DOI 10.20398/jscr.v12i2.27516

EFFECT OF ARRABIDEAE CHICA (CRAJIRU) EXTRACT IN A MODEL OF INDUCED EXPERIMENTAL COLITIS IN RATS

EFEITO DO EXTRATO DE ARRABIDEAE CHICA (CRAJIRU) EM MODELO DE COLITE EXPERIMENTAL INDUZIDA EM RATOS

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Study performed at Department of Surgery, Federal University of Rio Grande do Norte (UFRN), Brazil.
Financial support: CNPq, Grant:311625/2018/5.
Conflicts of interest: None.
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Submitted: nov 04; accepted after revision, dec 27, 2021.

ABSTRACT

Objective: This study aimed to investigate the effect of A. chica extract on the evolution of experimental rectocolitis in rats, and the expression of the pro-inflammatory cytokines TNF-a, IL-1β and IL-6 in colonic tissue. **Methods:** Wistar rats weighing 275±23g were distributed into 4 groups of 6 animals each. Rectocolitis was induced in rats by rectal administration of trinitrobenzene sulfonic acid (TNBS). Seventy-two hours after TNBS injection, animals were treated daily for 6 days. Groups: 1. Normal control group without induction of rectocolitis. Received 0.9% saline injection v.o. by gavage during treatment. 2. TNBS rectocolitis group, treated with normal saline (SN) by gavage (TNBS+SN); 3. TNBS rectocolitis group treated with A. chica extract (ACE), receiving a daily dose of 300 mg of A. chica extract by gavage (TNBS+ACE);4. TNBS rectocolitis group treated with mesalazine, receiving a daily dose of 100 mg/kg of mesalazine orally (TNBS+MEZ). Macroscopic examination of the colon and dosing of TNF-α, IL-1β and IL-6 in colon tissue were performed. **Results:** There was a reduction in weight in animals treated only with TNBS+NS. No difference in weight was observed comparing the animals treated with ACE and MEZ. In the control group no mucosal ulcers or edema of

the colon wall were observed. Several mucosal ulcers, edema and hyperemia occurred in the colon of rats in the TNBS+SN group. In two of the animals in this group there wascolon perforation, tamponated by omentum. A reduction of mucosal ulcers number in the TNBS+ACE (crajiru) group was seen, compared to the TNBS+SN and TNBS+MEZ group. There was a significant reduction of TNF-α, IL-1β and IL-6 in the colon tissue of animals treated with crajiru extract, TCBS+ACE group, when compared to the control group ($p<0.001$), TNBS+SN group, and TNBS+MEZ groups ($p<0.001$). **Conclusion:** This is the first study to show that A. chica extract positively influences the treatment of TNBS/induced rectocolitis through its antiinflamatory activity. More comprehensive studies are needed to understand the underlying mechanisms.

Keywords: Rectocolitis. TNBS. Arrabideae chica. Extract. Treatment. Cytokines. Rats.

RESUMO

Objetivo: Investigar o efeito do extrato de Arrabideae chica (crajiru) na evolução da retocolite experimental em ratos, e a expressão das citocinas pró-inflamatórias TNF-a, IL-1β e IL-6 no tecido colônico. **Métodos**: Ratos Wistar pesando 275±23g foram distribuídos em 4 grupos de 6 animais cada. A retocolite foi induzida nos ratos pela administração retal de ácido trinitrobenzenossulfônico (TNBS). Setenta e duas horas após a injeção de TNBS, os animais foram tratados diariamente durante 6 dias. Grupos: 1. Controle normal sem indução de retocolite. Recebeu injeção de solução salina a 0,9% v.o. por gavagem durante o tratamento. 2. Grupo retocolite com TNBS, tratado com solução salina normal (SN) por gavagem (TNBS + SN); 3. Grupo retocolite com TNBS tratado com extrato de A. chica (ACE), recebendo dose diária de 300 mg de extrato de A. chica por gavagem (TNBS + ACE); 4. Grupo retocolite TNBS tratado com mesalazina, dose diária de 100 mg/kg de mesalazina v.o. (TNBS + MEZ). O exame macroscópico do cólon e a dosagem de TNF-α, IL-1β e IL-6 no tecido do cólon foram realizados. **Resultados**: Houve redução de peso nos animais tratados apenas com TNBS+NS. Não foram observadas diferenças de peso comparando os animais tratados com ACE e MEZ. No grupo controle não havia úlceras mucosas ou edema da parede do cólon. Várias úlceras da mucosa, edema e hiperemia ocorreram no cólon de ratos no grupo TNBS+SN. Em dois dos animais deste grupo houve perfuração do cólon, tamponada por omento. Foi observada redução das úlceras da mucosa no grupo TNBS+ACE, em comparação com o grupo TNBS + SN e TNBS + MEZ. Houve redução significativa de TNF-α, IL-1β e IL-6 no tecido do cólon de animais tratados com extrato de crajiru, (grupo TCBS+ECA), quando comparados ao grupo controle ($p <0,001$), e grupos TNBS+SN, e TNBS+MEZ ($p <0,001$). **Conclusão**: Este é o primeiro estudo a mostrar que o extrato de A. chica influencia positivamente o tratamento de / retocolite induzida por TNBS, por meio de sua atividade antiinflamatória. Estudos mais abrangentes são necessários para compreender os mecanismos subjacentes.

