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Research Article

Humic Acid Ameliorates DSS-Induced Colitis by Enhancing MUC-2 Expression and Restoring Colonic Histoarchitecture in Rats

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ABSTRACT

Ulcerative colitis (UC) is a chronic idiopathic inflammatory disorder that involves any part of the colon. It typically presents with symptoms such as bloody diarrhea, abdominal pain and rectal urgency. Humic acid isa chemical produce by decaying organic matters, and has immune stimulating effect but its mechanistic role in treating UC remains underexplored. This study investigates the role of humic acid (HA) in attenuating Dextran Sulfate Sodium (DSS)-induced UC model in male Wistar rats. Twenty male Wistar rats were randomly assigned to groups (n = 6). Group 1 served as controls; Group 2 received 5% DSS alone; Group 3 received 5% DSS followed by humic acid (30 mg/kg); and Group 4 received 5% DSS followed by sulfasalazine (200 mg/kg). DSS was administered orally to induce colitis in Groups 2, 3, and 4. Colitis was induced for five days and drug treatment done for another five days. The disease activity index was assessed on days 1, 3, 5, and 10. Animals were euthanized by cervical dislocation and colon specimens harvested for macroscopic assessment and histological and biochemical assays. Humic acid treatment significantly attenuated DSS-induced colitis by reducing inflammation markers (MPO, TNF-α, interleukin-6, interleukin-1β, and arginase) and restoring colonic histological integrity. Significant improvements were observed in colonic tissue nitrite levels and MUC-2 expression. Conclusively, HA shows promise as an alternative therapeutic for UC, offering anti-inflammatory, and mucosal barrier-protective effects.

Keywords: *Inflammatory bowel diseases, humic acid, arginase, cytokines, Muc-2*

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that damages the lining of the colon and the rectum, causing inflammation, ulcers, and bleeding in the gastrointestinal tract (Ungaro et al., 2017). UC symptoms can range in severity and vary depending on the individual. Common symptoms are often diarrhea, abdominal spasms and pain, weight loss, exhaustion, and anemia (Perler et al., 2019). It is well established that UC is not an uncommon disease, as there are about a hundred thousand cases each year. The frequency and incidence of inflammatory bowel disease (IBD), a disease that affects people all over the world, are rising sharply (M'Koma, 2013). The global incidence is projected to reach 5 million cases by 2023 and is continuing to rise (Le Berre et al., 2023). Although the manifestation of IBD is higher in developed countries compared to African countries, there are increasing reported cases in Africa. This trend has been attributed to the adoption of western diets by Africans (Hodges and Kelleys, 2020). Western diets, which are often processed foods, are known to irritate the lining of the

colon, leading to inflammation and activation of a cascade of immune responses in UC patients (Gill et al., 2022).

The disease's etiology is specifically unknown, but a number of factors, including genetics, the environment, microbiology, diet, and immunity, have been linked to its pathogenesis, such that overreaction to the immune system may result in the release of mediators like cytokines and neutrophil infiltration, which may cause damage to the colon (Saez et al., 2023). By preserving the colon's protective mucus barrier, MUC2 plays a crucial part in the pathophysiology of ulcerative colitis. In UC, its deficiency or decreased secretion increases the colonic epithelium's exposure to microbiota, which causes inflammation (Kang et al., 2022). The integrity of the mucus layer is compromised by the reduction in MUC2 production, which is frequently associated with goblet cell loss. This allows bacteria to make direct contact with the epithelium and accelerates the course of the disease (Yao et al., 2021), this, therefore makes MUC-2 a therapeutic target in UC management.

Dietary changes and use of medications such as aminosalicylates, steroids, immunosuppressants, antibodies, and anti-tumor necrosis factor (TNF)-alpha have been helpful in the treatment of IBD (Cai et al., 2021; Souza et al., 2023) but they are not totally effective treatments. In addition, treatment response varies in individuals (Burri et al., 2020). Apart from the high costs of using these drugs, they also have great risks of side effects, which may outweigh their advantages. Till today, plants and other organic products continue to serve as therapeutic agents to cure human diseases (Dzobo, 2022), especially in the rural regions of sub-Saharan countries where there are limitations to accessing medical facilities and abject poverty, which makes the dwellers unable to afford orthodox medicine. Hence, there is a need for a conventional way to treat illnesses such as ulcerative colitis with readily available nutraceuticals and novel bioactive compounds, such as humic acid.

