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Dietary Supplementation with Green Fruits of *Musa* spp. AAA Ameliorates Acute, Subchronic, and Chronic Intestinal Inflammation with Relapse Induced by Trinitrobenzenesulfonic Acid in Rats

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ABSTRACT: *Musa* spp. AAA (green dwarf banana) fruit has many health benefits, including intestinal anti-inflammatory activity associated with the presence of resistant starch. Resistant starch is an indigestible polysaccharide source of short-chain fatty acids (SCFAs) after the colon fermentative process by gut microbiota. SCFAs are classified as metabolites of the gut microbiota fermentative process, which have the potential to control intestinal inflammatory processes. This work investigated the intestinal anti-inflammatory activity of dietary green fruits of *Musa* spp. AAA in the trinitrobenzenesulfonic acid (TNBS) model of intestinal inflammation using three different experimental designs: acute, subchronic, and chronic inflammation with relapse. The results revealed that 10% *Musa* dietary supplementation significantly attenuated the colonic damage induced by TNBS, as made evident by both histological and biochemical evaluations. This beneficial effect was associated with colonic oxidative status improvement based on *Musa* spp. AAA prevention of glutathione depletion and inhibition of myeloperoxidase activity. In addition, *Musa* effects were associated with decreased pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-17, and INF-γ) and gut microbiota modulation, increasing colonic SCFAs production. In conclusion, the intestinal anti-inflammatory activity exerted by *Musa* spp. AAA was related to its antioxidant, immune, mucosal healing, and gut microbiota modulation properties.

KEYWORDS: *green dwarf cavendish, inflammatory bowel disease, gut microbiota, short-chain fatty acids, mucosal healing*

1. INTRODUCTION

Inflammatory bowel disease (IBD) consists of Crohn's disease (CD) and ulcerative colitis (UC), two relapsing, chronic intestinal inflammatory processes, which are part of a group of immune-mediated diseases with pharmacological treatment and without cure.[1] Although their presentation and location may differ, CD and UC can cause symptoms like diarrhea, rectal bleeding, and abdominal pain.[2] In CD, lesions are transmural and discontinuous, appearing from the mouth to the anus, while in UC, lesions are restricted to the mucosa, affecting the colon and rectum with continuous lesions.[3,4]

Several aspects, including genetic predisposition and environmental factors, such as stress, sleeping patterns, antibiotic use, hygiene, diet, and smoking, are associated with the development of IBD.[2,5] IBD has higher rates in industrialized countries, and a rapid increase in case numbers is happening in newly industrialized areas such as South America and India.[2,4] This enhancement can be related to increased consumption of processed foods, sugars, and fats associated with unrestrained use of antibiotics, leading to dysbiosis, a disturbance of gut microbiota abundance and diversity.[6] There are several ways to beneficially modulate intestinal microbiota, including probiotics, prebiotics, symbiotics, and more recently fecal microbiota transplantation.[5] Resistant starch is an indigestible polysaccharide that after the colon fermentative process by gut microbiota generates short-chain fatty acids (SCFAs), which are classified as prebiotic products and have the potential to control intestinal inflammatory processes. *Musa* spp. AAA (green dwarf banana) fruit has many health benefits, including intestinal anti-inflammatory activity associated with the presence of resistant starch.

Our research group, over the years, has focused its research on modulating the gut microbiota using several prebiotics products, including *Typha angustifolia* (narrow-leaf cattail), *Musa* spp. AAA (dwarf cavendish banana), and the edible Amazon fruits *Euterpe oleracea* (assay berry), *Mauritia flexuosa* (burity palm), and *Theobroma grandiflorum* (cupuassu).[7−11] Among them, the green fruit of the dwarf banana was one of the most promising products with intestinal anti-inflammatory activity, modulating oxidative stress and gut microbiota dysbiosis in acute intestinal inflammation induced by the trinitrobenzenesulfonic acid (TNBS) model of intestinal inflammation.[8,9] Based on this, this study aimed to evaluate the *Musa* spp. AAA green fruit flour in acute, subchronic, and chronic inflammation with relapse in the TNBS model of intestinal inflammation, focusing on the effects of prolonged consumption of this product, its effectiveness in preventing intestinal inflammation and relapse,

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and its activity on the inflammatory mediators, oxidative stress, and short-chain fatty acid production.

2. MATERIALS AND METHODS

2.1. Chemicals. All chemicals were supplied by Sigma-Aldrich and were freshly prepared for each biochemical evaluation and gas chromatography/mass spectrometry (GC/MS) analysis. The enriched diet with 10% green dwarf Cavendish banana flour was manufactured at São Paulo State University (UNESP), São Paulo, Brazil.

2.2. Green Dwarf Banana Flour Preparation. Organic green dwarf cavendish banana (*Musa* spp. AAA) dehydrated and pulverized fruits were provided by TROPDAN (Indstria e Comércio de Produtos Alimentcios, Cajati, São Paulo, Brazil) and stored in the PhytoPharma-Tec laboratory until use. The banana flour was added to the Nuvilab CR-1 feed for rodents in the ratio of 10% by geometric dilution. Diets were pelletized using pelletizer equipment (MA362 Model, Marconi Ltd., Piracicaba, SP, Brazil) and stored at 4 °C. The ingredient composition of the diets was calculated from the major nutrients of the normal Nuvilab CR-1, considering the addition of 10% green fruits of *Musa* spp. AAA (Table 1), which is appropriate for human consumption.

Table 1. Ingredient Composition of the Diets Fed to Rats (g/1000 g)

ingredients	control diet	10% *Musa* diet
total protein	220.0	208.0
mineral mixture[a]	3.0	2.7
total fiber	70.0	63.0
vitamin mixture[b]	6.0	5.4
lipids	40.0	36.0
amino acids[c]	19.0	17.1
corn starch	320.0	288.0
soybean meal	25.0	22.5
wheat bran	30.0	27.0
ash	9.0	8.1
Musa spp. AAA flour[d]	0.0	100.0

[a]Mineral mixture provided the following amounts (in mg/kg): Mg (1.7), Mn (110.0), Co (2.0), Fe (180.0), Zn (10.0), Cu (30.0), Se (0.2), Na (2.8), P (8.5), and Ca (13.0). [b]Vitamin mixture provided the following amounts: vitamin A (25.500 UI/kg), vitamin D_3 (2.100 UI/kg), vitamin E (60 UI/kg), vitamin K (12.5 mg/kg), vitamin B_1 (14.40 mg/kg), vitamin B_2 (11.0 mg/kg), vitamin B_6 (12.0 mg/kg), vitamin B_{12} (60 μg/kg), folic acid (6.0 mg/kg); choline (2.400 mg/kg), biotin (0.26 mg/kg), niacin (600 mg/kg), thiamine (11.0 mg/kg), and calcium pantothenate (112.0 mg/kg). [c]Amino acids: lysine (14 g/kg), methionine (5 g/kg) [d]Green fruit of *Musa* spp. AAA contains starch (73.6−79.4%), amylose (20.9−23.5%), protein (2.61−2.99%), soluble fiber (2.29−2.49%), insoluble fiber (5.35−5.39%), ash (3.44−3.56%), and traces of lipids.[12]

