

ORIGINAL ARTICLE

Alternanthera brasiliana (L.) Kuntze decreases pain and inflammation in acute colitis model without hepatotoxicity

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ABSTRACT

Objective: To investigate antinociceptive and anti-inflammatory activities of the keto-alcoholic extract of *A. brasiliana* leaves in a mice model of acute colitis. **Methods:** The experiments were performed in Swiss mice; the keto-alcoholic extract of *A. brasiliana* leaves was prepared and flavonoids were quantified. The antinociceptive/analgesic activity was evaluated by the writhing test. The keto-alcoholic extract of *A. brasiliana*, saline and Dipyrone monohydrate were tested. Acute colitis was induced by 7.5% (v/v) acetic acid intrarectally and the animals were treated, by gavage, with keto-alcoholic extract of *A. brasiliana*, saline and Mesalazine. After euthanasia, intestinal segments of the mice were analyzed by macroscopic index and quantification of colonic edema. The mechanical hyperalgesia test was performed after induction of acute colitis with acetic acid and treatment of the mice using the electronic Von Frey test. Hepatotoxicity of the keto-alcoholic extract of *A. brasiliana* was tested by plasma dosage of the aspartate transaminase (AST) and alanine transaminase (ALT) enzymes. Data were analyzed by ANOVA followed by Tukey post-test considering $P < 0.05$ as significant. **Results:** *In vivo* results indicate that mice treated with the keto-alcoholic extract of *A. brasiliana* leaves at 100 and 300 mg/kg (0.872 and 2.616 mg total flavonoids/kg) showed a significant decrease in the number of writhing compared to the negative control. Between the two concentrations that were shown to be effective, no significant difference was observed. The Von Frey test points that mice treated with *A. brasiliana* extract at 30 (0.2616 mg of total flavonoids/kg), 100 and 300 mg/kg showed greater hyperalgesia. Regarding colitis, the mice treated with keto-alcoholic extract of *A. brasiliana* leaves at 10, 30, 100 and 300 mg/kg showed no lesions or lower-grade lesions when compared to the untreated group. In the quantification of edema no significant difference was observed among any experimental group. Plasma AST and ALT values were within normal reference values indicating no liver damage by the extract. **Conclusion:** The keto-alcoholic extract of *A. brasiliana* shows antinociceptive/analgesic and anti-inflammatory activity in an acute colitis model in mice without causing hepatotoxicity in the mice.

Key words: *Alternanthera brasiliana*, anti-inflammatory activity, antinociceptive activity, inflammatory bowel diseases, hepatotoxicity, medicinal plants

INTRODUCTION


In developing countries, a considerable part of the population uses folk medicine for the treatment of

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diseases, so medicinal plants stand out as an important part of the culture.^[1] Pharmaceutical industries and researchers have obtained phytotherapeutic agents from plants whose use has been proven safe and efficient for the purpose applied, through clinical and scientific studies.^[2] Therefore, medicinal plants are potential sources for the development of new therapies.

Inflammatory bowel diseases (IBD) are characterized by chronic and recurrent inflammation and their incidence has increased in the last decades, and the available therapeutic protocols try to reduce the symptomatology, since, so far, there is no cure for these pathologies.^[3] In Brazil, 46546 hospital admissions due to IBD were recorded between 2009 and 2019.^[4]

Epidemiological data point out that children, adolescents, and young adults are the most affected by IBD, with a great impact on the quality of life of sufferers.^[5] The impact is very much related to the symptomatology of these diseases. The ulcerative rectocolitis (UR) presents with bloody diarrhea, which may be associated with abdominal pain, colic, mucopurulent exudate in the stool, tenesmus, and evacuation urgency. In Crohn's disease (CD) the most common clinical symptom is abdominal pain, diarrhea, and intestinal obstructive symptoms formation of fistulas.^[6]

The recommended treatments for IBD are self-care and drug treatments.^[7] Drug treatment is performed by administering corticosteroids, 5-aminosalicylic acid, immunomodulators, and biologic drugs,^[5] which have several adverse effects,^[8] significantly affecting patients. The complexity in the treatment of IBD has increased the search for new and effective treatments. In this sense, the use of phytotherapy shows itself as an interventional therapeutic possibility.^[9]

Alternanthera brasiliana (L.) Kuntze (Amaranthaceae) is a plant native to subtropical and tropical regions of South America and Australia is commonly known as penicilina, perpétua-do-mato and perpétua-do-Brasil. This plant has intensely colored leaves and stems, for this reason, it is widely used as a natural dye, and is used as an analgesic and for the treatment of inflammation, cough and diarrhea.^[10]

Antibacterial, antioxidant, photoprotective, healing and antiproliferative properties have already been reported for *A. brasiliana*,^[11] with these activities attributed to compounds such as flavonoids, which are widely distributed in the plant kingdom.^[12]

