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ORIGINAL ARTICLE

Ulcerative Colitis Induced with Acetic Acid is Ameliorated by *Antrocaryon micraster* through Reduced Serum Levels of Tumor Necrosis Factor Alpha and Interleukin-6 in Sprague Dawley Rats

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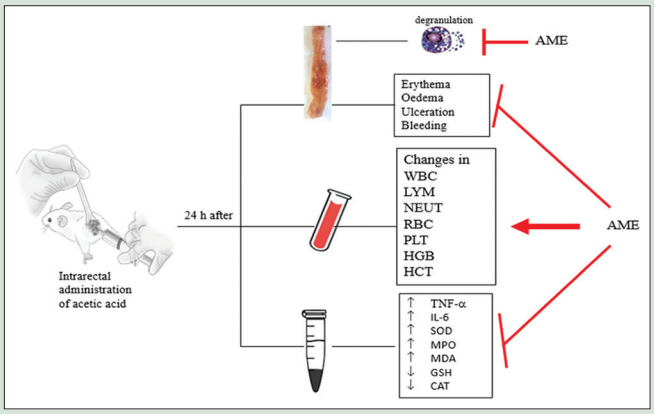
ABSTRACT

Background: *Antrocaryon micraster* possesses significant antioxidant and anti-inflammatory activities. These properties are shared by medicinal plants that have demonstrated beneficial effects in the treatment of ulcerative colitis. **Objectives:** To assess the effect of the total ethanol stem bark of *A. micraster* extract (AME) on damage to the rat colon in acetic acid-induced colitis. **Materials and Methods:** Rats were pretreated with sulphasalazine 500 mg/kg or AME 30, 100, and 300 mg/kg daily for 3 days after which colitis was induced in test animals with acetic acid (4% V/V). Rats were sacrificed 24 h later and blood samples were obtained for hematological and cytokine assays. Colons were dissected for assessment of macroscopic and microscopic damage. **Results:** AME treatment modified hematological parameters and reduced serum levels of tumor necrosis factor alpha and interleukin-6. Macroscopic assessment showed that AME reduced mucosal erythema, edema, erosions, bleeding, and ulceration. Histology showed preserved mucosal architecture, reduced inflammatory cell infiltration, decreased mucosal thickening, preserved goblet cell numbers, and inhibition of mast cell proliferation and degranulation in the colons. **Conclusion:** Taken together, the total ethanol stem bark AME exerts ameliorative effects on damage to the rat colon in acetic acid-induced colitis.

Key words: Acetic acid-induced colitis, *Antrocaryon micraster*, cytokines, inflammation, mast cells

SUMMARY

- The stem bark AME has ameliorative effect on acetic acid-induced colitis in rats. This is achieved through the extract's potential to modify the haematological and histopathological parameters while reducing serum expression of IL-6 and TNF.



Abbreviations Used: TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin 6; AME: *Antrocaryon micraster* extract; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; MPO: Myeloperoxidase; MDA: Malondialdehyde; ROS: Reactive oxygen species.

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INTRODUCTION

Ulcerative colitis is a chronic relapsing inflammatory condition that affects the mucosae of the rectum and colon. Although its exact etiology is still unclear, it is well established however that factors such as immunological, genetical, and environmental influences are largely implicated in the disease initiation as well as development.[1,2]

Transmural inflammation, epithelial ulceration, and tissue destruction characterize the disease.[3] The mucosae of the diseased colons are rife with inflammatory cells such as lymphocytes (LYMs), neutrophils (NEUTs), and macrophages. When these cells are activated, they produce excessive amounts of free radicals.[4] The generation of these reactive metabolites of oxygen and nitrogen results in the depletion of antioxidant defenses, oxidative stress and lipid peroxidation and ultimately cellular damage.[2] Another notable feature of the pathogenesis of ulcerative colitis is increased serum and mucosal amounts of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), a result of the effects of reactive oxygen species and reactive nitrogen species.[5] At the molecular level, these reactive species activate transcriptional factors which include nuclear factor kappa-light-chain-enhancer of activated B-cells and activator protein-1 which regulate expression of genes that encode for pro-inflammatory cytokines.[6] Production of TNF-α for instance then enhances the release of more of the reactive metabolites of oxygen and nitrogen and therefore sets up a vicious cycle of cytokine storm and oxidative stress.[7]

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Acetic acid-induced colitis produces an easily inducible and diffused colitis that shows clinical and histopathological features and a profile of inflammatory mediators that are similar to human ulcerative colitis.[8-10] The injury produced is confined to the area of contact with the acid and typically restricted to the mucosae of the colons. It is thought that there is a large deposition of protons (H+) in the intracellular space leading to a decreased pH. The increased acidity leads to an injury of the epithelial cells and a resultant detrimental inflammatory response associated with oxidative stress.[11,12] It stands to reason; therefore, an agent that reduces oxidative stress and/or the serum concentrations of pro-inflammatory cytokines would be important in the management of ulcerative colitis.[13,14] Indeed, the potential benefits of medicinal plants with anti-inflammatory and antioxidant properties in the therapy of ulcerative colitis is well documented.[15]

