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Original Research Article

Methanol Extract of *Chrysophylum albidum* Fruit Attenuates Dextran Sulfate Sodium-Induced Ulcerative Colitis

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ABSTRACT

Ulcerative colitis (UC) is a chronic bowel illness that causes inflammation by eroding the colon and rectum lining. The prevalence of inflammatory bowel disease (IBD) is on the rise worldwide. The available treatments have been reported to cause adverse effects such as nasopharyngitis, hypokalemia, and leukopenia. Hence, in the quest to manage ulcerative colitis, there is a need to investigate potential therapeutic bioactives in natural products such as *Chrysophylum albidum* fruit which have been reported to have anti-inflammatory properties. The aim of this study, therefore, was to evaluate the effects of *Chrysophylum albidum* fruit methanol extract (CAFÉ) on inflammation in the Dextran sulfate sodium (DSS) induced model of colitis in male Wistar rats. Twenty-five male Wistar rats were randomly divided into 5 groups of 5 animals each: Control (I); DSS-induced colitis (II); DSS-induced colitis + 200mg/kg of CAFÉ (III); DSS-induced colitis + 400mg/kg of CAFÉ (IV) and DSS induced colitis + 200mg/kg of Sulfasalazine (V). Colitis was induced by administering 3% (v/v) DSS for seven days. CAFE administration at 200 mg/kg and 400 mg/kg reduced the infiltration of inflammatory cells and alterations in the structure of colonic crypts. The extract significantly ($p<0.05$) attenuated the increased MDA, nitrite, TNF-α, and IL-6 levels. Activities of SOD, CAT, MPO, and GSH were also significantly ($p<0.05$) increased. Immunohistochemistry revealed increased colonic MUC-2 expression by CAFÉ following DSS-induced colitis. In conclusion, CAFÉ attenuated DSS-induced colitis via antioxidant and anti-inflammatory mechanisms and through the stimulation of MUC 2 expression in the colon.

Keywords: *Chrysophylum albidum*, Ulcerative colitis, MUC-2, Medicinal Plant.

Introduction

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) characterized by chronic inflammation of the colon and rectum which presents with symptoms such as abdominal pain, bloody diarrhea, weight loss, and weakness due to the breakdown of the intestinal epithelium that has a mucus gel layer cover and a series of remissions and relapses that leads to bad quality of life for millions worldwide.[1,2,3,4] The mucus gel layer is made up of mucins which are heavily glycosylated O-glycoproteins produced by secretory epithelial cells.[5] Alteration in mucin expression or composition can affect the function of the mucus gel layer.[6,7] Mucin 2, oligomeric mucus gel-forming, also known as MUC2, a protein encoded by the MUC2 gene is the only gel-forming mucin that is expressed in the colon from goblet cells at a physiologically relevant level.[8,9] It precise etiology is still obscure, but a complex interplay of genetic, environmental, gut microbiota and immune factors have been implicated.

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While the global prevalence of IBD is high; about 59.25 per 100 000 people in 2019 [29], the available treatment for UC mainly manages inflammation by inducing remission with aminosalicylates, corticosteroids, immunomodulators or biologic agents that can be effective but with adverse effects and a non-sustainable remission in a certain proportion of people, [30, 31, 32] thus prompting the need for safer and more effective therapeutic alternatives. Further, the World Health Organization (WHO) reported in 2019, that over 80% of the world's population basically uses herbs for their health challenges. [10] Folkloric uses of herbs in the treatment and management of disease conditions have enabled the continuity in extraction and identification of their bioactive as they have the advantages of lower adverse effects, accessibility, and affordability.[11, 12, 13]

Many animal models have been used to characterize the complexity of ulcerative colitis, identify underlying mechanisms, and evaluate potential therapeutics. [23] Dextran sodium sulfate (DSS), a chemical colitis agent, is a well-established and widely used experimental model for UC in Wistar rats that bears a striking resemblance to human IBD, with typical symptoms like as diarrhea, blood in the stool, and weight loss. Oral administration of DSS causes harm to the colon's epithelial monolayer lining, causing intestinal inflammation that is associated with a decrease in the production of Mucin 2 (MUC2), an oligomeric mucus gel-forming protein that is expressed in the colon from goblet cells at a physiologically relevant level. [8, 9, 34] These features make DSS an important means of assessing possible anti-inflammatory and or gut-protective agents,[33].*Chrysophyllum albidum* (C. albidum) G. Don, commonly known as the African star apple or "agbalumo" in Yoruba, is a fruit-bearing plant of the Sapotaceae family, commonly found in East and West Africa.[14] C. albidum plant

parts have been reported to possess some ethnomedicinal and pharmacological activities; leaves, bark, and fruits are used as anti-inflammatory, [15] antidiabetic, [16 17, 18] hypolipidemic, [19 20] blood pressure, [21] antiplasmodial, [22,23, 24] and ulcer. [25] Phytochemical analysis of *Chrysophylum albidum* fruit signifies saturated (palmitic acid, myristic acid ester) and unsaturated (linoleic acid, oleic acid) fatty acids [26] and the exocarp and pulp are very high in ascorbic acid, [27] Moreover, phenolic compounds in the fruit include; catechin, chlorogenic acid, caffeic acid, epicatechin acid, cyanidine-3–0-glycoside, rutin, quercitrin, quercetin and kaempferol.[28] Given the significant role of oxidative stress and inflammation in the pathogenesis of UC, the therapeutic potential of *C. albidum* fruit in colonic inflammation needs to be investigated. This is the first attempt to investigate the anti-inflammatory properties of *C. albidum* fruit in the management of ulcerative colitis and it therefore, aims to evaluate the effect of *Chrysophyllum albidum* fruit on DSS-induced ulcerative colitis in *Wistar* rats. Specifically, we hypothesize that oral administration of *C. albidum* fruit extract will ameliorate the disease activity, macroscopic, and microscopic signs of colitis, reduce inflammatory markers, and restore colonic MUC2 expression and tissue integrity in DSS-treated rats. The findings from this work could provide scientific validation for the folklore use of *C. albidum* and potentially identify a novel, natural therapeutic agent for the management of ulcerative colitis.

