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Research Article

Effect of *Fumaria Capreolata* Total Alkaloid Extract on Acetic Acid-induced Ulcerative Colitis in NMRI Mice

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I N F O

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A B S T R A C T

Background: Fumaria capreolata has been used in North African traditional medicine for its gastrointestinal benefits and anti-inflammatory effects.

Objective: This study explores the intestinal anti-inflammatory effects of total alkaloids extracted from the aerial parts of *Fumaria capreolata* (AFC) against acetic acid-induced experimental colitis in mice.

Methods: Intestinal anti-inflammatory effects of AFC (6.25, 12.5, and 25 mg/kg) were evaluated using the acetic acid-induced ulcerative colitis model in male NMRI mice. Colonic damage was assessed through colitis parameters and histological analysis of the colon.

Results: This study demonstrated that pre-treatment with AFC alleviates intestinal damage and clinical symptoms of ulcerative colitis. Histological analysis revealed that AFC helped preserve the integrity of the intestinal barrier. This finding was further supported by the colonic weight/length (W/L) ratio calculations.

Conclusion: AFC demonstrates potential as a promising candidate for preventing inflammatory diseases, such as colitis.

Keywords: Acetic-acid, Alkaloids, *Fumaria capreolata*, Anti-inflammatory, Colitis

Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions affecting the gastrointestinal tract, primarily categorized into two main disorders: Crohn's disease and ulcerative colitis.[1] The most common symptoms, such as intermittent abdominal pain, rectal bleeding, fever, weight loss, fatigue, and diarrhea, significantly diminish the quality of life for affected patients.[2] IBD is thought to have a complex etiology. In addition to environmental, infectious, and immunological influences, genetic factors are also considered to exert a significant effect.[3] There is growing evidence that the microbiota plays a crucial role in maintaining immunological homeostasis in the healthy gut and in the development of IBD. The prevailing hypothesis suggests that in genetically predisposed individuals, overly aggressive acquired (T cell) immune responses to certain commensal enteric bacteria, combined with environmental factors, trigger the onset or reactivation of the disease.[4,5]

During chronic intestinal inflammation, the frequency of apoptosis increases, which is thought to contribute to the disruption of the intestinal barrier function.[6] The damaged intestinal epithelial barrier then becomes a real "gateway" to many microorganisms, ions, and other molecules, thus induces the generation and maintenance of the inflammatory state causing IBD.[7] This response disrupts the regulation of the synthesis and release of pro-inflammatory mediators, such as interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ), as well as anti-inflammatory cytokines like IL-10 and transforming growth factor (TGF)-β.[8] Conventional pharmacological treatments for IBD currently involve the use of anti-inflammatory or immunosuppressive drugs, antibiotics, and supplements. However, these treatments are not curative and often come with various adverse side effects.[9]

Plants produce a wide range of metabolic products derived from both primary and secondary metabolism. Alkaloids are naturally occurring specialized metabolites, characterized by the presence of nitrogen in their molecular structures.[10,11] Isoquinoline alkaloids represent a significant class of pharmacologically active compounds, known for their diverse bioactivities, including antimicrobial, antifungal, antibacterial, and antitumor effects. They serve as vital raw materials in the quest for novel molecules, driving the development of more effective drugs with reduced side effects.[12] Among these medicinal plant rich in alkaloids, the Fumariacea family and more precisely the species of *Fumaria capreolata* which is a plant known for its antinociceptive, vasculo-protective and antioxidant properties because of its richness in isoquinoleic alkaloids.[13,14]

A previous study reported that the total alkaloid fraction of *Fumaria capreolata* demonstrated antioxidant activity and exhibited no significant toxicity when orally administered to mice at doses of up to 2000 mg/kg.[15] Additionally, the ethanolic extract of *Fumaria capreolata* has been reported to exhibit anti-nociceptive and anti-inflammatory properties effects against chemically induced nociception and against both inflammation and non-inflammation mediated nociception.[16] In this study, we evaluated the preventive effects of the total alkaloids from *Fumaria capreolata* in experimental ulcerative colitis induced by acetic acid *in vivo.*

Materials and methods

Drugs and chemicals

All substances, unless stated otherwise, were purchased from Sigma-Aldrich Chemical (Madrid, Spain). The test substances were dissolved in distilled water and freshly prepared daily for administration to the animals.