A. micraster, from our earlier work, was shown to have significant antioxidant and anti-inflammatory effects.[16] We could show that the administration of the ethanolic stem-bark extract reduced inflammatory cell infiltration and inflammation-associated tissue damage in a carrageenan-induced model of acute inflammation in experimental animals. *A micraster* reduced the expression of myeloperoxidase (MPO) and the level of malondialdehyde (MDA), exerting a significant antioxidant effect. Considering the involvement of these inflammatory events in the underlying mechanism of both experimentally induced and clinical colitis, we propose *A. micraster* as a potential candidate for screening against ulcerative colitis.

In this present study, we investigated the activity of *A. micraster* on damage to the colon and serum levels of the cytokines TNF-α and IL-6 in a 4% acetic acid-induced ulcerative colitis in rats.

MATERIALS AND METHODS

Materials

Sulphasalazine (Pfizer Inc, New York, USA), Rat TNF alpha (EK0526) and IL-6 (EK0412) Picokine™ ELISA kits (Boster Biological Technology, Pleasanton CA, USA), normal saline (Intravenous infusions ltd, Koforidua, Ghana), formalin (Kanoria Chemical and industries, Kolkata, India), hematoxylin and eosin (H and E) stain (Abcam, Cambridge, UK), Carnoy's solution fixative (Lab Alley, Austin, USA), toluidine blue (Thomas Scientific Co., NJ, USA), and acetic acid (Sigma-Aldrich Chemical Co, St Louis, USA) were purchased.

Animals

Healthy Sprague Dawley rats (200–250 g) of either sexes were acquired from the Animal House, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST). Rats were kept under hygienic conditions in wire mesh cages with wood shavings for bedding and allowed enough time to acclimatize. They were maintained at a 12-h light-dark cycle, fed on standard chow, and given clean water *ad libitum*. Throughout the experimental period, animals were handled humanely as directed by the Animal Welfare Regulations (USDA 1985; US Code, 42 USC § 289d) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS 2002). At the end of each experiment, rats were euthanized. The Ethics Committee of the Department of Pharmacology, KNUST gave approval and clearance for all rodent studies.

Methods

Preparation of Antrocaryon micraster extract

The stem bark of *Antrocaryon micraster* obtained from Kwahu Asakraka in the Eastern Region of Ghana (6°37'37.2072 N";0°41'23.0352"W) in February 2015 was identified and authenticated by Professor Abraham Yeboah Mensah of the Department of Pharmacognosy, KNUST. A specimen with voucher number (KNUST/HM1/2017/SB006) was deposited at the Department's herbarium. The plant material was air-dried and milled to a powder. Eight hundred and forty grams (840.0 g) of the powder was extracted with 2.5 L ethanol (70% v/v) by cold static maceration for 3 days. The filtrate was concentrated in a rotary evaporator (R-210, BUCHI Labortechnik AG, Flawil, Switzerland) and oven-dried at 50°C to obtain a paste with a yield of 7. 46% w/w. When needed, the extract was reconstituted in normal saline (0.9% NaCl) and referred to as *A. micraster* extract (AME) used in the experiments.

Induction of colitis

Rats were randomized into 6 groups (n = 5). Intervention groups received p. o. either normal saline 5 ml/kg, sulphasalazine 500 mg/kg, or AME (30, 100, and 300 mg/kg) daily for 3 days. The rats were deprived of water and food for 24 h, slightly anesthetized with ether after which bowel emptying was established via administration of 1 mL normal saline at 37°C intrarectally. While holding the animal in a head-down position, 1 mL acetic acid (4% v/v) was administered by the intrarectal route to induce colitis as previously described by MacPherson and Pfeiffer[9] after which rats were sacrificed 24 h later. Hematology, serum cytokine levels, colon weight-to-length ratio and macroscopic damage to the colon, histopathology, and mast cell stability were evaluated as described below.

Hematological assessment

Counts for total and differential leukocytes (white blood cell [WBC], LYM, NEUT), erythrocyte (red blood cell [RBC]), platelet (PLT) as well as estimation of hemoglobin (HGB) concentration and hematocrit (HCT) assessment were done with an automated hematology analyzer (KX-21N, Sysmex America Inc, Illinois, USA) on blood samples collected.

Assay of serum cytokines, tumour necrosis factor alpha and interleukin-6

To obtain sera, blood samples were centrifuged (Heraeus Megafuge 16 R, Thermo Scientific, Massachusetts, United States) at 600×g for 30 min at 4°C. According to the manufacturer's instructions, sera were assayed quantitatively for TNF-α and IL-6 using Rat TNF-α and IL-6 ELISA kits, respectively.