2.3. Animals and Experimental Design. Male Wistar rats (weighing 180−200 g) were obtained from ANILAB (Paulínia, São Paulo, Brazil) and housed in standard environmental conditions (21 ± 2 °C, 60−70% humidity) with 12 h light/dark cycles and air filtration. The animals had free access to water and food (Nuvilab). This study was carried out according to the Guidelines of Animal Experimentation and approved by the Animal Ethics Committee from UNESP (CEUA-922). Intestinal inflammation was induced by the method originally described.[13] The animals were fasted overnight and anesthetized with halothane. Under anesthesia, rats were given 10 mg of TNBS dissolved in 0.25 mL of 50% ethanol (v/v) using a Teflon cannula inserted 8 cm through the anus. Three different settings were as follows:

2.3.1. Acute Intestinal Inflammation. The rats were given a diet containing 10% green dwarf banana flour for 35 days before and 2 days after the induction of intestinal inflammation (Figure 1). The healthy rat group received standard rodent feed and 0.25 mL of phosphate-buffered saline, whereas, in a TNBS-control group, the rats received TNBS by intracolonic route and standard feed. The animal body weights, the occurrence of diarrhea, and the total food intake were recorded daily. Animals (n = 7 in each group) were killed 2 days after inflammation induction by an overdose of halothane.

2.3.2. Subchronic Intestinal Inflammation. Rats were given a diet containing 10% green dwarf banana flour for 35 days before and 7 days after the induction of intestinal inflammation (Figure 1). The healthy rat group received standard rodent feed and 0.25 mL of phosphate-buffered saline, whereas the TNBS-control group rats received TNBS by intracolonic route and standard feed. The animal body weights, the occurrence of diarrhea, and the total food intake were recorded daily. Animals (n = 7 in each group) were killed 7 days after inflammation induction by an overdose of halothane.

2.3.3. Chronic Intestinal Inflammation with Relapse. Rats were given a diet containing 10% green dwarf banana flour for 35 days before and 16 days after the first induction of intestinal inflammation. Intestinal inflammation was induced with 10 mg of TNBS ethanol as previously described. A second dose of TNBS was given 14 days after the first to mimic the common relapses in human IBD. The animals (n = 7 in each group) were killed 2 days after the second inflammation induction (Figure 1). The healthy rat group received standard rodent feed and 0.25 mL of phosphate-buffered saline, whereas, in a TNBS-control group, the rats received two doses of TNBS by intracolonic route and standard feed. The animals were killed 2 days after the second TNBS administration.

The number of animals in each group was based on the previous description.[8,9]

2.4. Intestinal Inflammatory Process Assessment. Animal body weights, diarrhea (detected by perianal fur soiling), and total food intake for each group were recorded daily. Once the rats were killed, the colon was removed aseptically and adhesions between the colon and adjacent organs were noted. Then, the luminal contents were collected for short-chain fatty acids analysis. After removal, colon samples were weighed, and their length was measured under a constant load (2 g), then opened longitudinally and scored for macroscopically visible damage on a 0−10 scale as previously described.[14] The colon was longitudinally divided into different pieces for the determination of myeloperoxidase (MPO) activity, total glutathione (GSH) content, and cytokine (IL-1α, IL1-β, IL-6, IL-17, TNF-α, and INF-γ) levels.

2.5. Biochemical Assays in Colonic Specimens. MPO activity was measured according to the technique previously described[15] with some modifications.[8,9] The results were expressed as MPO units per gram of wet tissue. Total GSH content was quantified with the recycling assay[16] with some modifications.[8,9] The results were expressed as nmol per gram of wet tissue.[8,9] Colonic samples for the determinations of IL-1α, IL-1β, IL-6, IL-17, TNF-α, and INF-γ were weighed, homogenized, minced on an ice-cold plate, and resuspended in a centrifugation tube containing 10 mM/L phosphate-buffered saline pH 7.4 (1:5 w/v). The tubes were placed in a shaker submerged in a 37 °C water bath for 20 min and then centrifuged at 9000g for 5 min at 4 °C. The supernatants were frozen at −80 °C until they were assayed. The cytokine levels were quantified by a DuoSet ELISA Kit to measure the concentration of natural and recombinant rat enzyme according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN). The results were expressed as pg/mL.

2.6. SCFAs Determination in Fecal Samples. The internal fecal samples were collected after animal death and stored in microtubes at −80 °C. The extraction process and SCFA determination were based on the previously described method.[9] Briefly, feces samples were thawed at room temperature, suspended using 1 mL of phosphoric acid 0.5% for 100 mg of feces samples, homogenized with a Vortex (Vortex MA, Marconi Ltd., Piracicaba, São Paulo, Brazil) for about 1 min, and centrifuged for 10 min at 18,000g. Each milliliter of water supernatant was extracted with 1 mL of ethyl acetate and centrifuged for 10 min at 17,950g. Before analysis, 1 mL of the organic phase was transferred into a tube containing octanoic acid (Sigma-Aldrich) as an internal standard at a final concentration of 755 μg/mL. Chromatographic analysis was carried out using a gas chromatography−mass spectrometry Thermo Scientific, model FOCUS equipped with an automatic liquid sampler

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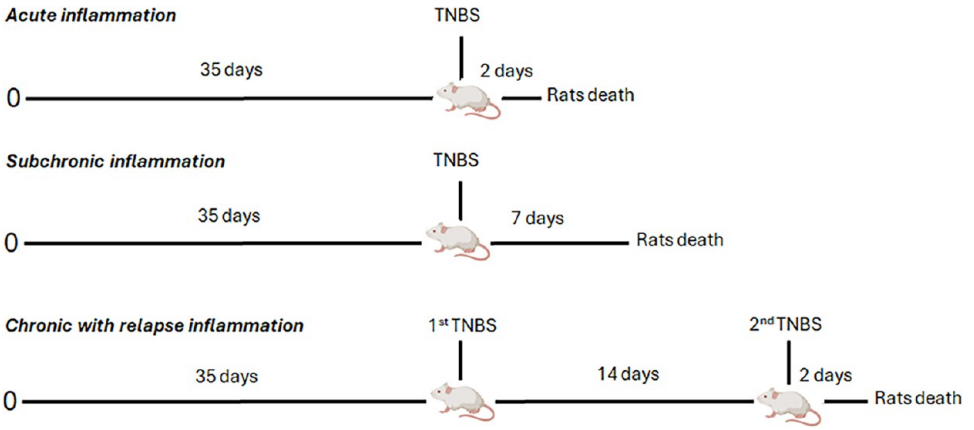

Figure 1. Experimental designs of acute, subchronic, and chronic inflammation with relapse induced by TNBS in rats.