Extraction of total alkaloids

The aerial elements of *Fumaria capreolata* were gathered from the northeastern Algerian province of Bejaia, a voucher specimen was deposited under Reference No. FC015. The alkaloid extract of *Fumaria capreolata* (AFC) was prepared according to a previously reported procedure.[17] In brief, the aerial parts of *Fumaria capreolata* were dried in an oven at 40°C overnight and then ground into a fine powder. The powdered samples were subjected to Soxhlet extraction with ethanol for 8 hours, followed by evaporation under reduced pressure. The resulting aqueous acidic solution was adjusted to pH 9.5 using concentrated ammonium hydroxide and then extracted with dichloromethane. The extracts were evaporated to obtain a crude total alkaloid extract.

Animals

Healthy male NMRI mice (25–30 g), obtained from the Pasteur Institute of Algiers, Algeria, were housed in Makrolon cages under standard experimental conditions, including a controlled temperature of 26 ± 1°C and a 12-hour light-dark cycle. The mice had free access to tap water and food but were fasted for 24 hours before the experimental procedure. The experimental protocol was conducted out in compliance with the directive number 2010/63/EU of 22 September 2010, and received approval from the local Ethics Committee of PBVE, (Ref. No.CE-LBVE-2020-106).

Induction of ulcerative colitis and AFC treatment

Colitis was induced as described previously by.[18] Briefly, mice were divided into five groups (n=6). Two groups, the non-colitic and control groups, received oral pretreatment with vehicle, while the other groups (treated groups) received AFC treatment (6.25, 12.5, and 25 mg/kg) for seven days. On the sixth day of treatment, 4% acetic acid was administered intracolonically to induce colitis. Daily measurements of body weight, the incidence of diarrhea, and the amount of food and water consumed were made during the experiment. On the eighth day, after a 48-hour treatment with acetic acid, the animals were sacrificed, and the colon was aseptically excised and placed on an ice-cold surface. The colon was then longitudinally opened. Tissue samples were collected from the inflamed regions adjacent to areas exhibiting macroscopic damage and fixed in 4% buffered formaldehyde for histological analysis. Corresponding colonic segments were also obtained from the non-colitic group for comparison.

Colon damage evaluation using macroscopic scoring and histological assessment

The internal bowel surface was visually inspected, the mucosal lesions were scored macroscopically according to

the following criteria: 0, no damage; 1, localized hyperaemia without ulcers; 2, linear ulcers with no significant inflammation; 3, linear ulcers with inflammation at one site; 4,two or more significant ulceration and inflammatory sites that run more than 1 cm along the colon's length; and 5-8, after the first 2 cm of ulceration, one point is added for every additional centimeter.[19] For the histological investigations, representative whole gut specimens were obtained from an area of the inflamed colon that corresponded to the segment next to the gross macroscopic damage. These specimens were then preserved in 4% buffered formaldehyde. The non-colitic group also provided equivalent colonic segments.

Statistical Study

The data are presented by graph pad as mean±SEM. Analyzes were conducted using the ANOVA test and flowed by Dunnet's test, used to compare the values of the treated groups with the values of the sick group ($p < 0.05$, $p < 0.01$, $p < 0.001$, n=6).

Results

Clinical improvement of colitis by AFC

We evaluated the preventive intestinal anti-inflammatory effects of the total alkaloid extract from *Fumaria capreolata* (AFC) in mice by administering it orally at different doses (6.25, 12.5, and 25 mg/kg). Following rectal injection of 4% acetic acid, all treated mice exhibited signs of illness and diarrhea, in contrast to the healthy control group. Acetic acid administration led to clinical symptoms, including bloody diarrhea, weight loss, and a significant increase in the colon weight/length ratio. Macroscopic damage was also evident compared to the non-colitic group (Figure 1). Moreover, pretreatment of colitic mice with three doses of AFC (6.25, 12.5, and 25 mg/kg) resulted in an improvement in their clinical condition compared to the untreated colitic group

Anti-inflammatory effect of AFC on W/L ratio

Colonic inflammation was evident after sacrifice, as the weight/length ratio was significantly higher in the colitic control group (57.80 ± 7.15) compared to the control group (39.80 ± 3.56). Pretreatment with AFC at doses of 25, 12.5, and 6.25 mg/kg induced a notable reduction of the colonic weight/length ratio (39 ± 7.41, 40 ± 6.32, and 44 ± 5.47, respectively) in contrast to the colitic group that was not treated (Figure 2).

