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Acetic acid-induced ulcerative colitis alleviation by *Xylopia aethiopica* (Dunal) A. Rich in Sprague Dawley rats

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ABSTRACT

Ulcerative colitis is a chronic idiopathic inflammatory disorder characterized by immune system dysregulation, with oxidative stress and humoral immunity significantly involved. While many advances in therapy exist, ongoing challenges highlight the need for new therapies. Medicinal plants, like *Xylopia aethiopica*, show promise due to their ability to modulate inflammation, reduce oxidative stress, and enhance tissue healing, all with fewer side effects and lower costs. This study sought to investigate the chemo-profile of the aqueous ethanol extract of the dried fruit of *X. aethiopica* and its alleviating potential of ulcerative colitis induced with acetic acid in rats. After extraction of the dried fruit of *X. aethiopica*, HPLC finger print was generated using established method. Sprague Dawley rats were treated with *Xylopia aethiopica* extract at doses of 30, 100 and 300 mg/kg for 8 days with colitis being induced on the 4th day of treatment, using normal saline and 500 mg/kg sulphasalazine as naïve and positive controls, respectively. The different compounds identified in the dried fruit extract of *X. aethiopica* were seven bands. Findings from this study also showed that treatment with *X. aethiopica* extract suppresses macroscopic and histologic damage, improves mucosal regeneration by reducing silver-stained nucleolar organizer regions, and significantly increases antioxidant enzymes; ascorbate peroxidase, catalase and superoxide dismutase. Thus, the hydroethanolic dried fruit extract of *Xylopia aethiopica* is effective in acetic acid-induced ulcerative colitis and may serve as alternate therapy or source of drug leads in the treatment of ulcerative colitis and this may be attributed to a myriad of compounds in the extract.

Background

Ulcerative colitis (UC), a type of inflammatory bowel (IBD) disease that affects the colon and rectum, is believed to occur as a result of a dysregulated interaction between the gut's immune system and its microbial flora, coupled with an impaired epithelial membrane that facilitates damages in the gut mucosa [1–3]. The disease is usually limited to the rectum but can extend proximally to the colon. Except for backwash ileitis, UC does not have small bowel involvement but may extend to the pancreas or the hepatobiliary system [4]. Characteristic findings of the disease include a relapsing-remitting inflammation with leucocytic infiltration of the lamina propria and crypts where microabscesses can be seen as the disease progresses [4–6].

In addition, increased mast cell activity in the gut exacerbates the inflammatory response and contributes to the recruitment of other cells, thus amplifying the inflammatory response [7,8]. The amplified

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inflammatory response is usually accompanied by a decrease in the activity of antioxidant enzymes and elevation in malondialdehyde (MDA), a marker of oxidative damage. Muscarà *et al.* [9] enhanced the understanding of UC through histopathological analyses using AgNOR staining. In their work and in the context of UC, an increase in AgNOR expression often correlates with heightened cellular activity as the damaged mucosa attempts to regenerate.

Current treatment approaches for UC primarily focus on inducing remission by reducing inflammation through the use of corticosteroids, aminosalicylates, and biological agents [10,11]. While these treatment strategies have improved disease outcome for most patients, they are often associated with significant limitations. For example, corticosteroids are effective in reducing inflammation but are linked to severe side effects such as osteoporosis, hyperglycaemia, and increased infection risk on chronic use. Aminosalicylates can also cause adverse reactions, including nephrotoxicity, hepatotoxicity, and increased susceptibility to infections. Additionally, biologics are expensive and can lose efficacy over time due to the development of antibodies, leading to treatment failure in a substantial subset of patients [11,12].

Given these limitations, there is a growing interest in the search for alternative therapies. In the search of new medicines, plants have been shown to offer potential novel therapeutic approaches often associated with fewer side effects and reduced cost [13,14]. One such plant of interest is *Xylopia aethiopica*, a plant rich composition of bioactive substances such as alkaloids, flavonoids, tannins, and terpenoids and traditionally used for numerous inflammatory diseases [15]. These phytochemicals have been shown to possess potent antioxidant, anti-inflammatory, and antimicrobial properties, making the plant a promising candidate for the treatment of inflammatory conditions such as UC.

Moreover, *Xylopia aethiopica* has been shown to modulate inflammatory pathways, reduce oxidative stress, and promote tissue healing [15,16]. These effects are particularly relevant in the context of UC, where inflammation and oxidative damage are central to the disease progression. Previous research by Osafo *et al.* [17] showed that, the compound xylopic acid (XA), isolated from *Xylopia aethiopica* demonstrated anti-colitis activity in rats induced with acetic acid colitis. While this isolated compound's activity has been tested in UC models, the crude extracts activity in experimental colitis remains unknown. Given this, the present study seeks to explore the activity of the hydroethanolic fruit extract of *Xylopia aethiopica* in acetic-acid induced colitis.

Materials and methods

Materials

Animals

Sprague Dawley rats (200 – 250 g) were obtained from the Centre for Plant Medicine Research, Akuapem-Mampong, Ghana, and maintained under standard laboratory conditions with alternating 12 h light-dark cycle with free access to food and water. All animal handling were done in accordance with the principles set by the UK Animals (Scientific Procedures) Act 1986 and National Research Council Guide for the Care and Use of Laboratory Animals (2011). Findings from this study are reported in adherence with the ARRIVE guidelines 2.0 to ensure transparent and thorough reporting [18].

Drugs and chemicals

Ascorbic acid from Holland and Barrett, Warwickshire, England; trichloroacetic acid from Amresco®, Solon, USA; haematoxylin and eosin Y from Abbey Color, Philadelphia, USA; liquid paraffin from KAMA Pharmaceutical Industries, Accra, Ghana; toluidine blue, xylene, picric acid, dipotassium monohydrogen phosphate, monosodium phosphate, tris(hydroxymethyl) aminomethane, o-dianisidine dihydrochloride, and pentobarbitone sodium, EDTA, formaldehyde, pyridine hydrochloride, thiobarbituric acid, tween-80, silver nitrate from Sigma-Aldrich Chemical Co., St. Louis, USA; sulphasalazine from Pfizer Inc., New York, USA; hydrogen peroxide from Bell's Healthcare, Cheshire, England; bluing solution from IHC World LLC, Maryland, USA; gelatin from Frey Scientific, Greenville, WI, USA.

Plant collection and authentication

The dried fruits of *Xylopia aethiopica* were obtained from the open market in Kumasi, Ghana. The fruits were authenticated using the Plant list (www.theplantlist.org) after which a voucher specimen was kept at the herbarium of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana (Specimen number: FP/09/77).

Extraction of plant material

About 3 kg of the dried fruit was milled and extracted by maceration with 5 L of 70 % v/v ethanol in a percolator. A filtrate was obtained after 5 days and concentrated using a vacuum rotary evaporator (R-210, BUCHI, Switzerland). A solid mass weighing 170 g was obtained which was emulsified using tween-80 (at a final concentration of 0.4 %) to obtain the administered extract (i.e., XAE) and was orally given to the treatment groups in the current study.

Preliminary phytochemical screening

The presence of alkaloids, sterols, saponins, glycosides, tannins, and flavonoids were determined using qualitative phytochemical analysis.

Alkaloids

The presence of alkaloids was assessed by adding 0.5 g of the XAE to 10 ml of dilute hydrochloric acid, followed by boiling the mixture for 5 min. After cooling, the solution was filtered, and a few drops of Dragendorff's reagent were added to the clear supernatant. The appearance of a reddish-orange precipitate was indicative of the presence of alkaloids [19,20].

Sterols

The presence of sterols in XAE was assessed using the Liebermann–Burchard test. Briefly, 100 mg of XAE was dissolved in 2 ml of chloroform, followed by the addition of 2 ml of acetic anhydride and 1 ml of concentrated sulphuric acid. The appearance of a blue-green colouration indicated the presence of sterols [21].