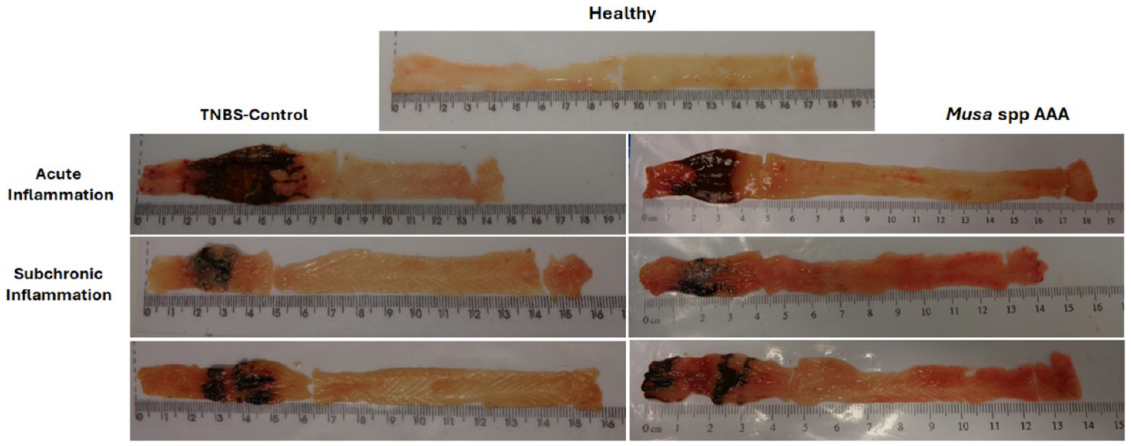

Figure 2. Representative pictures of the colon from healthy, acute, subchronic, and chronic with relapse inflammation.

Table 2. Effects of *Musa* spp. AAA Flour Supplementation on the Macroscopic Parameters in TNBS-Induced Intestinal Inflammation

experimental groups	macroscopic damage score (0−10)[a]	extension of lesion (cm)[b]	weight/length ratio (mg/cm)[b]	adhesions to adjacent organs (%)
		Acute Inflammation		
healthy	0[c]	0[c]	83.24 ± 5.77[c]	0
TNBS-control	9 (7−10)	5.18 ± 0.35	144.27 ± 5.11	100
Musa spp. AAA	8 (6−10)	4.15 ± 0.31[c]	153.83 ± 4.18	70
		Subchronic Inflammation		
healthy	0[c]	0[c]	80.52 ± 7.62[c]	0
TNBS-control	4.5 (1−8)	1.27 ± 0.32	150.55 ± 12.33	78.57
Musa spp. AAA	5 (1−7)	1.31 ± 0.37	158.27 ± 16.90	66.66
		Chronic with Relapse Inflammation		
healthy	0[c]	0[c]	89.81 ± 4.29[c]	0
TNBS-control	7 (2−9)	3.40 ± 0.30	157.92 ± 6.02	100
Musa spp. AAA	7.5 (4−9)	3.47 ± 0.66	178.55 ± 14.08	50

[a]Score data are expressed as the median (range). [b]Extension of lesion and weight/length ratio are expressed as mean ± SEM. [c]$p \leq 0.05$ vs TNBS-control group.

(Thermo-Triplus DUO, Thermo Fischer Scientific, Waltham, MA), and coupled to a Thermo-ISQ 230ST mass detector. The gas chromatography was fitted with a high-polarity, poly(ethylene glycol) fused silica capillary TG-WASMS column (30 m, 0.25 mm i.d., 0.25 m film thickness, Thermo Fisher Scientific), and helium was used as the carrier gas. The injection was made in a split mode with an injection volume of 1 mL and a split flow of 300 mL/min. The injector temperature was 250 °C. The column temperature was initially 80 °C, then increased to 120 °C at 50 °C/min, to 160 °C at 70 °C/min and kept at this temperature for 50 s, to 180 °C at 70 °C/min and kept at this temperature for 1 min, and finally to 210 °C at 70 °C/min and kept at this temperature for 1 min. The solvent delay was 3.5 min. Identification of the SCFA was based on the retention time of standard compounds (Sigma-Aldrich) and with the assistance of the NIST 08 library provided by the manufacturer and the NIST online (http://chemdata.nist.gov) library. The peaks were quantified as the relative abundance of the total ionic count, concerning the internal standard. The concentration (μg/g of feces) of each SCFA was calculated using the linear regression equations ($R^2 > 0.99$) from the corresponding standard curves obtained with 12 different concentrations.

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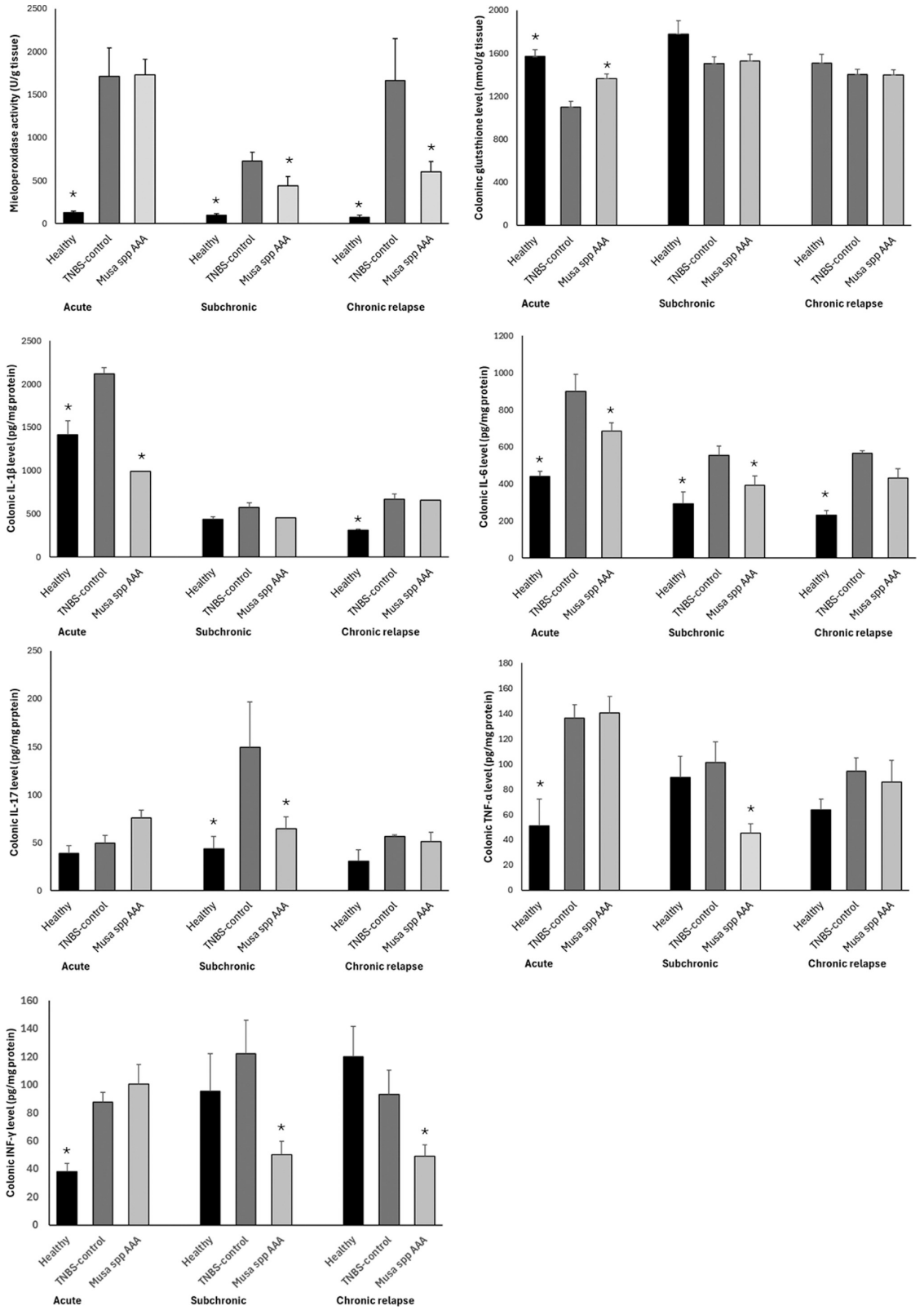