Colon weight-to-length ratio determination and extent of macroscopic damage on colon

Rats were dissected, and the distal 10 cm of the colon was excised and opened by longitudinal incisions. The mucosa weight was noted after it had been washed with normal saline to remove all fecal residues. The weight-to-length ratio (wet weight) was calculated as an index of disease-associated intestinal wall thickening.[17] The grading scale of Millar *et al.*[18] was adopted for macroscopic assessment of mucosal injury with a 5-point scale based on clinical presentation of the colon graded as follows: no macroscopic changes (score 0), mucosal erythema only (score 1), mild mucosal edema, slight bleeding or small erosions (score 2), moderate edema, slight bleeding ulcers or erosions (score 3), and severe ulceration, edema and tissue necrosis (score 4).

Histology (extent of microscopic damage on colon)

Segments of the colons were fixed in 10% formalin and embedded in paraffin. Transverse sections of 5 μm thickness were cut using a microtome (RM 2125 RTS, Leica Biosystems, GmBH, Wetzlar, Germany), mounted on slides, stained with H and E and viewed under a digital light microscope (DM 750, Leica Microsystems, GmBH, Wetzlar, Germany) fitted with a digital camera ICC 50 HD, Leica Microsystems, GmBH, Wetzlar, Germany). The degree of histological insult to the colon was graded using a cumulative semiquantitative scale of 0–11: loss of mucosal

architecture (0–3), cellular infiltration (0–3) muscle thickening (0–3), crypt abscess formation (0–1), and goblet cell depletion (0–1).[19]

Mucosal mast cell stability

Segments of the colons were fixed in Carnoy's fixative and embedded in paraffin. Transverse sections of 5 μm thickness were stained with 1% toluidine blue (pH 4.0) and examined under a light microscope for mast cell count. The total number of intact and degranulated mast cells were counted in 5 randomly selected fields.

Statistics

Data was analyzed with GraphPad Prism for Windows version 6.01 (GraphPad Software Inc, San Diego, CA, USA) and presented as mean ± standard error of mean. Differences between treatment groups were determined with one-way analysis of variance complemented with Dunnett's *post hoc* test. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of *Antrocaryon micraster* extract on hematological parameters

Hematological analysis of the naïve control animals showed a total WBC count of 4.5 ± 0.6 × 10^3 cells/μL, a LYM count of 3.7 ± 0.2 × 10^3 cells/μL, and a NEUT count of 0.4 ± 0.5 × 10^3 cells/μL as baseline levels of the WBC indices [Table 1]. The colitic control group showed significantly elevated levels of the white cell indices with a total WBC count of 17.6 ± 3.2 × 10^3 cells/μL, contributed to by a high LYM count of 14.8 ± 0.3 × 10^3 cells/μL, consistent with the chronicity of the condition and a NEUT count of 2.0 ± 0.4 × 10^3 cells/μL. At 100 and 300 mg/kg, AME-treated groups significantly reduced levels of the total WBC to 7.7 ± 1.3 × 10^3 cells/μL and 7.4 ± 1.2 × 10^3 cells/μL, respectively. LYM counts were also significantly reduced to 10.3 ± 0.9 × 10^3 cells/μL, 6.9 ± 1.4 × 10^3 cells/μL, and 6.3 ± 1.3 × 10^3 cells/μL, respectively, while NEUT counts of 0.3 ± 0.1 × 10^3 cells/μL, 0.4 ± 0.2 × 10^3 cells/μL, and 0.3 ± 0.1 × 10^3 cells/μL for the three doses of AME used, respectively, were obtained. However, no statistically significant changes in RBC, PLT, HGB, and HCT were noted [Table 1]. Sulphasalazine significantly reduced the elevated white cell indices compared to acetic acid control with no significant change in the other hematological indices [Table 1].

Effect of *Antrocaryon micraster* extract on serum levels of tumour necrosis factor alpha and interleukin-6

The serum level of TNF-α in the naïve control group of 16.3 ± 3.9 pg/mL was significantly increased in the acetic acid-induced colitic control group to 84.0 ± 2.9 pg/mL [Figure 1a]. This elevated concentration was significantly reduced with AME administered at 30, 100, and 300 mg/kg – 63.2 ± 3.8 pg/mL, 64.0 ± 3.3 pg/mL, and 52.2 ± 3.7 pg/mL, respectively, representing percentage reductions of 24.7% ± 3.8%, 23.8% ± 3.3%, and 37.9% ± 3.7%, respectively. Sulphasalazine reduced serum concentration of TNF-α to 46.7 ± 4.1 pg/mL, a percentage reduction of 55.6% ± 2.1% [Figure 1a]. On quantification, the serum concentration of IL-6 in the naïve control group was 3.7 ± 1.1 pg/mL, while the acetic acid-induced disease control group had a significantly increased level of 36.1 ± 3.0 pg/mL [Figure 1b]. At the same doses of AME used, there was significantly reduced serum concentrations of IL-6 to 26.6 ± 1.4 pg/mL, 22.1 ± 1.7 pg/mL, and 18.9 ± 1.5 pg/mL representing reduction of 26.4 ± 3.9%, 38.7 ± 4.8%, and 47.5 ± 4.0%, respectively, compared to acetic acid-induced colitic control. Sulphasalazine significantly reduced serum concentration of IL-6 to 17.5 ± 1.8 pg/mL, a reduction of 51.6% ± 4.8% [Figure 1b].