Histological improvement of colonic damage

Through the histological analysis of colon samples, the intestinal anti-inflammatory effect of AFC was confirmed, demonstrating that the treatment encouraged intestinal epithelial regeneration. A considerable drop in microscopic scores when compared to the acetic acid group that did not receive any of the three AFC dosages served as evidence of this (Figure 3). No histological alterations were observed in the colon tissue of non-colitic mice. In colitic mice, epithelial cell destruction resulted in degeneration of intestinal crypts and disorganization of epithelial tissue. The severity of these lesions corresponded to a score of 7.16 ± 1.47 (Figure 4). In contrast, oral administration of AFC facilitated tissue regeneration by restoring normal cellular architecture and preserving intestinal epithelial integrity. The severity of lesions was significantly reduced compared to the colitic group, with score values of 4.33 ± 1.36, 3.5 ± 0.5, and 3 ± 0.63 for AFC doses of 6.25, 12.5, and 25 mg/kg, respectively ($p < 0.001$ vs. colitic group).

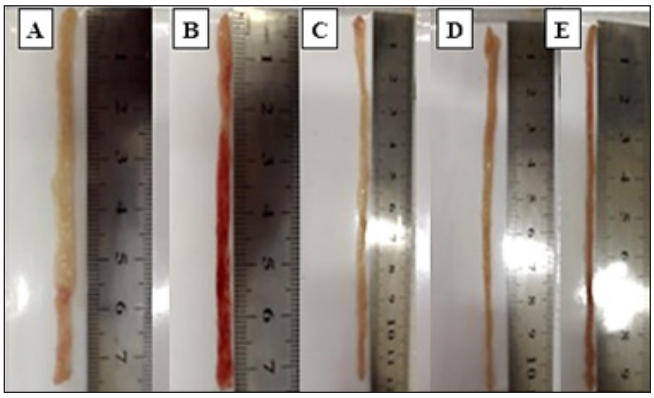

Figure 1.Macroscopic appearance of opened colons from various groups in the experimental colitis model in mice. A (non-colitic), B (colitic group), C (6.25mg/kg), D (12.5 mg/kg), E (25mg/kg)

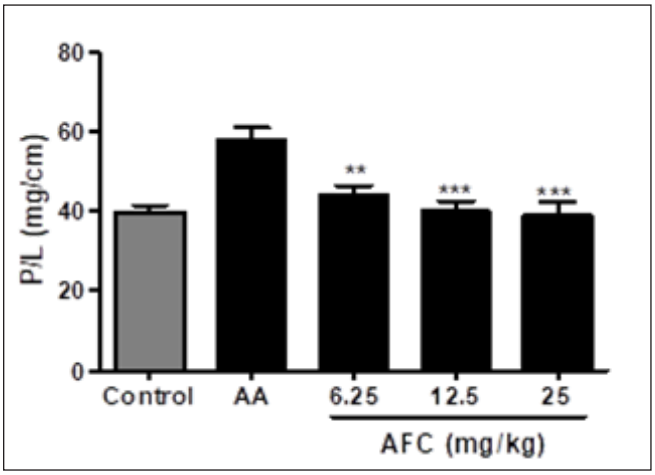

Figure 2.Effect of Fumaria capreolata alkaloid extract (6.25, 12.5, and 25 mg/kg) treatment on colon weight/length ratio in mice