Materials and Methods

Study Location

This study was carried out at the Department of Physiology, Faculty of Basic Medical Sciences, University of Medical Sciences, in Ondo state.

Materials, apparatus, drugs, and chemicals

Dextran sulfate sodium was procured from BIOSYNTH (Carbosynth Ltd, UK). Trichloroacetic acid (TCA), thiobarbituric acid (TBA), 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), and hexadecyltrimethylammonium bromide (HTAB) were purchased from Sigma (Sigma, Germany). Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were ELISA kits from BioLegend, USA. Primary antibody (Elabscience, USA), blocking reagent (Santa Cruz, USA), ImmPRESS™ HRP Anti-Rabbit IgG (Peroxidase) polymer reagent (Vector Labs, USA), and DAB Peroxidase (HRP) Substrate Kit (Vector Labs, USA). All other reagents used were of analytical grade.

Extraction of Chrysophyllum albidum fruit

Extracting the edible portion of the fruit, large quantities of Chrysophylum albidum fruits were purchased at a small-scale farmer's stand at the Bodija market in Ibadan, Oyo State, Nigeria. Fruit samples with stalks and leaves were brought to the Forest Research Institute's regional herbarium, Ibadan, Oyo State Nigeria for authentication and voucher number (FHI 110475). The fruits were washed under running water to get rid of impurities and waxes. In the lab, the entire fruit—seeds excluded—was chopped into tiny pieces and blended in an electrical mixer. 200g of the homogenized peel and pulp of *Chrysophyllum albidum* fruit was macerated in 500 mL of methanol (80%) for 72 hours in a glass jar bottle and was kept in a dark cupboard. The extract was filtered using muslin cloth and doubly filtered using filter paper. The filtrate was concentrated using reduced pressure in a rotary evaporator at 45ºC and the concentrate was dried to a constant weight in a vacuum oven to remove all traces of methanol and water. The percentage yield was 4.5 %. For the experiment, the extract was prepared freshly by using 800 mg in 10 ml of distilled water and diluting appropriately for respective doses.

Experimental animals

Twenty-five male Wistar rats (140-160 g) were purchased from Mctemmy Concepts Laboratory Ogbomosho, Oyo state, Nigeria. The animals were kept in plastic cages and maintained at standard laboratory conditions under photoperiods of the 12-h light and 12-h dark cycle. Rats were allowed to acclimatize for two weeks during which they had access to chow and drinking water ad libitum. All animal experimentation was conducted according to the internationally accepted principles for laboratory animal use and care as found in the United States Guidelines. [Institute for Laboratory Animal Research, 2011] Ethical approval was obtained from the University of Medical Sciences animal research ethical committee (AREC/ADV/2023/046).

Animal grouping and Experimental design

Twenty-five (25) male *Wistar* rats were randomly divided into five groups as listed below:

Control group.

Dextran Sulfate Sodium (DSS) induced colitis group.

Dextran Sulfate Sodium (DSS) induced colitis + 200mg/kg of methanol extract of *Chrysophyllum albidum* fruit.

Dextran Sulfate Sodium (DSS) induced colitis + 400mg/kg of methanol extract of *Chrysophyllum albidum* fruit.

Dextran Sulfate Sodium (DSS) induced colitis + 200mg/kg of Sulfasalazine.

Induction of Colitis

Colitis was induced by administration of 3 % DSS for 7 days alongside daily oral doses of different treatments depending on their grouping. (The rats in group 1 received only water as a vehicle throughout the study. On day eight of the study, they were euthanized by cervical dislocation, and colon samples were collected in plain sample bottles homogenized in phosphate buffer saline (pH 7.4), and stored at -4 degrees Celsius. Colon tissue samples were stored in 10% formalin for histological analysis.

Evaluation of Disease Activity Index (DAI)

Disease activity index (DAI) was accessed daily according to the method described earlier by [35] scoring Body weight loss (0: none, 1: 1–5%, 2: 5–10%, 3: 10–20%, 4: >20%); stool consistency (0: normal, 1: loosed stool that did not stick to the anus, 2: semi-loose stool that sticks to the anus, 3: very loose stool that does not stick to the anus, 4: diarrhea that sticks to the anus; rectal bleeding (0: none, 1: slight bleeding, 2: gross bleeding); the humane endpoint was set at DAI = 3, and at DAI ≥1.5, ulcerative colitis was diagnosed.

Macroscopic Assessment of Colon

Rats were euthanized on the eighth day of DSS-colitis, colons were excised, macroscopically examined with the naked eye by two independent observers, and photographs were taken. Colons were thereafter, washed with ice-cold phosphate-buffered saline and then processed for biochemical, histological, and immunohistochemical analysis.

Enzyme-linked immunosorbent assay (ELISA) for determination of TNF-α and IL-6

After being cleansed with one volume of phosphate buffer saline (PBS), colon tissues were homogenized using a Teflon glass homogenizer (Glass-Col, USA) in four volumes of ice-cold 0.1 M phosphate buffer (pH 7.4). Homogenates were further allowed to freeze-thaw in four cycles and centrifuged at 5000 g for 5 min at 4°C with an MV 16RF cold centrifuge (Inspiration Marvotech, China). Levels of TNF- α and IL-6 in colon tissues were tested according to protocols of the kit manufacturer, BioLegend ®, U.S.A, with a sensitivity limit of 4 ρg/mL. All quantification was done at room temperature by the manufacturer's guide and using a microplate reader (Labtech LT-4500, UK) with a 450nm filter. Concentrations of IL-6 and TNF-α from homogenates were extrapolated from standard curves of IL-6 and TNF-α contained in the test kits and expressed in ρg/mL.