Effect of *Antrocaryon micraster* extract on colon weight-to-length ratio in rat acetic acid-induced colitis

The mean colon weight-to-length ratio of 80.5 ± 8.5 g/cm for the naïve control group was significantly increased to 163.5 ± 15.5 g/cm when acetic acid was used to induce colitis in control animals [Figure 2]. AME-treated groups had mean colon weight-to-length ratio of 127.5 ± 7.5 g/cm, 118.5 ± 6.5 g/cm, and 88. 5 ± 3.5 g/cm, respectively, at 30, 100, and 300 mg/kg extract treatment albeit significant only with the 300 mg/kg dose. Compared to the colitic control group, sulphasalazine-treated rats had a significantly reduced mean colon weight-to-length ratio of 63.5 ± 10.5 g/cm [Figure 2].

Effect of *Antrocaryon micraster* extract on macroscopic damage in colons of acetic acid-induced colitic rats

The colons of the noncolitic control animals showed normal mucosa with no signs of hemorrhage or ulcerations [Figure 3a1] while that of the acetic acid-induced colitic control animals showed diffuse colitis characterized by extensive hyperemia and hemorrhagia [Figure 3a, B1]. The mucosae of the colons of the disease control animals had erosions, ulcerations, and necrosis along a greater length of the segment of the colon [Figure 3a, B2]. Treating rats prophylactically with AME at 30–300 mg/kg ameliorated colonic damage with relatively reduced erosion, ulceration, hemorrhagia, and no necrosis of the mucosa [Figure 3a, D-F]. Sulphasalazine reduced ulceration and erosion in the colons when compared to the colitic control [Figure 3a, C]. When quantified, a significantly increased mean macroscopic score

Table 1: Effect of *Antrocaryon micraster* extract on hematological parameters

Parameter	Naïve control	Acetic acid-induced colitic control	Sulphasalazine (500 mg/kg)	AME (mg/kg)		
				30	100	300
WBC×10^3 (cells/μL)	4.5±0.6	17.6±3.2$^{\#\#}$	9.3±0.6*	11.4±1.3	7.7±1.3**	7.4±1.2**
LYM×10^3 (cells/μL)	3.7±0.2	14.8±0.3$^{\#\#\#}$	8.7±0.5*	10.3±0.9*	6.9±1.4**	6.3±1.3**
NEUT×103 (cells/μL)	0.4±0.5	2.0±0.4$^{\#\#}$	0.7±0.3*	0.3±0.1**	0.4±0.2**	0.3±0.1**
RBC×106 (cells/μL)	6.9±0.9	6.6±0.2	7.0±0.3	7.2±0.2	7.2±1.1	7.5±1.1
PLT×103 (cells/μL)	650.0±79.5	845.0±17.5	720.0±43.0	697.0±15.0	678.0±48.0	779.0±52.5
HGB (g/dL)	12.3±1.7	11.7±0.4	12.3±0.6	13.6±0.1	13.2±0.9	13.7±1.4
HCT (%)	40.5±5.4	38.4±2.9	41.0±0.7	44.2±1.4	43.5±4.4	45.0±5.6

Rats received either normal saline, sulphasalazine 500 mg/kg or AME (30, 100, and 300 mg/kg p.o) for 3 days. Colitis was induced as described in the methods. Blood samples were obtained for hematological assessment after 24 h. Data is presented as mean±SEM. (n=5). *P<0.05 and **P<0.01 compared to the disease control group, $^{\#\#}P$<0.01 and $^{\#\#\#}P$<0.001 compared to the naïve control (One-way ANOVA followed by Dunnett's *post hoc* test). WBC: White blood cell; LYM: Lymphocyte; NEUT: Neutrophil; RBC: Red blood cell; PLT: Platelet; HGB: Hemoglobin; HCT: Hematocrit; AME: *Antrocaryon micraster* extract; ANOVA: Analysis of variance; SEM: Standard error of mean