Figure 3. Effects of *Musa* spp. AAA on the colonic myeloperoxidase activity and levels of glutathione, IL-1β, IL-6, IL-17, TNF-α, and INF-γ. Results represent the mean ± standard error of the mean. *$p < 0.05$ statistically significant compared to the TNBS-control group.

2.7. Histological Evaluation. Representative whole gut specimens were taken from a region of the inflamed colon corresponding to the segment adjacent to the gross macroscopic damage and were fixed in 4% buffered formaldehyde. The samples were subjected to hematoxylin and eosin staining for morphological analysis and PAS/Alcian Blue mucin analysis. After staining, images were subjected to analysis and photomicrography with a Leica microscope using Leica Qwin Plus versions 3.3 and 3.40.

2.8. Statistical Analyses. Parametric data are expressed as the mean ± S.E.M., and the differences between means were tested for

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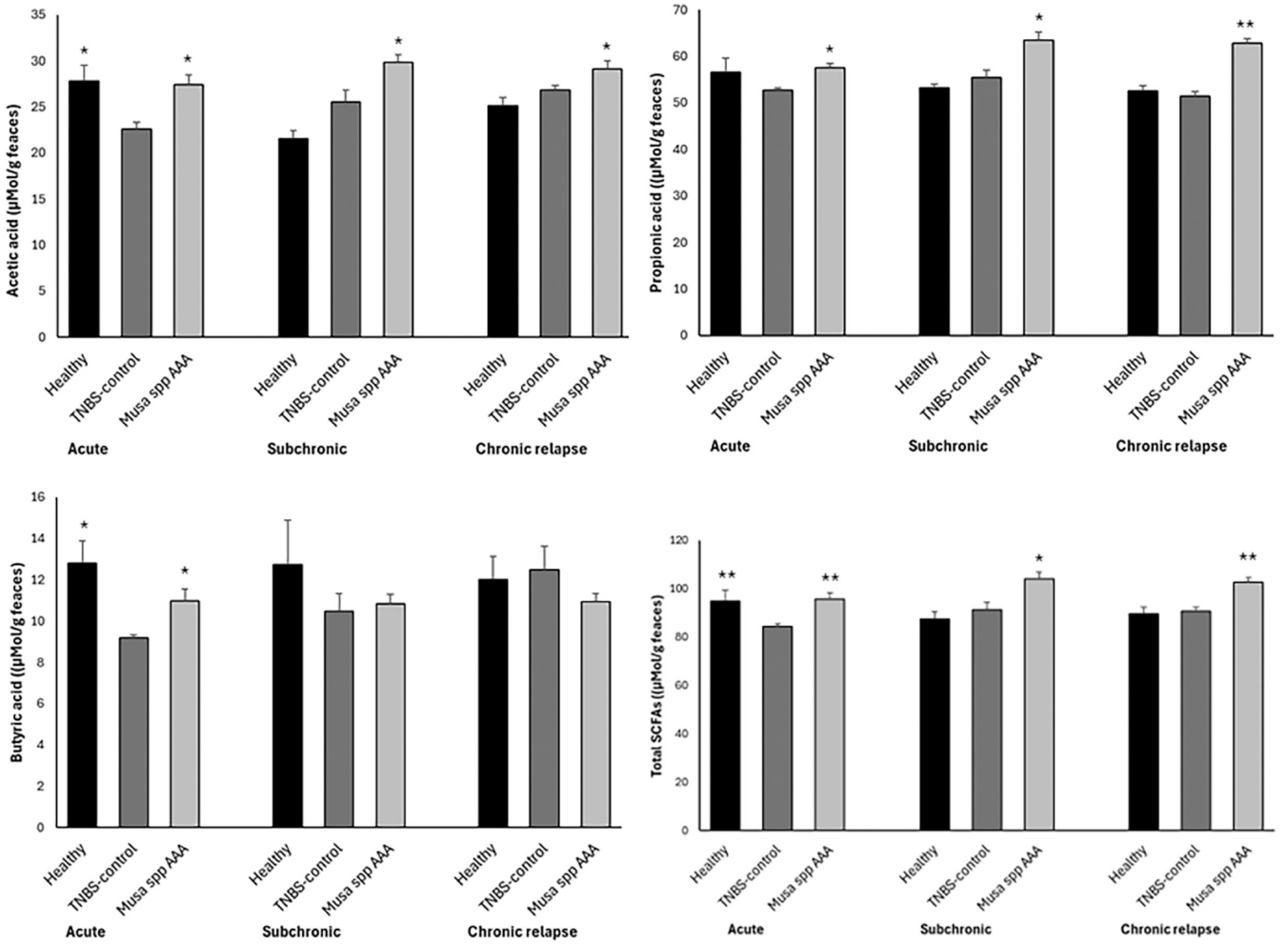

Figure 4. Effects of *Musa* spp. AAA on the colonic acetic, propionic, butyric, and total short-chain fatty acid production. Results represent mean ± standard error of the mean. *$p < 0.05$; **$p < 0.01$ statistically significant compared to the TNBS-control group.

statistical significance using one-way analysis of variance (ANOVA) and Dunnett's posterior test. Nonparametric data (score) are expressed as the median (range) and were analyzed with the Kruskal–Wallis test and Dunn's posterior test. Statistical significance was set at $p \leq 0.05$.

3. RESULTS

3.1. Clinical Analysis. Intracolonic administration of TNBS resulted in inflammation after 2 days, characterized by hyperemia, diarrhea, focal adhesions to adjacent organs, and severe necrosis of mucosa (Figure 2), extending from 3.5 to 7.7 cm with bowel wall thickening and an increase of colon weight/length ratio (Table 2).

The inflammatory process was associated with reduced food intake and weight gain (data not shown). In the subchronic process, there was a normal and gradual recovery, as evidenced by a lower macroscopic damage score and extension of the lesion (0.5–3.5 cm). On the other hand, after the second TNBS instillation, there was a new increase in the macroscopic damage score and lesion extension (1.8–4.7 cm), confirming the inflammatory process relapse (Table 2). This new inflammation is not as pronounced as acute inflammation due to tissue fibrosis. Supplementation with 10% *Musa* spp. AAA flour significantly decreased the extension of lesions in acute inflammation, with no effects in subchronic inflammation and chronic inflammation with relapse. *Musa* spp. AAA flour also reduced adhesions to adjacent organs in the acute, subchronic, and chronic intestinal inflammation with relapse (Table 2).