Assays to determine colonic tissue oxidative stress biomarkers

Tissues were homogenized in ice-cold phosphate buffer (0.1M, pH 7.4) with a power-driven homogenizer and a glass Teflon (Glass-Col, USA). Homogenates were centrifuged at 4ºC in a cold centrifuge at a speed of 10,000 rpm for 10 min to get the supernatant. The supernatants were aliquoted and refrigerated at -20ºC for later use. Reduced glutathione (GSH), a non-enzymic antioxidant fabricator was quantified using the method described by.[36] Catalase (CAT) activity was calculated using a colorimetric test based on the yellow compound with molybdate and H_2O_2 as described by.[37] Activity of superoxide dismutase (SOD) was also, estimated as described by. [38] The activity of glutathione-S-transferase (GST) was studied by the

method of [39] while the unit of the enzyme was stated as U/mg protein. Lipid peroxidation was done by estimating the level of malondialdehyde (MDA) as described by.[40] Nitrite was quantified as a marker of nitric oxide (NO) production according to the Griess method described by. [41] Myeloperoxidase (MPO) activity was tested by the method described by. [42] One unit of MPO activity was expressed as destroying one micromole of peroxide per minute at 25°C (U/mg protein).

Hematoxylin and Eosin staining
Colon tissues fixed in neutral buffer formalin were treated using routine paraffin embedding standard protocols and, thereafter, were subjected to routine Hematoxylin and Eosin staining. Photomicrographs were obtained from the stained slides using a Digital Microscope (OMAX microscopes, Irvine, CA, USA) at 400 magnifications.

Immunohistochemistry and image quantification
After being deparaffinized, colon sections of 5 µm thickness that were obtained from ordinary paraffin were heated for 30 minutes in a steamer in a citrate-based antigen-revealing solution (pH 6.0; Vector Labs, CA, USA) and allowed to cool at room temperature for another 30 minutes. In PBS (pH 7.4) containing 0.3% hydrogen peroxide, endogenous peroxidase blocking was carried out for 10 minutes. Following that, sections were incubated for two hours at room temperature in primary rabbit antibodies that had been diluted in UltraCruz® Blocking Reagent (Santa Cruz, USA), a universal antibody diluent. At 1:500, the MUC-2 polyclonal antibody (Elabscience, USA; #E-AB-70212) is the main antibody utilized. After being cleaned in PBS, the sections were incubated with Vector Labs' ImmPRESSTM HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, which was made in horses. Sections were counter-stained in hematoxylin after color was established using the DAB Peroxidase (HRP) Substrate Kit (Vector Labs, USA). [43, 44] After that, sections were examined using a digital brightfield microscope, and X400 magnification photomicrographs were taken. Image J software (NIH, USA) was used to conduct the image investigation. The Immuno Ratio plugin on Image J, which divides and measures the proportion of DAB (positive immunoreactivity) via digital color deconvolution, was used to count immunoreactivity. [43, 45]

Data analysis
Data was analyzed with statistical software; graph pad Prism version 8.0 (GraphPad Software, Inc. La Jolla, CA 92037 USA). Results are stated as Mean ± standard error of the mean (S.E.M) and statistical significance was evaluated using one-way analysis of variance (ANOVA). Differences at * $p< 0.05$ were taken to be significant.

Results and Discussion

Disease activity index (DAI)
As shown in Table 1, DAI was significantly increased in DSS only group compared with DSS + CAFE group thus, indicating decrease in body weight and diarrhea. According to the stool consistency score results, colitis animals scored higher than the controls, which is in line with the findings from changes in body weight. By day 8 which was the terminal day for the experiment, CAFÉ administration at 200 and 400 mg/ kg following DSS colitis induction significantly reduced disease activity index (DAI) compared to DSS colitis without treatment group (Table 1).

Table 1: Disease activity index (DAI)

DAYS	CONTROL	DSS	DSS+CAFE (mg/kg)	DSS+CAFE (mg/kg)	DSS+SFZ
Day 1	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Day 2	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Day 3	0.00 ±0.00	0.44±0.06**a**	0.40±0.04**a**	0.40±0.05**a**	0.40±0.04**a**
Day 4	0.00 ±0.00	0.69±0.03**a**	0.60±0.04**a**	0.47±0.06**ab**	0.40±0.07**ab**
Day 5	0.00 ±0.00	1.27±0.07	0.73±0.03**ab**	0.60±0.05**ab**	0.73±0.03**ab**
Day 6	0.00 ±0.00	1.33±0.07	0.53±0.08**ab**	0.53±0.08**ab**	0.47±0.08**ab**
Day 7	0.00 ±0.00	1.67±0.06	0.47±0.05**ab**	0.53±0.05**ab**	0.40±0.05**ab**
Day 8	0.00 ±0.00	1.83±0.07	0.34±0.06**ab**	0.31±0.06**ab**	0.27±0.02**ab**

Disease activity index (DAI) from day 1 through 8. DSS – Dextran sulfate sodium; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine. Values represent mean ± SEM (n=5). [a] $p < 0.05$ vs. control group; [b] $p < 0.05$ vs. DSS group.

Colon macroscopic appearance following DSS-induced colitis
Fig. 1 shows the effect of CAFE on macroscopic appearance of colon after dextran sulfate sodium-induced colitis in male *Wistar* rats.

CAFE reduced colon tissue oxidative stress markers
Myeloperoxidase was measured in all experimental groups so as to ascertain the level of neutrophil infiltration. DSS administration resulted in significant increase in MDA, NO and MPO concentration. This is indicative of lipid peroxidation and neutrophil infiltration respectively, (Fig. 2 A-C). However, CAFE treated rats showed decreased MDA, NO and MPO concentration at 200 mg/kg and 400 mg/kg doses, thereby decreasing oxidative stress markers.

CAFE improved colon tissue antioxidant markers in DSS-induced-colitis
Fig. 3. A-D shows the effect of CAFE on assays of tissue antioxidant markers of DSS-induced colitis in adult male *Wistar* rats. Exposure to DSS only resulted in a significant decrease in the activity of antioxidant

enzymes namely GSH, SOD, CAT, and GST when compared with the control. However, the administration of CAFE significantly increased the activities of these antioxidant enzymes.