Flavonoids, especially kaempferol, are found in abundance in *A. brasiliana*; this flavonoid has been attributed anti-inflammatory, antioxidant, antimicrobial, anticancer, cardioprotective, neuroprotective,

antidiabetic, anti-osteoporotic, anxiolytic, analgesic, and antiallergic activities.^[10] Other compounds described are saponins, with as immunostimulant, antineoplastic, cholesterol-reducing, and anti-inflammatory properties.^[12] Other bioactive found in *A. brasiliana* are betacyanin, triterpenoids, sitosterol, which together with other compounds act in the elimination of free radicals.^[13] This study aimed to analyze the anti-inflammatory and the antinociceptive potential of the keto-alcoholic extract of *A. brasiliana* leaves in the improvement of the symptomatology of acute inflammatory bowel disease induced in mice.

MATERIALS AND METHODS

Animals

Ethical clearance was obtained from the Ethics Committee for Animal Use of the Setor de Ciências Biológicas - UFPR/PR/Brazil (Protocol R.O. 04/2022). Male swiss mice weighing 25 to 30 g were housed in polypropylene boxes (41 cm × 33 cm × 16 cm) with free access to standard pellet food (commercial pelleted food specific for murines, Nuvilab CR) and chlorinated drinking water at a controlled temperature (22°C ± 2.0°C), in a 12-hour light/dark cycle.

Keto-alcoholic extract of *A. brasiliana* preparation and total flavonoids quantification

A. brasiliana was collected in Toledo/Paraná/Brazil, coordinates 24° 43' 12" S, 53° 44' 36" W, in July 2022. Specimens were analyzed by a biologist from the Universidade Federal do Paraná. The material was processed according to the previous protocol, with slight modifications. Leaves of *A. brasiliana* were sanitized, disinfected, washed and dried at 40°C for 72 hours, and then ground.^[14]

The ground material was mixed in a 1:1 Acetone/Methanol solution in a 1:10 plant/solution ratio, sonicated (5 cycles—5' on/5' off), incubated at room temperature for 24 hours, filtered, and evaporated. The entire extraction process was carried out in the dark. For the *in vivo* experiments, the extracts were solubilized in dimethyl sulfoxide (DMSO) 2% (v/v) in saline (NaCl 0.9% (w/v)). Quantification of total flavonoids in the keto alcoholic extract was performed by colorimetric method, using 5% (w/v) AlCl₃ methanolic solution and absorbance reading at 425 nm. Quercetin was used as the standard and the results were expressed as quercetin equivalents.^[15]

Antinociceptive test

The antinociceptive activity of the keto-alcoholic extract of *A. brasiliana* was evaluated by the writhing test.^[16] Nociception was induced by intraperitoneal adminis-

tration of acetic acid (0.8% (v/v) in saline (NaCl 0.9% (m/v)) (10 mL/kg). Groups of mice ($n = 8$) were treated with the keto-alcoholic extract of *A. brasiliana* at 10, 30, 100 and 300 mg/kg, saline (negative control), Dipyrone monohydrate 100 mg/kg (positive control) and DMSO 2% (v/v) (vehicle control) by gavage, and 30 minutes later acetic acid was administered. Individually, the mice were housed in glass cylinders and the number of writhing performed in a period of time from 0 to 20 minutes was quantified. The intensity of the twitch response was expressed by the cumulative number of twitches performed in a total time period of 20 minutes.^[17,18]

Induction of experimental acute colitis

The duration of the experiment was 14 hours. After 24 hours of solid fasting the mice ($n = 8$) were anesthetized with Isoflurane (1.5% diluted in 100% oxygen). In hours 0 and 6 of the experiment, mice were pre-treated with the keto-alcoholic extract of *A. brasiliana* (10, 30, 100 and 300 mg/kg), Mesalazine 200 mg/kg (positive control), saline (negative control) and a group of animals in which there was no colitis induction or treatment was used. At hour 9, a saline enema was performed on the mice. At time 10, acute experimental colitis was induced with 7.5% (v/v) acetic acid intrarectally. The mice were kept upside down for 3 minutes in order to avoid extravasation of fluid. The intracolonic injection was performed with a 3 cm long polyethylene cannula.^[19] At hour 12 the treatment was performed under the same conditions as the pre-treatment. One hour after treatment, the animals were subjected to the nociception test induced by mechanical hyperstimulation (Von Frey test). At hour 14 the mice were euthanized by an overdose of Isoflurane, followed by cervical dislocation. The distal colon segment of the animals was collected and washed with saline for removal of fecal remains and subsequent macroscopic evaluation of the degree of lesions according to a score index (from 0 to 6)^[20] and for verification of edema.^[19,21] To infer the presence of edema a 2 cm colonic segment was weighed on a precision digital scale.

