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Original Article

Efficacy of *Baccharis dracunculifolia* in the treatment of diversion colitis in rats

Arthur Medeiros Lima[a], *Carlos Eduardo Costa Nascimento*[a], *Carlos Henrique Marques dos Santos*[a,b,*], *Doroty Mesquita Dourado*[a,b], *Gabriel Elias Cardoso Siqueira*[a], *Giovana Maria Rigo*[a], *Lauren Umpierre Bernardi*[a], *Paulo Otávio Souza Leonel*[a], *Rosemary Matias*[a], *Vitor Caldas Ferreira*[a], *Vitor Cruz Rosa Pires de Souza*[a]

[a] *Universidade Anhanguera (Uniderp), Campo Grande, MS, Brazil*
[b] *Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil*

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ABSTRACT

Rationale: Disuse colitis is frequent in our country and the most effective treatment is high cost and there is a need for effective and low cost therapy.
Objective: To evaluate the efficacy of *Baccharis dracunculifolia* (field rosemary) in the treatment of exclusion colitis in rats.
Method: Eighteen Wistar rats were anesthetized and submitted to colostomy; they were then distributed into two groups: Control Group, receiving intrarectal saline infusion (n = 8) and Group BD receiving intrarectal infusion of*Baccharis dracunculifolia* extract (n = 10); after 21 days of treatment they were euthanized, the intestinal segment excluded from intestinal transit was resected and submitted to histopathological study, classifying the degree of inflammation and degree of vascular congestion from 0 to 3.
Results: Mean inflammation was 2.7 in Control Group versus 2.1 in BD Group (p = 0.049), while mean vascular congestion was 2.3 and 2, respectively, in Control and BD groups (p = 0.1642).
Conclusion: Intra-rectal infusion of*Baccharis dracunculifolia* extract significantly minimized the inflammatory process in the exclusion colitis of rats submitted to colostomy, without altering the degree of vascular congestion.

* *Corresponding author.*
E-mail: chenriquems@yahoo.com.br (C.H. Santos).
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Eficácia da *Baccharis dracunculifolia* no tratamento da colite de desuso em ratos

Palavras-chave:
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RESUMO

Racional: A colite de desuso é frequente em nosso meio e o tratamento de maior eficácia é de alto custo, havendo necessidade de se encontrar uma terapêutica eficaz e de baixo custo.
Objetivo: Avaliar a eficácia da *Baccharis dracunculifolia* (alecrim-do-campo) no tratamento da colite de exclusão em ratos.
Método: Utilizou-se 18 ratos Wistar, os quais foram anestesiados e submetidos à colostomia; em seguida distribuídos em 2 grupos: Grupo Controle, recebendo infusão intrarretal de solução salina (n = 8) e Grupo BD, recebendo infusão intrarretal de extrato de *Baccharis dracunculifolia* (n = 10); após 21 dias de tratamento foram submetidos a eutanásia, o segmento intesinal excluso de trânsito intestinal foi ressecado e submetido a estudo histopatológico classificando-se o grau de inflamação e grau de congestão vascular de 0 a 3.
Resultados: Verificou-se média de inflamação 2,7 no Grupo Controle vs. 2,1 no Grupo BD (p = 0,049), enquanto as médias de congestão vascular foram 2,3 e 2, respectivamente, nos grupos controle e BD (p = 0,1642).
Conclusão: A infusão intrarretal do extrato de *Baccharis dracunculifolia* minimizou significantemente o processo inflamatório na colite de exclusão de ratos submetidos à colostomia, sem alterar o grau de congestão vascular.

Introduction

Intestinal transit diversion is still common worldwide, particularly in urgent surgical procedures such as trauma, intestinal obstruction, perforations, and inflammatory diseases. These stomata are mostly performed on a temporary basis, but it can often become definitive due to patients' clinical conditions. When temporary, an average of three months is estimated for intestinal reconstruction, however, this timeframe may be extend into years due to multiple factors.[1]

The colostomy using Hartmann's procedure, which involves sigmoid colon exteriorization and rectum exclusion from intestinal transit, accounts for half of the total intestinal transit deviations performed, which also makes this type of stoma the one with the highest number of complications, especially in the long run. Thus, in cases where intestinal transit diversion remains for more than 6 months, the number of complications increases, including Disuse Colitis (DC).[2]

