Original Research

Passiflora edulis peel intake and ulcerative colitis: Approaches for prevention and treatment

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Abstract

Inflammatory bowel disease is a chronic relapsing disease that affects millions of people worldwide; its pathogenesis is influenced by genetic, environmental, microbiological, and immunological factors. The aim of this study was to evaluate the effects of short- and long-term *Passiflora edulis* peel intake on the antioxidant status, microbiota, and short-chain fatty acids formation in rats with 2,4,6-trinitrobenzenesulphonic acid-induced colitis using two ''*in vivo*'' experiments: chronic (prevention) and acute (treatment). The colitis damage score was determined using macroscopic and microscopic analyses. In addition, the antioxidant activity in serum and other tissues (liver and colon) was evaluated. Bifidobacteria, lactobacilli, aerobic bacteria and enterobacteria, and the amount of short-chain fatty acids (acetic, butyric, and propionic acids) in cecum content were counted. Differences in the colon damage scores were observed; *P. edulis* peel intake improved serum antioxidant status. In the treatment protocol, decreased colon lipid peroxidation, a decreased number of aerobic bacteria and enterobacteria, and an improvement in acetic and butyric acid levels in the feces were observed. An improvement in the bifidobacteria and lactobacilli was observed in the prevention protocol. These results suggested that *P. edulis* peel can modulate microbiota and could be used as source of fiber and polyphenols in the prevention of oxidative stress through the improvement of serum and tissue antioxidant status.

Keywords: *Passiflora edulis* peel, ulcerative colitis, antioxidant status, microbiota, short-chain fatty acids

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Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing disease that encompasses Crohn's disease (CD) and ulcerative colitis (UC) and affects millions of people worldwide. The difference between CD and UC is related to the location and severity of the mucosa; CD is a transmural inflammation that can affect the entire gastrointestinal tract, and UC is a nontransmural inflammation restricted to the colon.[1] The pathogenesis of IBD is influenced by genetic, environmental, microbiological, and immunological factors.[2–4] An imbalance in the intestinal microbiota has been postulated to be responsible for the initiation and progression of this inflammation, especially when the pathogenic bacteria grow faster than nonpathogenic bacteria.[5] The use of probiotics and prebiotics has been shown to have a modulatory effect on IBD, through direct microbiota modulation and by modulation of their end-products, such as short-chain fatty acids (SCFAs).[6,7] Experiments using orally administration, enema, and *in vitro* assays have shown that butyric acid has an anti-inflammatory effect by reducing neutrophil migration and by modulating interleukin production.[8–10] Many inflammatory mediators, including reactive oxygen species (ROS) and cytokines, contribute to the inflammatory cascade that modulates the immune system in UC.[11–14] In contrast, depletion of glutathione enzyme in chronic inflammation, which can improve the damage in tissue, has been reported.[15,16]

In addition to prebiotics and probiotics, some polyphenolic compounds have shown anti-inflammatory activity in UC, especially by inhibiting proinflammatory markers.[17,18] Moreover, in the last few years, many studies have used microbiota substrates that improve the formation

of SCFAs and protect the mucosal tissue against inflammatory damages.[6,19–22] *Passiflora edulis* is largely cultivated in Brazil and for the production of juices and pulps. However, a great amount of waste is generated by this industry because half of the fruit is peel, which is a source of pectin[23] and flavonoids.[24] The consumption of *P. edulis* peel has been shown to improve insulin sensitivity[25] and blood pressure[26]; additionally, its polysaccharides have antitumor properties *in vitro* and its flavonoids have antioxidant activity.[24]

P. edulis is a great source of polyphenols, soluble and insoluble fiber, which could modulate inflammatory markers of UC. Thus, the aim of the present study was to evaluate the effects of *P. edulis* peel consumption in experimental model of rat colitis induced by 2,4,6-trinitrobenzenesulphonic acid (TNBS).

Materials and methods

Passion fruit flour

Organic *P. edulis* (passion fruit) crops from Torre de Pedra/SP harvested in June 2010 were used to produce the flour. The fruit was cleaned and separated into pulp and peel (flavedo + albedo). The peels were cut into small pieces and dried in an oven with air circulation at 50°C (Marconi, Piracicaba/SP, Brazil) until they reached a moisture content of 10% or less. The dried peel was ground into a fine powder using a hammer mill (20 mesh), and the flour was stored in an amber flask at room temperature (24°C).

Animals and experimental design

This study was carried out in accordance with the ''Guide for the Care and Use of Laboratory Animals'' proposed by the Brazilian College of Animal Experimentation (COBEA). The study was approved by the Animal Research and Ethic Committee of the University of Campinas (Brazil) (protocol 2257–1/2010).

Male Wistar rats were housed under standard temperature (22°C ± 2), humidity (60–70%), and light–dark cycle (19–07 h). Forty-eight rats were randomized into two experiments: the treatment and prevention protocols. In the treatment protocol, 24 rats (77 days old) were divided into control and passion fruit flour (PFF) groups ($n = 12$). They were fed with control and experimental diet, respectively, for a week. The diets were prepared in accordance with the American Institute of Nutrition[27] with 50% of the cellulose substituted with dietary fiber from PFF in the experimental diet. After seven days, colitis was induced in six animals from each group. The animals were maintained for seven days; afterwards they were anesthetized with ketamine and xylazine and sacrificed by exsanguinations through cardiac puncture. In the prevention protocol, 24 rats (21 days old) were divided into control and PFF groups ($n = 12$). They were fed with the control or experimental diets for 84 days, at which point colitis was induced in six animals per group. After seven days, they were sacrificed as described above. Access to food and drink was *ad libitum* and monitored three times a week.