Humic acid (HA) is a chemical produced by decaying plants and occurs naturally in water, peat, soil, and brown coal. Humic substances have been reported to possess anti-inflammatory as well as pro-inflammatory properties (Ahfeethah et al., 2023; Hriciková et al., 2023). In recent times, the ethnopharmacological potency of humic acid has been reported, especially in other lower animals (Marcinčák et al., 2023). In addition, HA has been utilized to treat a number of illnesses, such as gastritis, diarrhea, and stomach ulcers. According to earlier research, broiler hens infected with Clostridium can have improved growth performance, nutrient digestibility, blood biochemistry, and intestinal morphology when given HAs and lincomycin in their diet (Saleh et al., 2022). Despite the increasing corpus of research on humic acid, its therapeutic role on ulcerative colitis, most especially on MUC-2 and comparative effect with standard drug have reamined underexplored. The purpose of this study was to determine whether humic acid could provide therapeutics to ulcerative colitis caused by dextran sulfate by reducing inflammation, improving immunological response, and improving protective effects on mucosal integrity. This contributes to our growing knowledge of humic acids as a comprehensive and potential alternative UC therapeutic option.

MATERIALS AND METHODS

Drugs and chemical

Different chemicals and drugs used were purchased from accredited vendors. Ketamine hydrochloride (Ciron Drugs & Pharmaceuticals, India), triochloroacetic acid (TCA, Sigma, Germany), thiobarbituric acid (TBA, Sigma, Germany), 5, 50 dithio-bis-2-nitrobenzoic acid (DTNB, Sigma, Germany), hexadecyltrimethyl ammonium bromide (HTAB, Sigma, Germany), and adrenaline (Sigma, Germany). ELISA kits for tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Biolegend, USA.), dextran sodium sulfate (DSS) obtained from Sigma-Aldrich (USA). Sulfasalazine was purchased from an accredited pharmaceutical store in Ondo State, Nigeria.

Extraction and purification of humic acid

Humic acid was extracted and purified using a slight modification of the method described by Chaitra *et al*. (2018). One kilogram (1 kg) of each soil sample into conical flasks. 0.1 M NaOH was added to each soil sample, and the samples were stirred with a shaker for about half an hour. Following a 24-hour period, the aqueous solution was filtered and centrifuged for 15 minutes at 4000 rpm in order to eliminate any suspended particles. The dirt residue was centrifuged at 4000 rpm, agitated for 15 minutes, and then cleaned with 500 mL of 0.1 M NaOH. Until the clear supernatant was achieved, the operation was repeated. In order to cause the precipitation of humic acids, the supernatant was acidified to pH 2 with 0.1 M HCl and let to stand for the entire night. Centrifugation was used to separate the fulvic acid-containing supernatant from the precipitate. After dissolving the humic acid in 0.1 M NaOH, clay impurities were entirely removed by centrifuging the mixture. Humic acid was precipitated from the mixture by acidifying it with 0.1 M HCl. To get rid of any remaining contaminants, distilled water was used to wash the precipitate. Ultimately, the precipitated humic acids were dialyzed in a dialysis bag against distilled water until the AgNO3 solution ceased to contain chloride ions. The dialyzed humic acids were dissolved in ethanol to extract the insoluble humic acid from the mixture's soluble hymatomelanic acid, and the insoluble humic acid was then extracted by centrifugation. After being refined, the humic acid was dried at 40 degrees Celsius in a controlled oven and kept for later use.

Experimental animals

Twenty (20) adult male Wistar rats with an average weight of 160 ± 10 g were purchased from Mctemmy Animal Farm. Animals were acclimatized for two weeks and allowed access to food and water freely. Animals were kept in

ventilated plastic cages cushioned with wood shavings and housed in the animal facilities of the University of Medical Sciences, Ondo, Ondo State, Nigeria. All procedures involving the use of animals conformed with the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines (2010) and ethical standards of the University of Medical Sciences Research and Ethics Committee on Animal Use and Care (UAREC), under approval reference number UNIMED-AREC/Apv/2023/033.