Visceral mechanical hyperalgesia

Visceral mechanical hyperalgesia was assessed using the electronic Von Frey test.^[13] Mice were allocated to boxes in a temperature-controlled room for at least 45 minutes before the start of measurements. The test consisted of eliciting a withdrawal response from the animal with a portable force transducer (Digital Analgesimeter EFF 310 - Insight Ltda, SP/Brazil) fitted to a 0.7 mm² polypropylene tip, which was applied to the lower abdomen up to the mid-abdomen area. After removal, the pressure intensity was recorded automatically, with values from the average of three measurements. The results were expressed as the delta (Δ) of the withdrawal

threshold (in grams), calculated by subtracting the average of the test values taken one day before the experiment and 3 hours after the induction of acute colitis.

Hepatotoxicity analysis

At euthanasia of the mice in the colitis experiment blood was collected by cardiac puncture for plasma quantification of alanine transaminase (ALT) and aspartate transaminase (AST) to verify whether the keto-alcoholic extract of *A. brasiliana* at the doses tested induced acute hepatotoxicity. The enzymatic activity of AST and ALT was quantified using commercial AST/TGO Liquiform (Cat. #109-4/30) and ALT/TGP Liquiform (Cat. #108-4/30) tests (Labtest Diagnóstica S.A./Minas Gerais/Brazil), respectively. All assays were performed in duplicate and the manufacturer's recommendations were followed. Samples were analyzed by UV/Vis Multiskan Sky spectrometer (Thermo Fisher Scientific Inc.)

Statistical analysis

The means of the values obtained for each group of animals, in each experiment, were calculated and analyzed with the help of the Prism 3.0 One-Way analysis of variance (ANOVA) program, which was used to compare the groups in each treatment dosage, in order to distinguish dose-effect, followed by Tukey's test. Results with $P < 0.05$ were considered significant.

RESULTS

Total flavonoids

Total flavonoids were detected $8.72\% \pm 0.0077$ quercetin equivalents/g dry mass of *A. brasiliana* leaves by keto-alcoholic extraction, or 8.72 ± 0.0077 mg total flavonoids/g dry mass. Therefore, the tested extract doses were 0.0872 (10), 0.2616 (30), 0.872 (100) and 2.616 (300) mg of total flavonoids/kg.

Acetic acid-induced writhing test

No significant difference in the mean number of writhing was observed between the negative control group and the group treated with DMSO 2% (v/v). The *A. brasiliana* extract at 100 and 300 mg/kg significantly reduced the number of writhing compared to the negative control group, 48% and 40%, respectively. In the mice treated with Dipyrone, a reduction of 83% in the number of writhing was observed; this value was significant in relation to the negative control and the groups treated with *A. brasiliana* extract at 100 and 300 mg/kg (Figure 1A). Although there was no statistical difference in the antinociceptive activity between *A. brasiliana* extract at 100 and 300 mg/kg, clinically the extract at 100 mg/kg proved to be better. Despite Dipyrone showing better results in relation to the *A. brasiliana* extract, when compared with the extract at 100

mg/kg no statistical difference in dose-dependent activity was observed (Figure 1B).