CAFE suppressed proinflammatory cytokines in DSS-induced Colitis
Fig. 4 A and B show the effect of CAFE on the proinflammatory mediators in male *Wistar* rats. There was a significant elevation in the

levels of TNFα and IL-1β in rats exposed to DSS only when compared with the control group. Treatment with CAFE significantly prevented the increase in these proinflammatory mediators.

Effect of CAFE on colon histopathology in DSS-induced colitis.
The photomicrograph of the control displays intact histology of the colon; well-defined mucosa, sub-mucosa, and muscular layers, the lining epithelium and crypts in the mucosa layer also, appear intact. DSS-induced colitis group without any treatment, however, reveals damaged crypts and lining epithelia, with marked inflammatory infiltrates which appear to be necrotic in the sub-mucosa. Also, necrotic

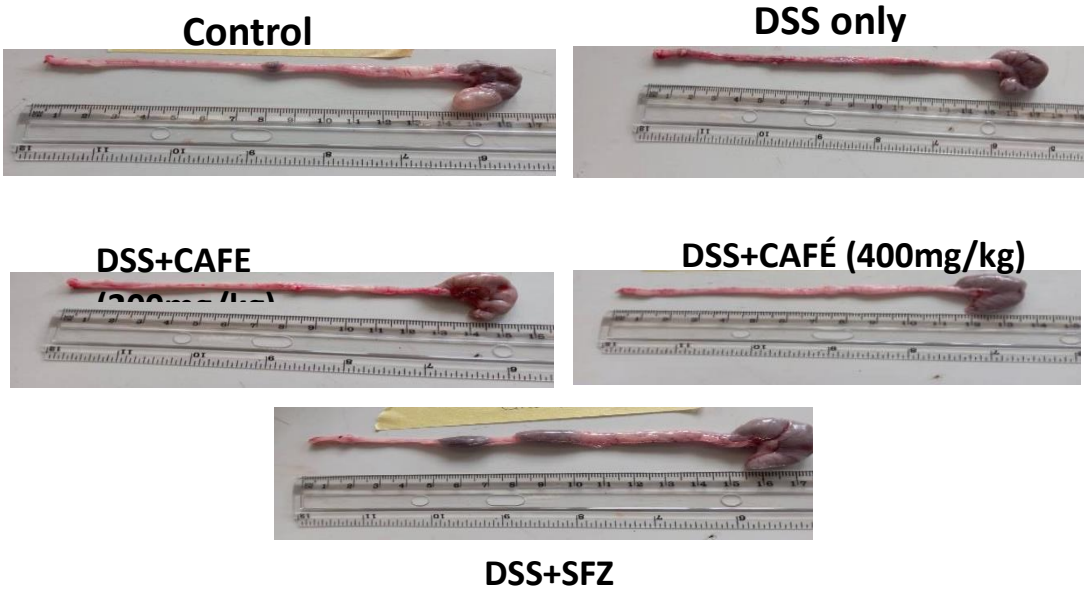

Figure 1: Macroscopic appearance of rat's colon after DSS-induced colitis.

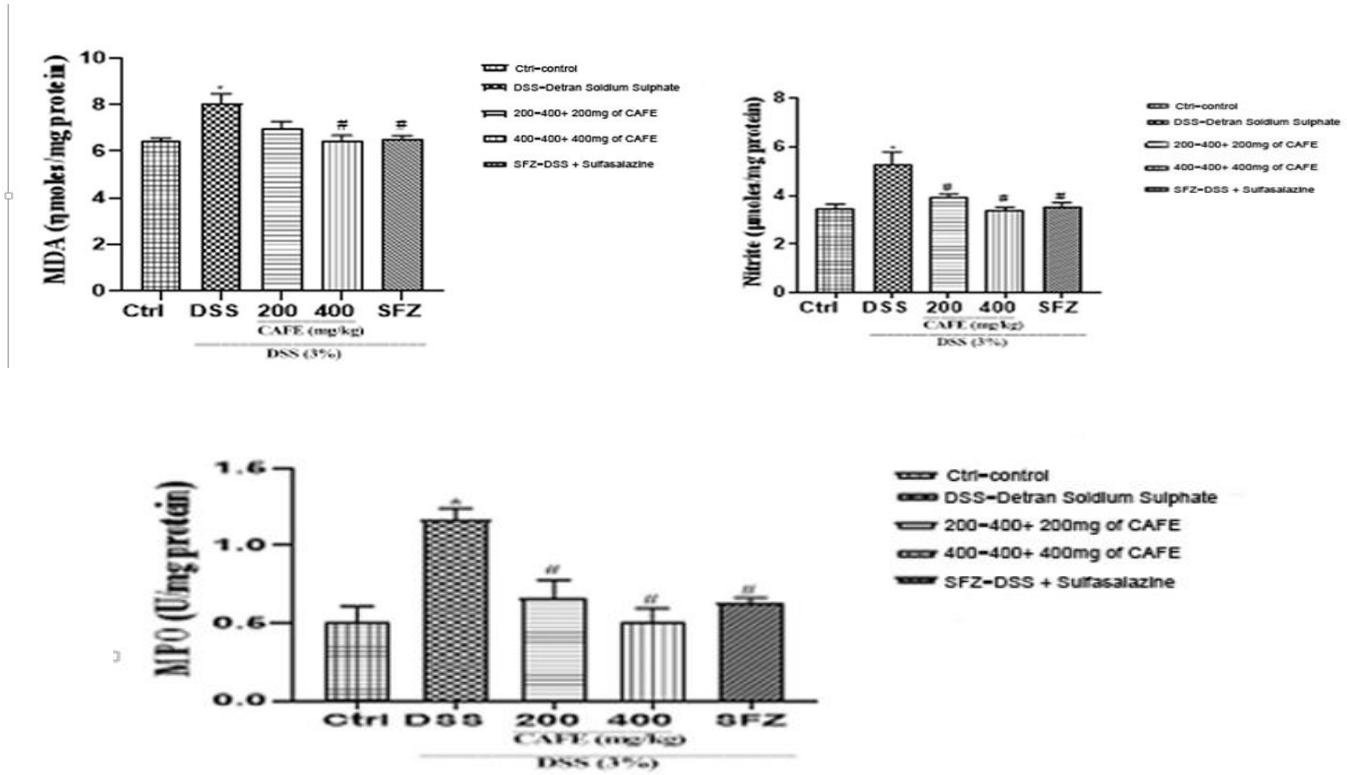

Figure 2: CAFE reduced tissue oxidative stress markers after DSS-induced colitis. Malondialdehyde (A), Nitrite (B), and Myeloperoxidase (C). CTRL – Control; DSS – Dextran sulfate sodium; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine. Values represent mean ± SEM (n=5). * $p < 0.05$ vs. control group; # $p < 0.05$ vs. DSS group.