Experimental design

Two weeks after acclimatization, twenty (24) adult male Wistar rats were divided into 4 groups (6 per group), as shown in the table below:

Table 1. Animal grouping

Groups	Treatment
1	Distilled water only
2	Dextran sulfate sodium (DSS) then Distilled water only.
3	DSS then Humic acid (30 mg/kg)
4	DSS then Sulfasalazine (200 mg/kg)

As shown in Table 1 above, the rats in Group 1 served as normal control and received distilled water throughout the study. The rats in groups 2, 3, and 4 received 5% DSS daily for five (5) days according to their body weights. Thereafter, they received distilled water, humic acid, and sulfasalazine for the next five (5) days, respectively. Humic acid and sulfasalazine were solubilized in distilled water and administered via oral gavage. The dose of HA was based on the preliminary toxicological study and previous study as reported by Omolaso *et al*. (2024).

On the day eleven (11), they were euthanized by cervical dislocation, and colons carefully dissected for macroscopic assessment, then had the fecal materials cleaned and colon samples collected in plain sample bottles. Tissue samples of the colon for histological and immunohistochemistry studies were collected and stored in 10% formalin.

Induction of colitis

Colitis induction done in accordance with slightly modified method as described by Chassaing *et al*. (2014), using 5% (weight per volume) dextran sodium sulfate. DSS was added to the drinking water for 5 days. Each drinking solution was administered to the rats in a bottle containing an equal number of moles of the compounds under study.

Disease activity index (DAI)

The Disease Activity Index (DAI) was assessed on days 1, 3, and 5 of DSS treatment, as well as on day 10, equivalent to the post-drug treatment period. Parameters used for assessment included stool consistency, diarrhea, and bleeding scores, which were obtained from observations. Consistent with the methodology described by Cooper *et al*. (1993).

$$DAI = \frac{(\text{body weight drop} + \text{stool consistency} + \text{rectal hemorrhage})}{3}$$

The humane endpoint was defined as DAI = 3, while a diagnosis of ulcerative colitis (UC) was established when DAI was ⩾ 1.5.

Biochemical assessment of inflammation

Excised colon sections of rats were homogenized in phosphate buffer solution (pH 7.4, 0.1M) and centrifuged at 4 ^{o}C at a speed of 1 x 10^4 rpm to obtain the supernatants, which were thereafter stored at -20 ^{o}C.

Determination of Nitrite levels in the Colon tissue homogenate

Nitrite in the colon tissue homogenate was measured as an indicator of nitric oxide (NO) production according to the Griess method as described by Green *et al.* (1982). The concentration of nitrite was determined from the sodium nitrite standard curve and expressed as µmoles/mg protein.

Determination of myeloperoxidase (MPO) activity in colon tissue homogenate

Tissues were suspended in extraction buffer (0.5% hexadecyltrimethylammonium bromide) and 50 mM potassium phosphate buffer (pH 6.0) and frozen at 20 °C. The process of freeze-thaw and sonication for a 10-second cycle was repeated three times. The suspension was finally centrifuged at 15,000 rpm at 4°C for 15 min. MPO activity was assayed by adding 20 µL of supernatant to a 96-microtiter plate, then 180 µL of reaction buffer (containing 0.167 mg/mL O-dianisidine in 50 mM potassium phosphate buffer and 0.15 mM H_2O_2) was added. The change in absorbance at 450 nm was monitored over 5 minutes in a microplate reader (LT4500, UK). One unit of MPO was defined as that giving a change in absorbance of 0.001 per min, and the specific activity was expressed as a unit of MPO per milligram of protein.

Enzyme linked immunosorbent assay (ELISA) for determination of IL-6 and TNF-α in the colon tissue.

Colon tissue levels of IL-6 and TNF-α were determined by the Biolegend ELISA kit (USA) specific to the cytokines of interest, with a sensitivity limit of 4 pg/mL. All the measurements were done at room temperature in accordance with Biolegend instructions using a microplate reader with a 450nm filter. The concentrations of IL-6 and TNF-α in the serum were extrapolated from the standard curves of the IL-6 and TNF-α standards included in the assay kits and expressed as pg/mL. The assay was carried out according to the protocol ELISA kit manufacturer, Biolegend ®, U.S.A.

Histological assessment of the colon

This was done in accordance with the previously described method by Owen *et al.* (2011). In brief, the tissues were

fixed in formalin (10%). Water was removed in graded alcohol. Thereafter, it was cleared in xylene and fixed in paraffin wax. The tissues were later cut into sections (four micrometers thick) by a microtome, embedded on the slides, and stained with hematoxylin and eosin (H&E). The resultant slides were examined underneath a light microscope (Olympus, Japan), and photomicrographs were taken with a DM750 camera (Leica, Germany) at 100 magnifications.