Briefly, colitis induction was carried out using TNBS. The animals were anaesthetized with halothane and 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.[28] The same procedure was performed on noncolitic animals, which were referred to as saline group, through intracolonical administration of 0.25 ml of phosphate-buffered saline instead of TNBS.

Blood sampling

Blood samples were obtained by cardiac puncture under anesthesia, collected in tubes sprayed internally with clot accelerator (SiO_2), and centrifuged at 2600 g for 20 min. Serum was stored at −80°C until the analyses were performed. Serum albumin and total proteins were measured using commercial kits (Laborlab, Guarulhos, São Paulo, Brazil). Serum was treated with ethanol:ultrapure water (40:20, v/v) and 0.75 mol L^{-1} metaphosphoric acid,[29] and these extracts were used to determine the antioxidant activity of the samples using hydrophilic oxygen radical absorbance capacity (ORAC)[30] and to measure the ability of the antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe(III)\text{-}(TPTZ)2]^{3+}$ (FRAP).[31]

The ferric reducing antioxidant power (FRAP) method was developed by Benzie and Strain.[31] This methodology is based on the capacity to reduce the complex ferric ion and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ). The binding of Fe2+ to the ligand creates a very intense blue color. Changes in this color are monitored by measuring the absorption at 593 nm.

The ORAC assay measures the antioxidant scavenging activity against the peroxyl radical. 2,2-Azobis 2-amidopropane dihydrochloride (AAPH) is used to generation of free radical and the loss of fluorescence is recorded, which indicates the extent of the radical scavenging.[32]

Tissue sampling

The liver and colon were quickly removed, cleaned with physiologic solution (0.9% NaCl), and weighed. The colon's length was measured, and the macroscopically visible damage was evaluated on a 0–10 scale according to previously reported criteria.[33] Afterwards, the specimens were frozen in liquid nitrogen and kept at −80°C. Tissue homogenates were prepared in 50 mmol phosphate buffer (pH 7.4) using a Polytron homogenizer (MA102/Mini, Marconi, Piracicaba/SP, Brazil), and the supernatant was kept at −80°C until analyses.

Lipid peroxidation was determined in the tissue homogenates based on thiobarbituric acid reactive substances (TBARS) assay,[34] reduced glutathione (GSH) activity,[35] glutathione reductase (GR) activity,[36] glutathione peroxidase (GPx) activity,[37] and superoxide dismutase (SOD) activity.[38]

Myeloperoxidase activity (MPO) in the colon tissue homogenates was measured by the method proposed by Krawisz *et al.*[39]; the results were expressed as MPO units per mg of protein, where 1 unit of MPO activity was defined as the amount able to degrade 1 μmol hydrogen peroxide·min^{-1} at 25°C.

Colonic histology

For the histological analyses, freshly dissected colon samples were washed with physiologic solution (0.9% NaCl) and cryoconserved in a freezing medium (Jung; Leica Instruments, Nussloch, Germany). Serial cross sections (7 μm) were prepared using a cryostat (CM 1850 Cryostat, Leica) and fixed on silanized slides. After staining the samples with Haematoxylin-Eosin, the severity of each colitis case was analyzed (Eclipsi 80i microscope, Nikon) by a pathologist according to the criteria described by Chinen *et al.*[40]

pH and cecal microbiota

Fecal samples were collected from the cecum, diluted with deionized water (1 mg mL^{-1}), and homogenized to measure the fecal pH using a pH meter (Tecnal model TEC-5, Piracicaba/SP, Brazil).[41]

The cecal microbiota was evaluated in fecal samples homogenized in peptone water (100 mg mL^{-1}) followed by 10-fold serial dilutions in the same medium. Aliquots of 0.1 ml of the appropriate dilution were spread onto the MRS agar media for the Lactobacillus count and supplemented MRS agar (0.5 mg L^{-1} dicloxacillin, 1 g L^{-1} LiCl, and 0.5 g L^{-1} L-cysteine hydrochloride) for Bifidobacterium. The cultured plates were incubated in anaerobic condition at 37°C for 24–48 h. Similarly, 1 mL of each diluted sample was spread onto a specific count plates Petrifilm (3M®, São Paulo, MN) for Enterobacteriaceae and total aerobic bacteria. The plates were incubated at 37°C for 24–48 h. After the incubation, the specific colonies grown on the selective culture media were counted, and the number of viable microorganism g^{-1} feces (CFU g^{-1}) was calculated. The mean and standard error were calculated from the log 10 values of the CFU g^{-1}.

Cecal SCFA content

SCFAs were analyzed by gas chromatography[42] using Agilent 6890N equipment with a flame ionization detector (FID) and an autosampler N10149 (Agilent, EUA). A 30 m × 0.25 mm i.d. × 0.25 μm Nukol™ capillary column (Supelco, Bellefonte, PA, USA) was used. The chromatographic conditions were as follows: injector and detector temperatures, 250°C; injected volume, 1 μL with split ratio set to 1:10; and carrier gas, hydrogen at 1.0 mL min^{-1}. The column oven was programmed as follows: the temperature was kept at 100°C for 0.5 min, then heated at 8°C min^{-1} until it reached 180°C, held for 1 min, heated 20°C min^{-1} until it reached 200°C and then held for 5 min.

Statistical analysis

The statistical analyses were performed using one-way analysis of variance (ANOVA) followed by a Tukey multiple comparisons test. All data were expressed as the means ± SEM, and the difference was considered to be statistically significant when $P < 0.05$. Statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software.