Saponins

Saponins were detected by adding 6 ml of distilled water to 0.5 g of XAE in a test tube, followed by vigorous shaking. The mixture was then observed after a few minutes for the presence or absence of persistent foam, which indicates the presence of saponins [20].

Glycosides (General test)

Glycosides were detected by adding dilute sulphuric acid (5 ml) to 0.5 g of XAE, and bringing to boil for 2 min. the mixture was then filtered and a few drops of 20 % sodium hydroxide were added to the filtrate. Fehling's A and B solution were gently added to the filtrate and the formation of reddish-brown precipitates was assessed [20,22].

Tannins

Tannins were tested by boiling 0.5 g of XAE in 10 ml of water in a test tube, followed by filtration. A few drops of 0.1 % ferric chloride solution were then added to the filtrate, and the mixture was observed for the development of a dark green colouration, indicative of the presence of tannins [20,22].

Flavonoids

A small volume of the filtrate was treated with 5 ml of dilute ammonia solution, followed by the careful addition of concentrated sulphuric acid. The mixture was then observed for the appearance of a yellowish colouration, which indicated the presence of flavonoids [20].

High-performance liquid chromatography (HPLC) analysis of Xylopia aethiopica extract

About 0.5 g of XAE was weighed and dissolved in 10 ml of acetonitrile. A volume of 1 ml of the solution was measured and diluted to 10 ml, making a final solution concentration of 5 mg/ml. A Phenomenex C-18 reversed phase column (150 mm × 4.6 mm, 5 μm) was employed as a stationary phase in this method development. Isocratic elution was employed, utilizing a mixture of 0.1 % formic acid and Acetonitrile in the proportions of 20:80 (v/v), to achieve separation, eluting at ambient temperature. The formic acid was purified via a membrane filter with a pore diameter of 0.2 μm before its use. Compounds were detected using a UV detector set to detect across a variable wavelength between 230 nm and 350 nm, with 245 nm selected as it showed the best resolution peaks and separation

Induction of colonic injury and body weight assessment

Sprague Dawley rats weighing between 200 and 250 g were randomly assigned to nine groups (n = 5). Group I served as the control and received oral saline (0.9 % w/v) for 8 days. On the 4th day, Group II received 1 ml of 4.0 % acetic acid (v/v) intrarectally using a size 6 Ch/Fr pediatric catheter (Flexicare Medical Ltd, Mid Glamorgan, UK). Group III was pre-treated with sulphasalazine (500 mg/kg, *p.o.*) for 8 days, followed by the same acetic acid administration on the 4th day. Groups IV to VI were pre-treated with 30, 100 and 300 mg/kg *p.o.* respectively for 8 days, after which 1 ml of 4.0 % acetic acid was administered similarly to Group III. Treatment for all groups continued until day 8, during which body weight changes were monitored. The overall effect of XAE on body weight was quantified using the area under the time-course curve (AUC).

Macroscopic and microscopic colonic damage

Colonic injury was induced in rats using acetic acid, as outlined in the section above on disease induction. At the conclusion of the 8-day period, the colons were removed and evaluated for weight, stool consistency, gross macroscopic appearance, and length, measured from 1 cm above the anus to the top of the cecum. Macroscopic damage was assessed following the method established by Kimball et al. [23], which involved calculating the disease activity index (DAI). For stool condition, a score of 0 indicates normal, well-formed faecal pellets, while a score of 1 reflects loosely shaped moist pellets, a score of 2 denotes amorphous, moist, sticky pellets, a score of 3 represents diarrhoea, and a score of 4 signifies the presence of blood in the stool. Regarding colon damage, a score of 0 indicates no inflammation, a score of 1 shows reddening with mild inflammation, a score of 2 corresponds to moderate or more widely distributed inflammation, and a score of 3 reflects severe or extensively distributed inflammation. For colon weight change, a score of 0 represents a change of 5 %, scores of 1, 2, 3, and 4 correspond to weight changes of 5–14 %, 15–24 %, 25–35 %, and greater than 35 %, respectively. Lastly, for colon length shortening, a score of 0 indicates a change of 5 %, with subsequent scores reflecting changes of 5–14 %, 15–24 %, 26–35 %, and more than 35 %.

To assess microscopic colon damage using light microscopy, samples of the distal colon were promptly fixed in 10 % formaldehyde solution. These samples were then embedded in paraffin, cut into transverse 3 μm sections, and mounted on glass slides. The sections underwent deparaffinization and were stained with haematoxylin and eosin (H&E). For each specimen, three (3) transverse sections and three (3) microscopic fields per section were analyzed by two independent observers, who were blinded to the sample identity, examining six randomly selected fields of view.

Argyrophylic nucleolar organiser region (AgNOR) staining

To evaluate nucleolar organizer regions (NORs) using silver nitrate staining, we followed the protocol established by Romão-Corrêa et al. [24]. Paraffin-embedded 3 μm sections were first deparaffinized in xylene for 30 min, then gradually dehydrated with ethanol over 10-minute intervals. After washing with distilled water, the slides were immersed in a staining solution containing 50 % silver nitrate, 2.0 % gelatin, and 1.0 % formic acid, kept in the dark for 60 min, and subsequently rinsed in deionized water. The sections were then dehydrated, cleared with xylene, and mounted using distrene, plasticizer, and xylene (DPX). The presence of brown or black dots within the nucleus or near the nucleolus was assessed and counted under a Leica ICC50 HD light microscope (Jos. Hansen & Soehne GmbH, Hamburg, Germany). Data were reported as the average number of AgNORs per nucleus.

Haematology

Colitis was induced in experimental animals using acetic acid as previously described. The rats were euthanized, and blood samples were collected from the jugular vein. A complete blood count was performed on the collected samples using a haematology analyzer (BC-2800, Mindray, Shenzhen, China).

Mast cell proliferation

At the end of the 8th day, the rats were sacrificed, and their colons were excised. Full-thickness segments of the colons were fixed in Carnoy's fixative and subsequently stained with 1 % toluidine blue for morphological assessment. Mast cells were counted in coded sections at × 40 magnification using a micrometer grid (0.032 mm^2). For each rat, 12 contiguous, non-overlapping mucosal areas above the muscularis mucosae were evaluated.

Determination of enzyme activity

At the end of the experiment, tissue samples were homogenized in ice-cold 0.01 M Tris–HCl buffer (pH 7.4) using a Potter-Elvehjem homogenizer (Ultra-Turrax T25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) to produce a 10 % homogenate. superoxide dismutase, catalase, ascorbate peroxidase, myeloperoxidase activities and malondialdehyde levels were measured in tissue homogenate

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assessed as previously described by Misra and Fridovich [25]. Briefly, 0.5 ml of tissue homogenate was combined with 0.75 ml of 96 % ethanol (v/v) and 0.15 ml of chilled chloroform, and the mixture was centrifuged at 2000 rpm for 20 min. To the resulting 0.5 ml supernatant, 0.5 ml of 0.6 mM EDTA solution and 1 ml of carbonate-bicarbonate buffer (0.1 M, pH 10.2) were added. The reaction was initiated by introducing 0.05 ml of 1.3 mM adrenaline, and the increase in absorbance at 480 nm, corresponding to the formation of adrenochrome, was measured using a spectrophotometer. The percentage inhibition of adrenaline autoxidation was calculated using the following formula:

$$\%\text{inhibition} = \left[\frac{\text{Absorbance}_{\text{test}} - \text{Absorbance}_{\text{reference}}}{\text{Absorbance}_{\text{test}}}\right] \times 100$$

One unit of SOD activity is defined as the amount of protein that produces 50 % inhibition of adrenaline autoxidation at 25°C. This value was calculated using the following formula:

$$\text{Units of activity per mg protein} = \left[\frac{\%\text{inhibition}}{\text{50x weight of protein}}\right]$$

Catalase activity

Catalase (CAT) activity was assessed by measuring the decrease in hydrogen peroxide (H_2O_2) concentration at 240 nm over a 60-second period, with readings taken at 20-second intervals. The reaction medium included 130 μl of 50 mM potassium phosphate buffer (pH 7.0) mixed with the enzyme extract and 65 μl of 10 mM H_2O_2. For the blank, 65 μl of potassium phosphate buffer was combined with 130 μl of the sample. The concentration of H_2O_2 was calculated from the absorbance using the following formula [26]:

Table 1
Qualitative phytochemical screening of aqueous ethanol dried fruit extract of *Xylopia aethiopica*.