Immunohistochemistry and image quantification

Sections of 5 µm thickness obtained from routine paraffin were deparaffinized and subjected to antigen retrieval by heating in a citrate-based antigen unmasking solution, pH 6.0 (Vector Labs, CA, USA) for 30 mins in a steamer and allowed to cool on the bench at room temperature for 30 mins. Endogenous peroxidase blocking was performed in 0.3% hydrogen peroxide in phosphate-buffered saline (PBS, pH 7.4) for 10 min. Sections were then incubated at room temperature for 2 hours in primary rabbit antibodies diluted in a universal antibody diluent and blocking reagent, UltraCruz® Blocking Reagent (Santa Cruz, USA). Primary antibody used is MUC2 polyclonal antibody (Elabscience, USA; #E-AB-70212) at 1:500. Sections were washed in PBS and incubated in ImmPRESSTM HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse (Vector Labs, USA). The color was developed with the DAB Peroxidase (HRP) Substrate Kit (Vector Labs, USA), and sections were counter-stained in hematoxylin (Ijomone *et al*., 2018).

Statistical analysis

This study utilized GraphPad Prism version 9.0.5 (GraphPad Software, San Diego, USA) for the analysis of collected data. The data was presented as mean ± standard error of mean (SEM) with n = 5. An analysis of variance (one-way ANOVA) was employed to examine mean differences, and the Tukey post hoc test was used for multiple comparisons. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of humic acid on the disease activity index of dextran sulfate sodium-induced colitis in adult male wistar rats.

As shown in table 2 below, the results of the Disease Activity Index (DAI) across the days in each group indicated the progressive effect of DSS on colitis induction and the effect of humic acid as well. There was statistical significance between the disease activity index of days 3, 5, and 10 post-colitis induction. However, treatment with humic acid and sulfasalazine significantly reduced the DAI by day 10.

Table 1. Disease activity index (DAI) in rat groups

Days	Control	DSS only	DSS+HA (30 mg/kg)	DSS+Sulfasalazine (200 mg/kg)
1 post colitis induction	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3 post colitis induction	0.00 ± 0.00	0.7330 ± 0.1400[a]	0.6328 ± 0.1332[a]	0.796 ± 0.1231[a]
5 post colitis induction	0.00 ± 0.00	2.266 ± 0.1496[a]	2.200 ± 0.1334[a]	2.194 ± 0.1334[a]
10 (treatment)	0.00 ± 0.00	2.346 ± 0.0988	0.230 ± 0.00[b]	0.5666 ± 0.1666[ab]

[a]statistically different ($p < 0.05$) from control; [b]statistically different ($p < 0.05$) from DSS only across the groups for each day.

Effect of humic acid on the colon macroarchitecture of dextran sulfate sodium-induced colitis in adult male wistar rats.

As shown in figure 1 below, a colon sample of a rat in the control group did not show any sign of colitis. Oral administration of Dextran sulfate sodium (DSS) evoked colonic inflammation, as shown. In contrast to the Dextran sulfate sodium-induced colitis rats, rats treated with 30 mg/kg of humic acid showed no observable signs of colonic inflammation with well-formed fecal material. Similarly, rats treated with 200 mg/kg of sulfasalazine exhibited no colonic inflammation.

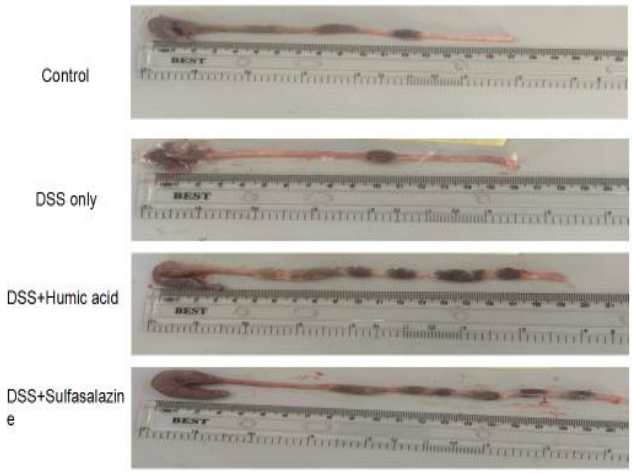


Figure 1. Effect of the humic acid on the macroarchitecture of the colon of Dextran sulfate sodium-induced ulcerative colitis rats.