Results and discussion

The consumption of fruits and vegetables are important to diminish the risk of UC, as they are rich sources of fibers and antioxidants.[43,44] The consumption of *P. edulis* peel could improve the intake of fibers and antioxidants and could play a role in UC.[23,24] Thus, flour (PFF) from *P. edulis* peel was prepared and incorporated into the rats' diet by using it to replace 50% of the standard source of fiber (cellulose). The effects of PFF intake in a short term (treatment protocol) and in a long term (prevention protocol) were evaluated in a TNBS-induced colitis model.

The symptoms of colitis were observed immediately after the administration of TNBS when the animals started to show bleeding on the rectum and in the feces. During the seven days after colitis induction, all of the animals showed colitis symptoms, such as bloody feces, diarrhea, weight loss, and a decrease on food intake. This decrease in food intake observed in the colitis groups had an important influence on the body weight loss (Table 1).

The short-term intake of the PFF diet in the treatment protocol did not promote changes in food intake and weight gain. However, in the prevention protocol, the long-term intake of the PFF diet resulted in a decreases in food intake and body weight gain that could be attributed to the changes in the types of fibers presents in the diet (soluble and insoluble). In contrast to the control diet, in which cellulose was the only source of dietary fiber, the PFF diet included cellulose and soluble and insoluble fibers from the *P. edulis* peel flour. Thus, this modification of the dietary fiber composition could have an influence on the satiety, gastric emptying, bowel transit time, and in the digestion and absorption of fat and carbohydrates, all of which can contribute to decreases in weight gain.[45–47]

Malnutrition occurs very frequently in IBD patients,[48] and they often have a lower level of serum albumin in the acute phase compared to healthy individuals, likely due to a decrease in serum albumin synthesis, an increase in transcapillary losses, and degradation.[49] Therefore, evaluating serum albumin could be a simple and inexpensive way to predict the nutritional status of IBD patients.[50] Consumption of the PFF diet increased the levels of serum albumin in both healthy and colitis animals in the prevention protocol, which led us to believe that these animals, although they exhibited less weight gain compared to the control group, most likely were not malnourished (Table 1).

The macroscopic evaluation of the inflamed mucosa was used to determine the presence and extension of the hyperemia and ulceration in the tissue compared to normal colon. TNBS-induced colitis is characterized by severe necrosis of the mucosa, typically extending 4–6 cm along the colon, bowel wall thickening, hyperemia, and focal adhesions to adjacent organs (Figure 1). In addition, transmural intense inflammatory infiltrations of lymphocytes and neutrophils, edema in the mucosa, and a focal loss of crypts and superficial epithelium were observed, similar to previous studies.[28,51]

Consumption of the PFF diet did not attenuate the macroscopic tissue damage observed in the TNBS-induced colitis model; the miscroscopic score was similarly

Table 1 Food intake, body weight changes, and serum analyses of rats fed control and PFF diets in the treatment and prevention protocols

	Control saline	Control colitis	PFF saline	PFF colitis
Treatment protocol				
Food intake daily (g)	24.9 ± 1.02^{a}	13.8 ± 1.16^{b}	22.8 ± 1.46^{a}	14.6 ± 1.97^{b}
Total weight gain (g)	41.8 ± 2.86^{a}	13.8 ± 4.00^{b}	36.5 ± 4.27^{a}	9.5 ± 1.78^{b}
Colon weight/length ratio	0.1 ± 0.00^{b}	0.2 ± 0.02^{a}	0.1 ± 0.01^{b}	0.2 ± 0.01^{a}
Serum total protein (mg/dL)	5.2 ± 0.15^{b}	5.5 ± 0.22^{ab}	5.9 ± 0.21^{a}	5.5 ± 0.12^{ab}
Serum albumin (mg/dL)	3.0 ± 0.04^{c}	3.4 ± 0.17^{abc}	3.4 ± 0.03^{a}	3.1 ± 0.03^{b}
Serum FRAP (μM TE/mL)	598.9 ± 32.95^{a}	504.1 ± 35.17^{abc}	481.8 ± 12.75^{c}	550.1 ± 17.36^{b}
Serum ORAC (μM TE/L)	265.0 ± 13.72^{b}	429.0 ± 4.76^{a}	438.1 ± 38.55^{a}	387.2 ± 23.84^{a}
Prevention protocol				
Food intake daily (g)	24.9 ± 0.38^{a}	22.7 ± 0.53^{b}	10.2 ± 0.20^{c}	10.0 ± 0.31^{c}
Total weight gain (g)	391.7 ± 4.15^{a}	312.2 ± 14.01^{b}	164.8 ± 8.10^{c}	141.8 ± 11.77^{d}
Colon weight/length ratio	0.1 ± 0.00^{b}	0.3 ± 0.05^{a}	0.1 ± 0.00^{b}	0.2 ± 0.03^{a}
Serum total protein	5.9 ± 0.13^{a}	5.1 ± 0.12^{bc}	5.6 ± 0.19^{ab}	5.1 ± 0.13^{c}
Serum albumin	3.7 ± 0.08^{c}	3.0 ± 0.13^{d}	5.7 ± 0.17^{a}	5.1 ± 0.13^{b}
Serum FRAP (μM TE/mL)	465.5 ± 49.38^{b}	496.8 ± 32.67^{b}	563.1 ± 66.53^{ab}	649.6 ± 57.90^{a}
Serum (ORAC (μM TE/L)	420.3 ± 16.24^{a}	402.2 ± 35.52^{ab}	398.1 ± 22.16^{ab}	349.6 ± 21.96^{b}

PFF: passion fruit flour; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity. Values are expressed as the mean ± SEM ($n = 6$ rats per group). Different superscript letters in the same line denote significant differences ($p < 0.05$) between the groups.