DC can cause rectal pain in addition to mucopurulent discharge, discomfort to patients, as well as difficult anastomoses at the time of intestinal reconstruction, when it is possible. The best therapy for this condition is to infuse short-chain fatty acid enemas in order to have direct lumen nutrition of intestinal cells.[3] However, the major obstacle to this therapy is the cost, which is quite high and not feasible when the therapy needs to be extended for long periods.[4,5]

Thus, there is a growing number of studies seeking a therapeutic option as effective as short chain fatty acids with a lower cost and no adverse effects, which, in theory, would be achieved with herbal medicine, as many have shown a significant anti-inflammatory effect in other situations.[6,7]

Baccharis dracunculifolia extract may inhibit elastase enzyme activity in inflammatory processes involving exacerbated neutrophil stimulation without altering the degranulation process, which would be beneficial in reducing the exacerbated inflammatory process.[8] The anti-inflammatory effect of Baccharis dracunculifolia extract has also been observed in the inflamed colonic mucosa of rats by reducing oxidative stress, decreasing myeloperoxidase enzyme activity, and maintaining endogenous antioxidants, such as glutathione, at normal levels.[9]

Thus, due to evidence of the anti-inflammatory effect of Baccharis dracunculifolia and the lack of specific studies on its efficacy in disuse colitis, the present study is justified.

Objective

To evaluate the efficacy of Baccharis dracunculifolia (rosemary-of-the-field) in the treatment of exclusion colitis in rats.

Method

Animals

All animal procedures were performed in accordance with the standards provided by the Brazilian Council of Animal Experimentation (COBEA) and with the authorization of the Anhanguera Educacional Committee Ltd – CEUA/EASA No. 3046, approved on 11/07/2017. Eighteen male Wistar Rattus norvegicus, body weight 250–300 g, from the Federal University of Mato Grosso do Sul were used.

Formed groups

The animals were divided into the following groups:

Control Group: 8 rats were submitted to colostomy and after 7 days received intra-rectal saline infusion;

Baccharis dracunculifolia Group: 10 rats underwent colostomy and after 7 days received intra-rectal infusion of Baccharis dracunculifolia extract.

Preoperative period

The animals underwent 12 h of fasting immediately before surgery under intraperitoneal injection of 2:1 solution of Ketamine hydrochloride (Cetamin®), 50 mg/mL, and Xylazine hydrochloride (Xilazin®), 20 mg/mL, respectively, in dose of 0.1 mL/100 g (Flecknell, 1996).

Surgical procedure

After anesthesia, the animals were immobilized to the operating table in the supine position, followed by abdominal hair scraping and antisepsis with 2% chlorhexidine diglyconate for a median laparotomy with length of approximately 5 cm.

A number-6 urethral tube was introduced rectally, graduated in millimeters to standardize the amount of intestine remaining in the distal stump after section. The distal colon was sectioned at the 6 cm mark and closed by continuous single-stitching suture with seromuscular stitches and coaptation of the edges using suture 5–0 polyglactin 910.

The proximal portion of the colon was exteriorized through the abdominal wall with an approximately 0.5 cm incision and fixed by four single stitches separately placed, joining the seromuscular layer to the skin using suture 5–0 polyglactin 910.

Incision closure was performed in two planes, first closing the aponeurosis with continuous stitches using suture 5–0 polyglactin and then closing the skin with simple separate stitches using suture 4–0 nylon.

Preparation of Baccharis dracunculifolia extract

The aerial parts of the vegetal raw material (Baccharis dracunculifolia) were collected at the Três Barras School Farm of Anhanguera University – Uniderp. The samples were air dried, ground, packaged and identified. Subsequently, the ground plant underwent material extraction with ethanol (99.5%) until depletion. After extraction and filtration, the solvent was evaporated (water bath at 50 °C) to obtain ethanolic crude extracts. Subsequently, 0.9% sodium chloride solution was added until reaching the concentration of 200 mg/mL of the Baccharis dracunculifolia.

Treatment

Seven days after the surgical stoma, enema infusion was started daily in all rats for 21 days, with the aid of a 10-millimeter disposable syringe and # 6 urethral tube, according to group distribution:

Control Group: 8 rats underwent intra-rectal infusion of 5 mL saline solution;

Baccharis dracunculifolia Group: 10 rats underwent to intra-rectal infusion of 5 mL Baccharis dracunculifolia extract.

Euthanasia and histopathological study

After 21 days of treatment, the animals were euthanized with intraperitoneal administration of anesthetic overdose combined with Xylazine (5 mg/kg) and Ketamine (30 mg/kg). After laparotomy, samples of the rectum were removed, weighed, measured, and fixed in 10% buffered formalin, imbibed in paraffin to be sectioned at 5 μm, stained with hematoxylin and eosin, and evaluated by conventional optical microscopy by a blind histologist.