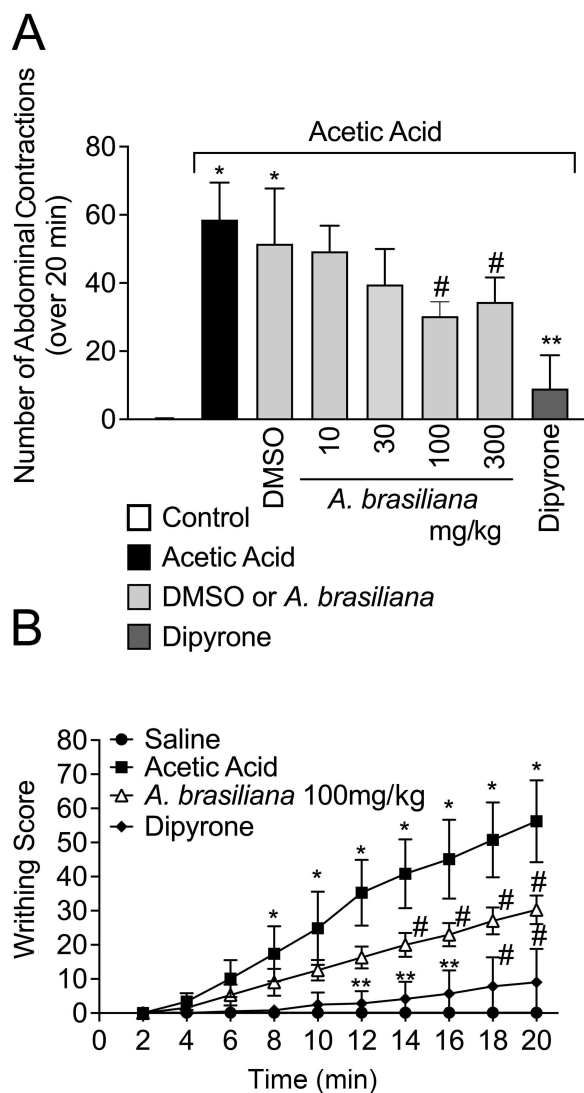


Figure 1. The antinociceptive activity of keto-alcoholic extracts of *A. brasiliiana* leaves in the writhing test. **A.** Comparison among the experimental groups. **B.** Time-dependent activity of the keto-alcoholic extract of *A. brasiliiana* leaves at 100 mg/kg. Results were presented \pm representative error of 8 animals per group. * $P < 0.05$ compared to the saline group. # $P < 0.05$ compared to DMSO 2% (v/v) and acetic acid groups. ** $P < 0.05$ compared to all groups except the saline group. One-way ANOVA followed by Tukey's t test.

Acetic acid-induced acute colitis

In the induced colitis experiment, the keto-alcoholic extract of *A. brasiliiana* leaves at 100 mg/kg showed a moderately lower macroscopic index when compared to the other dosages of the extract; most of the mice presented score of 0 (normal colon) after the induction of acute colitis (Figure 2B). Both positive control groups, treated with Mesalazine 200 mg/kg, and treated with keto-alcoholic extract of *A. brasiliiana* at 300 mg/kg showed no significant difference in their results, since

half of the animals presented score of 0 and a half score of 1 (localized hyperemia, without ulcers). The other groups of mice (*A. brasiliiana* extract at 10 and 30 mg/kg) did not show a significant results in reducing macroscopic damage generated by colitis. In the negative control group of the experiment (without colitis induction) all mice presented a score of 0. No significant difference was observed among the experimental groups regarding the reduction of colonic edema, although clinically, the extract of *A. brasiliiana* was more efficient than Mesalazine (Figure 2A).

Von Frey test

A significant decrease in nociceptive sensitivity was observed in mice treated with the keto-alcoholic extract of *A. brasiliiana* at concentrations of 30 (36%), 100 (84%) and 300 (70%) mg/kg, and with Mesalazine (80%), compared to the negative control group. The *A. brasiliiana* extract at 100 and 300 mg/kg, and Mesalazine also showed greater antinociceptive activity than the *A. brasiliiana* extract at 30 mg/kg. No significant difference was observed among the three treatments (Figure 3).

Acute hepatotoxicity

The values of both plasma AST and ALT of the mice treated with the keto-alcoholic extract of *A. brasiliiana* were higher compared to the control group (untreated) (Table 1). However, all values were below normal reference values (no liver damage), including the ALT of mice treated with 300 mg/kg of the extract, which showed a much higher value (235.27 ± 0.28 U/L) than the other experimental groups.

DISCUSSION

In this study, it was observed that, by keto-alcoholic extraction, 8.72 ± 0.0077 mg of total flavonoids per gram of dry mass from the leaves of *A. brasiliiana* were acquired, while by acidified aqueous extraction only $35.243 \mu\text{g/g}$ (0.035243 mg/g) of polyphenols were obtained from the leaves of the plant, among the polyphenols flavonoids were more abundant.^[10] This demonstrates the effectiveness of the extraction methodology used in this study, a relevant point, since flavonoids are *bioactive* with diversified activity, being able to act on the immune system, against inflammation, due to their pharmacological potential.^[12]

In humans, the sensitization of some nociceptors leads to hyperalgesia, a clinical condition that is defined by the exacerbated response to a painful stimulus. This response occurs through the sensitization of primary afferent neurons, which are induced by the action of final inflammatory mediators; these act by directly sensitizing the peripheral neurons responsible for nociception.^[22] Pain covers several mechanisms, being

Table 1: Aspartate transaminase and alanine transaminase plasma values of mice treated with the keto-alcoholic extract of *A. brasiliensis* leaves

	Dosage (mg/kg)				
	Negative control	10	30	100	300
AST (U/L)	21.48 ± 0.16*	93.21 ± 0.72	62.83 ± 0.12	27.91 ± 0.28	42.87 ± 0.04
ALT (U/L)	29.11 ± 0.24	51.90 ± 0.06	68.14 ± 0.36	42.78 ± 0.10	235.27 ± 0.28