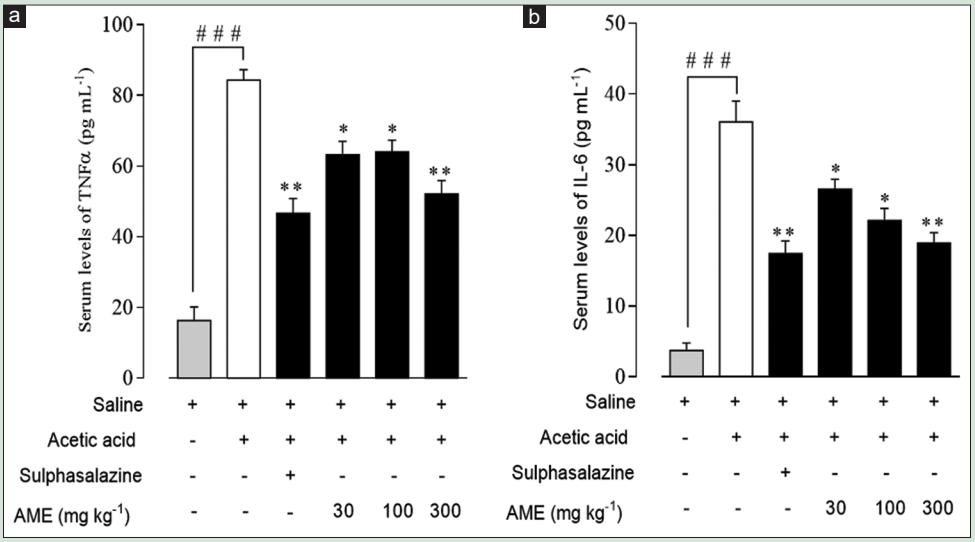

Figure 1: Effect of *Antrocaryon micraster* extract on serum levels of Tumor necrosis factor alpha and IL-6. Rats received daily administration of either normal saline, sulphasalazine 500 mg/kg, or *Antrocaryon micraster* extract (30, 100, and 300 mg/kg p.o) for 3 days. Colitis was induced as described in the methods. Blood samples were obtained after 24 h and the sera assayed for levels of tumor necrosis factor alpha (panel a) and IL-6 (panel b). Data are presented as mean ± standard error of mean ($n = 5$). *$P < 0.05$ and **$P < 0.01$ compared to acetic acid control group (AA); ###$P < 0.001$ compared to naïve control (one-way analysis of variance followed by Dunnett's *post hoc* test)

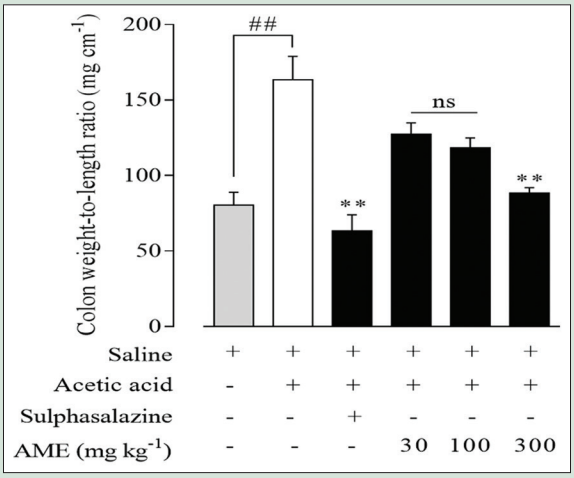

Figure 2: Effect of *Antrocaryon micraster* extract on colon weight-to-length ratio in rat acetic acid-induced colitis. Rats received either normal saline, sulphasalazine 500 mg/kg, or *Antrocaryon micraster* extract (30, 100, and 300 mg/kg p.o) for 3 days. Colitis was induced as described in the method. Colons were removed after 24 h and the weights and lengths taken. Data are presented as mean ± standard error of mean ($n = 5$). **$P < 0.01$ compared to the acetic acid control group (AA); ##$P < 0.01$ compared to the naive group (one-way analysis of variance followed by Dunnett's *post hoc* test). NS not statistically different

of 3.8 ± 0.3 in the acetic acid-induced disease group was obtained relative to zero scored for the naïve control group [Figure 3b]. Administration of 100 and 300 mg/kg *A. micraster* significantly reduced the mean macroscopic scores to 2.0 ± 0.4 and 1.5 ± 0.3, respectively, when compared to the colitic control. However, at 30 mg/kg, the mean macroscopic score of 2.8 ± 0.6 was not significant when compared to the colitic control group [Figure 3b]. Sulphasalazine as expected significantly reduced the mean macroscopic score to 1.0 ± 0.4 [Figure 3b].

Effect of *Antrocaryon micraster* extract on microscopic injury in acetic acid-induced colitis in rats

Microscopic observation of the H and E-stained sections of the colons of the noncolitic-naïve control animals revealed normal mucosal architecture with normal wall thickness and no inflammatory cell infiltrates [Figure 4a, A]. On the other hand, the mucosa of the colitic control animals showed distorted architecture with ulceration and focal areas of complete mucosal loss, severe cellular infiltration with thickened muscles, and goblet cell depletion [Figure 4a, B]. AME-treated rats at 30–300 mg/kg showed dose-dependent preservation of mucosal architecture, reduced inflammatory cell infiltration, decreased mucosal thickening, and preserved goblet cell numbers compared to colitic control animals [Figure 4a, D-F]. Oral administration of 500 mg/kg Sulphasalazine protected against colon damage induced with acetic acid. There was reduced number of cell infiltration with no loss of mucosa architecture, muscle thickening crypt abscess formation, or goblet cell depletion [Figure 4a, C]. On quantification, the acetic acid-induced colitic control group had a significantly increased mean microscopic score of 9.3 ± 0.7 relative to the naïve control group [Figure 4b]. AME-treated groups when compared to the acetic acid-induced disease control rats had significantly reduced mean microscopic scores of 5.0 ± 0.7, 3.0 ± 0.6, and 1.7 ± 0.3 at 30, 100, and 300 mg/kg, respectively. Sulphasalazine-treated group had a significantly reduced mean microscopic score of 1.0 ± 0.6 when compared to the disease control group [Figure 4b].