Figure 1 Macroscopic changes in the architecture of the colon tissue in the TNBS model of colitis. (a) Normal mucosa; (b) typically inflamed mucosa observed in this experimental model, characterized by bowel wall thickening, hyperemia, and areas of necrosis. (A clolor version of this figure is available in the online journal)

unaffected (Figures 2 and 3). In addition, there were no decreases in the colonic weight/length ratio in the PFF groups compared to the control groups in either protocols (Table 1).

The concept of oxidative stress is defined globally as an imbalance between pro-oxidants and antioxidants, but it has been discussed based on the inconclusive results shown in interventional trials, where limited increases in protection after an increase in antioxidants supply were reported. Based on those results, Jones[52] stated that the definition of oxidative stress should be changed to a condition in which there is a disruption in redox signaling and control. In the present study, these changes were investigated in the serum and colonic and hepatic tissue of the rats in the treatment and prevention protocols.

The level of lipid peroxidation in the colon mucosa was evaluated using the TBARS assay and by measuring the antioxidants and myeloperoxidase (MPO) enzymes activities (Figures 4 and 5). The TBARS assay quantifies the level of malondialdehyde (MDA) synthesis, which is a validated marker for evaluating the lipid peroxidation caused by the action of ROS. PFF consumption in the treatment protocol protected the animals' colon tissue from lipid peroxidation; however, it did not affect the levels of endogenous antioxidants enzymes or decrease MPO synthesis.

MPO is an enzyme found in neutrophils that, following stimulation, causes an increase in ROS production and proinflammatory mediators. In a study of patient rectal biopsies, Dagli *et al.*[53] observed an increase in ROS concentration, and neutrophil-derived MPO activity, which was in accordance with an increase in MDA levels and a decrease in SOD enzyme. Silva *et al.*[54] observed a dose-dependent decrease in MPO, and a significant reduction in carrageenan-induced paw edema in rats that received pectin isolated from the peels of *P. edulis*. In addition, the flavonoid isoorientin extracted from *P. edulis* peel was shown to be more effective in MPO activity reduction than the methanolic extract from the pulp. The technique that was used provided evidence that MPO inactivation might be related

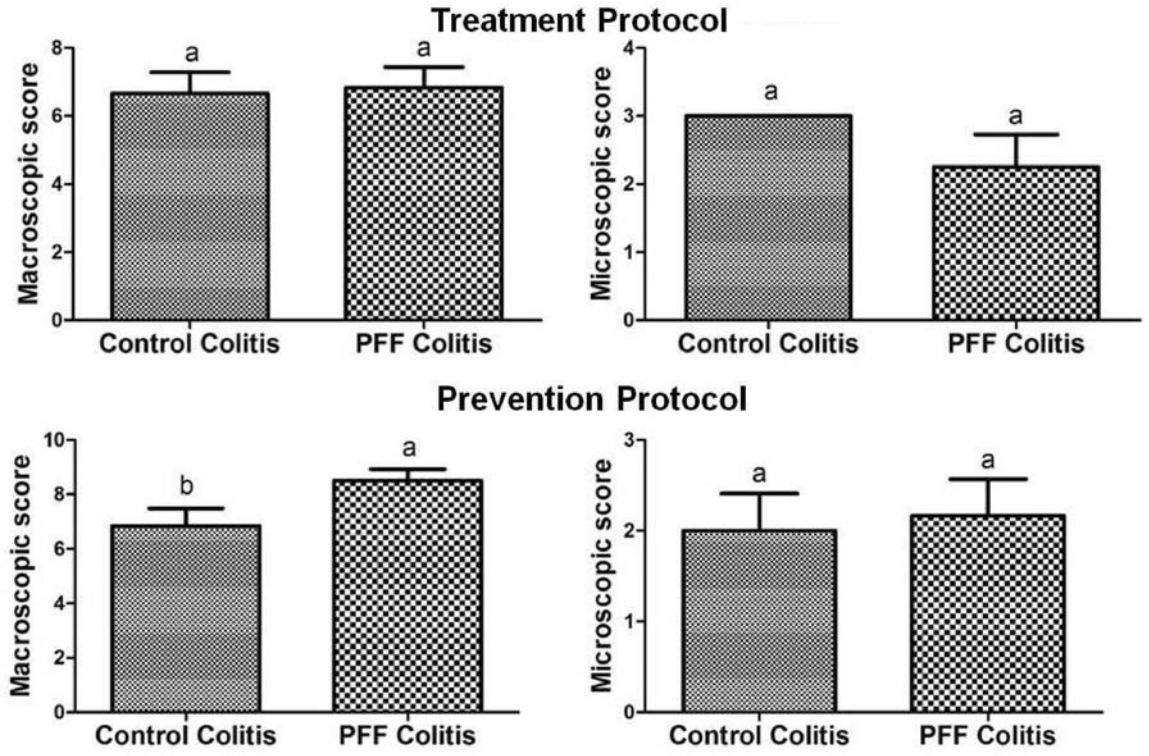

Figure 2 Evaluation of the macroscopic and microscopic scores in inflamed colonic mucosa in the TNBS-induced colitis model. Inflammation severity is scored in comparison to healthy animals (score = zero). Different superscript letters in the columns denote significant differences ($p < 0.05$) between the groups. PFF: passion fruit flour