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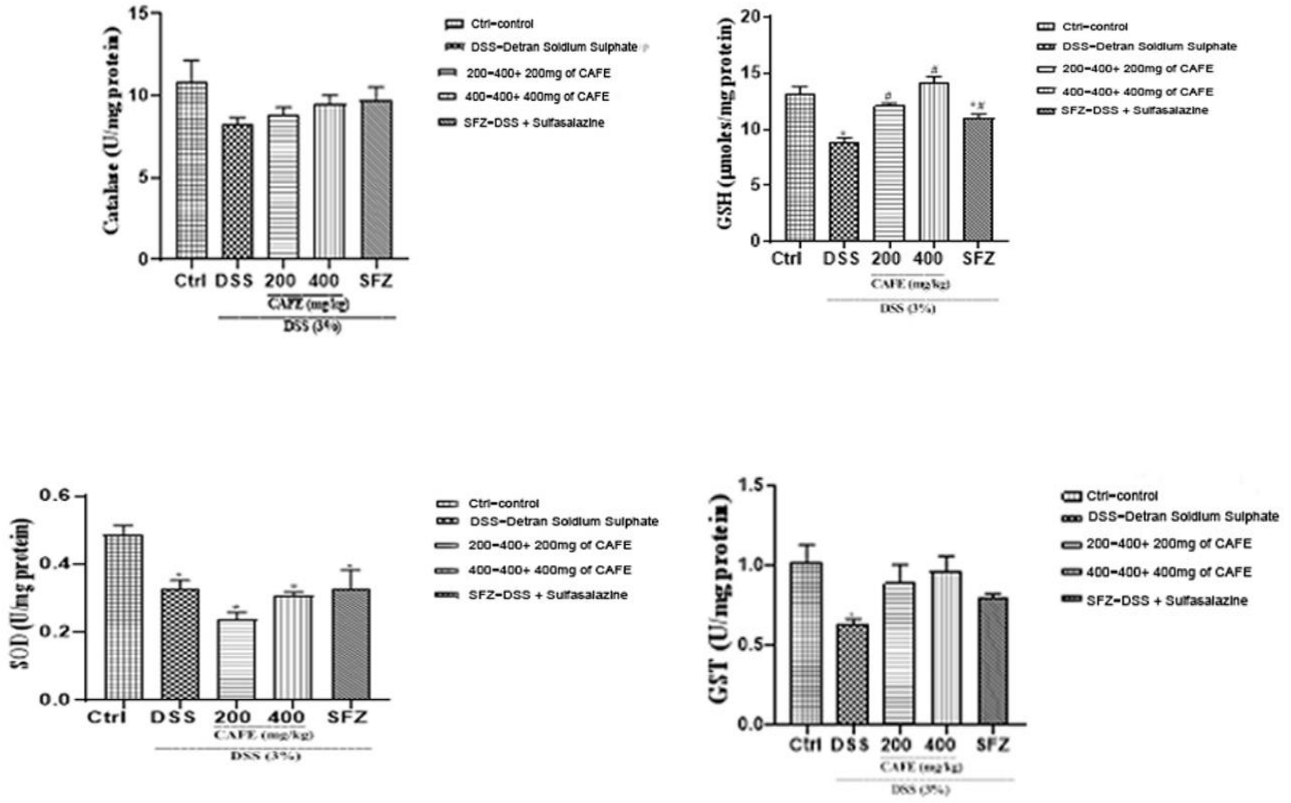

Figure 3: Effect of CAFE on colon tissue antioxidant markers in Dextran sulfate sodium-induced colitis. Glutathione concentration (A), Catalase activity (B), Superoxide dismutase (C), and Glutathione S-Transferase (D). CTRL – Control; DSS – Dextran sulfate sodium; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine. Values represent mean ± SEM (n=5). * $p < 0.05$ vs. control group; # $p < 0.05$ vs. DSS group.