Effect of *Antrocaryon micraster* on mucosal mast cell stability

Both intact and degranulated mast cells were observed when toluidine blue dye was used to stain the colon sections from all the treatment groups [Figure 5a]. On quantification, the colon sections of the noncolitic group had mean degranulated mast cells of 1.6% ± 0.5% while those of the colitic control group had a mean of 55.6% ± 2.9%. Administration of AME dose dependently inhibited mast cell degranulation to mean values of 42.2% ± 3.2%, 38.8% ± 2.4%, and 38.6% ±1.9%, respectively,

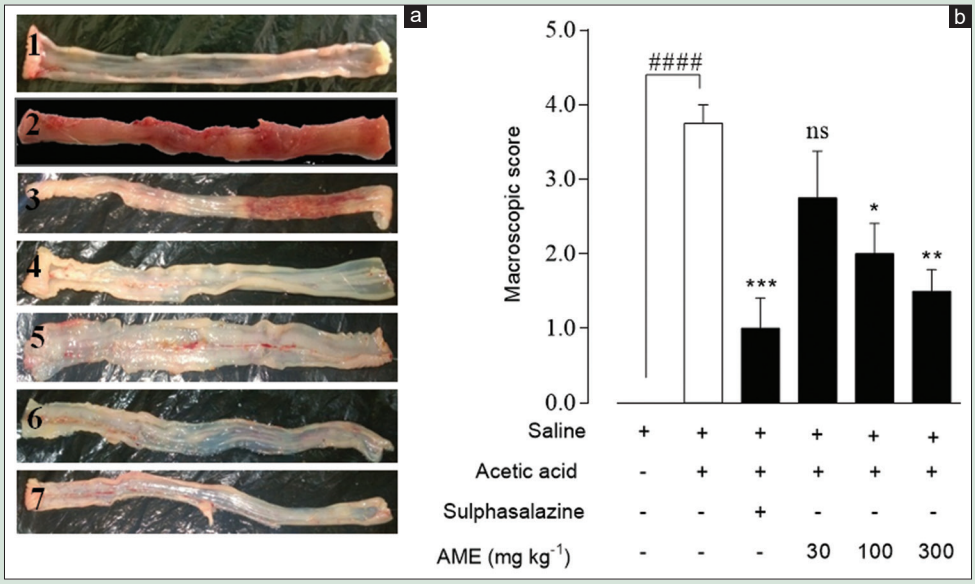

Figure 3: Effect of *Antrocaryon micraster* extract on macroscopic damage in colons of acetic acid-induced colitic rats. Sprague Dawley rats received either normal saline, sulphasalazine 500 mg/kg, or *Antrocaryon micraster* extract (30, 100, and 300 mg/kg p.o) for 3 days. Colitis was induced as described in the method. Colons were removed after 24 h, opened by longitudinal incision and photographed (a). 1: Naive control, 2: Colitic control showing diffuse erosion, 3: Colitic control showing necrosis, 4: Sulphasalazine 500 mg/kg, 5-7: *Antrocaryon micraster* extract 30, 100, and 300 mg/kg, respectively, and quantified (b). Data are presented as mean ± standard error of mean ($n = 5$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to the acetic acid control group; ####$P < 0.001$ compared to the naive group (One-way analysis of variance followed by Dunnett's *post hoc* test). NS not statistically different