Descritores: Retocolite. TNBS. Extrato. Arrabideae chica. Tratamento. Citocinas. Ratos.

INTRODUCTION

Inflammatory bowel disease, often in the forms of Crohn's disease and ulcerative rectocolitis, is a chronic idiopathic inflammatory disorder that affects the gastrointestinal tract of children and adults. The precise etiology of IBD remains uncertain[1]. However, current research agrees that arachidonic acid metabolites such as leukotrienes and prostaglandins and also reactive oxygen species (ROS) contribute to the development of the disease. It is well established that chronic intestinal inflammation results from the overproduction of reactive oxygen and nitrogen species (ROS and RNS)[2]. Complex interactions between several factors, including genetic factors, the host's immune system and environmental factors, cause disturbances in intestinal homeostasis, leading to dysregulated inflammatory responses in the intestine[1-3]. Basic and translational research[4,5] have led to a better understanding of the role of inflammatory mediators, including cytokines in the intestine of patients with IBD. Cytokines transmit signals between immune, epithelial and mesenchymal cells and play a central role in the development of ulcerative rectocolitis. 5-aminosalicylic acid, (mesalazine) is used in the traditional treatment of rectocolitis. The in vivo administration of mesalazine can decrease the inflammatory response through several mechanisms that include the inhibition of the activity of the nuclear factor κB (NF-κB), inhibition of cyclooxygenase-2 (COX-2), and of eicosanoids[6,7]. Although mezalasin and other available agents have shown clinical benefits to some degree, they are not fully effective and have multiple adverse effects. Furthermore, the treatment of rectocolitis requires long-term use, which often leads to refractoriness or intolerance[8].

Therefore, it is necessary to develop new therapeutic approaches. Several natural products have been shown to safely suppress the pro-inflammatory pathway and control rectocolitis. In vivo and/or in vitro studies suggest that the anti-rectocolitis effects exhibited by natural products are mainly caused by their ability to modulate the production of cytokines, such as TNFa, IL-1b and IL-6[9-11]. Ko et al[12] demonstrated that orally and locally administered extract from the roots of *Astragalus membranaceus* can protect rats against hapten-induced colitis through attenuation of TNF-a and IL-1b and up-regulation of IL-10.

The antioxidant effect of *Arrabidaea chica*, known as crajiru, a shrubby plant that is widely distributed from southern Mexico to central Brazil, mainly in the Amazon region, has been investigated. This plant belongs to the Bignoniaceae family, and its extract has been used as an anti-inflammatory and astringent agent, as well as a remedy for intestinal cramps, diarrhea, leukorrhea, anemia, and leukemia. A literature review indicated that this plant is a source of anthocyanins, flavonoids, tannins and phytosterols[13-18]. However, there are very few experimental and clinical studies

regarding the use of *A. chica* extract to control diseases, more specifically ulcerative rectocolitis.

Thus, the present study aimed to investigate the effect of *A. chica* extract on the evolution of experimental rectocolitis in rats, and the expression of the pro-inflammatory cytokines TNF-a, IL-1β and IL-6 in colonic tissue.