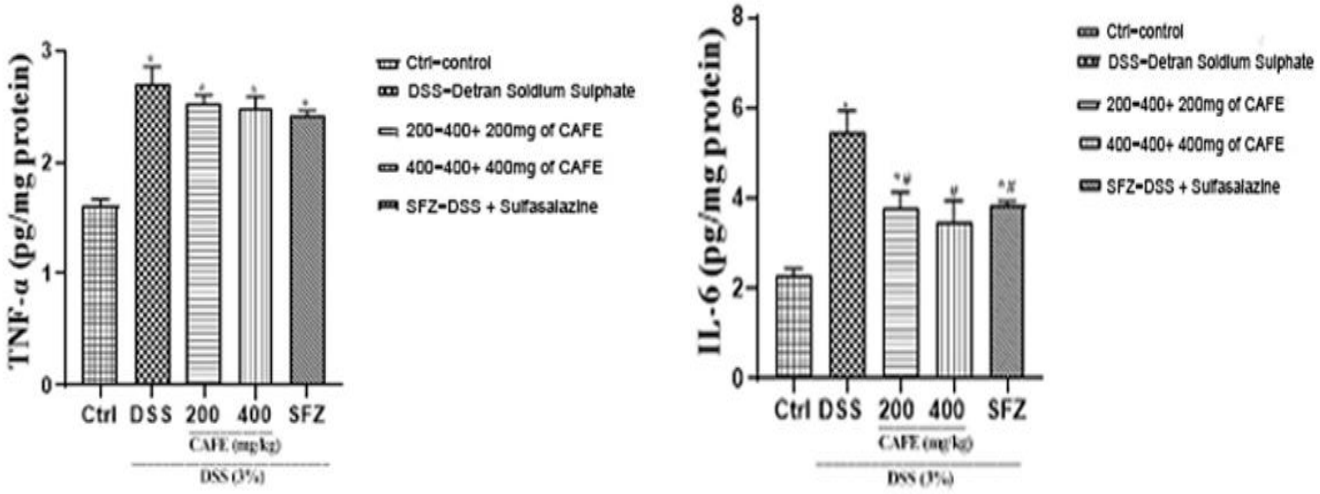

Figure 4: Effect of CAFE on pro-inflammatory proteins in DSS-induced colitis. Tumor Necrosis Factor Alpha (TNF-α) (A) and Interleukin-1β (IL-1 β) (B). CTRL – Control; DSS – Dextran sulfate sodium; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine. Values represent mean ± SEM (n=5). * $p < 0.05$ vs. control group; # $p < 0.05$ vs. DSS group.

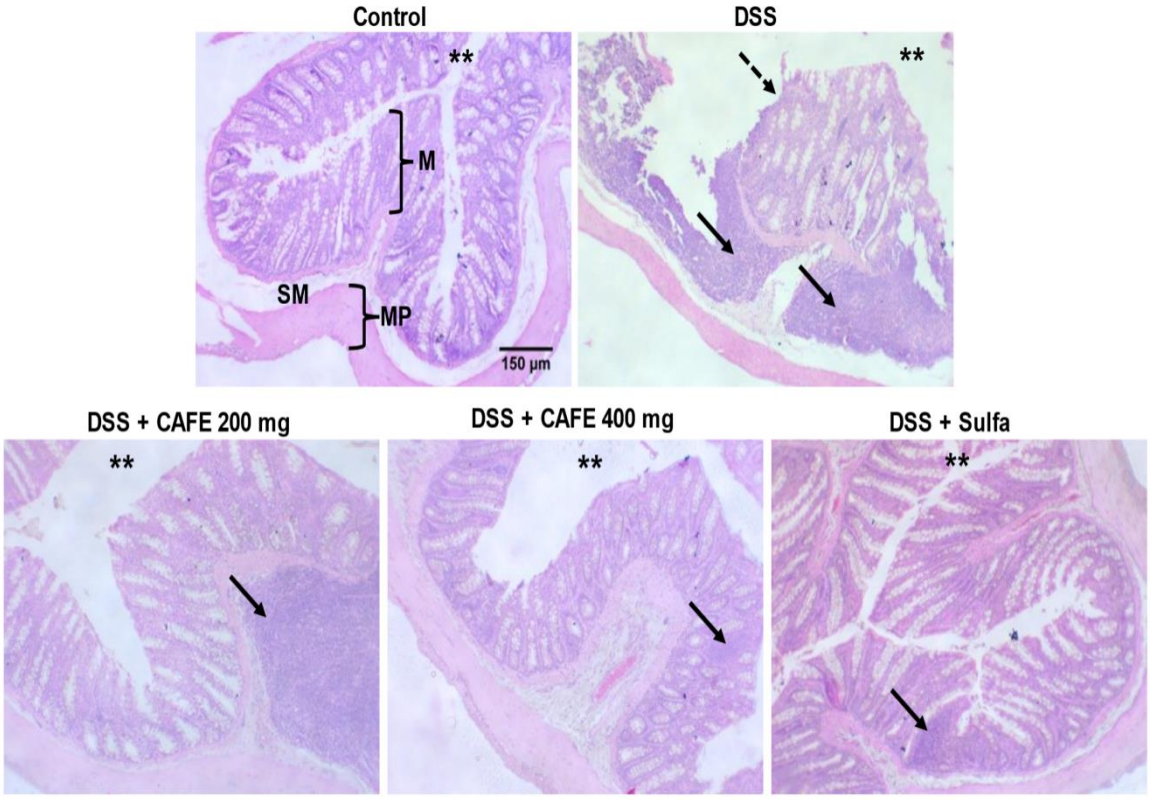

Figure 5: Histology of colon sections day 8 after DSS-induced colitis. Photomicrographs show colon section from; the control having normal tissue architecture, DSS-induced colitis group revealing crypt and lining epithelial damage with inflammatory cell infiltration, DSS + CAFE 200 mg/kg-treated group showing normal histological architecture with infiltration of inflammatory cells, DSS + CAFE 400 mg/kg-treated group show normal histological architecture with infiltration of inflammatory cells, and DSS + Sulfa-treated group show normal histological architecture with slight infiltration of inflammatory cells. Colon sections are H&E stained and photomicrographs are captured at x400. M – mucosa layer; SM – submucosa layer; MP – muscularis propria; Arrows – infiltrate of inflammatory cells; Dashed arrow – crypt and lining epithelial damage; CAFE – *Chrysophylum albidum* fruit extract; Sulfa – Sulfasalazine.

inflammatory tissues persist in DSS + CAFE groups (200 mg/kg and 400 mg/kg CAFE), although to a lesser extent, with moderately damaged crypts. Lastly, the DSS + Sulfa group shows mild damage to crypts and epithelial lining only.

CAFÉ Improved Positive Immunoreactivity for MUC-2.

As revealed by Immunohistochemical staining, MUC-2 expression is increased in the control group when compared with the DSS-induced colitis without any treatment group, which shows a decreased expression of MUC-2 (Fig. 6 and 7) suggesting the harmful effects of DSS. Administration of CAFÉ (200 mg/kg and 400 mg/kg) conversely, significantly prevented DSS mediated decrease in the expression of MUC-2. Lastly, DSS + Sulfasalazine-treated group also, exhibit a marked increased expression of MUC-2 compared with DSS-induced colitis without any treatment group.