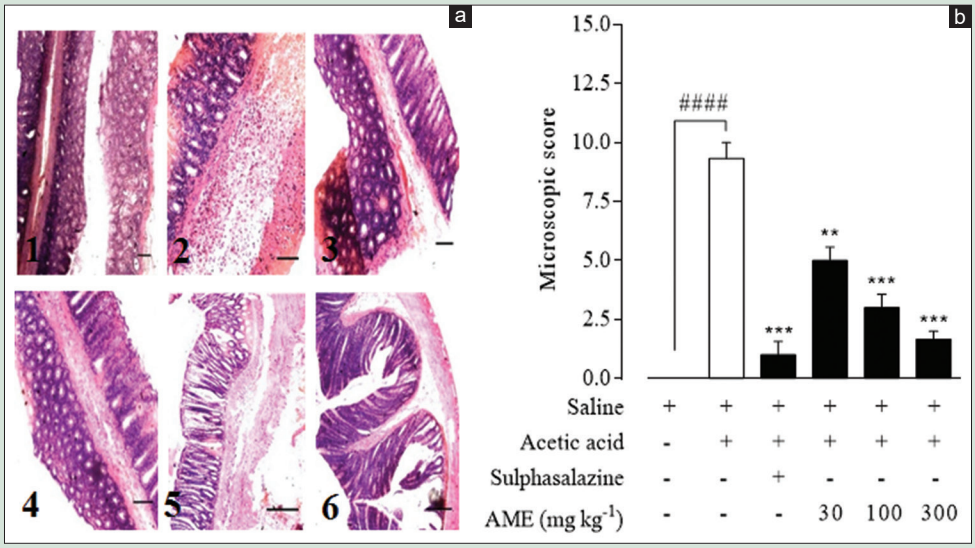

Figure 4: Effect of *Antrocaryon micraster* extract on microscopic injury in acetic acid-induced colitis in rats. Sprague Dawley rats received either normal saline, sulphasalazine 500 mg/kg, or *Antrocaryon micraster* extract (30, 100, and 300 mg/kg p.o) for 3 days. Colitis was induced as described in the method. Colons were removed after 24 h and photomicrographs obtained at ×400 (a). 1: Naive control, 2: Colitic control, 3: Sulphasalazine 500 mg/kg, 4-6: 30, 100, 300 mg/kg *Antrocaryon micraster* extract, respectively, and quantified for extent of microscopic injury (b). Data are presented as mean ± standard error of mean ($n = 5$). **$P < 0.01$ and ***$P < 0.001$ compared to the acetic acid control group (AA); ####$P < 0.001$ compared to the naive group. (One-way analysis of variance followed by Dunnett's *post hoc* test). Scale bar represents 200 μm of tissue

at 30–300 mg/kg. Sulphasalazine-treated rats showed a significantly reduced mast cell degranulation of 38.6% ± 1.9% [Figure 5b].

DISCUSSION

In this study, evaluation of the activity of the ethanolic extract of *A. micraster* on acetic acid-induced colitis was made. This is a reproducible model that shows similar histological characteristics to ulcerative colitis and thus a useful model for the study of the disease mechanisms.[20] Ulcerative colitis is a chronic inflammatory condition largely restricted to the colon and rectum and has a high disease burden.[21] With no established etiology documented, current evidence however proposes a link with the immune, genetic, and environmental factors.[22] Induced immune responses increase vascular permeability to enhance the release of inflammatory cells and mediators such as pro-inflammatory

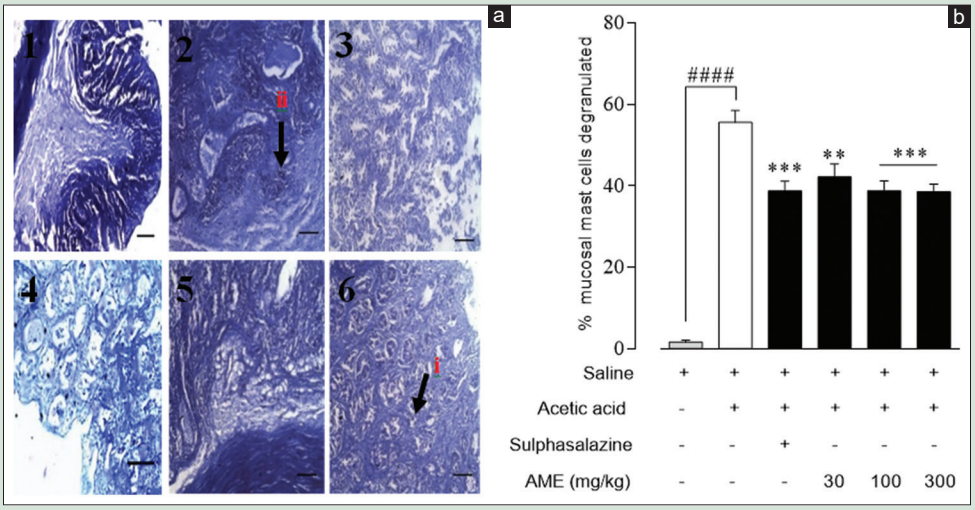

Figure 5: Effect of *Antrocaryon micraster* on mucosal mast cell stability. Rats received daily administration of either normal saline, sulphasalazine 500 mg/kg, or *Antrocaryon micraster* extract (30, 100, and 300 mg/kg) for 3 days. Colitis was induced as described in the method. Colons were removed after 24 h and stained with toluidine blue. Photographs were obtained at ×400. (a) Representative photomicrographs of noncolitic control (1), acetic acid control (2), sulphasalazine 500 mg/kg (3), *Antrocaryon micraster* extract 30 mg/kg (4), *Antrocaryon micraster* extract 100 mg/kg (5), and *Antrocaryon micraster* extract 300 mg/kg (6). Percentage degranulated mast cells were calculated (b). Data are presented as mean ± standard error of mean ($n = 5$). $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the acetic acid control group (AA); $^{\#\#\#\#}P < 0.001$ compared to the naive group (One-way analysis of variance followed by Dunnett's *post hoc* test). i: Intact mast cell, ii: Degranulated mast cell. Scale bar represents 200 μm of tissue