Effect of humic acid on the assays of colon tissue nitrite and arginase level on dextran sulfate sodium-induced ulcerative colitis

As shown in Fig. 2a, our data indicated a significant ($p < 0.05$) decrease in the colon tissue level of nitrite in the DSS-treated rat group compared to the control group. However, treatment with humic acid significantly ($p < 0.05$) increased

the nitrite level compared with the DSS-only group. Also, in Fig. 2b, our data indicated a significant ($p< 0.05$) increase in the colon tissue level of arginase in DSS-treated rats compared with the control group. On the other hand, treatment with humic acid significantly ($p< 0.05$) decreased the level of arginase compared with the DSS-only group.

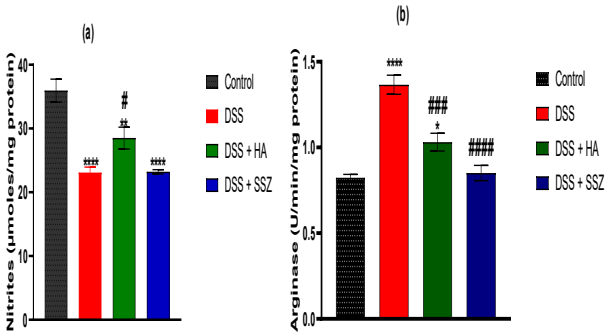
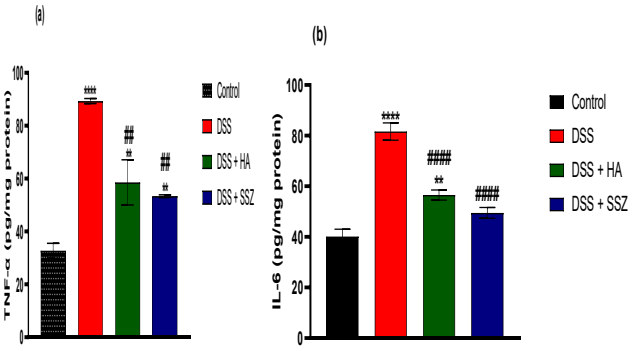


Figure 2. Effect of treatment with Humic acid on the colon level of nitrite in rats induced with colitis.

Bars represent Mean ± Standard Error of Mean (SEM), (n = 5) (one-way ANOVA followed by *Tukey post* hoc test). *p<0.05, **p<0.01, ****p<0.0001 in comparison with control; #p<0.05, ###p<0.001, ####p<0.0001 vs. DSS.

Effect of humic acid on levels of inflammatory markers in the colon tissues of Dextran sulfate sodium-induced colitis rats

As shown in Fig. 3a to 3d, our data indicated a significant ($p< 0.05$) increase in the colon levels of markers of inflammation (TNF-α, interleukin-6, interleukin-1β, and MPO) respectively in DSS-treated rats compared with the control group. On the other hand, treatment with humic acid significantly ($p< 0.05$) decreased the levels of TNF-α, interleukin-6, interleukin-1β, and arginase compared with the DSS-only group.



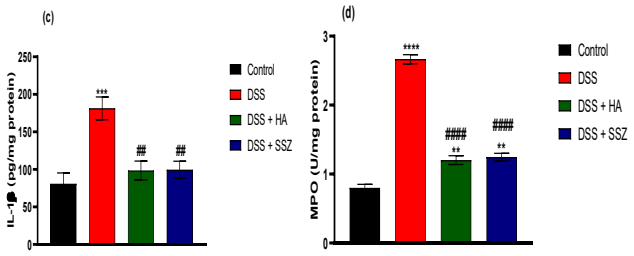


Figure 3: Effect of treatment with Humic acid on the colon levels of (a) TNF-α, (b) interleukin-6, (c) interleukin -1β, and (d) arginase in rats induced with colitis.

Bars represent Mean ± Standard Error of Mean (SEM), (n = 5) (one-way ANOVA followed by *Tukey post* hoc test). **p<0.01, ***p<0.001, ****p<0.0001 in comparison with control; ##p<0.01, ####p<0.0001 vs. DSS.

Effect of humic acid on MUC2 gene expression in DSS induced ulcerative colitis in wistar rats

As shown in figures 4 and 5, the immunochemistry of MUC-2 expression colon tissues revealed a significant reduction ($p<0.05$) in MUC-2 expression compared with the control. Results also show obvious MUC2 expression in the colon of the humic acid-treated group as compared with the DSS group.