*Standard deviation

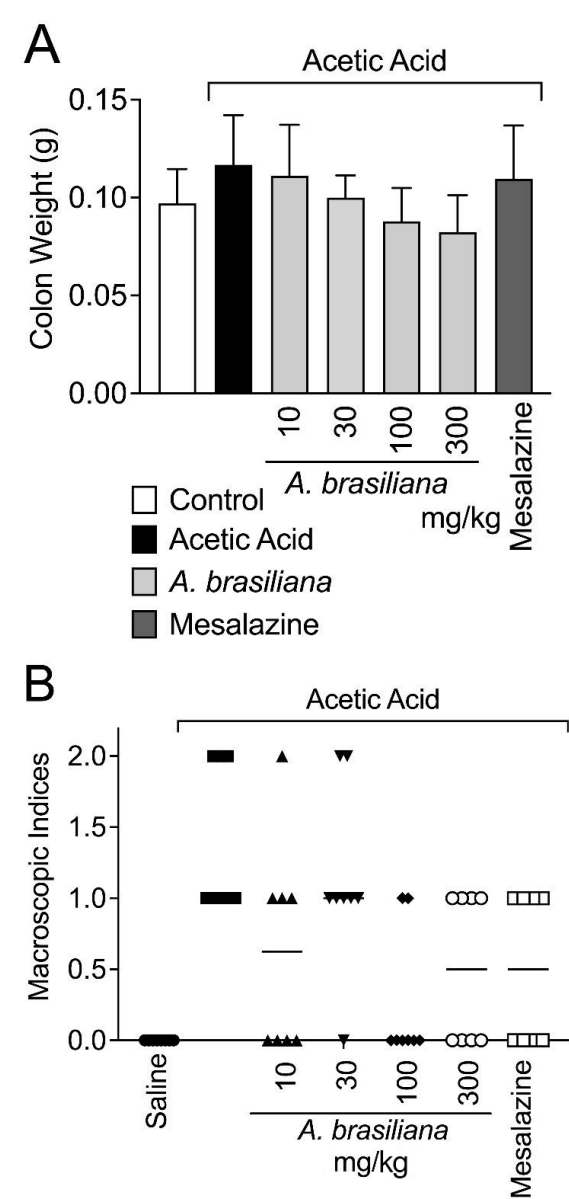


Figure 2. Anti-inflammatory activity of the keto-alcoholic extract of *A. brasiliensis* leaves on acute colitis. **A.** Colonic edema obtained by the measurement of colon segment weight, there was no difference between the groups. Results were presented ± representative error of 8 animals per group. One-way ANOVA followed by Tukey's *t* test. **B.** Macroscopic indices of colonic segments from mice with induced colitis post-treatment. Scores: 0 = no lesion; 1 = hyperemia without ulceration; 2 = ulceration without hypertrophy of the intestinal wall, according to Wallace *et al.*^[20].

considered multidimensional, with various origins such as psychosocial, behavioral and pathophysiological. This makes pain an essential factor in the development of morbidities or as a symptom associated with these morbidities, which can consequently lead to a temporary or permanent disability of the individual.^[23] In mice, the inflammatory stimulus induces pain sensitivity, which is expressed by behaviors of marked retraction of the abdomen, licking or scratching immediately, jumping and recoiling.^[13]

The writhing test is an experiment with sensitivity for screening the analgesic potential of bioactive compounds, and the nociception produced in this type of model occurs due to the sensitization of central and peripheral nociceptors, through inflammatory mediators of pain.^[24] When suffering some kind of tissue injury, leading to a noxious stimulus, the tissues and cells release chemical mediators that are able to stimulate the C-type neuronal fibers, causing local pain. Acetic acid can trigger this mechanism by activating macrophages and basophils from the abdominal cavity, which release pro-inflammatory cytokines, such as IL-1 β , TNF- α and IL-8.^[25] This study demonstrated that flavonoids of *A. brasiliensis* have a significant action in inhibiting this type of painful stimulus. More expressively, it was noted that the keto-alcoholic extract at 100 mg/kg showed considerable results in reducing the number of writhing in mice.

Dipyrone is considered a pharmacological compound with strong analgesic, antipyretic, and spasmolytic properties.^[26] Therefore, it can be inferred in the current study that these effects were demonstrated in a relevant way to decrease the response to the painful stimulus in mice.

The study evaluated the analgesic and anti-inflammatory activity of the aqueous extract of *A. brasiliensis*^[12], which induced a significant reduction in the number of writhing in all doses tested in the study, when compared to the negative control group; the dose of 400 mg/kg showed the greatest reduction (96.55%). Comparatively, in this study, lower doses of *A. brasiliensis* extract induced a significant inhibition—48% (100 mg/kg) and 40% (300 mg/kg) of writhing in mice.