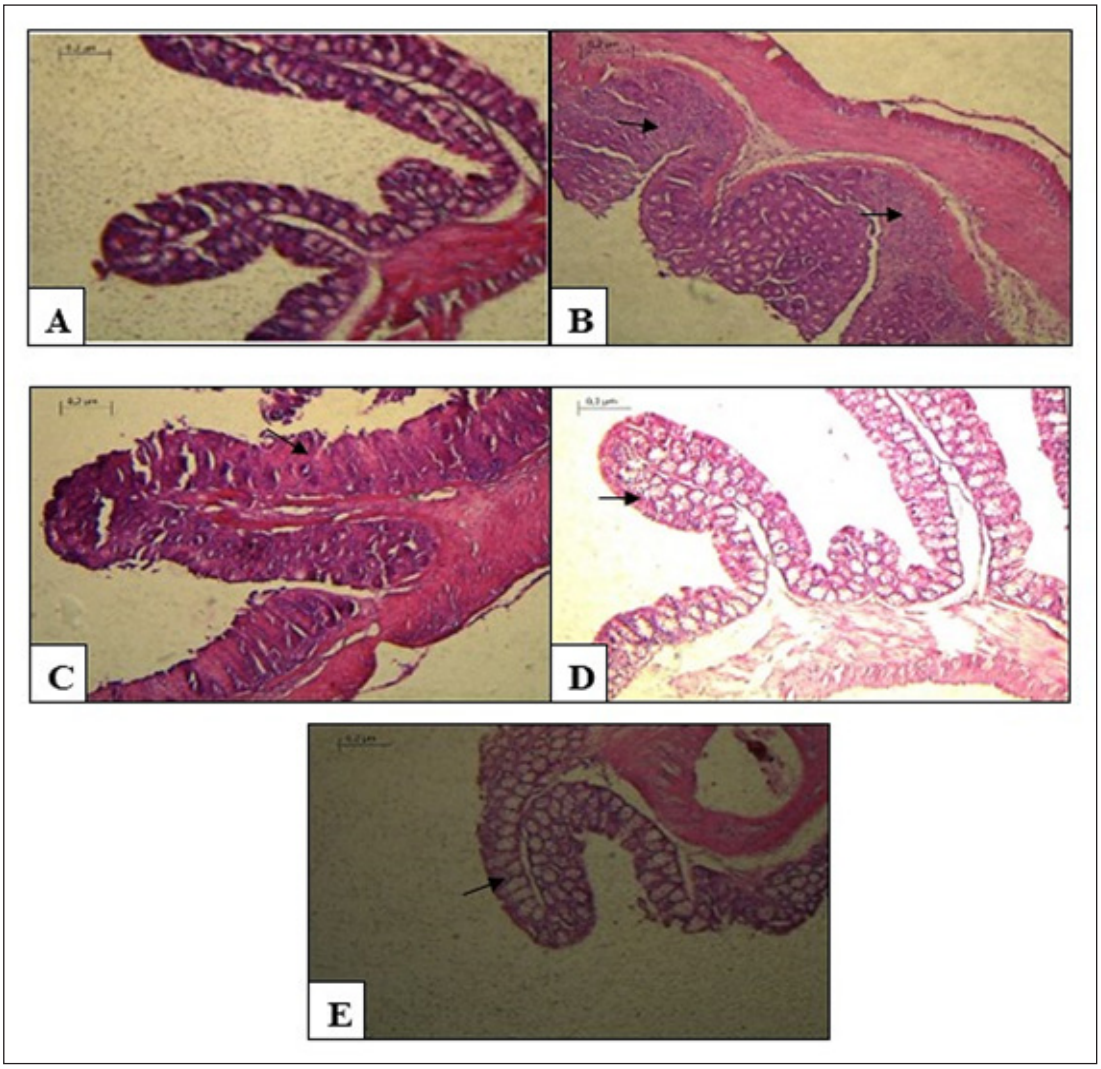

Figure 3. Effects of the total alkaloid fraction from Fumaria capreolata (AFC) in acetic acid induced colitis. Histological sections of colonic mucosa stained with hematoxylin and eosin (40 x magnification): Control group (A), acetic acid group (B), AFC (6.25, 12.5 and 25 mg/kg) (C-E respectively). AFC: Alkaloids of Fumaria capreolata