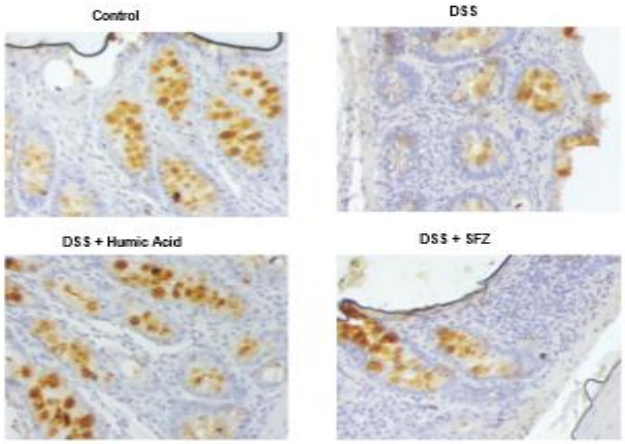


Figure 5: Effect of humic acid on Muc2 Gene Expression in DSS induced Ulcerative Colitis in wistar rats.

Photomicrographs were taken at x400 magnification. Image analysis was performed using Image J software (NIH, USA). Positive immune-expression of MUC2 is noted by brown colour in tissues.

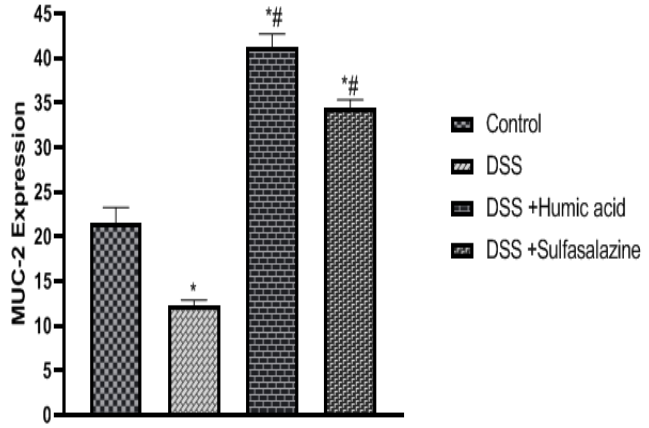


Figure 6: Image analysis of the effect of treatment with humic acid on the MUC-2expression in rats induced with colitis.

Bars represent Mean ± Standard Error of Mean (SEM), (n = 5) (one-way ANOVA followed by *Tukey post* hoc test). *p<0.05 vs. control; #p<0.05 vs. DSS.

Histological assessment of humic acid effects on the colon of DSS-induced ulcerative colitis.

As shown in the micrograph, H&E histological staining at x 100 magnifcation reveals inflammatory infiltrate, crypt, and lining epithelial damage in the colon of the DSS-treated rat, while the cellular architecture of the colon of the humic acid-treated rat was fully restored.

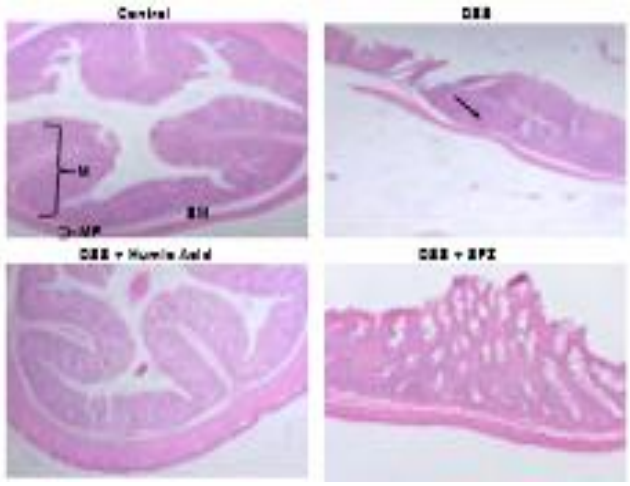


Figure 7: Micrographs of colon of control and treated rats.

H&E x 100. M – mucosa layer; SM – submucosa layer; MP – muscularispropria; Arrows – inflammatory infiltrate; Dashed arrow – crypt and lining epithelial damage.

DISCUSSION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by relapsing and remitting inflammation of the colonic mucosa (Saez *et al*., 2023). The incidence of ulcerative colitis (UC), a chronic inflammatory bowel disease (IBD) affecting the colon and rectum, is rising quickly in developing nations (Ungaro *et al*., 2017) Current therapeutic strategies aim to reduce inflammation and maintain remission, but side effects and the need for long-term medication pose significant challenges. Humic acid, a complex mixture of organic compounds that is gotten from decaying organic matters used in this study provides evidenced based effects in DSS model of ulcerative colitis in rats.