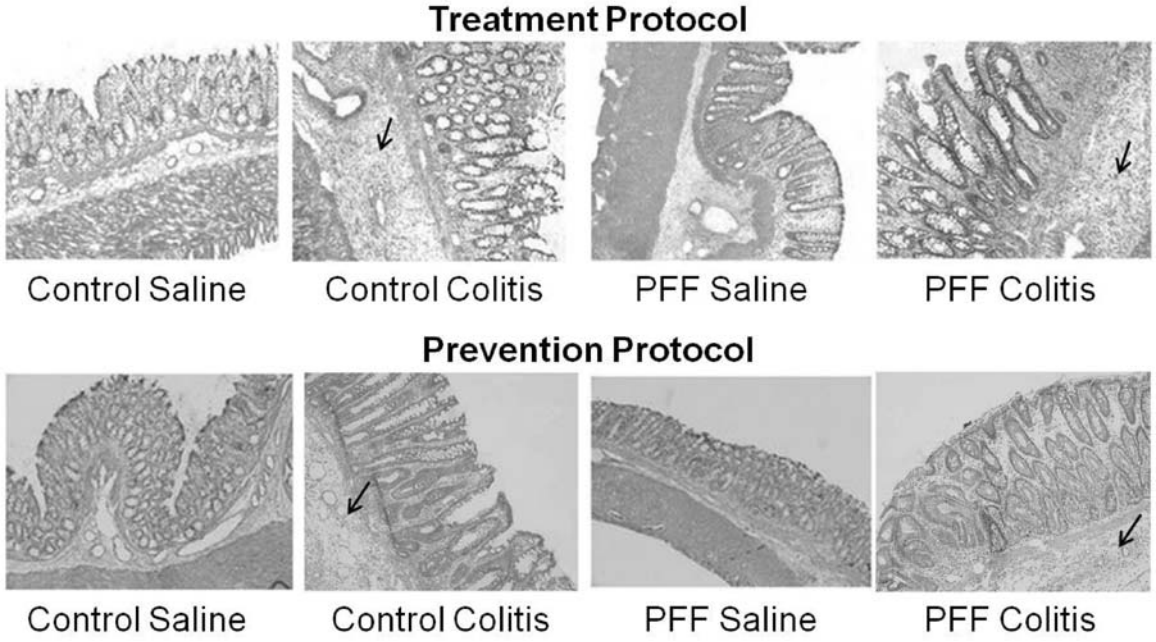

Figure 3 Effects of PFF on the histological evaluation of colon tissues from rats submitted to TNBS-induced colitis (original magnification, ×100). This experimental model is characterized by a continuous lesion, restricted to the distal colon, with a large area of necrosis and the transmural involvement of the mucosa. The colon of colitis groups showed loss of crypts and epithelial integrity, submucosal edema (indicated by arrows), and intense inflammatory cellular infiltration in all layers. PFF: passion fruit flour

to a structural modification or linkage between the flavonoid and the active site on the enzyme's protein chain.[55]

In contrast, in the prevention protocol, the consumption of PFF tended to improve the level of the antioxidant enzymes, but there was no significant difference between the control and PFF colitis groups. The increase in the MDA level of the PFF colitis group could be responsible for the significant decrease in the SOD enzyme. This enzyme