All 28 slides with histological material were analyzed under light microscope, and the variables assessed were vascular congestion and inflammatory infiltrate, classified using a scale rate of 0–3, where:

Inflammatory infiltrate rating: 0 = no inflammatory focus/field; 1 = mild, 1 inflammatory focus/field; 2 = moderate, 2 inflammatory foci/fields; 3 = severe, 3 inflammatory foci/fields (Fig. 1).

Were each focus is considered as an infiltrate of mononuclear cells that include macrophages, lymphocytes and plasma cells, resulting from continuous recruitment from blood circulation and local proliferation.

Vascular congestion ratting: 0 = absent; 1 = mild; 2 = moderate; 3 = severe (Fig. 2).

Where each grade is evaluated as representing stasis, which is the consequence of increased vascular permeability caused by endothelial cell contraction, in which protein-rich fluid leaks into extravascular tissues causing red blood cells to become more concentrated, thus increasing blood viscosity and slowing circulation, represented by the presence of small dilated vessels filled with red blood cells—the criteria for determining the intensity of vascular congestion were pronounced dilation and engorgement of arterioles and capillaries with peripheral leukocytes in the laminar flow—"margination, pavement, and diapedesis of leukocytes".

BioEstat 5.3 software was used for statistical tests. ANOVA was performed using Kruskal–Wallis and Mann–Whitney tests for nonparametric and unpaired data, followed by Tukey test with statistical significance at $p < 0.05$.

Results

The means found in the evaluation of the inflammatory process were 2.7 in Control Group and 2.1 in Baccharis dracunculifolia extract Group ($p = 0.049$). The results are presented in Table 1.

Regarding the assessment of vascular congestion there was no difference between groups, once in the Control Group the observed mean was 2.3, while in the Baccharis dracunculifolia extract Group the mean was 2 ($p = 0.1642$) (Table 2).

Discussion

In the present study, it was found that the exclusion of fecal transit in the colonic segment of rats for 21 days actually promoted an inflammatory process, considering the mean of