cytokines which effectively further recruit more cells to the site of tissue injury. The presence of these numerous cells leads to an augmented production of cytokines such TNF-α and IL-6.[23] There is also the release of oxygen-derived free radicals which are associated in disease development.[24] The significant roles played by the pro-inflammatory responses and oxidative stress in the etiology of ulcerative colitis makes these processes great research interests, especially in the screening of the effects of natural products on colitis. An agent that has both anti-inflammatory and antioxidant effects will therefore have synergistic effects in the management of ulcerative colitis.[14]

Medicinal plants that offer protection against ulcerative colitis have been shown to have significant antioxidant activity through reduction of oxidative stress to augmenting antioxidant defenses. They also demonstrate anti-inflammatory activity by reducing serum levels of pro-inflammatory cytokines.[15]

Pre-treatment with AME offered protection against mucosal injury showed by improved macroscopic and microscopic scores. This was not particularly surprising since AME was previously shown to augment the antioxidant capacity of tissues in a carrageenan-induced pleurisy study where we demonstrated that AME exerts significant antioxidant effects. We could show in that study that AME increased the activities of the antioxidant enzymes superoxide dismutase and catalase and the level of Glutathione, while reducing the activity of MPO (consistent with the reduction of inflammatory cell infiltrates in the colon tissues, as MPO is an index of NEU count, a component of the WBC) and the level of lipid peroxidation measured as MDA.[16] This property is the key to the observed protective effect of AME in colitis since the overproduction of ROS and the resultant oxidative stress is a notable factor in the pathogenesis of ulcerative colitis.[2,15] Ulcerative colitis tends to lead to increased blood loss, the bowel ulceration leading to decreased RBC, PLT, HGB, and HCT. With AME treatment, there are no significant changes in RBC, PLT, HGB, and HCT from the baseline which can be attributed to the potential of AME to prevent increased blood loss from the colonic ulcers induced with acetic acid.

Attenuation of cellular infiltration and edema shown by decreased weight-to-length ratio confirms the inhibitory effect of AME on vascular events in the inflammatory process reported on in our previous publication.[16] The proliferation of mast cells and the increased release of their granular contents is implicated in the etiology of colitis.[25,26] Mast cells are located within the gut tissue and express mediators of the inflammatory process such as histamine, cytokines, and arachidonic acid metabolites.[27] Microscopic analyses of toluidine blue-stained sections of the colons of AME-treated rats showed fewer numbers of degranulated mast cells compared with colons of the colitic control group, indicating that the extract confers a mast cell-stabilizing effect in the study model. Iba *et al.*[28] demonstrated that a reduction in mast cell proliferation and degranulation may play an important role in the recovery process of an induced colitis model. Again, the role of mast cell in the symptoms of the disease model was shown by La *et al.*[29] where the rate of degranulation was correlated with severity of symptoms. Thus, the inhibitory effect of AME on mast cell degranulation partly accounts for the extract's ability to mitigate the injurious effect of acetic acid.

Oversecretion of TNF-α and IL-6 have been implicated in the pathogenesis of colitis, and the levels of these cytokines correlate with the grade of inflammation and disease activity.[30-32] These cytokines induce the production and activation of pro-inflammatory factors which eventually lead to exacerbation of the condition. Administration of AME significantly reduced the serum expression of these cytokines with a resultant decrease in the severity of the disease in this study. Our finding suggests that the ameliorative effect of AME is partly due to the reduction in the serum levels of the measured pro-inflammatory cytokines. Another notable feature of acetic acid-induced colitis is the depletion of goblet cells[33] which secrete mucin that serves as a protective barrier to the intestinal epithelium. When this role is compromised, the protective barrier is removed, predisposing the epithelium to further damage.[33,34] The histopathological analysis revealed an inhibition of the depletion of goblet cells with AME administration. This beneficial effect suggests that AME preserved the epithelium of the colons through sustaining goblet cell numbers and activity.

We reported previously that phytochemical analyses showed that *A. micraster* contained secondary metabolites such as saponins, alkaloids, triterpenoids, and tannins,[16] and therefore, it is not surprising that it exhibited anti-inflammatory activity in various disease models of

inflammation.[35-38] Preliminary characterization of the extract indeed confirms the presence of phenolic compounds. While our data could only predict the nature of the compounds, the exact structures of the compounds that are present in AME are beyond the scope of our work.

CONCLUSION

Taken together, the data from this present study demonstrate that AME exerts protective effects against colitis via mechanisms of reducing serum TNF-α and IL-6 levels and free radical scavenging given its antioxidant properties.

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Conflicts of interest

There are no conflicts of interest.

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