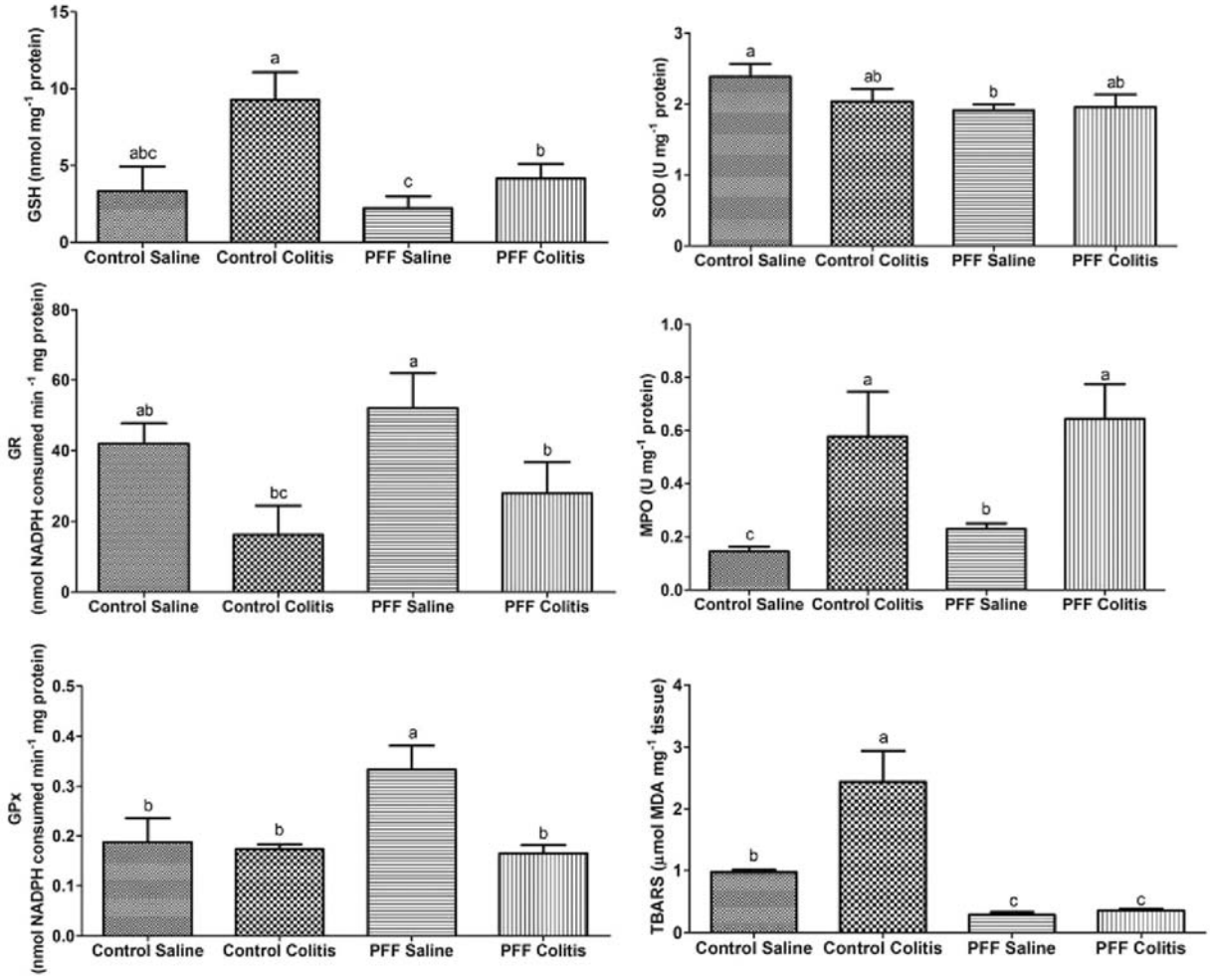

Figure 4 Evaluation of the effects of the treatment protocol (PFF short-term intake) on the endogenous antioxidant enzymes activity and the level of lipid peroxidation in inflamed colonic mucosa in the TNBS-induced colitis model. The data are expressed as the mean ± SEM ($n = 6$ rats per group). Different superscript letters in the columns denote significant differences ($p < 0.05$) between the groups. PFF: passion fruit flour; GSH: glutathione; GR: glutathione reductase; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; GPx: glutathione peroxidase; SOD: superoxide dismutase; MPO: myeloperoxidase activity; TBARS: Thiobarbituric Acid Reactive Substances

converts the O_2^- radical into O_2 and H_2O_2 and then, the H_2O_2 is converted into H_2O by GSH oxidation.[11] These findings suggest that long-term PFF intake could have a protective effect on the antioxidant status of the colonic tissue.

Antioxidant status was also evaluated in the serum, and changes in serum antioxidant activity were observed in both protocols (Table 1). In the prevention protocol, the PFF colitis group exhibited an improvement in FRAP serum antioxidant activity compared to the control colitis group. The serum antioxidant activity measured by FRAP in a previously study was inversely associate with the level of serum C-reactive protein.[56] In addition, the FRAP assay does not measure the sulfydryl (SH)-group present on the antioxidants,[57] which reinforces the effect of long-term PFF intake on the improvement of antioxidant status in colitis animals. The main source of SH groups in plasma is albumin, which has been reported to be increased in the PFF groups in the prevention protocol.[58]

The consumption of *P. edulis* juice did not improve antioxidant enzyme status but did decrease the blood TBARS levels, indicating that the antioxidant capacity may be related to the addition of exogenous antioxidants present in the juice.[59] However, although Rudnicki *et al.*[60] did not observe changes in liver MDA levels, the consumption of the hydroalcoholic extracts of both *P. alata* and *P. edulis* leaves had significant protective effects against carbonyl protein formation and advanced glycation end-products. In addition, those authors observed a linear relationship between total phenolic content and total antioxidant activity, indicating that these compounds might be the major contributors to animal's antioxidant activities.

Animals from the PFF intake group showed changes in their liver antioxidant status (Table 2), with a decrease in GPx level and, an increase in GR and MDA levels, but no changes in the hepatic reduced glutathione level. GPx catalyzes the reduction of hydroperoxides, including hydrogen peroxides, by reducing glutathione, which can be recycled by GR; GR reduces the oxidized glutathione (GSSG) using nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor. Thus, the level of GPx is often discussed in parallel with GR, which maintains a constant supply of GSH from GSSG for enzymatic activity. GSSG