S/ No.	Bioactive compounds	Aqueous ethanol extract of *Xylopia aethiopica*
1	Alkaloids	+
2	Saponins	+
3	Flavonoids	+
4	Glycosides	+
5	Tannins	+
6	Sterols	+

+ Present

$$[H_2O_2mM] = \left[\frac{A_{240nm} \times 1000}{39.4 \quad mol^{-1}cm^{-1}}\right]$$

Where A_{240} is the absorbance at wavelength of 240 nm. Molar extinction coefficient for H_2O_2 is 39.4 $mol^{-1}cm^{-1}$ is the. CAT activity was expressed as U/mg protein.

Ascorbate peroxidase activity

Ascorbate peroxidase (APx) activity was evaluated by monitoring the decrease in absorbance at 290 nm (extinction coefficient: 2.8 $mM^{-1}cm^{-1}$) over a 60-second period, with measurements taken every 10 s. The assay mixture comprised 600 µl of 50 mM potassium buffer (pH 7.0), 100 µl of 0.1 mM EDTA, 100 µl of 1.25 mM H_2O_2, 100 µl of 0.5 mM ascorbic acid, and 100 µl of the enzyme extract. The blank contained all the components except the enzyme extract. The reaction was initiated by the addition of H_2O_2 [27].

Myeloperoxidase activity

Myeloperoxidase (MPO) activity was assessed using a modified *o*-dianisidine method [28]. The assay mixture consisted of 0.3 ml of 0.1 M phosphate buffer (pH 6.0), 0.3 ml of 0.01 M H_2O_2, 0.5 ml of freshly prepared 0.02 M *o*-dianisidine in deionized water, and 10 µl of supernatant, bringing the total volume to 3.0 ml. The supernatant was added last, and the change in absorbance at 460 nm was monitored for 10 min, with readings taken every minute. All measurements were conducted in duplicate. One unit of MPO activity was defined as the amount that produced an increase in absorbance of 0.001 min^{-1}, with specific activity expressed as U/mg protein.

Assay of malondialdehyde levels

This measurement followed the method established by Heath and Parker [29]. Specifically, 3 ml of 20 % trichloroacetic acid containing 0.5 % thiobarbituric acid was combined with a 1 ml aliquot of the supernatant. The mixture was then heated at 95°C for 30 min and rapidly cooled in an ice bath. Following this, the sample was centrifuged at 10000 ×g for 10 min, and the absorbance of the supernatant was recorded at 532 nm. The nonspecific absorbance at 600 nm was subtracted from the reading at 532 nm. The concentration of MDA was determined using its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Table 2
Detection and quantification data of constituents in XAE using HPLC.

Peak	Retention time (Min)	Area (mAU*s)	Height (mAU)	Area %
1	1.187	47.19879	4.28970	3.591
2	1.449	152.35742	14.40503	11.5937
3	1.640	119.32223	7.27834	9.0800
4	2.012	93.19088	6.07136	7.0915
5	2.818	747.0821	56.71428	56.8471
6	3.288	48.18941	3.62486	3.6671
7	3.659	106.81399	5.56333	8.1289

Statistical analysis

Data were expressed as mean ± SEM and analyzed with one-way ANOVA and Dunnett's *post hoc* test. All statistical analyses were done using GraphPad Prism for Windows Version 5.01 (San Diego, CA, USA).

Results

Preliminary phytochemical screening

The phytochemical screening identified the presence of glycosides, sterols, saponins, alkaloids, tannins, and flavonoids in the *Xylopia aethiopica* extract (Table 1).

HPLC analysis data of Xylopia aethiopica extract

The chromatogram in Fig. 1 and its corresponding data in Table 2 indicates the elution sequence and profile of compunds present in XAE. Seven different chemical groups were found in the extract with the most abundant compound occurring at a retention time of 2.828 min with a corresponding area of 56.8471 %.

Effect of XAE on changes in body weight

In the present study, the effect of colitis progression on body weight, DAI and histopathology were assessed. The study's findings indicated that the group exposed solely to acetic acid experienced a consistent decline in body weight throughout the experiment (Fig. 2a), with a significant overall weight loss compared to the non-colitis control group (Fig. 2b). In contrast, rats treated with sulphasalazine showed a gradual increase in body weight during the study (Fig. 2a), although this increase was not statistically significant when compared to the colitis control group (Fig. 2b). Animals receiving XAE treatment also exhibited a gradual rise in body weight over the study duration (Fig. 2a), but there were no significant ($p > 0.05$) differences in overall weight compared to the colitis control at dosages of 30 mg/kg and 100 mg/kg. However, a significant ($p < 0.01$) increase in overall body weight was noted at the

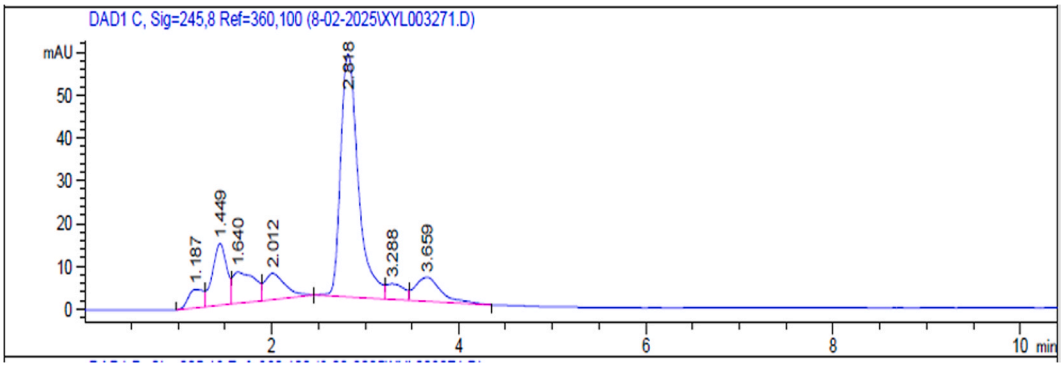

Fig. 1. HPLC Chromatogram of *Xylopia aethiopica* extract solution at 245 nm.

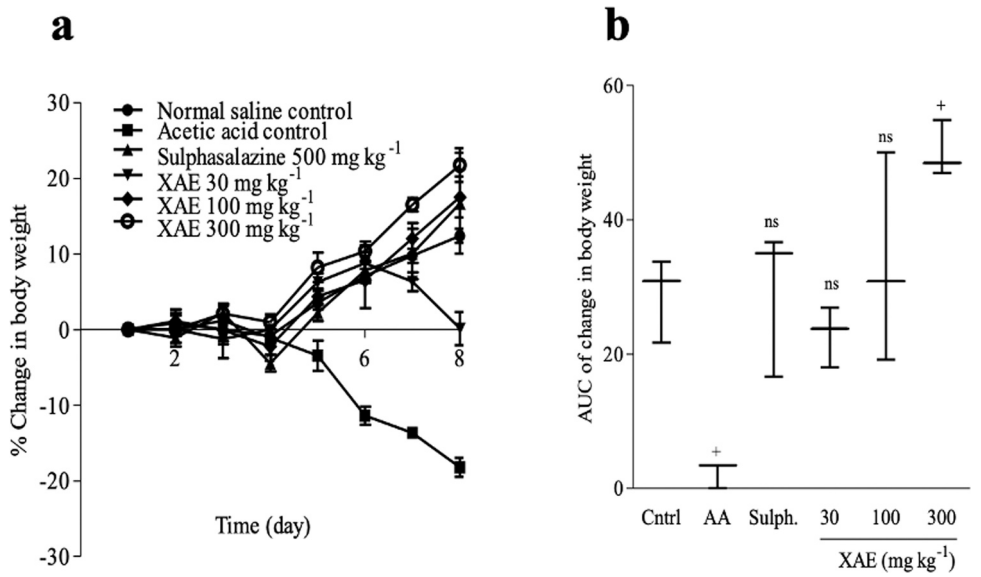

Fig. 2. ***Xylopia aethiopica*** **suppresses colitis-induced weigh changes.** Rats were pretreated prior to colon injury induction with acetic acid on day 4, followed by continued treatment until day 8 after which body weight of rats was measured. **a,** Percentage change in body weight; **b,** Area under the time course curves of total changes in body weight measured during the study period. $^{+}p < 0.01$, $^{ns}p > 0.05$ when compared to con-colitis control.