In this present study, after inducing ulcerative colitis with DSS, animals exhibited similar symptoms to IBD, including mucosal ulceration and inflammation (Lee et al., 2023). While DSS is not the cause of the human model of UC, we chose the Dextran sodium sulfate (DSS) UC induction model because it is frequently used for chemical induction of colitis in animals. This model is widely used because of its ease of induction, reproducibility, and well-characterized mucosal injury resembling the effects that occur in human colitis; the onset, duration, and severity of the disease can be effectively controlled (Eichele and Kharbanda, 2017; Tie et al., 2024).

We obtained the Disease Activity Index (DAI), a composite score that evaluates the severity of colitis based on weight loss, stool consistency, and rectal bleeding. As shown in Table 2, DSS administration induced colitis in rats, evidenced by a progressive increase in DAI scores. By days 3, 5, and 10 post-colitis induction, the DAI scores reflected the acute inflammatory response and tissue damage induced by DSS. This is consistent with the previous studies where DSS treatment was used to induce colitis (Gerges et al., 2020). We also observed better performance of HA than sulfasalazine in reducing the DAI. The results of the notable increase in the DAI of this study as presented brought great insights that the severity of colitis progresses with time and, when untreated, could have impacted greatly on the gastrointestinal membrane architecture. Also, as seen in the result, macroscopic assessment of the colon revealed distinct differences between the groups. The control group showed no signs of colitis, with healthy and intact colonic mucosa and well-formed fecal material. In contrast, DSS administration resulted in pronounced colonic inflammation, characterized by mucosal erythema, edema, and ulcerations.

As shown in Figure 3, DSS treatment increased MPO levels in colonic tissues compared with the control, reflecting heightened neutrophil infiltration. Similarly, the results show DSS treatment elevated the levels of these cytokines (TNF-α, IL-6, and IL-1β) in colonic tissues, reflecting the inflammatory milieu characteristic of UC. Also, DSS-induced colitis decreased the nitrite levels compared with the control group, likely due to the consumption of NO during the inflammatory process. In order to understand the role of arginase in the nitric oxide pathway of the UC model, we investigated the level of colonic arginase as well. Just like the pro-inflammatory markers, DSS treatment elevated the levels of arginase, implicating arginase as relevant in the UC pathogeneis and therapeutic approach.

The pathogenic mechanisms of inflammatory bowel diseases are multifaceted and associated with oxidative stress, an unbalanced gut microbiota, and an aberrant immune response (Ungaro *et al*., 2017; Saez *et al*., 2023). Myeloperoxidase (MPO) is not an enzyme produced by

neutrophils during inflammation, serving as a marker for neutrophil infiltration (Herrero-Cervera *et al*., 2022**)**. MPO levels are correlated with the severity of inflammation in UC. Higher MPO activity indicates more intense inflammation and mucosal damage (Khan *et al*., 2020)**.** Monitoring MPO levels provides insights into disease activity and response to treatment. MPO produces reactive oxygen species (ROS) during inflammation, which contribute to oxidative stress and tissue damage (Lin *et al*., 2024)**.** Elevated MPO activity can therefore be associated with increased oxidative damage in the colonic tissue, which exacerbates UC pathology.

The expression of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, plays pivotal roles in the inflammatory cascade of UC (Souza et al., 2023). Also, nitrite levels in colonic tissues are indicative of nitric oxide (NO) production, a mediator of inflammation and tissue damage in UC (Waltz et al., 2015). Increased nitrite can be beneficial in colitis, particularly by converting to nitric oxide (NO) to reduce inflammation and helps the epithelial cells of the colon heal from damage (Jädert et al., 2018). A linkage between NO and arginase, an enzyme that catalyzes the conversion of arginine into ornithine and urea, has been established in the cascades of inflammatory reactions (Li et al., 2022). This reaction competes with nitric oxide synthase (NOS), which converts arginine into nitric oxide (NO). Inflammatory conditions often see increased arginase activity, which can reduce NO production. The balance between NO and ornithine affects inflammatory processes, NO has anti-inflammatory and immunomodulatory effects, whereas increased ornithine can lead to the production of polyamines that promote tissue repair but also contribute to chronic inflammation when dysregulated (Martí et al., 2021).

