435 [PMID: 20573566 DOI: 10.1016/j.jep.2010.04.047]

36 **Pereira JL**, Hughes LE, Young HL. Spleen size in patients with inflammatory bowel disease. Does it have any clinical significance? *Dis Colon Rectum* 1987; **30**: 403-409 [PMID: 3595356 DOI: 10.1007/BF02556485]

37 **Lima de Albuquerque C**, Comalada M, Camuesco D, Rodríguez-Cabezas ME, Luiz-Ferreira A, Nieto A, Monteiro de Souza Brito AR, Zarzuelo A, Gálvez J. Effect of kale and papaya supplementation in colitis induced by trinitrobenzenesulfonic acid in the rat. *E Spen Eur E J Clin Nutr Metab* 2010; **5**: e111-e116 [DOI: 10.1016/j.eclnm.2009.12.002]

38 **Veljaca M**, Lesch CA, Pllana R, Sanchez B, Chan K, Guglietta A. BPC-15 reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. *J Pharmacol Exp Ther* 1995; **272**: 417-422 [PMID: 7815358]

39 **Alex P**, Zachos NC, Nguyen T, Gonzales L, Chen TE, Conklin LS, Centola M, Li X. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. *Inflamm Bowel Dis* 2009; **15**: 341-352 [PMID: 18942757 DOI: 10.1002/ibd.20753]

40 **Shi XZ**, Winston JH, Sarna SK. Differential immune and genetic responses in rat models of Crohn's colitis and ulcerative colitis. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G41-G51 [PMID: 20947704 DOI: 10.1152/ajpgi.00358.2010]

41 **Zhu MY**, Lu YM, Ou YX, Zhang HZ, Chen WX. Dynamic progress of 2,4,6-trinitrobenzene sulfonic acid induced chronic colitis and fibrosis in rat model. *J Dig Dis* 2012; **13**: 421-429 [PMID: 22788928 DOI: 10.1111/j.1751-2980.2012.00607.x]

42 **Witaicenis A**, Luchini AC, Hiruma-Lima CA, Felisbino SL, Garrido-Mesa N, Utrilla P, Gálvez J, Di Stasi LC. Suppression of TNBS-induced colitis in rats by 4-methylesculetin, a natural coumarin: comparison with prednisolone and sulphasalazine. *Chem Biol Interact* 2012; **195**: 76-85 [PMID: 22119283 DOI: 10.1016/j.cbi.2011.11.004]

43 **Ogryzko NV**, Renshaw SA, Wilson HL. The IL-1 family in fish: swimming through the muddy waters of inflammasome evolution. *Dev Comp Immunol* 2014; **46**: 53-62 [PMID: 24690566 DOI: 10.1016/j.dci.2014.03.008]

44 **Li P**, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell* 1995; **80**: 401-411 [PMID: 7859282 DOI: 10.1016/0092-8674(95)90490-5]

45 **Jones-Hall YL**, Grisham MB. Immunopathological characterization of selected mouse models of inflammatory bowel disease: Comparison to human disease. *Pathophysiology* 2014; **21**: 267-288 [PMID: 24935242 DOI: 10.1016/j.pathophys.2014.05.002]

46 **Schreiber S**, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* 1995; **108**: 1434-1444 [PMID: 7729636 DOI: 10.1016/0016-5085(95)90692-4]

47 **Arab HH**, Salama SA, Eid AH, Omar HA, Arafa el-SA, Maghrabi IA. Camel's milk ameliorates TNBS-induced colitis in rats via

downregulation of inflammatory cytokines and oxidative stress. *Food Chem Toxicol* 2014; **69**: 294-302 [PMID: 24788059 DOI: 10.1016/j.fct.2014.04.032]

48 **Mahadevan U**, Loftus EV, Tremaine WJ, Sandborn WJ. Safety of selective cyclooxygenase-2 inhibitors in inflammatory bowel disease. *Am J Gastroenterol* 2002; **97**: 910-914 [PMID: 12008668 DOI: 10.1111/j.1572-0241.2002.05608.x]

49 **Prelich G**, Stillman B. Coordinated leading and lagging strand synthesis during SV40 DNA replication in vitro requires PCNA. *Cell* 1988; **53**: 117-126 [PMID: 2894900 DOI: 10.1016/0092-8674(88)90493-X]

50 **Fukui T**, Takeda H, Shu HJ, Ishihama K, Otake S, Suzuki Y, Nishise S, Ito N, Sato T, Togashi H, Kawata S. Investigation of Musashi-1 expressing cells in the murine model of dextran sodium sulfate-induced colitis. *Dig Dis Sci* 2006; **51**: 1260-1268 [PMID: 16944023 DOI: 10.1007/s10620-006-8046-3]

51 **Sánchez-Fidalgo S**, Cárdeno A, Sánchez-Hidalgo M, Aparicio-Soto M, de la Lastra CA. Dietary extra virgin olive oil polyphenols supplementation modulates DSS-induced chronic colitis in mice. *J Nutr Biochem* 2013; **24**: 1401-1413 [PMID: 23337347 DOI: 10.1016/j.jnutbio.2012.11.008]

52 **Kannan N**, Guruvayoorappan C. Protective effect of Bauhinia tomentosa on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. *Int Immunopharmacol* 2013; **16**: 57-66 [PMID: 23538025 DOI: 10.1016/j.intimp.2013.03.008]

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