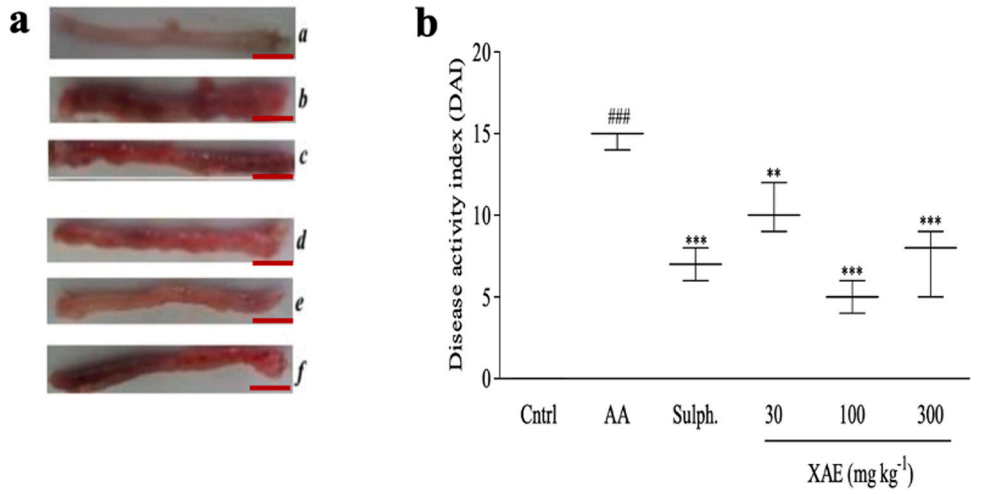

Fig. 3. ***Xylopia aethiopica*** **modulates acetic acid-induced colonic damage in rats.** Rats were pretreated prior to colon injury induction with acetic acid on day 4, followed by continued treatment until day 8 after which colons were removed and disease activity indices were determined.*$p < 0.01$, **$p < 0.001$ ***$p < 0.0001$ when compared with colitis control. $^{###}p < 0.0001$ when compared with non-colitis control. **a;** Representative slides of colon (a, untreated control; b, AA treatment only; c, AA + 500 mg kg^{-1} sulphasalazine; d, AA + 30 mg kg^{-1} XAE; e, AA + 100 mg kg^{-1} XAE; f, AA + 300 mg kg^{-1} XAE. **b;** Disease activity index of XAE (micron bar = 60 mm).

300 mg/kg dosage (Fig. 2b).

Effect of XAE on macroscopic and microscopic colonic damage

The results indicated that the rats in the acetic acid control group displayed significant colonic injury on a macroscopic level, evidenced by a notably high Disease Activity Index (DAI) compared to the non-colitis control group (Figs. 3a and 3b). Treatment with sulphasalazine led to a significant ($p < 0.001$) improvement in the disease's macroscopic profile, as reflected by a reduced DAI in comparison to the colitis control group (Figs. 3a and 3b). Similarly, animals treated with XAE showed considerable macroscopic improvement in their disease profile, with a significant ($p < 0.0001$) decrease in DAI compared to the acetic acid-treated group at doses of 30, 100, and 300 mg/kg (Figs. 3a and 3b).

Microscopically, the colon of non-colitis control animals showed intact goblet cells (green arrow) and no signs of inflammatory cell infiltration, fibrosis, or necrosis (Fig. 4a). In contrast, the colitis control rats displayed histological ulcers characterized by necrosis in the colonic mucosa (red circle, Fig. 4b) and inflammatory cell infiltration (green circle). Moreover, other histological sections showed submucosal inflammation in the colitis control, primarily involving neutrophils and lymphocytes. Also observed were crypt abscesses, granulomatous inflammation with fibrosis, and significant thickening of the submucosa. The crypts in the acetic acid group were elongated and distorted, accompanied by epithelial cell loss, ulceration, lymphocyte infiltration, thickening of the bowel wall, and depletion of goblet cells (red circle, Fig. 4b). Sulphasalazine treatment resulted in reduced mucosal damage, with decreased inflammatory cell infiltration of the lamina propria (green circle, Fig. 4c). All doses of XAE effectively lessened the mucosal injury caused by acetic acid, showing decreased epithelial cell loss and reduced granulomatous inflammation, especially in the XAE 300 mg kg^{-1} – treated group which showed intact epithelial structures

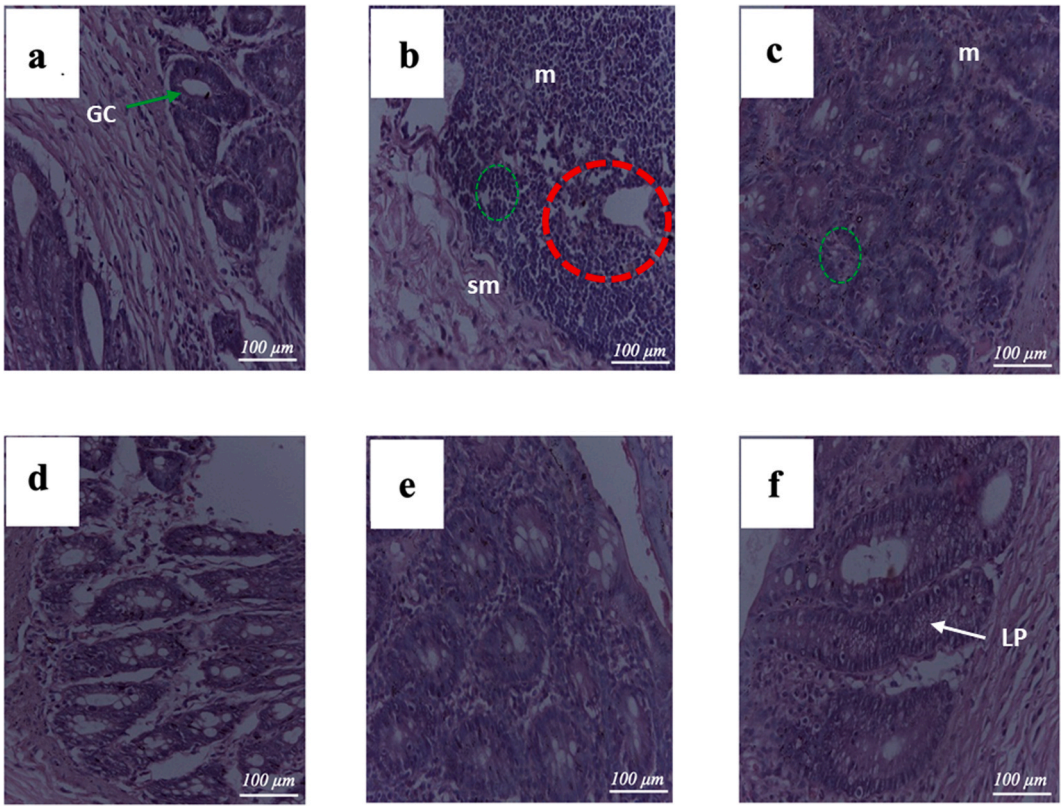

Fig. 4. ***Xylopia aethiopica*** **fruit extract reduces histological damage in acetic acid-induced colitis in rats.** Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were harvested and examined histologically for microscopic damage. Representative histological images of rat colons; Control (untreated) (a), acetic acid control (b), sulphasalazine 500 mg kg^{-1} (c), XAE 30 mg kg^{-1} (d), XAE 100 mg kg^{-1} (e), XAE 300 mg kg^{-1} (F) (micron bar = 100 μm). Green arrow, goblet cell (GC); red circle, necrosis and goblet cell depletion; green circle, inflammatory cell infiltrate; sm, submucosa; m, mucosa; LP, lamina propria.