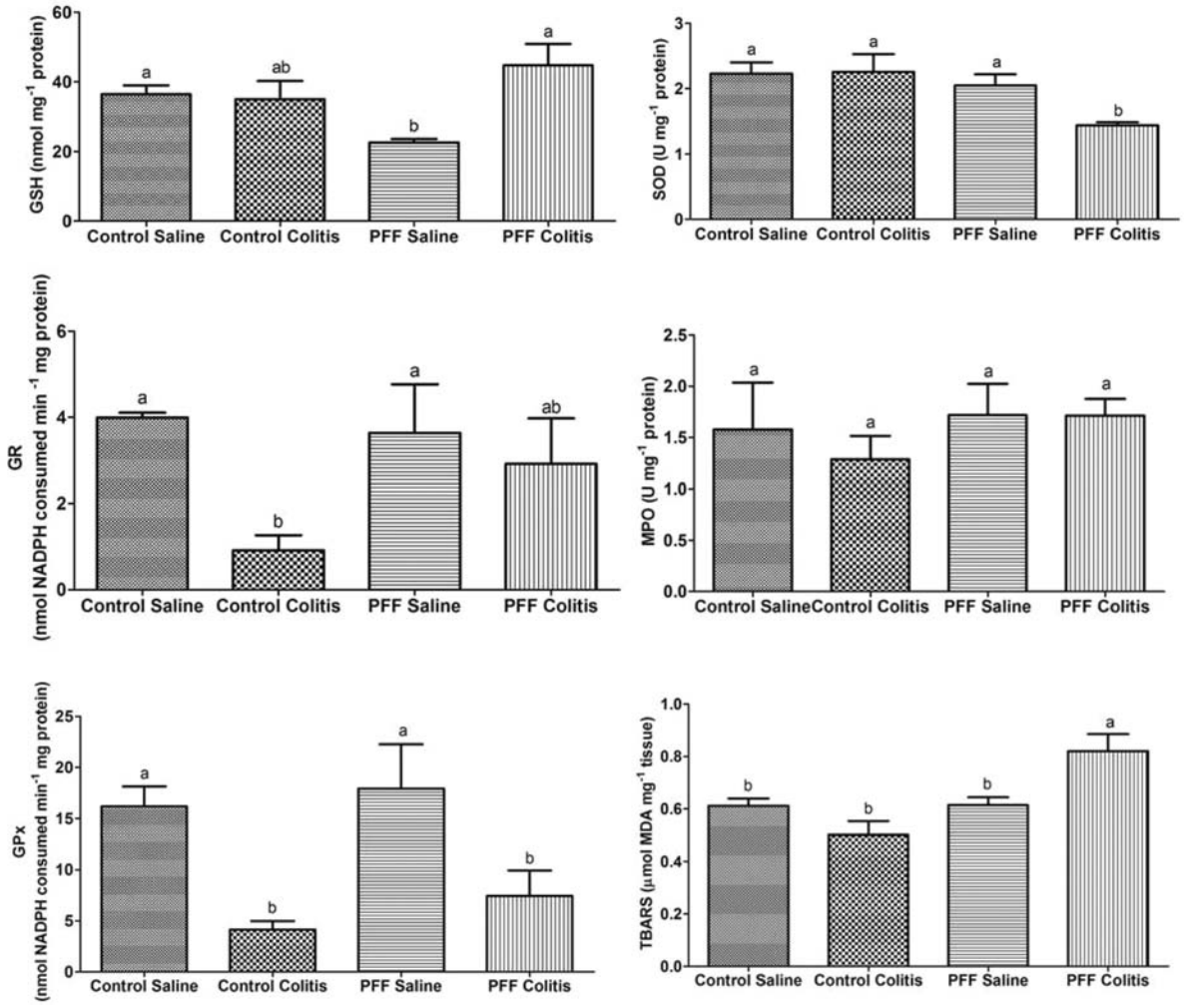

Figure 5 Evaluation of the effects of the prevention protocol (PFF long-term intake) on the endogenous antioxidant enzymes activity and the level of lipid peroxidation in inflamed colonic mucosa in the TNBS-induced colitis model. The data are expressed as mean ± SEM ($n = 6$ rats per group). Different superscript letters in the columns denote significant differences ($p < 0.05$) between the groups. PFF: passion fruit flour; GSH: glutathione; GR: glutathione reductase; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; GPx: glutathione peroxidase; SOD: superoxide dismutase; MPO: myeloperoxidase activity; TBARS: Thiobarbituric Acid Reactive Substances

Table 2 Effects of PFF on hepatic antioxidant status in TNBS-induced colitis rats

	Control saline	Control colitis	PFF saline	PFF colitis
Treatment protocol				
Liver weight (g)	13.7 ± 0.58^{a}	14.4 ± 0.82^{a}	16.1 ± 0.85^{a}	15.2 ± 0.73^{a}
GSH (µM GSH/mg protein)	40.0 ± 9.46^{a}	35.2 ± 3.38^{a}	35.4 ± 5.50^{a}	35.0 ± 3.95^{a}
GPx*	10.7 ± 0.77^{ab}	12.0 ± 0.82^{a}	8.9 ± 0.80^{bc}	6.28 ± 1.30^{c}
GR*	9.6 ± 1.16^{b}	10.3 ± 0.79^{b}	12.9 ± 1.37^{ab}	18.1 ± 1.35^{a}
SOD (units/mg protein)	10.5 ± 1.88^{a}	6.9 ± 1.36^{b}	10.8 ± 1.97^{a}	6.5 ± 1.82^{b}
TBARS (µM MDA/mg tissue)	1.2 ± 0.07^{b}	0.8 ± 0.05^{c}	1.5 ± 0.16^{ab}	1.5 ± 0.11^{a}
Prevention protocol				
Liver weight (g)	15.4 ± 1.32^{a}	14.0 ± 0.76^{a}	8.7 ± 0.34^{b}	8.7 ± 0.52^{b}
Reduced GSH (µM GSH/mg protein)	59.7 ± 2.48^{a}	69.4 ± 8.54^{a}	69.4 ± 7.07^{a}	71.1 ± 11.17^{a}
GPx*	10.6 ± 1.12^{a}	12.0 ± 1.42^{a}	6.4 ± 1.29^{b}	7.3 ± 1.15^{ab}
GR*	21.3 ± 2.13^{b}	21.1 ± 3.21^{b}	41.3 ± 3.82^{a}	31.0 ± 3.29^{a}
SOD (units/mg protein)	2.0 ± 0.18^{b}	4.6 ± 0.81^{a}	3.4 ± 0.33^{a}	2.4 ± 0.18^{b}
TBARS (µM MDA/mg tissue)	1.1 ± 0.05^{c}	1.2 ± 0.11^{b}	2.0 ± 0.06^{a}	1.9 ± 0.10^{a}

GSH: glutathione; GR: glutathione reductase; GPx: glutathione peroxidase; SOD: superoxide dismutase; TBARS: Thiobarbituric Acid Reactive Substances. *nmol NADPH consumed/min mg protein. Values are expressed as mean ± SEM ($n = 6$ rats per group). Different superscript letters in the same line denote significant differences ($p < 0.05$) between the groups.