METHODS

Animals

Wistar rats weighing 275±23g were distributed into 4 groups of 6 animals each. They were kept at a 22°C room and a relative humidity of 45-65%. The rats were housed in polypropylene cages with *ad libitum* access to rodent food (Prevence®) and water, in a room with a 12:12 light/dark cycle. All care with the animals were in accordance with the Guidelines of the Brazilian Law 11,794/08 for animal experimentation. The protocol was approved by the Ethics Committee on the Use of Animals of the institution (CEUA/HUOL), protocol nº 03/2020. All animals were acclimated for one week at the Experimental Surgery Center before the start of the study.

Rectocolitis Induction

Rectocolitis was induced in rats by rectal administration of trinitrobenzene sulfonic acid (TNBS) (purchased from Sigma-Aldrich) dissolved in 50% ethanol solution. The animals were anesthetized with 70 mg/kg ketamine + 7 mg/kg xylazine intraperitoneally. About 3 ml/kg of TNBS-ethanol solution (50 mg/ml) was administered into the colon at a distance of 8 cm from the anal margin using a soft polyethylene catheter. The rats were positioned in the Trendelenburg position for one minute, in order to avoid the loss of the TNBS solution through the rectum.

Preparation of A. chica extract (Crajiru)

The alcoholic extract was prepared by maceration of 200 g of dried *A. chica* leaves. Ethanol at a ratio of 1:3 was added to the percolation process at room temperature. The material was filtered and concentrated on a rotary evaporator at a temperature of 60°C. The extract was weighed and diluted, and an alcoholic extract at a concentration of 10% was obtained, being stored at 4ºC until used.

Experimental design

Seventy-two hours after TNBS injection, animals were treated daily for 6 days.

1. Normal control group without induction of rectocolitis. Received 0.9% saline injection v.o. by gavage during treatment.

Rats with TNBS-induced rectocolitis were randomly divided into 3 treatment groups (6 animals each):

2. TNBS rectocolitis group, treated with normal saline (SN) by gavage (TNBS+SN);

3. TNBS rectocolitis group treated with *A. chica* extract (ACE), receiving a daily dose of 300 mg of *A. chica* extract by gavage (TNBS+ACE) for 6 days;

4. TNBS rectocolitis group treated with mesalazine, receiving a daily dose of 100 mg/kg of mesalazine orally (TNBS+MEZ) for 6 days.

Animals were then recovered from anesthesia and observed daily in polypropylene cages for the 6 days of the study.

The rats were weighed at the beginning and at the end of the experiment, to assess the evolution of weight with the treatments.

Twenty-four hours after the last treatment, all animals were euthanized with overdose of sodium thiopental 100 mg/kg via I.P. The distal colon extending 8 cm to the anal margin was removed, opened with surgical scissors along the longitudinal mesenteric edge, and washed with isotonic saline solution.

Macroscopic evaluation of the colon

The pieces were visually examined with the aid of a magnifying glass. Macroscopic colonic damage was analyzed for: mucosal hyperemia without ulcers; linear ulcer without colon wall thickening; linear ulcer with thickening of the colon wall; colonic ulcer in multiple areas; extensive ulcers and perforation.

Proinflammatory cytokine assays

To quantify colonic tissue cytokines, 100 mg of colonic tissue from each animal was homogenized and extracted using 500 µl of 5 M guanidine HCl and 50 mM Tris–HCl (pH 8.0) with a protease inhibitor. The extracts were centrifuged at 3,000g for 30 minutes at 4°C to remove insoluble materials. The supernatant fractions were analyzed by the ELISA kit (PeproTech-Brasil – Ribeirão Preto, SP), according to the manufacturer's instructions. To quantify TNF-α, IL-1β and IL-6 in colon tissue, 1:20 dilutions of colon tissue homogenate supernatants were added into 96-well microplates. These microplates were coated with antibodies specific for rats. After the enzyme-substrate reaction, the absorbances of the samples were measured at 450 nm with a microplate reader. Standard curves were prepared with the samples using diluted standard solutions. These curves were used to provide comparisons for the calculations of TNF-α, IL-1β, IL-6 in the samples. All dosages were performed in duplicate.