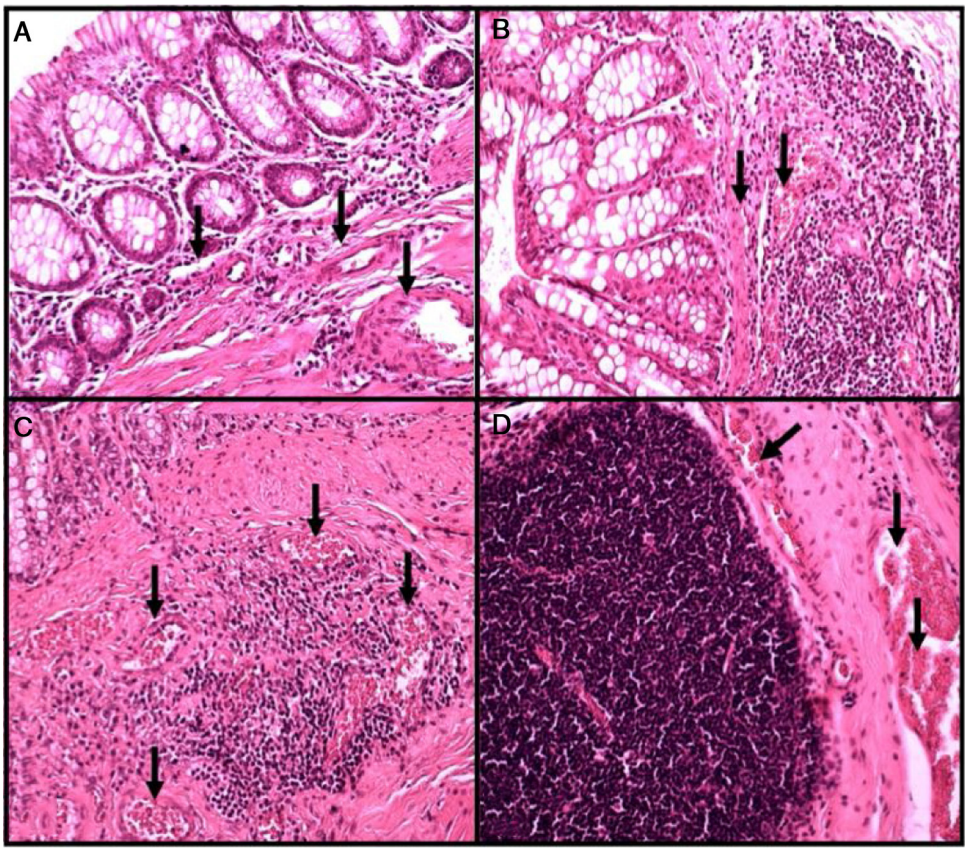

Fig. 1 – Classification of inflammatory infiltrate. (A) Normal epithelium, absence of inflammation; (B) one inflammatory focus per field; (C) two inflammatory foci per field; (D) more than two inflammatory foci per field; Inflammatory foci are characterized by the presence of lymphocytes, macrophages, dendritic cells, T lymphocytes and B lymphocytes, which have been observed in hyperplastic lymphoid follicles exhibiting colonic mucosa and submucosa atrophy (arrows); Hematoxylin-Eosin staining (200×).

Table 1 – Evaluation of the inflammatory process in the excluded intestinal segment.

Rats	Groups	
	Control	*Baccharis dracunculifolia*
1	3	2
2	3	2
3	2	3
4	3	2
5	3	3
6	3	2
7	3	2
8	2	1
9	–	2
10	–	2
Mean	2.7	2.1
$p < 0,05$		

Table 2 – Evaluation of the degree of vascular congestion in the excluded intestinal segment.

Rats	Groups	
	Control	*Baccharis dracunculifolia*
1	0	2
2	1	2
3	3	3
4	3	2
5	3	3
6	2	1
7	3	2
8	3	1
9	–	2
10	–	2
Mean	2.3	2.1
$p > 0,05$		

2.7 for the degree of inflammation on a scale ranging from 0 to 3, which characterizes moderate to severe inflammation. The same had already been found by other authors, which is the starting for evaluating the therapeutic efficacy of Baccharis dracunculifolia.[3–5] The histological evaluation method was also the same, which facilitates the comparison as it is well characterized by infiltration with mononuclear cells, which include macrophages, lymphocytes and plasma cells; tissue destruction induced by inflammatory cells; and healing attempts by replacing damaged tissue with connective tissue through small vessel proliferation (angiogenesis) and fibrosis.[10–17]

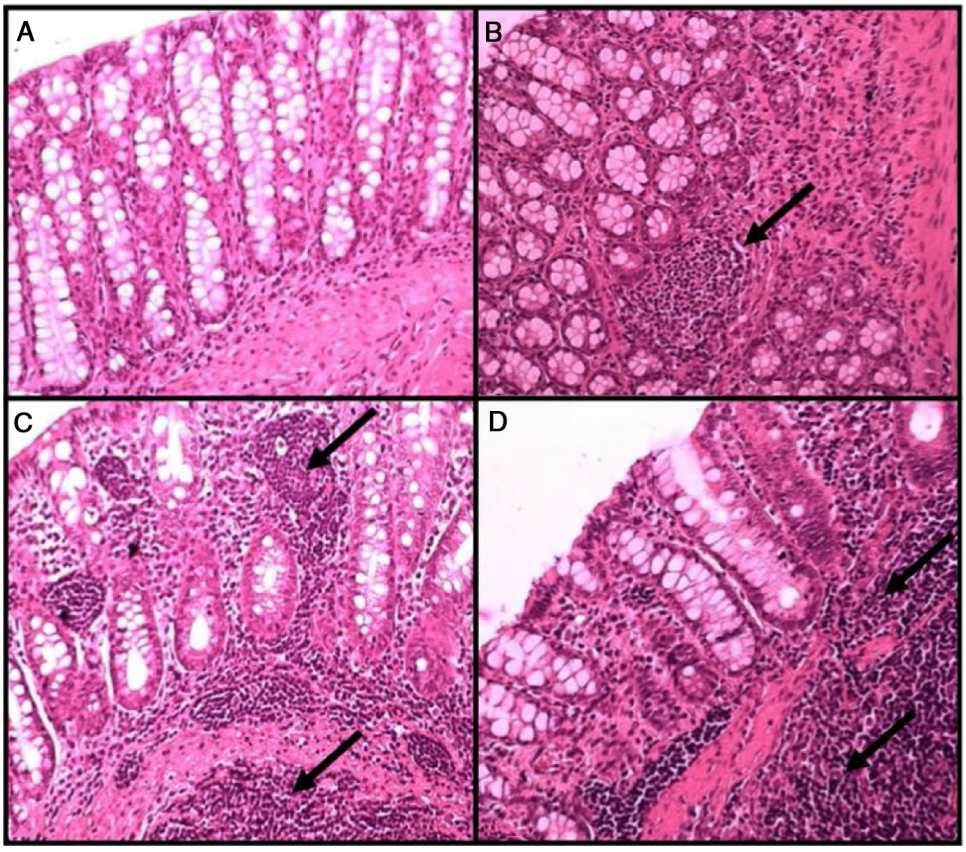

Fig. 2 – Classification of vascular congestion. (A) Absent of vascular congestion; (B) mild vascular congestion (arrows); (C) moderate vascular congestion; D, intense vascular congestion; Hematoxylin-Eosin staining (200×).