The DSS-induced colitis model is widely used due to its potential to reveal pathophysiological structures and histological changes observed in humans. [46] In the present study therefore, the DSS experimental colitis model was used to show the same occurrence observed in humans, thereby enabling a translational outlook, and mechanisms involved in the treatment of DSS-induced experimental colitis with *Chrysophylum albidum* fruit extract (CAFE) were investigated. Disease activity index (DAI) which is a scored macroscopic parameter including weight loss, stool consistency, and presence of occult blood used for evaluating the severity of colitis. A decrease in this index can be directly linked with improvement in the general appearance of the colitis.

DAI was increased in the DSS-induced colitis group without treatment, thus reflecting the damaging effect of DSS and the severe state of colitis. Treatment with CAFE at 200 mg/kg and 400 mg/kg, and sulfasalazine after DSS-induced colitis, however, considerably decreased DAI. This observed reduction in DAI is a significant finding, suggesting a potent therapeutic effect of CAFE in easing the severity of inflammatory bowel disease (IBD))-symptoms.

During experimental colitis, malondialdehyde (MDA) and nitric oxide (NO) concentrations together with myeloperoxidase enzyme activity which are valuable markers for assessing the severity of inflammation are usually on the increase. [48, 49, 50] Increased lipid peroxidation which usually results from oxidative stress, causes elevation of MDA concentration and reduction of endogenous antioxidants. [51] In this present study, it was observed that CAFE administration at 200 mg/kg and 400 mg/kg decreased MDA concentration, suggesting its reactive oxygen species (ROS) scavenging potentials. Normally, NO enhances anti-inflammation while its overproduction can induce inflammation and thereby contribute to an increase in the severity of diseases like UC [52, 50]. The increase in NO concentration observed in the DSS without treatment group was reversed by CAFE administration at 200 mg/kg and 400 mg/kg. This is signifying that CAFE's rich natural antioxidants scavenged free radicals and reactive oxygen species.

Myeloperoxidase (MPO) is an enzyme that is expressed in the granulocyte of neutrophils and which can indicate injury as a result of inflammation. [51] In the present study, an increase in colonic MPO activity was observed in the DSS without treatment group. This finding could be attributed to the

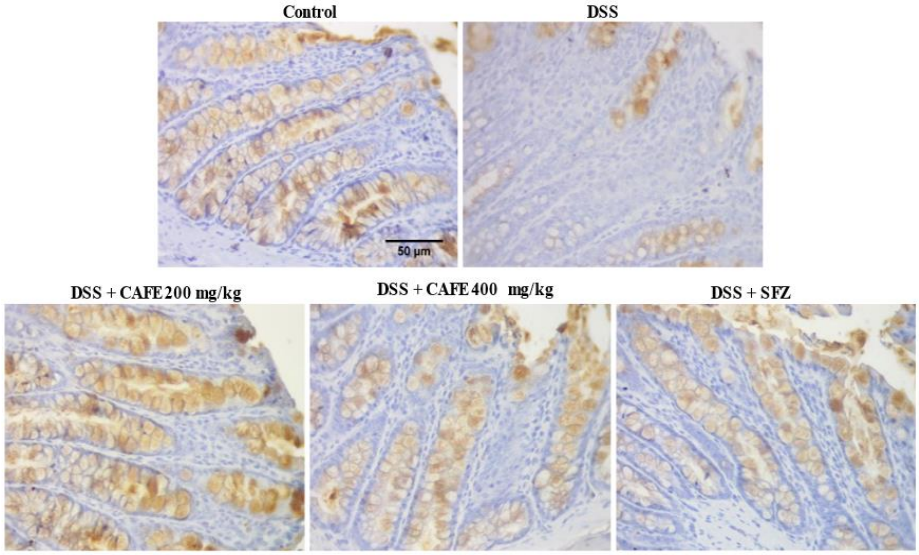

Figure 6: Photomicrographs of colon sections on day 8 after DSS-induced colitis taken at x400 magnification show the expression of MUC-2 after Immunohistochemical staining. Control rats, dextran sulfate sodium group (DSS)-induced rats, DSS + CAFE 200 mg/kg-treated rats, DSS + CAFE 400 mg/kg-treated rats, and DSS + Sulfa-treated rats. Positive immune expression of MUC-2 is noted by the brown color in colon tissues displaying obvious MUC-2 expression in all groups except the DSS group, which appears reduced. Image analysis was performed using Image J software (NIH, USA). CAFE – *Chrysophylum albidum* fruit extract; Sulfa – Sulfasalazine. M – mucosa layer; SM – submucosa layer; MP – muscularis propria; Arrows – infiltrate of inflammatory cells; Dashed arrow – crypt and lining epithelial damage; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine.

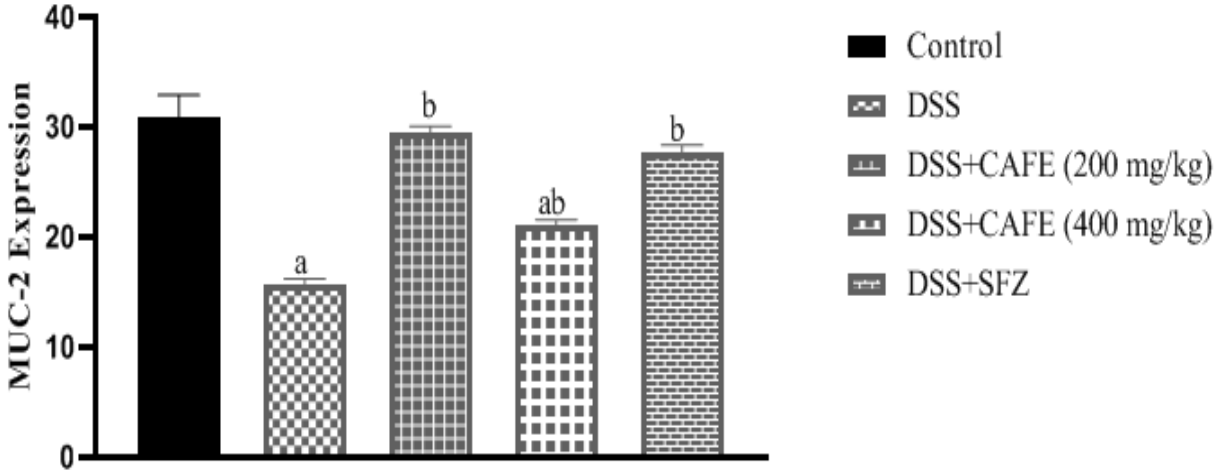

Figure 7: Image analysis of positive immunoreactivity for MUC-2 was performed using Image J software (NIH, USA). CTRL – Control; DSS – Dextran sulfate sodium; CAFE – *Chrysophylum albidum* fruit extract; SFZ – Sulfasalazine. Values represent mean ± SEM (n=5) [a] $p < 0.05$ vs. control group; [b] $p < 0.05$ vs. DSS group.