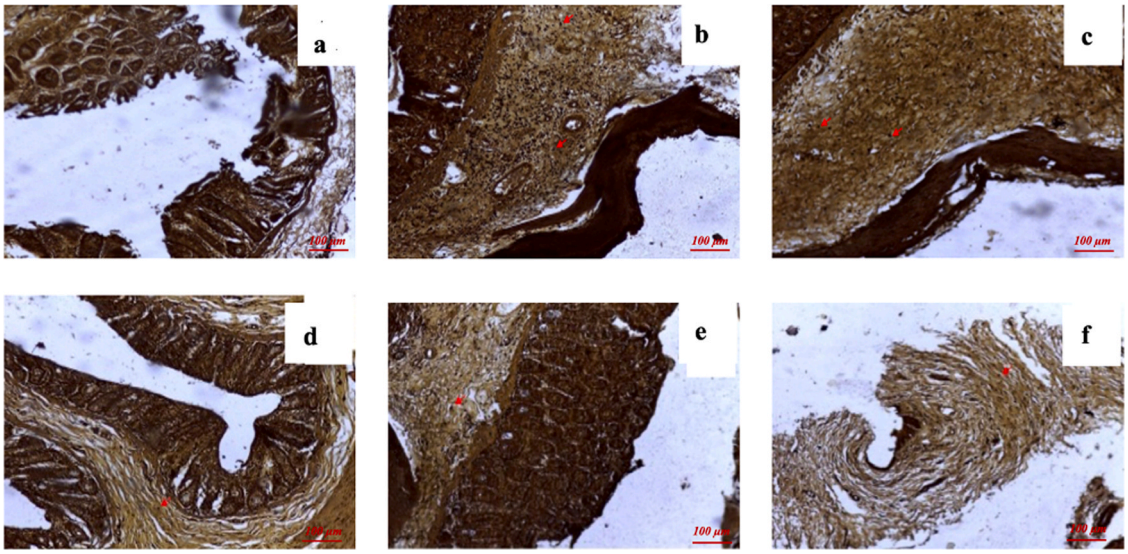

Fig. 5. ***Xylopia aethiopica*** **fruit extract reduces silver nitrate-stained nucleolar organizer regions in rat colon.** Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were harvested and examined for silver nitrate-stained nucleolar organizer regions. Non-colitis control (a), colitis control (b) sulphasalazine 500 mg kg^{-1} (c), XAE 30 mg kg^{-1} (d), XAE 100 mg kg^{-1} (e), XAE 300 mg kg^{-1} (f) (micron bar = 100 μm). Red arrows indicate nucleolar organizer regions.

(Figs. 4d, 4e, and 4f).

Argyrophylic nucleolar organiser region (AgNOR) staining

The non-colitis control animals presented with reduced expression of AgNOR in cells (Fig. 5a). The AgNORs-to-nucleus ratio did not show an

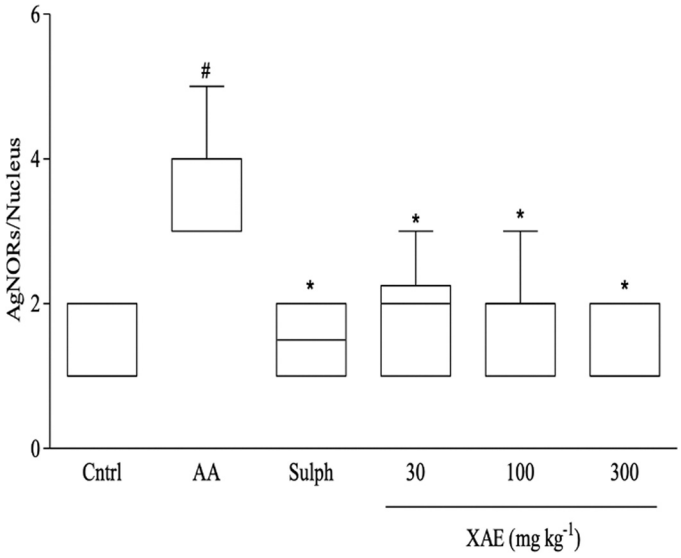

Fig. 6. Effect of *X. aethiopica* fruit extract on argyrophilic nucleolar organiser region/nucleus ratio in acetic acid-induced colitis in rats. Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were harvested and examined for silver nitrate-stained nucleolar organizer regions. The presence of brown or black dots within the nucleus or outside the nucleolus was assessed through silver staining. These dots were counted, and the average count per 10 nuclei was calculated and expressed as Mean ± SEM. $^{*}p < 0.05$, treatment compared with colitis control. $^{\#}p < 0.05$ when compared with non-colitis control.

increase when compared to the colitis control group, as quantified (Fig. 6). The colitis control exhibited high AgNOR proliferation in the cells (Fig. 5b), which corresponded to a significant increase in the AgNORs-to-nucleus ratio (Fig. 6). Treatment with sulphasalazine led to a notable reduction in AgNOR proliferation within the colonic epithelial cells (Fig. 5c), resulting in a significant decrease in AgNOR counts per nucleus compared to the colitis control group (Fig. 6). Additionally, the XAE-treated groups at doses of 30, 100, and 300 mg kg^{-1} showed a marked reduction in AgNOR expression (Figs. 5d, 5e, and 5f). Quantitative analysis of the AgNORs-to-nucleus ratio revealed a significant decline ($P < 0.0001$) with XAE treatment, bringing it to levels similar to those observed in the non-colitis control group (Fig. 6).

Haematology

In the non-colitis control group, typical levels of white blood cells (WBC), lymphocytes (LYM), neutrophils (NEU), haemoglobin (HGB), red blood cells (RBC), haematocrit (HCT), and platelets (PLT) were observed. After induction of colitis with acetic acid, significant increases were seen in WBC, HCT, and HGB levels, while LYM, NEU, RBC, and PLT levels significantly decreased (Table 3). Rats treated with sulphasalazine exhibited marked increases in LYM, HGB, and RBC compared to those treated with acetic acid alone (Table 3). XAE treatment resulted in an increase in PLT and a notable reduction in HGB across doses of 30, 100, and 300 mg/kg, when compared to the acetic acid-treated control group. Additionally, WBC levels decreased at 30 and 300 mg/kg doses of XAE, while RBC levels were reduced at 30 and 100 mg/kg doses (Table 3).

Mast cell proliferation

Toluidine blue-stained colon sections were evaluated for proliferation of mast cells following colitis induction. The results showed that, in the non-colitis control group, there was no significant ($p > 0.05$) increase in mast cell influx into the colonic segments (Fig. 7a), and the mast cell count was not elevated when compared to the colitis control group (Fig. 8). In contrast, the acetic acid-treated group exhibited a pronounced influx of mast cells (Fig. 7b), with a significantly ($p < 0.0001$) higher mast cell count in the colon compared to the non-colitis control group (Fig. 8). Treatment with sulphasalazine led to a reduction in mast cell proliferation within the colon (Fig. 7c), showing a significantly ($p < 0.0001$) lower mast cell count compared to the acetic acid-induced colitis group (Fig. 8). Rats treated with XAE at doses ranging from 30 to 300 mg/kg demonstrated a reduction in mucosal mast cell infiltration (Figs. 7d, 7e, and 7f), with a significant ($p < 0.0001$) decrease in mast cell count compared to the acetic acid-treated colitis control group (Fig. 8). This suggests that XAE treatment reduced both cellular activity and mast cell proliferation.