It is relevant to point out that none of the doses of the keto-alcoholic extract of *A. brasiliana* tested in this study caused liver damage to the mice, ruling out acute toxicity. In fact the reference values for AST and ALT in Swiss mice are 60.00 to 395.00 U/L and 20.00 to 335.00 U/L, respectively.^[27] Different values were reported in another study^[28] 44.5 to 394.5 U/L for AST and 27.5 to 162.0 U/L for ALT. Such variations in animals of the same species and strain are due to environmental and animal management differences. In addition, no behavioral signs indicative of intoxication were observed in the mice, such as prostration, apathy, and raised hairs.^[29]

Nociception was also evaluated by the visceral mechanical hyperstimulus test, which aims to provoke a withdrawal response from the animal when faced with a painful induction that occurs due to abdominal compression.^[13] It was noted that the groups of mice treated with *A. brasiliana* extract at 100 and 300/kg showed a significant results in reducing pain sensitivity, however, no significant difference was observed between the two doses when compared to the positive control treated with Mesalazine 200 mg/kg.

Conventional treatment for IBD consists of several classes of drugs, such as corticoids, 5-aminosalicylic acid, immunomodulators, and biological drugs,^[5] which present a number of adverse effects.^[8] In this sense, the use of medicinal plants, their parts or extracts, has been implemented for decades as alternative medicine.^[30] In this study, it was possible to demonstrate the possibility of using medicinal plants, specifically the keto-alcoholic extract of *A. brasiliana*, for the treatment of experimental acute colitis in murine. This plant was chosen due to their antibacterial, antioxidant, photoprotective, healing and antiproliferative biological active properties.^[11] The promising results of the preclinical tests indicates the possibility of extrapolation to studies for use in humans.

The experimental induction of ulcerative colitis by the administration of intrarectal acetic acid is considered a widely used method in order to mimic the pathophysiology and progression of the disease.^[31] Chemical agents, such as acetic acid, have the ability to reproduce colitis diffusely, mainly in the distal portion of the colon, and this induction is dose-dependent, as demonstrated in the present study.^[21]

Inflammation is characterized as a physiological process, being a response to stimuli such as infections, physico-chemical and antigenic changes, or occurrence of traumatic injuries, and may be present in various dysfunctions and pathologies,^[32] being that the key to the resolving event of acute inflammation is the decrease in the presence of neutrophils in inflamed sites, because these are the cells of initial migration in large numbers,

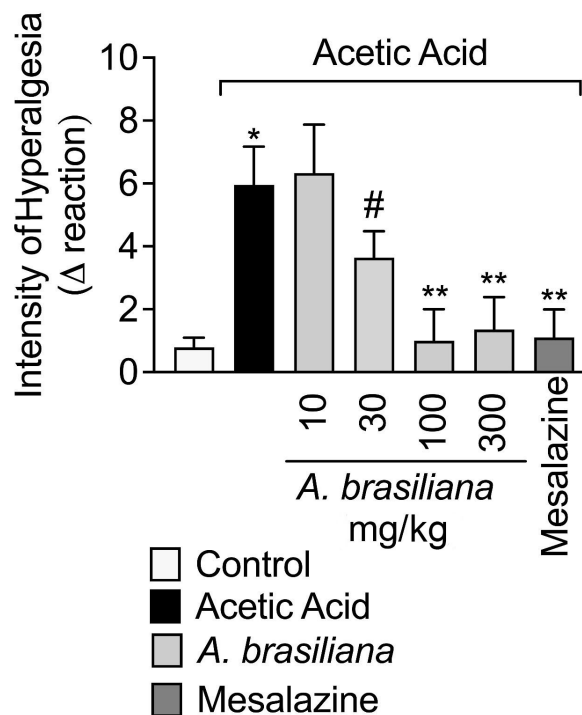


Figure 3. Inhibition of hyperalgesia by the keto-alcoholic extract of *A. brasiliana* (doses) leaves in acute colitis model using Von Frey. Results were presented \pm representative error of 8 animals per group. * $P < 0.05$ compared to the saline group. # $P < 0.05$ compared to the acetic acid group. ** $P < 0.05$ compared to all groups except the saline group. One-way ANOVA followed by Tukey's t test.

which leads to loss of tissue homeostasis, triggering the inflammatory process.^[33] In view of these facts, through this study, it can be demonstrated that the keto-alcoholic extract of *A. brasiliana*, was able to reduce damage to the intestinal mucosa, which indicate the presence of local inflammation, in an expressive manner, especially at concentrations of 100 and 300 mg/kg.

Edema can be used as a marker of the presence of inflammation in colitis induction models in mice.^[21] In the current study, there was no expressive distinction in the quantification of edema among the groups of mice in the experiment.

Mesalazine or 5-aminosalicylic acid is the most commonly prescribed drug for IBD treatment.^[3] In this study, mice treated with Mesalazine (positive control) showed higher rates of edema when compared to the groups treated with keto-alcoholic extract of *A. brasiliana*. The low efficiency of this drug may be attributed to the fact that its use is assigned for the treatment of chronic inflammatory bowel disease, as established by the Brazilian guidelines,^[6,34] different from the acute model used in this study.