Statistical analysis

Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test, with a significance level of 5% were used, with the statistical program BioEstat 5.0, Belém, Pará, Brazil..

RESULTS

All animals survived the treatments. There was a reduction in weight in animals treated only with TNBS+NS. No differences in weight were observed comparing the animals treated with ACE and MEZ.

The macroscopic evaluation of the colon wall and mucosa showed that in the control group there were no mucosal ulcers or edema of the colon wall. Several mucosal ulcers, edema and hyperemia occurred in the colon of animals in the TNBS+SN group. In two of the animals in this group there was colon perforation, tamponated by omentum. In the TNBS+ACE (crajiru) group there was a significant reduction in the number of mucosal ulcers and colon wall edema, compared to the TNBS+SN group, a finding that was repeated in the animals of the mezalasin-treated group (TNBS+MEZ).

A. chica extract (ACE) had an important effect in reducing the inflammatory process in the colon of animals with TNBS-induced rectocolitis. There was a significant reduction of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in the colon tissue of animals treated with crajiru extract, TCBS+ACE group, when compared to the control group ($p<0.001$). A reduction in cytokine dosage was also observed in the group with rectocolitis treated with A. chica (crajiru) extract (group TNBS+ACE) compared to the TNBS+SN group. At the same time, these values were significantly lower than in the group of rats with rectocolitis, treated with mezalasin ($p<0.001$). These data are summarized in table 1..

Table 1 – Results of measurements of proinflammatory cytokines in the colon wall, on groups. Descriptive and inferential statistics.

Citokines	Groups				P-value
	CONTROL	TNBS+NS	TNBS+ACE	TNBS+MEZ	
TNF (pg/mL)	45.8 ± 1.8§	425.4 ± 5.8§	167.4 ± 4.8§	183.8 ± 9.2§	<0.001
IL-1 (pg/mL)	28.2 ± 0.8§	119.3 ± 2.9§◆	29.6 ± 1.7◆	38.8 ± 3.2§◆	<0.001
IL-6 (pg/mL)	23.5 ± 1.92§	91.6 ± 3.7§	56.4 ± 2.6§	68.1 ± 3.4§	<0.001

Mean ± standard deviation. TNBS, trinitrobenzene sulfonic acid; NS, normal saline; ACE, Arrabideae chica extract; MEZ, mezalasin.
Values on the same line followed by identical symbols were significantly different. p-value ANOVA

DISCUSSION

TNBS-induced experimental retocolitis in murine is one of the most widely used animal model for inflammatory intestinal disease research. Its various histological changes and characteristics, including distinct thickening of colon wall, ulceration, infiltration of polymorphonuclear leukocytes, and increased production of inflammatory and profibrogenic mediators, are similar to human disease[20,21].

In the present work, it was found that the TNBS-treated retocolitis rats showed more body weight loss, higher macroscopic colon lesions as compared with the control rats. In contrast to the TNBS+NS group, the treatment of rats with ACE and MEZ significantly attenuated the loss of body weight, and remarkably recovered the colon macroscopic lesions observation.

Results from the study of Siraichi et al (2013) showed a high concentration of antioxidants on *A. chica* extract[22]. The phenolic composition of A. chica leaves included isoscutellarein, 6-hydroxyluteolin, hispidulin, scutellarein, luteolin, and apigenin. When the antioxidant activities of scutellarein and apigenin isolated from *A. chica* were compared, scutellarein showed higher antioxidant activity than apigenin. This is in agreement with the findings of Kandasamy and Rathinam[23]. These authors suggested that the higher antioxidant potential of scutellarein is attributed to the electronic parameters present on the ring of the molecular structure of scutellarein, enabling it to scavenge more reactive oxygen species.

To our knowledge, this work was a pioneering effort to explore the therapeutic potential of *Arrabideae chica* extract (ACE) against TNBS-induced murine experimental rectocolitis. We contributed to elaborate the potential mechanism of ACE in regulating inflammation events related to proinflamatory cytokine expression in colon wall of rectocolitis rats. In conclusion, this is the first study to show that *A. chica* extract positively influences the treatment of TNBS/induced rectocolitis through its antiinflamatory activity. More comprehensive studies are needed to understand the underlying mechanisms.

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