**ORIGINAL ARTICLE**

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

Jennerf S. Luz¹, Gabrielle C. Peiter², Luiz H. C. Piovezani³, Cleison F. Silva¹, Matheus N. Tsutumi¹, Augusto F. Chaves¹, Edson L. Michalkiewicz Jr.¹, Raphael H. Chappuis¹, Rafael M. Luiz¹, Cinthia F. Wendel¹, Ana C. Zarpelon-Schutz¹,², Kadima N. Teixeira¹,²,³

¹Universidade Federal do Paraná - Campus Toledo, Toledo, Paraná 85.919-899, Brazil
²Universidade Federal do Paraná - Setor Palotina, Programa Multicêntrico de Pós-graduação em Bioquímica e Biologia Molecular, Palotina, Paraná 85.950-000, Brazil
³Universidade Federal do Paraná - Setor Palotina, Programa de Pós-graduação em Biotecnologia, Palotina, Paraná, 85.950-000, Brazil

**ABSTRACT**

**Objectives:** 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 diseases, so medicinal plants stand out as an important part of the culture. 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.[8] 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.[9] 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.[9] 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.[9]

The recommended treatments for IBD are self-care and drug treatments.[9] Drug treatment is performed by administering corticosteroids, 5-aminosalicylic acid, immunomodulators, and biologic drugs,[9] which have several adverse effects,[9] 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 interventive therapeutic possibility.[9]

_Altéranthera brasiliána_ (L.) Kuntze (Amaranthaceae) is a plant native to subtropical and tropical regions of South America and Australia is commonly known as penicilina, perpêta-do-mato and perpêta-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. brasiliána_,[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. brasiliána_; this flavonoid has been attributed anti-inflammatory, antioxidant, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, anti-osteoporotic, anxiolytic, analgesic, and antiallergic activities.[12] Other compounds described are saponins, with as immunostimulant, antineoplastic, cholesterol-reducing, and anti-inflammatory properties.[13] Other bioactive found in _A. brasiliána_ 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. brasiliána_ 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. brasiliána_ preparation and total flavonoids quantification**

_A. brasiliána_ 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. brasiliána_ 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 vitro_ 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. brasiliána_ was evaluated by the writhing test.[14] Nociception was induced by intraperitoneal administration 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. brasiliána_ at...
10, 30, 100 and 300 mg/kg, saline (negative control), Dipyrrone 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 intracolic 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 noception 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 (Labetest Diagnostica 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% ± 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 Dipyrrone, 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 Dipyrrone 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).

**Acetic acid-induced acute colitis**

In the induced colitis experiment, the keto-alcoholic
Figure 1. The antinociceptive activity of keto-alcoholic extracts of A. brasiliana leaves in the writhing test. A. Comparison among the experimental groups. B. Time-dependent activity of the keto-alcoholic extract of A. brasiliana leaves at 100 mg/kg. Results were presented ± 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.

A significant decrease in nociceptive sensitivity was observed in mice treated with the keto-alcoholic extract of A. brasiliana at concentrations of 30 (36%), 100 (84%) and 300 (70%) mg/kg, and with Mesalazine (80%), induction) all mice presented a score of 0. No a significant difference was observed among the experimental groups regarding the reduction of colonic edema, although clinically, the extract of A. brasiliana was more efficient than Mesalazine (Figure 2A).
compared to the negative control group. The *A. brasiliana* extract at 100 and 300 mg/kg, and Mesalazine also showed greater antinociceptive activity than the *A. brasiliana* extract at 30 mg/kg. No significant difference was observed among the three treatments (Figure 3).

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. Pain covers several mechanisms, being 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. 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.

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. 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-1β, TNF-α and IL-8. This study demonstrated that flavonoids of *A. brasiliana* 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 spasmyloytic properties. 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. brasiliana*, 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 (96.5%). Comparatively, in this study, lower doses of *A. brasiliana* extract induced a significant inhibition—48% (100 mg/kg) and 40% (300 mg/kg) of writhing in mice.

**DISCUSSION**

The values of both plasma AST and ALT of the mice treated with the keto-alcoholic extract of *A. brasiliana* 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 the 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.

**Acute hepatotoxicity**

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. brasiliana* were acquired, while by acidified aqueous extraction only 35.243 μg/g (0.035243 mg/g) of polyphenols were obtained from the leaves of the plant, among the polyphenols flavonoids were more abundant. 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.

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. brasiliana* were acquired, while by acidified aqueous extraction only 35.243 μg/g (0.035243 mg/g) of polyphenols were obtained from the leaves of the plant, among the polyphenols flavonoids were more abundant. 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.

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. brasiliana* were acquired, while by acidified aqueous extraction only 35.243 μg/g (0.035243 mg/g) of polyphenols were obtained from the leaves of the plant, among the polyphenols flavonoids were more abundant. 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.
Table 1: Aspartate transaminase and alanine transaminase plasma values of mice treated with the keto-alcoholic extract of *A. brasiliana* leaves

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

*Standard deviation

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.[30] It was noted that the groups of mice treated with *A. brasiliana* extract at 100 and 300 mg/kg showed a significant result 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-aminosalicyclic acid, immunomodulators, and biological drugs,[31] which present a number of adverse effects.[32] In this sense, the use of medicinal plants, their parts or extracts, has been implemented for decades as alternative medicine.[33] 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.[34] The promising results of the preclinical tests indicate 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.[35] 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.[36]

Inflammation is characterized as a physiological process, being a response to stimuli such as infections, physicochemical and antigenic changes, or occurrence of traumatic injuries, and may be present in various dysfunctions and pathologies,[37] 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, which leads to loss of tissue homeostasis, triggering the inflammatory process.[38] 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.[39] 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.[40] 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. brasiliana* 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. brasiliana* 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 elucidate the action mechanism and the mediators involved.

DECLARATIONS

Author contributions

Jeniffer 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; Jeniffer 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

28. Lima CS. Estudo da toxicidade não clínico em ratos submetidos ao tratamento com écloresina de Copaifera ducie Dwyer (subcrônico e