3.2. Biochemical Evaluation. This TNBS-induced inflammatory process was associated with increased MPO activity in acute and chronic inflammation with relapse settings compared with healthy animals (Figure 3). The MPO activity increment was associated with a GSH depletion in acute and chronic inflammation with relapse and a gradual recovery in GSH levels in chronic inflammation with relapse (Figure 3). Supplementation with *Musa* spp. AAA flour decreased MPO activity by 40% in subchronic inflammation and by 64% in chronic inflammation with relapse, compared to the respective TNBS-control group ($p < 0.05$). Additionally, it prevented GSH depletion induced by TNBS in the acute inflammation (Figure 3).

Intestinal inflammation induced by TNBS also promoted a significant increase in IL-1β, IL-6, TNF-α, and INF-γ colonic levels ($p < 0.05$) in acute inflammation, and an increase in IL-6 and IL-17 levels in subchronic inflammation (Figure 3). Moreover, TNBS significantly increased IL-1β and IL-6 levels ($p < 0.05$) in chronic inflammation with relapse (Figure 3). The beneficial effects of 10% *Musa* spp. AAA flour were evidenced by a significant decrease in 53% of the IL-1β and 24% of the IL-6 colonic levels in the acute inflammation compared with the TNBS-control group ($p < 0.05$). In the subchronic inflammation, *Musa* spp. AAA flour significantly reduced 30, 57, 55, and

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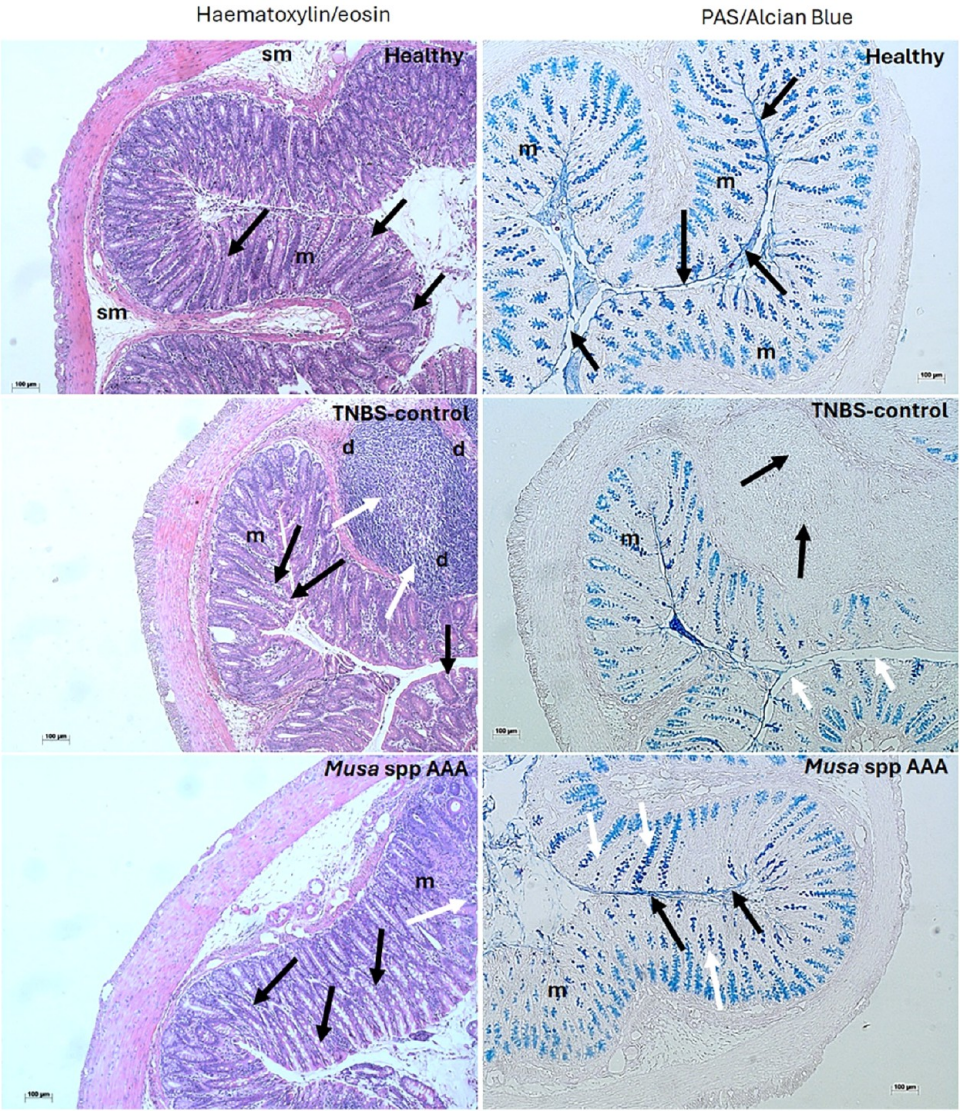

Figure 5. Hematoxylin/eosin (HE) and PAS/Alcian Blue (PAS/AB) photomicrographs of rat colon treated with *Musa* spp. AAA in the acute protocol of intestinal inflammation induced by TNBS (5×). HE: In healthy rats, the mucosa (m) contains numerous straight tubular glands (black arrows) with normal crypt, submucosa (sm), and luminal epithelium intact with typical morphology. In the TNBS-control group, the tubular glands (black arrows) were reduced with a cluster of inflammatory cells in the submucosa reaching the mucosa (white arrows), and goblet cells are atypical; a disruption of the epithelium (d). In the *Musa* spp. AAA-treated group, the colonic epithelium is in the recovery process with the restoration of tubular glands (black arrows) containing goblet cells; the edema was reduced, but infiltration of inflammatory cells (white arrows) is still present in the mucosa (m). PAS/AB: In the healthy group, there is a thin layer in the membrane, representing the membrane-bound mucin (black arrows) and a smooth blue staining in the mucosa (m), indicating the presence of mucin in the goblet cells. In the TNBS-control group, there is a great mucin depletion in the mucosa (m) and cell surface (white arrows) associated with low blue staining; cell infiltration (black arrows) is observed. In the *Musa* spp. AAA-treated group, there is a thin blue layer indicating the membrane-bound mucin (black arrows) associated with the mucin in goblet cells in the mucosa (m) represented by higher blue staining (white arrows).

59% the colonic levels of IL-6, IL-17, TNF-α, and INF-γ ($p < 0.05$), respectively, with no effects in the chronic inflammation with relapse (Figure 3).

3.3. Short-Chain Fatty Acid Determination. TNBS also decreased acetic acid, butyric acid, and total SCFA production in acute inflammation; however, it did not affect SCFA production in subchronic inflammation and chronic inflammation with relapse (Figure 4). On the other hand, when the animals were treated with *Musa* spp. AAA flour, there was a significant increase in the acetic, propionic, and total SCFAs in acute and chronic inflammation with relapse (Figure 4). *Musa* spp. AAA flour also increased butyric acid production in the acute inflammation ($p < 0.05$), with no effects on subchronic and chronic inflammation with relapse (Figure 4).