In conclusion, the data presented here suggest that *A.*

brasiliensis keto-alcoholic extract inhibits hyperalgesia and inflammatory effects acetic-acid induced in acute-colitis model. This effect is probably mediated by flavonoids content on extract that allows analgesic and antinociceptive activity on overt-pain like behavior and colitis model, and no behavioral signs indicative of intoxication were observed in the mice. These data show for the first time that *A. brasiliensis* could be used in inflammatory pain, therefore, suggesting that this drug is a promising therapeutic approach as an analgesic and anti-inflammatory. Further investigation in other medical models will be required to elucidated the action mechanism and the mediators involved.

DECLARATIONS

Author contributions

Jennefer S Luz, Luiz HC Piovezani, Cleison F Silva, Gabrielle C Peiter, Edson L Michalkiewicz Junior, Matheus N Tsutumi, Augusto F Chaves, Raphael H Chappuis, Rafael M Luiz and Cinthia F Wendel performed the experiments; Jennefer S Luz analyzed the results and wrote the manuscript; Ana C Zarpelon-Schutz and Kádima N Teixeira performed the critical analysis of data, corrected the manuscript and coordinated the study; all authors approved the final version of the manuscript.

Conflicts of interest

All authors declare no potential conflicts of interest.

REFERENCES

- de Moraes Mello Boccolini P, Siqueira Boccolini C. Prevalence of complementary and alternative medicine (CAM) use in Brazil. *BMC Complement Med Ther*. 2020;20:51.
- Calixto JB. The role of natural products in modern drug discovery. *An Acad Bras Cienc*. 2019;91 Suppl 3:e20190105.
- Veloso PM, Machado R, Nobre C. Mesalazine and inflammatory bowel disease - From well-established therapies to progress beyond the state of the art. *Eur J Pharm Biopharm*. 2021;167:89–103.
- Brito RCV, Peres CL, Silveira KAF, Arruda EL, Júnior MPA. Doenças inflamatórias intestinais no Brasil: perfil das internações, entre os anos de 2009 a 2019. *Revista Educação em Saúde*. 2020;8:127–135. DOI: 10.29237/2358.
- Wright EK, Ding NS, Niewiadomski O. Management of inflammatory bowel disease. *Med J Aust*. 2018;209:318–323.
- Brasil Secretaria de assistência à saúde/Ministério da saúde. Protocolo clínico e diretrizes terapêuticas para o tratamento de doença de Crohn. Portaria 858, 04 de novembro. DOU, Brasília, n. 214, seção I, p. 77-82, 2002a. Available from: <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=05/11/2002&jornal=1&pagina=77&totalArquivos=116>
- Seyedian SS, Nokhostin F, Malami MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *J Med Life*. 2019; 12:113–122.
- Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet*. 2007;369:1641–1657.
- Akram M, Hamid A, Khalil A, Ghaffar A, Tayyaba N, Saeed A, Ali M, Naveed A. Review on medicinal uses, pharmacological, phytochemistry and immunomodulatory activity of plants. *Int J Immunopathol Pharmacol*. 2014; 27:313–319.
- Deladino L, Alvarez I, De Ancos B, Sánchez-Moreno C, Molina-García AD, Schneider Teixeira A. Betalains and phenolic compounds of leaves and stems of *Alternanthera brasiliana* and *Alternanthera tenella*. *Food Res Int*. 2017; 97:240–249.
- de Alencar Filho JMT, Teixeira HAP, Sampaio PA, Pereira ECV, Amariz IAE, Rolim Neto PJ, Rolim LA, Araújo ECDC. Phytochemical analysis in *Alternanthera brasiliana* by LC-MS/MS and GC-MS. *Nat Prod Res*. 2020;34:429–433.
- Formagio EL, Mendel MT, Fracasso R, Knobloch JG, Teixeira PW, Kehl L, Maluf RW, Picoli SU, Ardenghi P, Suyenaga ES. Evaluation of the pharmacological activity of the *Alternanthera brasiliana* aqueous extract. *Pharm Biol*. 2012;50:1442–1447.
- Pereira DF, Zanon RB, Dos Santos M, Boligon AA, Athayde ML. Antioxidant activities and triterpenoids isolated from *Alternanthera brasiliana* (L.) Kuntze leaves. *Nat Prod Res*. 2013;27:1660–1663.
- Albuquerque ER, Gossler SC, Piovezani M, Conto LC. Extração e Determinação de Flavonóides, Taninos Condensados e Totais da casca do pinhão (*Araucaria angustifolia*). Material didático - Instituto Federal de Santa Catarina/SC/Brasil 2017. In: Material didático - IFSC [Internet] Available from: <http://docente.ifsc.edu.br/michael.nunes/MaterialDidatico/Analises%20Quimicas/TCC%2011/TCC%202017%201/Eduarda%20e%20Sheili.pdf>
- Dutra RP, Nogueira AMC, Marques RRO, Costa MCP, Ribeiro MNS. Avaliação farmacognóstica de geoprópolis de *Melipona fasciculata* Smith (túbia) em municípios da Baixada maranhense, Brasil. *Rev Bras Farmacogn*. 2008;18:557–562.
- Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother*. 1968;32:295–310.
- Valério DA, Cunha TM, Arakawa NS, Lemos HP, Da Costa FB, Parada CA, Ferreira SH, Cunha FQ, Verri WA Jr. Anti-inflammatory and analgesic effects of the sesquiterpene lactone budlein A in mice: inhibition of cytokine production-dependent mechanism. *Eur J Pharmacol*. 2007;562:155–163.
- Verri WA Jr, Cunha TM, Magro DA, Domingues AC, Vieira SM, Souza GR, Liew FY, Ferreira SH, Cunha FQ. Role of IL-18 in overt pain-like behaviour in mice. *Eur J Pharmacol*. 2008;588:207–212.
- Guazelli CF, Fattori V, Colombo BB, Georgetti SR, Vicentini FT, Casagrande R, Barakat MM, Verri WA Jr. Quercetin-loaded microcapsules ameliorate experimental colitis in mice by anti-inflammatory and antioxidant mechanisms. *J Nat Prod*. 2013;76:200–208.
- Wallace JL, MacNaughton WK, Morris GP, Beck PL. Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease. *Gastroenterology*. 1989;96:29–36.
- Colombo BB. A vimpocetina reduz a colite induzida por ácido acético em camundongos. M.Sc. Thesis, Universidade Estadual de Londrina 2017. Available from: <http://www.bibliotecadigital.uel.br/document/?view=vtls000211231>
- Júnior JOO, Junior CSAP, Cohen CP. Inflammatory mediators of neuropathic pain. *Revista Dor*. 2016;17 suppl 1:35–42.
- Raffaelli W, Arnaudo E. Pain as a disease: an overview. *J Pain Res*. 2017;10:2003–2008.
- Dai G, Li B, Xu Y, Li Z, Mo F, Wei C. Synergistic interaction between matrine and paracetamol in the acetic acid writhing test in mice. *Eur J Pharmacol*. 2021;895:173869.
- Guimarães HA, Nascimento MVM, Tavares A, Galdino PM, DePaula JR, Costa EA. Effects of ethanolic extract of *Lafroensia pacari* A. St.-Hil., Lythraceae (pacari), in pain and inflammation model. *Braz J Pharmacogn*. 2010;20:328–333.
- Lampl C, Likar R. [Metamizole (dipyrone): mode of action, drug-drug interactions, and risk of agranulocytosis]. *Schmerz*. 2014;28:584–590.