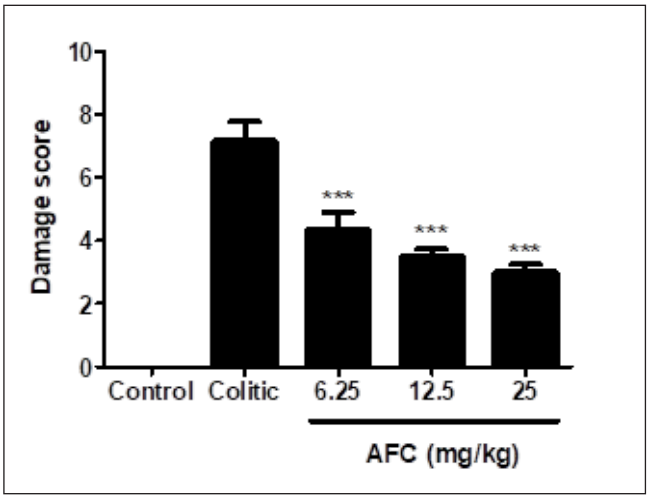

Figure 4.Effects of different doses (6.25, 12.5, and 25 mg/kg) of AFC extract on microscopic damage score in the acetic acid mice experimental colitis.

Discussion

The results of this study indicate that pre-treatment with the alkaloids from *Fumaria capreolata* exerts a preventative effect *in vivo.* Acetic acid leads to the release of protons, and intracellular acidification which causes damage to the intestinal barrier, epithelial damage, accompanied by bleeding, and the release of pro-inflammatory mediators involved in the increased permeability of the intestinal epithelial barrier during IBD. These effects appear quickly after one administration of acetic acid.[20] The mucus layer and intestinal epithelium, which preserve the separation between the gut lumen and the mucosal immune system, serve as the primary physical and chemical barriers against intestinal bacteria, food antigens and pathogens.[21] The intestinal epithelium is composed of a single layer of columnar epithelial cells held together by tight junctions.[22] The alteration of the architecture and composition of

tight junctions and the excessive production of oxidative species has several consequences during chronic intestinal inflammation because it causes an increase in intestinal water and electrolyte secretion and leads in the formation and development of ulcers.[6] The chemical barrier consists mainly of antimicrobial molecules which are synthesized by epithelial cells which destroy or inhibit the growth of bacteria and/or yeast. It is clear that persons with IBD must have barrier disruption. This observation is further supported by the microscopic image, which shows a decrease in goblet cells, in addition to the endoscopic view,[23] defective defensin production, decreased mucus layer thickness and increased permeability of the epithelial barrier,[24] causing direct contact of pathogenic bacteria and immune cells which leads to excessive activation of the immune system and the onset of chronic inflammation.[25] This explains the redness; pain; heat and swelling leading to morphological change in the colon and an increased weight/length ratio resulting in the formation of necrosis and fissures.

Appropriate characterization of the composition of AFC has revealed the existence of a combination of isoquinoline alkaloids, which have previously been identified as the main secondary metabolites.[13] *Fumaria capreolata* contains 23 molecules of isoquinoleic alkaloid. The two components which were identified according to previous study to the study are stylopine and protopine,[26] which exert immunomodulatory effects responsible for intestinal anti-inflammatory properties which have been shown to have no toxic effect on Bone Marrow Derived Macrophage cells (BMDM).AFC significantly alleviated both macroscopic and microscopic signs of intestinal inflammation in DSS-induced ulcerative colitis. Furthermore, it restored the colonic expression of both pro-inflammatory and anti-inflammatory mediators and enhanced the expression of intestinal barrier markers.[27] These results suggest that Fumaria capreolata, known for its richness in alkaloids, exerts a marked preventive intestinal anti-inflammatory activity *in vivo* judged by the general improvement in clinical condition of mice, significant reversal of the increase in the weight/length ratio and the attenuation of the histological lesions observed in the treated mice. However, additional research is needed to explore a broader dose range, understand the mechanisms of action, and conduct long-term toxicity studies.

Conclusion

In conclusion, this study demonstrates that the total alkaloid fraction from *Fumaria capreolata* exhibits significant preventive intestinal anti-inflammatory effects in a mouse model of acetic acid-induced colitis, which closely mimics human inflammatory bowel disease (IBD). These effects appear to be associated with the modulation of the intestinal immune response, as AFC effectively preserved intestinal barrier integrity, as evidenced by histological analysis. Therefore, the total alkaloid fraction of *Fumaria capreolata* shows promising potential for the prevention of IBD due to its anti-inflammatory properties.

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