Baccharis dracunculifolia extract was able to reduce the inflammatory process in the excluded segment, as shown in Table 1, since the average inflammatory process in this group was significantly lower than in the control group. This may be explained by a probable reduction of oxidative stress as already demonstrated by Cestari et al.[9] These authors induced intestinal inflammatory process in rats with trinitrobenzene sulfonic acid and found that in the group treated with B extract there was less tissue production of myeloperoxidase and prevented glutathione depletion, inhibiting lipid peroxidation and reducing myeloperoxidase activity. This protective action in the so-called oxidative stress may explain the mechanism of action of Baccharis dracunculifolia.

Figueiredo-Hinhel et al.[8] also evaluated the anti-inflammatory effects of Baccharis dracunculifolia but in an in vitro study and found that the plant extract acts as a neutrophil modulator and reduces the activity of toxic oxygen species. In the present study, a lower neutrophil concentration was also observed in the group treated with Baccharis dracunculifolia, demonstrating an effect similar to the one published by Figueiredo-Hinhel et al.[8]

There are few publications on Baccharis dracunculifolia, perhaps because it is more frequent in South America, especially in Brazil, a plant that grows in the savanna and adapts very well to the tropical climate, widely used for food seasoning.

Jaramillo-Garcia et al.[18] studied another rosemary species, Baccharis trinervis, to evaluate the toxicity and mutagenic properties of the plant, confirming some of these harmful effects with this plant. Munari et al.[19] demonstrated that Baccharis dracunculifolia prevented DNA damage and aberrant crypt formation in the intestinal mucosa of rats exposed to 1.2-dimethylhydrasine, an inducer of genotoxicity and preneoplastic lesions. In the present study no cellular alterations that could suggest mutations or other type of damage were observed, but new researches should be performed to confirm the safety of the extract.

Vascular congestion is a common finding in the inflammatory bowel process regardless of origin. It is also one of the earliest conditions, occurring even in mild inflammation.[20] Thus, the fact that there is no statistically significant difference between the studied groups does not detract the effect of Baccharis dracunculifolia, as the extract did not eliminate inflammation but decreased it. Perhaps, in a longer treatment model, we would see even less inflammation and also reduced vascular congestion, which should be assessed in future studies.

Azari et al.[21] studying the inflammatory process in the intestinal mucosa of rats also considered that vascular congestion would already be present in a mild inflammatory process, which is in accordance with our understanding; that is, since the Baccharis dracunculifolia Group had a moderate grade of inflammation, in contrast to the Control Group that was closer

to the severe grade, it would indeed be expected that the animals in the treatment group still had vascular congestion.

There are no publications evaluating the degree of vascular congestion in intestinal mucosa inflammation using Baccharis dracunculifolia, which precludes a direct comparison of the results obtained; however, there are a few other publications confirming the anti-inflammatory effect. Baggio et al.[22] studied Baccharis illinita DC in gastric inflammation of rats and found that it not only protects against alcohol-induced inflammation but also maintained the natural protective effects, such as mucus, bicarbonate and blood flow.

Thus, in the situation presented here and still very common in many parts of the world, in which patients remain with long-term intestinal transit diversion, resulting in the effects of disuse colitis, the use of an easily cultivated plant and low preparation cost could be helpful in decreasing the inflammatory process and, in principle, causing no deleterious effects.

The benefits seen in the model used here, trying to reproduce a real clinical situation, still need to be verified with different plant concentrations and different treatment periods in order to verify the best result to be obtained with Baccharis drancunculifolia, as the good results obtained show that this product is a promising treatment for disuse colitis.

Conclusion

The results allow us to conclude that the daily application of *Baccharis dracunculifolia* enemas for 21 days in rats undergoing intestinal transit diversion was able to decrease the inflammatory process in the excluded segment.

Conflicts of interest

The authors declare no conflicts of interest.

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