27. Lima FEG. Perfil bioquímico e hematológico de ratos e camundongos do biotério central da Universidade Federal do Ceará. M.Sc. Thesis, Universidade Federal do Ceará. 2018. Available from: <http://www.repositorio.ufc.br/handle/riufc/32198>
28. Supramaniam A, Bielefeldt-Ohmann H, Rudd PA, Webster J, Ferro V, Herrero LJ. PG545 treatment reduces RRV-induced elevations of AST, ALT with secondary lymphoid organ alterations in C57BL/6 mice. *PLoS One*. 2019;14:e0217998.
29. Lima CS. Estudo da toxicidade não clínico em ratos submetidos ao tratamento com óleos de Copaíba (subcrônico e reprodutivo). D.Sc. Thesis, Universidade Federal do Amapá. 2014. Available from: https://www2.unifap.br/ppgbio/files/2012/02/TESE-COM-FICHA-CATALOGRAFICA_CLARISSA.pdf
30. Azab A, Nassar A, Azab AN. Anti-Inflammatory Activity of Natural Products. *Molecules*. 2016;21.
31. Ahmedy OA, Ibrahim SM, Salem HH, Kandil EA. Antiulcerogenic effect of melittin via mitigating TLR4/TRAF6 mediated NF- κ B and p38MAPK pathways in acetic acid-induced ulcerative colitis in mice. *Chem Biol Interact*. 2020;331:109276.
32. Chamusca FV, Reis SRA, Lemaire D, Medrado AP. Mediators of the systemic effects of inflammation and photobiomodulatory therapies: a literature review. *Rev. Ci Med Biol*. 2012;11:70–78.
33. Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM. Resolution of Inflammation: What Controls Its Onset? *Front Immunol*. 2016;7:160.
34. Brasil Secretaria de assistência à saúde/Ministério da saúde. Protocolo clínico e diretrizes terapêuticas para o tratamento de retocolite ulcerativa. Portaria 861, 04 de novembro. DOU, Brasília, n. 214, seção I, p. 87-98, 2002b. Available from: <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=05/11/2002&jornal=1&pagina=87&totalArquivos=116>