Enzyme assay

To explore the effect of XAE on these enzymes, colonic tissue samples were homogenized, centrifuged, and the supernatant analyzed for the levels of SOD, CAT, APx, and MPO.

In the non-colitis control group, SOD activity was detectable but significantly reduced in rats treated with acetic acid (Fig. 9a). Sulphasalazine treatment increased SOD activity in colonic tissues, while XAE treatment resulted in a significant ($p < 0.0001$) increase in SOD activity across doses of 30–300 mg/kg (Fig. 9a). Similarly, CAT activity was significantly higher in the non-colitis control group, but decreased in rats treated with acetic acid (Fig. 9b). Sulphasalazine treatment restored CAT activity, and XAE treatment showed significant increase in CAT activity at doses of 100 and 300 mg/kg compared to the acetic acid-treated group (Fig. 9b).

APx activity was also significantly higher in the non-colitis control rats but was reduced following acetic acid treatment (Fig. 9c). Sulphasalazine treatment restored APx activity, and XAE treatment significantly increased APx activity (Fig. 9c). For MPO, detectable levels were observed in the non-colitis control group, but these were significantly elevated in rats treated with acetic acid (Fig. 9d). Sulphasalazine reduced MPO activity significantly compared to the acetic acid-treated group, and XAE treatment at doses of 100 and 300 mg/kg significantly reduced MPO activity (Fig. 9d).

Table 3
Effect of *Xylopia aethiopica* fruit extract on haematological parameters in acetic acid-induced ulcerative colitis in rats.

Treatment	WBC x10^3/μL	LYM%	NEUT%	HGB g dL^{-1}	RBC x10^6/μL	HCT %	PLT x10^3/μL
Control	10.6 ± 0.10$^{\#}$	62.5 ± 7.35	10.3 ± 1.37	13.7 ± 0.01	8.31 ± 0.2$^{\#}$	44.0 ± 1.23	626 ± 13.72$^{\#}$
Acetic acid	14.7 ± 0.23*	44.1 ± 9.25*	5.7 ± 0.75*	15.0 ± 0.00*	5.93 ± 0.01*	49.0 ± 0.02*	432 ± 10.92*
Sulphasalazine (mg/kg)							
500	15.1 ± 0.10*	62.1 ± 3.48*$^{\#}$	4.0 ± 0.43*	17.1 ± 0.20*#	9.11 ± 0.11*$^{\#}$	47.9 ± 0.23*	356 ± 3.45*
XAE (mg/kg)							
30	12.3 ± 0.11*$^{\#}$	70.9 ± 3.91*$^{\#}$	7.4 ± 1.35*$^{\#}$	12.5 ± 0.08*	7.21 ± 0.05*$^{\#}$	37.3 ± 0.12*$^{\#}$	1213 ± 20.92*$^{\#}$
100	18.6 ± 0.22*$^{\#}$	51.8 ± 2.75*	6.7 ± 0.92*$^{\#}$	12.4 ± 0.10*	6.99 ± 0.15*$^{\#}$	38.0 ± 0.07*$^{\#}$	1062 ± 15.31*$^{\#}$
300	7.3 ± 0.12*$^{\#}$	39.0 ± 1.78*	5.19 ± 0.63*$^{\#}$	10.1 ± 0.17*$^{\#}$	5.44 ± 0.42*	29.3 ± 0.75*$^{\#}$	1146 ± 21.45*$^{\#}$

* $p < 0.0001$ when compared with untreated control; $^{\#}p < 0.0001$ when compared with acetic acid

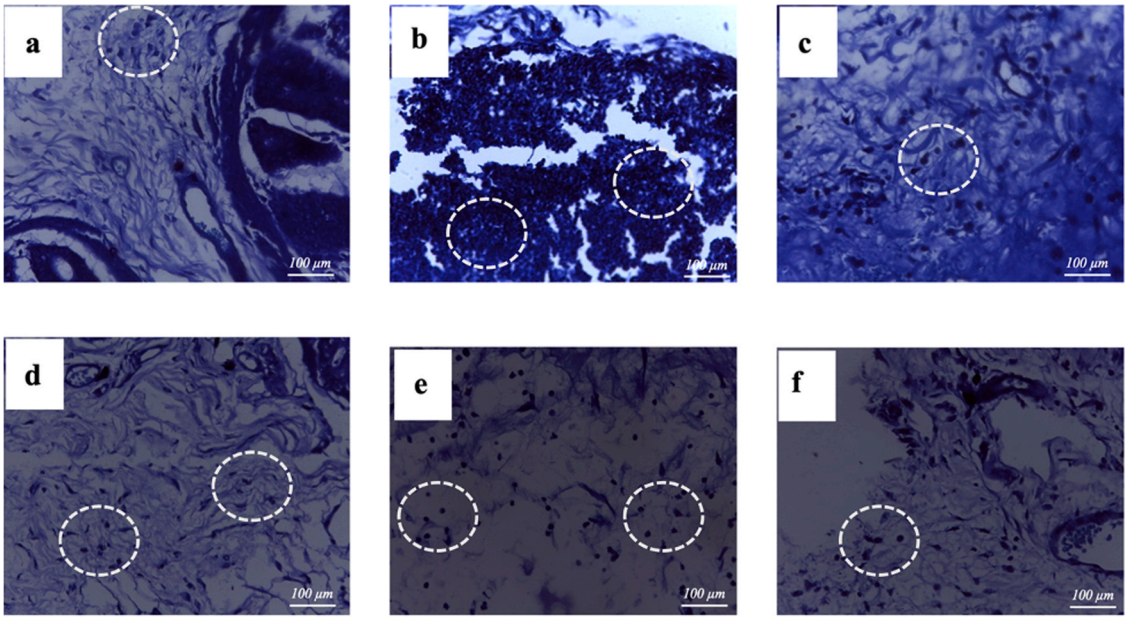

Fig. 7. Mast cell proliferation in colon of rats. Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were extirpated and examined for mast cells proliferation. Non-colitis control (a), acetic acid-treated control (b), sulphasalazine 500 mg/kg (c) XAE 30 mg/kg (d), XAE 100 mg kg^{-1} (e), XAE 300 mg/kg (f) (micron bar = 100 μm). White circle shows regions with mast cells.

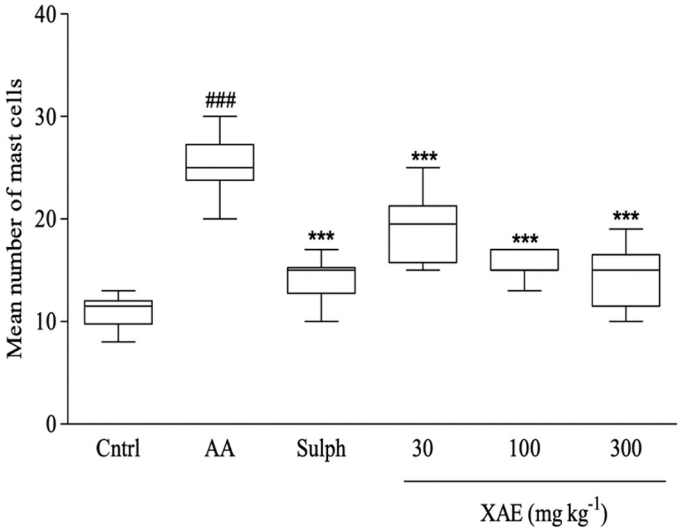

Fig. 8. Treatment with *X. aetiopica* hydroethanolic fruit extract reduces mast cell count in acetic acid-induced rat ulcerative colitis. Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were extirpated and examined for mast cells proliferation. Data presented as Mean ± SEM. $^{***}p < 0.0001$ when compared with colitis control. $^{###}p < 0.0001$ when compared with non-colitis control.