damaging effect of DSS as a result of inflammation, and it is a consistent observation in line with earlier reports from UC patients and experimental colitis. [51, 53, 54] Administration of CAFE at 200 mg/kg and 400 mg/kg, however, reversed the increase in MPO activity. This observation could be due to CAFE's rich phytochemical content which can neutralize excessive ROS production, which is correlating with decreased cellular infiltrates seen in the histology.

Endogenous antioxidant enzymes such as SOD, CAT, and GST work to reduce the effects of reaction oxygen species (ROS) that usually cause oxidative damage. [50] These enzymes can, however, become depleted by large quantities of ROS produced during inflammatory conditions as in UC. As observed in the DSS without treatment group, induction of colitis with DSS resulted in a reduction of colonic SOD, CAT, GST activities, and GSH levels. CAFE administration at 200 mg/kg and 400 mg/kg, however, reversed the decrease in antioxidants. The present study's observations of *C. albidium's* antioxidant activity in rats with ulcerative colitis show its potential to restore the antioxidant system and this is in line with findings published by. [55]

The severity of inflammation in experimental colitis can be assessed by the level of expression of inflammatory mediators such as TNF-α and IL-6. TNF-α and IL-6 which are pro-inflammatory cytokines found in the colon tissues of IBD patients activate macrophage

infiltration in an attempt to resist inflammation and induce the expression of endothelial cell adhesion molecules resulting in tissue necrosis or injury [56]. In the current study, administration of CAFE at 200 mg/kg and 400 mg/kg and sulfasalazine however, decreased levels of TNF-α and IL-6 following induction of colitis with DSS. This observation may be attributed to the anti-inflammatory properties of CAFE, which was previously reported [51] and this is indicative of CAFE's likely ability to reduce production and release of TNF-α and IL-6.

Previously reported research linked the action of *Chrysophylum albidum* to the presence of myricetin rhamnoside, one of its constituents. Reportedly, myricetin scavenges over 50% of reactive oxygen species (ROS) and suppresses the effect of inflammatory cytokines such as IL-2 and IL-6, TNF-α, and IFN-γ. [57] Since the action of *Chrysophylum albidum* fruit methanol extract is comparable to that of myricetin, even though our study did not screen the extract for myricetin, it is possible that this could be the cause of the ant-inflammatory activity seen in our study.

Ulceration, infiltration of cells, and crypt distortion are some common histological features that have been reported in both experimental colitis and UC patients. [58] In this study also, following Hematoxylin and Eosin staining of colon tissue sections of rats' post-DSS colitis, numerous cytoplasmic infiltrates between crypts were observed. *Chrysophylum albidum* fruit methanol extract administration at 200 mg/kg and 400 mg/kg however, reduced the histopathological damages; infiltration of inflammatory cells in the lamina propria and submucosa, and alterations in the structure of colonic crypts (Fig. 5). The capacity of *Chrysophylum albidum* fruit methanol extract to reduce colonic histopathological damage after DSS-induced colitis is a further indication of its anti-inflammatory potentials. To further investigate the effect of *Chrysophylum albidum* fruit methanol extract on the expression of MUC-2 following DSS-induced colitis, we used immunohistochemical staining. MUC-2 is the main mucin produced specifically in goblet cells, forming a thick layer of mucus covering the gastrointestinal epithelium. [59] Colon of both UC patients and healthy individuals express MUC-2 [60], however, in UC, the colon is depleted of goblet cells leading to a reduction in the expression of MUC-2 with resultant damage to the totality of intestinal epithelium, thereby, causing increased bacterial antigens translocation into the mucosa, innate immune system alteration, irregular signal transduction, and induction of more degrading inflammatory responses. [60] Disruption of the colonic barrier by DSS is associated with a decrease in MUC-2 production.[61] In the present study, reduction in the expression of MUC-2 was observed in DSS colitis without treatment group conversely, after DSS-induced colitis, 200 mg/kg and 400 mg/kg methanol extract of *Chrysophylum albidum* fruit increased colon MUC-2 expression in a dose-dependent way thereby reversing the effect of DSS on colon MUC-2 (Fig 7). This observation is an exciting finding, suggesting a beneficial role of *Chrysophylum albidum* fruit in restoring and maintaining gut barrier integrity.

Conclusion

In conclusion, the significant increase in MUC-2 expression observed in DSS-induced experimental colitis treated with *Chrysophyllum albidum* fruit extract strongly supports its potential as a natural therapeutic agent for inflammatory bowel diseases. This effect was mediated by its potent anti-inflammatory, antioxidant, and mucosal protective properties as observed in the colon tissue levels of nitrite, TNF-α, IL-6, SOD, CAT, MPO, and GSH. These findings provide a strong basis for further research into the therapeutic potential of *Chrysophyllum albidum* and its active constituents in managing inflammatory conditions of the gut. Future research would focus on isolating specific compounds responsible for mucin-enhancing effect, and elucidating the precise molecular pathways involved. While this is a promising experimental model for studying the therapeutic potential of *Chrysophyllum albidum* fruit, further studies are needed to assess its efficacy and safety in human IBD.

Conflict of Interest

The authors declare no conflict of interest.

Authors` Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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