3.4. Histological Analyses. *Musa* spp. AAA produced protective effects on cell morphology and cytoarchitecture in acute, subchronic, and chronic inflammation with relapse protocols, showing clear mucosa healing. In acute inflammation, the colonic epithelium showed a recovery process with the restoration of tubular glands, reduction of edema and cell infiltration, and increased mucus production (Figure 5), while in subchronic inflammation, the recovery process was better observed, mainly increasing the membrane-bound mucin barrier (Figure 6). In the chronic inflammation with relapse protocol,

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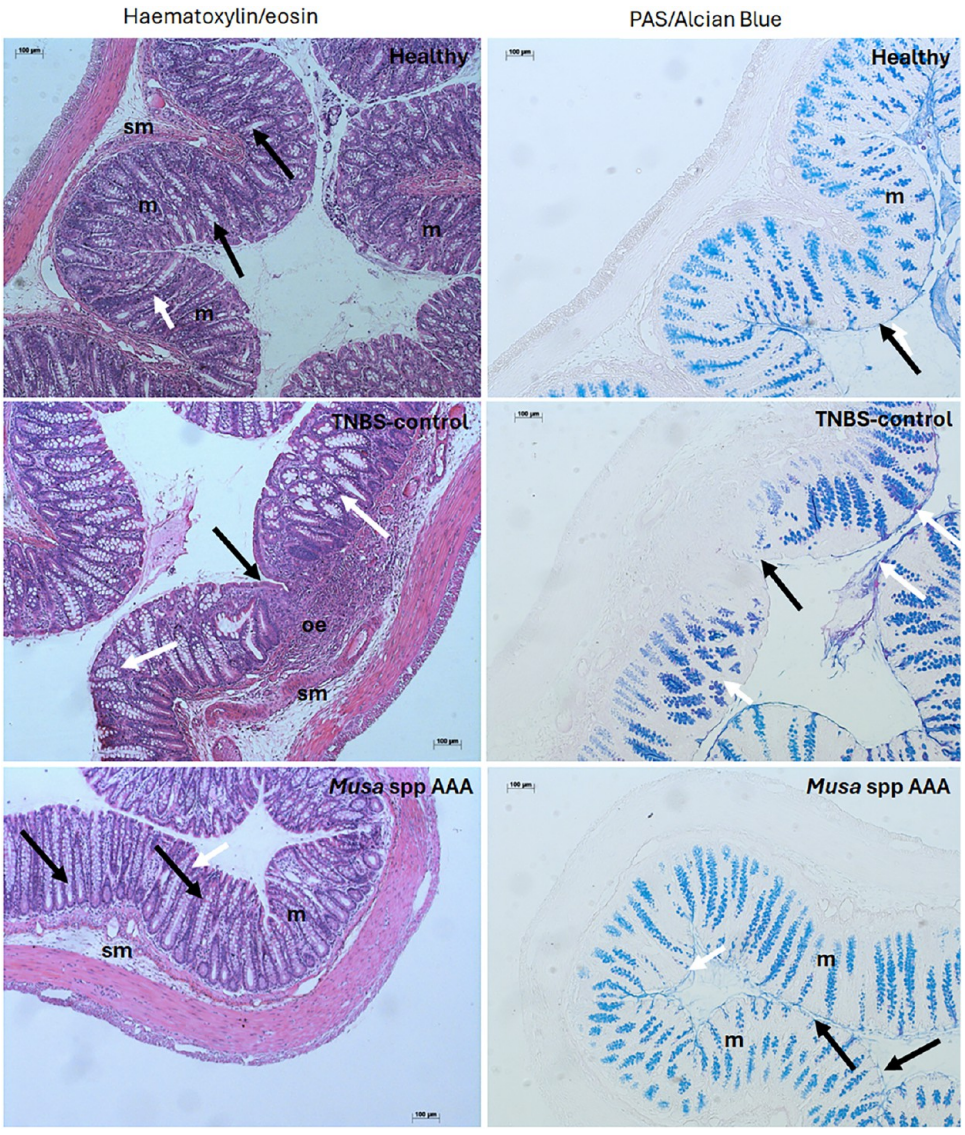

Figure 6. Hematoxylin/eosin (HE) and PAS/Alcian Blue (PAS/AB) photomicrographs of rat colon treated with *Musa* spp. AAA in the subchronic protocol of intestinal inflammation induced by TNBS (5×). HE: Healthy animals with normal crypt and goblet cells (black arrow), mucosa (m), submucosa (sm), and luminal epithelium are intact, presenting typical morphology. In the TNBS-control group, the tubular glands are reduced (white arrows) with membrane disruption (black asterisk) and large edema (oe) in the submucosa (sm). In the *Musa* spp. AAA-treated group, the colonic epithelium is recovered with regular tubular glands (black arrows) containing goblet cells, with no infiltration of inflammatory cells in the mucosa (m); the submucosa (sm) is recovered with no edema. PAS/AB: The healthy group presents a thin blue layer in the membrane, representing the membrane-bound mucin (black arrow) and smooth blue staining in the mucosa (m), indicating the presence of mucin in the goblet cells. In the TNBS-control group, disruption of the membrane (black arrows) with less mucin in surrounding goblet cells can be noted and a gradual recovery of mucin within the cells (white arrow). In the *Musa* spp. AAA-treated group, there is a recovery of the membrane-bound mucin barrier (black arrows), and in the mucosal goblet cells mucins in the mucosa (m), indicating a recovery of the colonic area.

Musa spp. AAA, cell cytoarchitecture, and membrane-bound mucin were like those of healthy animals (Figure 7).

4. DISCUSSION

The green *Musa* spp. AAA flour produced intestinal anti-inflammatory activity in the TNBS model, modulating gut SCFA production and reducing oxidative stress and colonic inflammatory cytokines. It had evident protective effects on colonic cell morphology and function, representing a promising natural ingredient with mucosa healing, anti-inflammatory, antioxidant, immunomodulatory, and prebiotic properties.