Malondialdehyde levels

In this study, acetic acid-induced colitis was associated with significant increase in lipid peroxidation, reflected by elevated MDA levels compared to the non-colitis control group (Fig. 10). Sulphasalazine treatment significantly reduced MDA levels when compared to the acetic acid-treated rats (Fig. 10). Similarly, XAE treatment (at doses of 30, 100, and 300 mg/kg) resulted in a reduction in MDA levels compared to the acetic acid group, suggesting a decrease in neutrophil infiltration and inflammation. This also indicates that XAE treatment reduces lipid peroxidation in the colon compared to the acetic acid-induced control group (Fig. 10).

Discussion

The present study evaluated the effects of XAE on chronic colonic inflammation. As previously asserted, xylopic acid, a compound isolated from *Xylopia aethopica* was shown to attenuate colonic inflammation induced by acetic acid [17]. While this is only but a single compound, the chemical diversity within the extract allows for further evaluation of the plant's activity. A widely used model in assessing the disease severity and treatment approaches is the acetic acid-induced colitis [23]. Induction by acetic acid triggers several immune responses through neutrophil and macrophage activation. Colitis induced by intrarectal administration of acetic acid serves as a relevant model for inflammatory bowel disease (IBD), closely mirroring human IBD in terms of pathogenesis, histopathological characteristics, and inflammatory mediator profiles [28,29]. The intrarectal administration of a diluted acetic acid solution leads to non-transmural inflammation, marked by heightened neutrophil infiltration in the intestinal tissue, extensive necrosis in the mucosal and submucosal layers, vascular dilation, oedema, and submucosal ulceration. These features are significant indicators of human colitis and can be collectively evaluated using the disease activity index (DAI) [31,32]. This process can also contribute to lipid peroxidation, increased vascular permeability, and infiltration of mucosal tissue in diseased colons.

The effects observed in the disease control group are consistent with previous research linking colonic inflammation to malnutrition, anorexia, and weight loss [6,33]. The severity of these issues is associated with the extent of disease and the level of colonic damage. Colonic inflammation triggers nausea and vomiting through the serotonergic nerves that activate the chemoreceptor trigger zone [6]. XAE has been shown to enhance fluid and glucose absorption in the jejunum, which may help reduce water loss and alleviate diarrhoea [34]. Anorexia might also result from serotonin's influence on the anorexigenic melanocortin pathway, leading to a decreased appetite and subsequent weight loss [35]. The overall improvement in weight, particularly the significant increase at 300 mg/kg compared to the control, suggests that XAE may promote tissue healing and help manage inflammation. Given the moderate effectiveness of sulphasalazine, XAE presents a promising

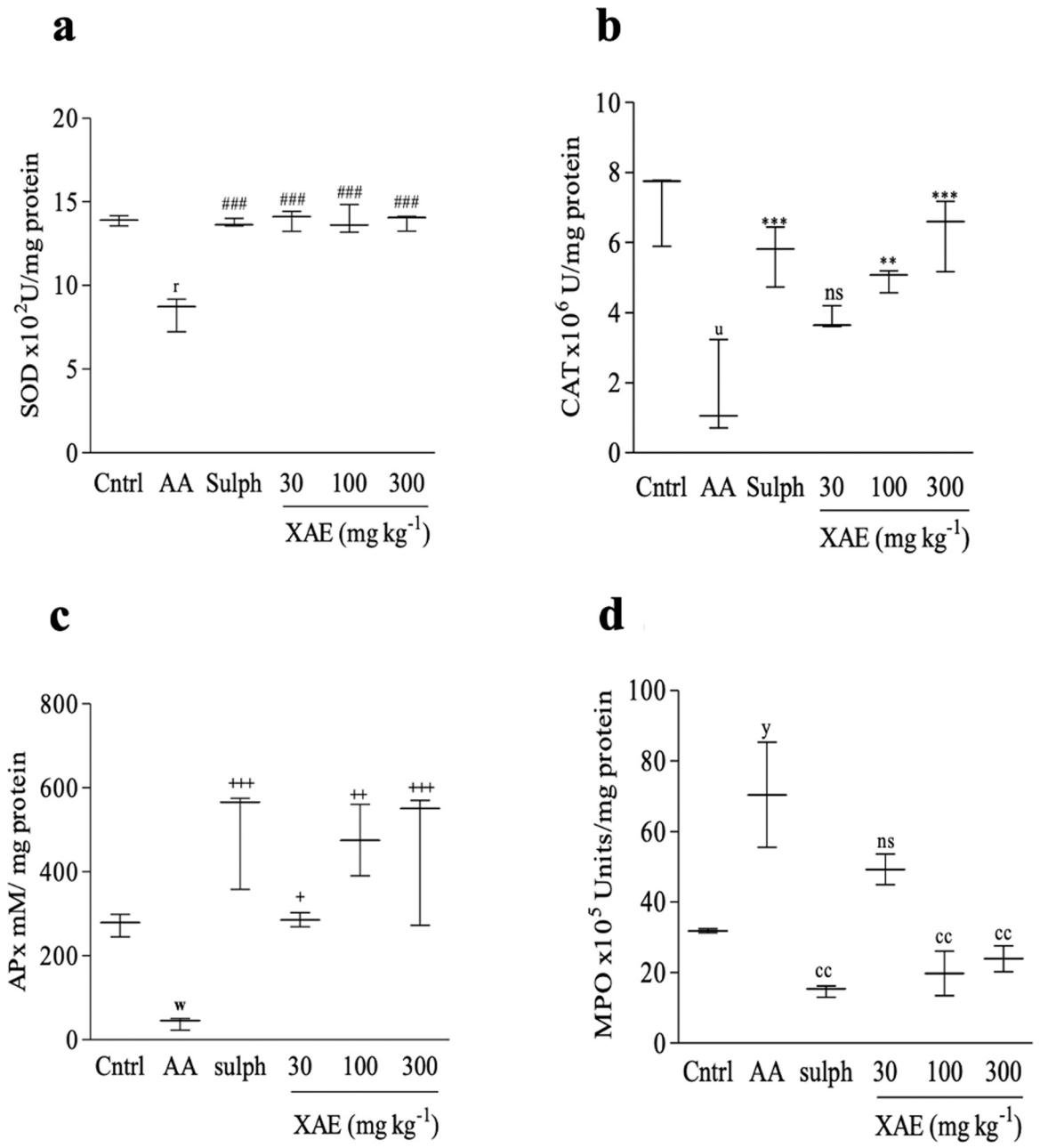

Fig. 9. *Xylopia aetiopica* hydroethanolic fruit extract enhances activity of antioxidant enzymes in rat colitis. Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were removed, homogenized and assayed for: SOD (a), CAT (b), APx (c) and MPO (d). $^{\#\#\#}p < 0.0001$, $^{**}p = 0.001$, $^{***}p = 0.0001$, $^{+}p < 0.05$, $^{++}p = 0.01$, $^{+++}p = 0.001$, $^{cc}p = 0.0029$, $^{ns}p > 0.05$ when compared with colitis control. $^{r}p < 0.0001$, $^{u}p = 0.0001$, $^{w}p < 0.05$ when compared with non-colitis control.

option for combination therapy, especially for patients who do not adequately respond to standard treatments.

Moreover, significant improvement in disease severity across both macroscopic and microscopic profiles suggest a strong mucosal protective property of XAE. Notably, the reduction in DAI in the XAE-treated groups was comparable to that observed in the sulphasalazine-treated group, suggesting that XAE may have potent anti-colitis effects similar to the current standard therapy. Decreased epithelia loss and reduction of inflammation points out a potential role in modulating inflammatory pathways. These findings suggest that XAE can protect against the inflammatory and fibrotic changes that characterize colitis, possibly through modulation of immune cell infiltration and suppression of pro-inflammatory mediators. This observation was consistent with the pharmacological effects of some compounds isolated from *X. aethiopica*, especially xylopic acid and kaurenoic acid [30,36]. Through reductions in colon epithelial argyrophylic nucleolar organizer region (AgNOR) expression, AgNORs/nucleus ratio, MPO and MDA levels, xylopic acid reduces colon damage induced with acetic acid [17]. Additionally, xylopic acid increases the activity of superoxide dismutase (SOD), ascorbate peroxidase (APx), and catalase (CAT), contributing to the anti-inflammatory effect of XAE [17].