Interestingly, treatment with HA reduced MPO levels, indicating its potent anti-inflammatory and anti-oxidative treatment properties. This reduction in MPO aligns with previous report where HA mitigated oxidative stress in the inflammation associated with traumatic spinal cord injury (Kinali et al., 2024). Similarly, HA treatment significantly decreased the levels of TNF-α, IL-6, IL-1β, and arginase compared to the DSS-only group, highlighting its anti-inflammatory potential. Treatment with HA significantly increased nitrite levels compared to the DSS-only group, suggesting a restoration of NO balance and potential modulation of inflammatory pathways. Possibly, HA could also have positvely modulate the gut microbiota there by reducing the levels of pro-inflammatory lipopolysaccharides (LPS) and subsequent inflammatory signaling via the TLR4-NF-κB pathway (Huang et al., 2023).

These results are in agreement with the report of Khan et al., (2023) where α-terpineol; a naturally occurring mitigates DSS-Induced colitis in rats as well by attenuating inflammation and apoptosis. Also, HA was reported to have protective effect on gastric ulcer disease model in rats by alleviating inflammation (Şehitoğlu et al., 2022). Other bioactive compounds from natural sources have been reported to be effective in the inhibition of the level of inflammatory factors and oxidative stress and histological inflammation, such as inflammatory cell infiltration and goblet cell depletion, in different animal models of UC (Sakai et al., 2019; Gerges et al., 2020; Xuan et al., 2020; Huang et al., 2022). Notably, treatment with HA culminated in the reduction of the DAI by day 10 as shown in table 2. Beyond the DAI, treatment with HA also markedly attenuated the macroscopic signs of inflammation, this further strengthened our claim of the therapeutic effects of HA in DSS-induced ulcerative colitis models.

Ulcerative colitis pathogenesis hinges on an imbalance where protective mucosal mechanisms fail to counter aggressive inflammatory responses in the colon (Kang et al., 2022). MUC-2 is a key component of the mucosal barrier and a major therapeutic target in inflammatory bowel diseases, its expression is critical for maintaining gut integrity (Yao et al., 2021; Kang et al., 2022). In UC, MUC-2 expression is often reduced, compromising the mucosal barrier and exacerbating inflammation. Immunohistochemical analysis from our study revealed a significant reduction in MUC-2 expression in the DSS-treated group compared to controls. However, HA treatment restored MUC-2 expression, suggesting a protective effect on the mucosal barrier. This finding shows that HA has the potential of enhancing mucosal barrier function and reducing intestinal permeability. Previous researches have reported that natural products could enhance mucosal barrier function and reduce intestinal permeability (López-Cauce et al., 2022; Li et al., 2023).

Apart from the obvious protective effects on the mucosa, the macroscopic restoration of the colonic histoarchitecture, the antioxidant and anti-inflammatory effects of HA, we looked further into the histology of the colon. Histological examination of colonic tissues gives insights into the cellular and structural changes induced by DSS and the protective effects of HA treatments. Hematoxylin and eosin (H&E) staining not only revealed significant inflammatory infiltrate but also crypt damage and epithelial disruption in the colon of the DSS-treated group (micrograph). In contrast, HA-treated rats showed restored cellular architecture with reduced inflammation and crypt damage. In other similar studies, nutraceuticals have been shown to restore gut epithelium morpho-histology (Bastaki et al., 2016; Nehmi-Filho et al., 2023; Xu et al., 2024). These histopathological findings corroborate the macroscopic and biochemical data, underscoring the therapeutic potential of HA in UC. HA does not clearly show to be more efficacious than the sulfasalazine but it has proven to have potential especially in the expression of MUC-2 gene and it might be more efficacious at higher dose. Hence, the need for a further study on the dose-dependent effect of HA.

CONCLUSION

In conclusion, the results of our study demonstrate that HA holds potential as an alternative therapeutic agent for UC, offering antioxidant, anti-inflammatory, mucosal barrier-protective properties, and histoarchitectural-restoring effects.

AUTHORS' CONTRIBUTIONS

OBO and AGA: Conceptualization, methodology, writing—review and editing, supervision; OOE: methodology, supervision, writing—original draft preparation; formal analysis, AA, EK, AOT, and AO: methodology, project administration and AJK: methodology, project administration. All author has read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

There is no conflict of any kind to declare

DATA AVAILABILITY

All data supporting the findings of this study are available within the paper.

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