Acute inflammation induced by TNBS instillation decreased acetate, propionate, butyrate, and total SCFAs levels in the TNBS-control group, and besides, unripe fruits of *Musa* spp. AAA prevented this depletion in acute protocol, as similarly observed,[9] also prevented the SCFAs depletion in subchronic and chronic inflammation with relapse, indicating a long-lasting modulation of gut microbiota. SCFAs are metabolites that can regulate epithelial and immune cells and participate in the enteric nervous system and gut motility regulation, which is interesting during IBD treatment.[16] Green banana-resistant starch from *Musa paradisiaca* has been reported to increase SCFA levels in a model of DSS(dextran sulfate sodium)-induced intestinal inflammation in C57BL/6 mice, suggesting that the intestinal anti-inflammatory activity of *Musa* spp. AAA is related to resistant starch presence.[17]

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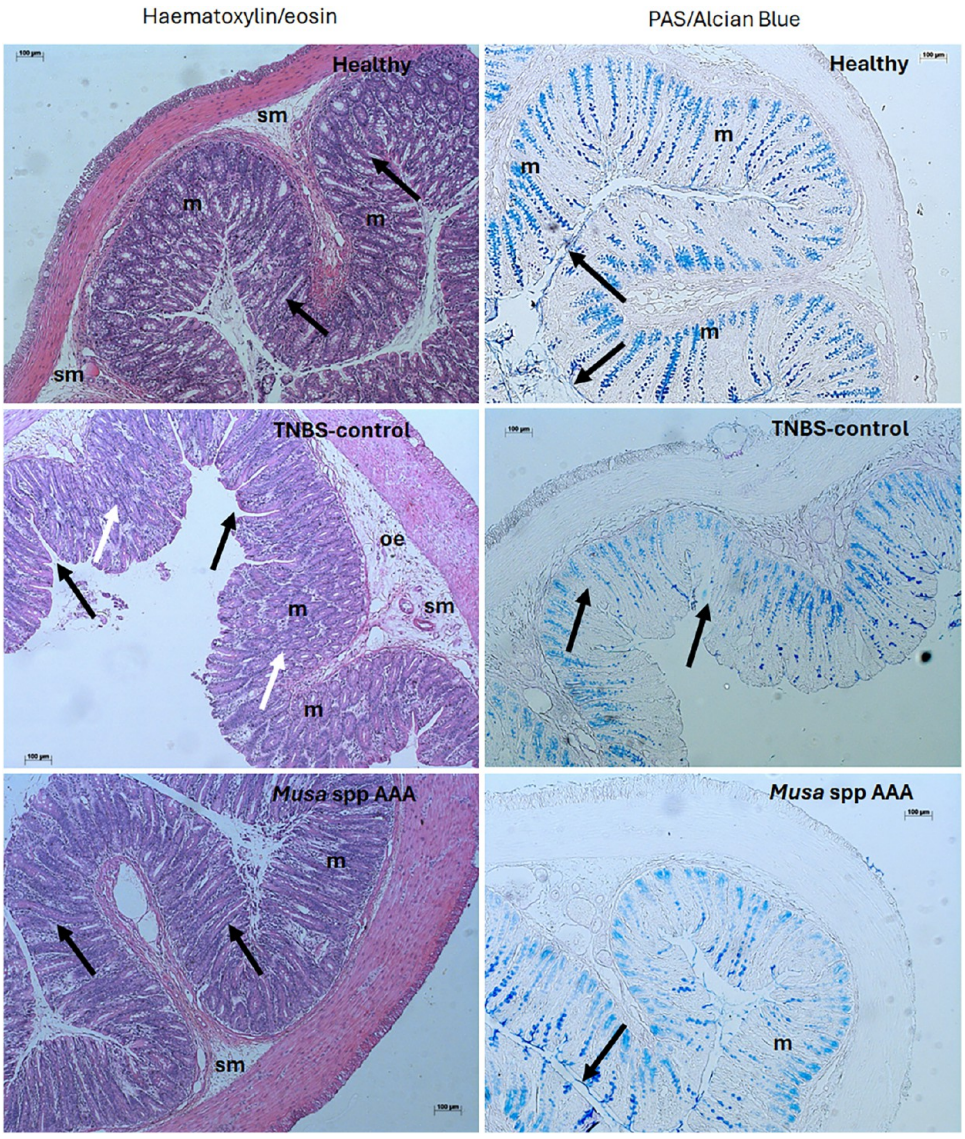

Figure 7. Hematoxylin/eosin (HE) and PAS/Alcian Blue (PAS/AB) photomicrographs of rat colon treated with *Musa* spp. AAA in the chronic with relapse protocol of intestinal inflammation induced by TNBS (5×). HE: In the healthy group, the luminal epithelium containing goblet cells (black arrows) is intact, with typical morphology in the mucosa (m) and submucosa (sm). In the TNBS-control group, there is a disruption of the epithelium (black arrow) with no normal goblet cells (white arrows) in the mucosa (m) and a large edema (oe) in the submucosa (sm). In the *Musa* spp. AAA-treated group, the colon cytoarchitecture was recovered with minor inflammatory cell infiltration in the mucosa (m); the epithelium is intact, with a restoration of tubular glands (black arrows) containing goblet cells, and submucosa (sm) recovery, which are like healthy animals. PAS/AB: The healthy group shows mild blue staining, indicating the presence of mucins both in the mucosa (m) and on the cell surface (black arrows). The TNBS-control group had no blue layer on the cell surface, and the goblet cells were damaged and depleted (black arrows). In the *Musa* spp. AAA-treated group, there was a thick blue layer in the cell surface representing the membrane-bound mucin (black arrows) and mucosal goblet cell mucins still present themselves in a reduced form (m).

IBD etiology is unclear, but several factors have been related to its occurrence and development, such as dysregulated immune response, dysfunctional intestinal barrier function, and genetic and environmental aspects, mainly dysbiosis.[1,2,18] Dysbiosis is a reduction in microbial diversity and abundance, and/or a combination of the loss of beneficial bacteria and an increase in pathogenic ones, affecting SCFA levels in the gut due to both the disruptions in gut microbiota composition and the reduction of dietary fibers and resistant starch fermentation.[17,19]

The unripe bananas, including the genotype *Musa* spp. AAA, contain 70−80% resistant starch, a fraction of starch that resists breakdown in the small intestine and arrives intact in the large intestine, where it is highly fermented by the gut microbiota, acting by its prebiotic properties.[20−24] Based on its resistant starch content, unripe banana flour is reported as a good and low-cost food ingredient whose consumption is booming due to its potential and physiological benefits to human health.[25] Besides resistant starch, unripe bananas are a good source of fibers, vitamins, minerals, and bioactive compounds such as phenolic compounds, and are classified as a functional food.[25] The modulation of gut microbiota evidenced by the increase in SCFA production corroborates the previously described action of *Musa* spp. AAA in the acute phase of intestinal inflammation.[8,9] However, this increase was also evidenced in acute, subchronic, and chronic inflammation with relapse, showing a prolonged effect. The prebiotic properties of unripe

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ACS Food Sci. Technol. 2025, 5, 2776−2786

bananas have been related to highly resistant starch content, serving as a substrate for gut microbiota fermentation being used as an energy source, and producing the SCFAs as acetate, propionate, and butyrate.[26,27] SCFAs are fatty acids with 2−6 carbon atoms produced spontaneously in the liver; however, the main production route is by the gut microbiota fermentative process of resistant starch and other fiber types.[26,28] Acetate is usually the most abundant SCFA, followed by propionate and butyrate, reaching relative molar ratios of approximately 60−20−20, depending on the available substrates, with higher concentrations in the proximal colon.[28,29] Once produced, SCFAs are absorbed by the colonic epithelial cells, contributing significantly to the energy needs of these cells and supporting their proper function and maintenance.[28]