In this study, treatment with the hydroethanolic fruit extract of *X. aethiopica* alleviated colon inflammation and decreased AgNORs count. Nucleolar organiser region (NOR) was used in this model to determine cellular behaviour and proliferation levels [37]. Studies have shown that the potential thickening of colon epithelium may stimulate epithelial cell proliferation secondary to the release of excess mucin in the colonic area [9,38]. Colitis is associated with an increased

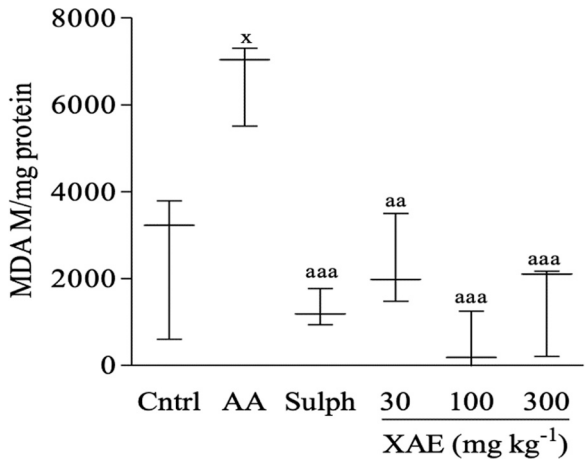

Fig. 10. Hydroethanolic fruit extract of *Xylopia aethiopica* inhibits lipid peroxidation in ulcerative colitis induced by acetic acid in rats. Rats were pretreated before colon injury was induced with acetic acid on day 4, after which treatment continued until day 8, when the colons were removed, homogenized and the supernatant assayed for malondialdehyde concentration. $^{aa}p < 0.002$, $^{aaa}p < 0.0002$ when compared with colitis control. $^{x}p < 0.002$ when compared with non-colitis control.

expression of AgNORs in colon tissues, which is attributed to heightened transcriptional and cellular activity.

Moreover, increased AgNOR/nuclear ratio is indicative of aggressive regenerative response in colonic tissue and has been highlighted as a common feature in colitis where continuous damage triggers excessive cellular changes as the body attempts to restore the mucosal integrity [39]. This hyperproliferation has been shown to exacerbate inflammation and lead to crypt distortion and other histological changes. The ability of XAE and its active principle, xylopic acid, to decrease levels of the AgNOR/nucleus ratio in the experimental animals highlights the ability of the extract and its isolates to inhibit aggressive epithelial regeneration in colitis and associated histopathological changes. It is also evident that, XAE does not only alleviate inflammation but also helps normalize cellular behaviour within the colon. This is particularly important, as prolonged hyperproliferation can result in tissue dysfunction and may contribute to neoplastic changes in chronic colitis.

Haematological analysis revealed an inflammatory response that is consistent with literature [40,41]. These alterations align with the well-documented haematological disturbances seen in colitis, where inflammatory processes lead to leucocytosis and altered erythropoiesis, often accompanied by anaemia and thrombocytopenia due to impaired absorption and systemic inflammation [42,43].

XAE produced dose-dependent effects with some potential drawbacks to its use. At doses of 30 and 300 mg/kg, WBC levels were decreased, indicative of a reduced inflammatory response which could enhance its tissue repairing ability. However, there were reductions in RBC at 30 and 100 mg/kg, which might indicate potential toxicity on red blood cell formation or blood volume. A notable increase in platelet count at all doses indicates a possible stimulation of platelet production, which could be beneficial in mitigating thrombocytopenia observed in colitis. However, the observed decrease in haemoglobin concentration across all XAE doses raises concerns about the XAE influence on the red blood cell turnover. The findings of this study, when compared to parallel studies of natural extracts products in colitis reveal similar findings on the haematopoietic system [42]. The observed reduction in lymphocyte population may be attributed to the immunomodulatory effect of XAE [43]. The reduction in these inflammatory cells in XAE-treated animals suggests that the extract can inhibit immune cell recruitment and activation in response to colon injury, thereby reducing the extent of inflammation and tissue damage.

Mast cells, upon activation, release a range of mediators that can promote inflammation and influence immune cell responses. These cells accumulate at sites of injury, which underscores their role in the inflammatory process [44]. The observed reduction in mast cell infiltration at the injury site following XAE treatment aligns with the decreased mast cell proliferation, indicating a potential anti-inflammatory effect of the extract. This finding supports earlier studies, such as those by Cavalcanti et al. [45], which demonstrated that kaurenoic acid, a kaurane diterpene found in *X. aethiopica* fruit extract, can inhibit cell proliferation.

In addition, *X. aethiopica* fruit extract reduced oxidative damage in diseased colons by enhamcing the activity of superoxide dismutase (SOD), ascorbate peroxidase (APx), and catalase (CAT). Although, the exact cause of ulcerative colitis remains unclear, studies such as those by Thippeswamy et al. [46], have identified elevated levels of myeloperoxidase (MPO) and malondialdehyde (MDA) as key biomarkers for colon damage. These changes are often accompanied by a reduction in the activity of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APx), and catalase (CAT) [44]. These enzymes play a crucial role in protecting against oxidative stress. Their reduction suggests a compromised ability to detoxify harmful reactive oxygen species (ROS), which contributes to the inflammatory environment characteristic of colitis. CAT, primarily located in peroxisomes, is essential for converting hydrogen peroxide into water and oxygen [45]. SOD helps mitigate oxidative stress by dismutating superoxide radicals into hydrogen peroxide, thereby reducing inflammation in conditions like inflammatory bowel diseases [46,47]. APx, a key enzyme in the glutathione-ascorbate cycle, helps neutralize peroxides using ascorbate [48].

The significant increase in the activity of these antioxidant enzymes following XAE treatment suggests that it helps restore the redox balance in colonic tissues, which may reduce the severity of inflammation. This restoration of enzymatic activity, comparable to that seen with sulphasalazine treatment, further supports the therapeutic potential of XAE in counteracting oxidative stress and providing cellular protection in the context of colitis.

Moreover, the levels of MPO, an enzyme associated with inflammation and primarily produced by neutrophils, was also reduced following treatment with XAE. According to Oladele et al. [49], elevated MPO levels contribute to increased MDA production, a by-product of lipid peroxidation. This process triggers a free radical chain reaction that exacerbates inflammation and eventually leads to the depletion of cellular antioxidants. The reduction in both MPO activity and MDA levels following XAE treatment is likely due to its anti-inflammatory and antioxidant properties. XAE may modulate MPO activity, which is involved in the production of hypochlorous acid, a reactive species that contributes electrons required for lipid peroxidation. By reducing lipid peroxidation, XAE treatment effectively lowers the production of MDA, thus mitigating oxidative damage and inflammation.

Conclusion

Given the above, the hydroethanolic fruit extract of *Xylopia aethiopica* (XAE) demonstrates significant therapeutic potential in ulcerative colitis. The results suggest that it effectively reduces colitis-associated weight loss, improves both macroscopic and microscopic colonic damage, and enhances antioxidant enzyme activity in rats. These may be attributed to the myriad of phytocompounds present in the fruit extract, making it a promising source of drug leads in the management of ulcerative colitis.

CRediT authorship contribution statement

Ellis Jeff Aidoo: Software, Investigation, Formal analysis. **Mavis Sersah Nyarko:** Writing – original draft, Validation, Project administration. **Aaron Opoku Antwi:** Methodology, Investigation, Formal analysis, Data curation. **Michael Hagan:** Writing – original draft,

Software, Investigation. **Newman Osafo:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Kofi Oduro Yeboah:** Investigation, Formal analysis, Data curation.

Ethical approval

The research was conducted in accordance with the international principles for animal and laboratory care. Animals were handles well throughout the experiment and the experiment approved by the Department of Pharmacology, KNUST Ethics committee (PCOL/15/1.17)

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Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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