The dysbiosis process is associated with oxidative stress, which boosts ROS generation and production of pro-inflammatory cytokines.[4] When a cell is exposed to excessive ROS levels, the redox balance cannot be maintained, leading to lipids, proteins, and DNA damage.[30] In acute and subchronic protocols, TNBS instillation decreased total GSH levels and increased MPO activity, modulating the oxidative state in the gut. In the subchronic protocol, there was a normal tissue recovery and consequent reduction in inflammation and neutrophil recruitment, as demonstrated by MPO activity inhibition compared with the control group from the acute protocol. The tripeptide GSH is the most important intracellular antioxidant with a key role in mucosal protection against oxidative stress, generally decreased in the mucosa of IBD patients,[31,32] and MPO is the most toxic enzyme found in granules of neutrophils and monocytes generating reactive intermediates, such as hypochlorous acid, increasing the intestinal oxidative stress and inducing lipid peroxidation as well as proteins and DNA damage.[1,33] Diet supplementation with *Musa* spp. AAA flour avoided GSH depletion in acute inflammation, accompanied by a reduction of MPO activity in subchronic and chronic inflammation with relapse protocols, indicating its potential as an antioxidative agent. A combination of acetate, propionate, and butyrate could regulate the antioxidant system in skeletal muscle *in vitro* by upregulation of GSH levels,[30] indicating that SCFAs produced after *Musa* spp. AAA fermentation could be acting as a regulator of the endogenous antioxidant system in the colon. Different banana species have been reported as sources of several bioactive compounds with high antioxidant properties, increasing antioxidant defense enzymes and glutathione levels and inhibiting myeloperoxidase activity.[34]

The intestinal anti-inflammatory activity of *Musa* spp. AAA was also related to differential inhibition of several pro-inflammatory cytokines, as evidenced by the reduction of IL-1β, IL-6 in acute inflammation, and IL-6, IL-17, TNF-α, and INF-γ in subchronic inflammation. In TNBS-induced intestinal inflammation, IL-1β and TNF-α orchestrate the inflammatory response within the lamina propria.[35] In the colon, IL-1β induces the differentiation of naïve T cells, which produce IL-17 or IFN-γ,[36] suggesting that inhibition of IL-1 β by *Musa* spp. AAA could represent a blockage in the perpetuation of the inflammatory process. On the other hand, IL-6, a pleiotropic cytokine, can bind to both the membrane-bound IL-6 receptor and the soluble IL-6R receptor on target cells.[37,38] During the onset of inflammation, IL-6 acts on mesenchymal and epithelial cells, inducing the recruitment of polymorphonuclear leukocytes and macrophages and promoting mucosal healing.[37] Studies in experimental intestinal inflammation indicate suppression of chronic intestinal inflammation after treatment with an IL-6 receptor antibody associated with a reduction of pro-inflammatory cytokine production and apoptosis,[38] suggesting that inhibitory action of IL-6 production leads to a decrease in the other studied cytokines.

TNF-α is a pro-inflammatory cytokine produced by several cell types, such as macrophages, T cells, dendritic cells, and adipocytes. After binding to receptors, TNF-α induces pro-inflammatory processes including neo-angiogenesis and activation of macrophages.[38] Excessive TNF-α alters epithelial integrity and induces apoptosis, thus weakening barrier function,[37] while the overexpression of TNF-α aggravates experimental colitis.[38] Several anti-TNF-α agents have been approved for IBD therapy, placing this pro-inflammatory cytokine as a pharmacological target to reduce intestinal inflammation.[38] On the other hand, IFN-γ is a pro-inflammatory cytokine primarily produced by mucosal T cells that contribute to mucosal inflammation through several mechanisms, including apoptosis, driving IBD-associated mucosal inflammation by disrupting the vascular barrier through the adherent junction protein VE-cadherins.[38] This way, a decrease of IFN-γ levels promoted by *Musa* spp. AAA flour associated with lower TNF-α production may have led to lower barrier disruption and lower inflammatory cell infiltration in the mucosa and submucosa, as evidenced by histological analysis.

Although the role of IL-17 in intestinal inflammation remains controversial, this cytokine generally induces the recruitment of immune cells, producing several pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β.[37] Reduction of IL-17 related to a decrease in IL-6 and TNF indicates a great intestinal anti-inflammatory potential of *Musa* spp. AAA flour, with a consequent reduction of edema, goblet cell abundance, cytoarchitecture recovery, and inflammation, as described in histological analysis. A recent study demonstrated that the goblet cell's appearance is closely related to the mucosal healing process in human IBD.[39] Moreover, the reduced production of pro-inflammatory cytokines promoted a decreased accumulation of inflammatory cells, such as neutrophils, thus reducing MPO secretion.

Based on the results described, the intestinal anti-inflammatory property of *Musa* spp. AAA was closely related to an increase in SCFA production, which was associated with the inhibition of oxidative stress and pro-inflammatory cytokines. SCFAs regulate immunity affecting neutrophil and macrophage recruitment and inhibit NF-κB, one of the most important transcription factors that regulate the transcription of TNF-α and IL-1β.[40−42] Moreover, SCFAs are known to trigger G protein-coupled receptors (GPCRs) in immune cells, mostly GPR41 and GPR43, which subsequently initiate an immune response.[40] The GPR43 receptor reduces the level of NF-kB through the β-arrestin 2 signaling pathway and the amount of the two NF-kB subunits inhibiting the transcription of cytokines as IL-1β and IL-6.[43] Furthermore, it has been found that SCFAs, mainly propionate, reduce IL-17 and IL-22 production by intestinal $\gamma\delta$T cells, which protect the epithelial barrier, corroborating data here demonstrated.[44]

Dietary supplementation with green dwarf banana (*Musa* spp. AAA) fruit flour showed prebiotic intestinal anti-inflammatory activity in the TNBS-induced intestinal inflammation model in rats, acting as prebiotic products by stimulating a prolonged production of SCFAs, mainly acetate and propionate, blocking the production of important mediators of the intestinal inflammatory process, such as IL-1β, IL-6, IL-17, TNF-α, and

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IFN-γ, acting as mucosal healer as evidenced by reduced inflammatory cell migration to the intestine, mucosal damage and edema, increasing mucus production, and reducing colonic oxidative stress. Therefore, green fruits of *Musa* spp. AAA can be classified as a functional food with prebiotic, immunomodulatory, antioxidant, and anti-inflammatory properties. Our results suggest that the consumption of functional food based on green dwarf banana fruits in the diet could benefit intestinal inflammation, particularly as a complementary product to the medications used in IBD treatment.

Although the present study demonstrated the effects of *Musa* spp. AAA in the acute, subchronic, and chronic inflammation with relapse induced by TNBS, acting as an antioxidant, immunomodulatory, and prebiotic properties, further studies are necessary using other experimental models like the DSS model of intestinal inflammation in mice, and *in vitro* assays including cell cultures, fermentation process, and antioxidant methods for detailed evaluation of the mechanisms of action of *Musa* spp. AAA as an intestinal anti-inflammatory product. Moreover, preclinical and clinical studies of safety are necessary before clinical trial evaluation *of Musa* spp. AAA.

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ABBREVIATIONS

CD–Chron disease
GSH–glutathione
IBD–inflammatory bowel disease
MPO–myeloperoxidase
SCFA–short-chain fatty acids
TNBS–trinitrobenzenesulfonic acid
UC–ulcerative colitis

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