Table 3 Effects of PFF on the microbiota and short-chain fatty acids in TNBS-induced colitis rats

	Control saline	Control colitis	PFF saline	PFF colitis
Treatment protocol				
pH	8.46 ± 0.14^{b}	8.80 ± 0.03^{a}	7.98 ± 0.13^{c}	8.44 ± 0.23^{ab}
Bifidobacterias (CFU/g wet feces)	9.86 ± 0.20^{ab}	10.34 ± 0.23^{a}	9.22 ± 0.22^{c}	9.35 ± 0.29^{b}
Lactobacilli (CFU/g wet feces)	10.35 ± 0.11^{ab}	10.52 ± 0.29^{ab}	10.33 ± 0.13^{b}	10.74 ± 0.16^{a}
Total aerobic (CFU/g wet feces)	9.64 ± 0.12^{ab}	9.81 ± 0.16^{a}	9.08 ± 0.26^{bc}	8.77 ± 0.15^{c}
Enterobacterias (CFU/g wet feces)	9.56 ± 0.23^{a}	9.28 ± 0.22^{a}	8.67 ± 0.29^{b}	8.28 ± 0.18^{b}
Acetic acid (μM/mg wet feces)	0.08 ± 0.01^{c}	0.06 ± 0.01^{d}	0.16 ± 0.02^{a}	0.13 ± 0.01^{b}
Propionic acid (nM/mg wet feces)	14.20 ± 1.89^{ab}	11.96 ± 1.52^{b}	21.05 ± 3.27^{a}	12.01 ± 1.73^{b}
Butiric acid (nM/mg wet feces)	11.09 ± 1.28^{c}	10.27 ± 1.53^{c}	23.21 ± 1.97^{a}	17.28 ± 1.53^{b}
Prevention protocol				
pH	8.53 ± 0.09^{b}	8.60 ± 0.20^{ab}	8.26 ± 0.23^{b}	9.00 ± 0.15^{a}
Bifidobacterias (CFU/g wet feces)	9.50 ± 0.03^{b}	8.72 ± 0.08^{c}	10.09 ± 0.06^{a}	9.42 ± 0.18^{b}
Lactobacilli (CFU/g wet feces)	9.40 ± 0.20^{b}	8.87 ± 0.13^{c}	10.20 ± 0.12^{a}	9.52 ± 0.25^{b}
Total aerobic (CFU/g wet feces)	8.63 ± 0.40^{a}	8.29 ± 0.16^{a}	8.43 ± 0.13^{a}	8.48 ± 0.24^{a}
Enterobacterias (CFU/g feces)	7.83 ± 0.44^{a}	8.02 ± 0.23^{a}	8.43 ± 0.37^{a}	8.35 ± 0.27^{a}
Acetic acid (μM/mg wet feces)	0.22 ± 0.01^{a}	0.14 ± 0.03^{c}	0.19 ± 0.01^{ab}	0.17 ± 0.01^{bc}
Propionic acid (nM/mg wet feces)	4.20 ± 0.33^{a}	4.25 ± 0.66^{a}	4.80 ± 0.24^{a}	4.28 ± 0.38^{a}
Butiric acid (nM/mg wet feces)	3.72 ± 0.44^{ab}	2.39 ± 0.67^{b}	4.55 ± 0.35^{a}	2.50 ± 0.42^{b}

Data are expressed as mean ± SEM ($n = 6$ rats per group). Different superscript letters in the same line denote significant differences ($p < 0.05$) between the groups.

can be translocated to the blood across the liver, and its plasmatic levels may reflect ROS activity *in vivo*.[61] Thus, this increase in GR in the PFF groups could be related to an influx of circulating GSSG. The decrease in liver weight observed in the PFF groups in the prevention protocol requires further investigation, specifically with respect the lipids and glycogen contents and histological modifications of the liver. A reduction in the liver weight has been observed in others studies,[62,63] in which the level of fiber in the diet was modified, but the reason why long-term consumption of PFF decreases the liver weight remains undetermined.

PFF is a source of flavonoids and fibers that can act as a substrate to the microbiota, yielding high levels of SCFAs that improves gastrointestinal health.[64] Changes in the microbiota and SCFA levels were observed in both protocols (Table 3). Consumption of PFF in the treatment protocol decreased the count of total aerobic bacteria and enterobacterias and improved the acetic and butyric acid levels in the feces. When PFF was used in the prevention protocol, an increased count of bifidobacterias and lactobacilli was observed but modifications in SCFA levels were not. Increases in butyrate concentrations have been suggested to contribute to the down-regulation of pro-inflammatory cascades and oxidative stress in IBD.[65,66]

A recent investigation showed that UC patients did not exhibit differences in mucosa-associated lactobacilli compared to healthy patients; however, a reduction in the bifidobacteria count was observed compared to healthy patients' microbiota.[5] Divergent results pertaining to these findings have been reported in the literature, especially because many different techniques are used to evaluate the microbiota. Nevertheless, an imbalance between the commensal protective and pathogenic bacteria in the microbiota has been postulated to be a factor in the pathogenesis of IBD. Long-term consumption of PFF improves the count of beneficial bacterias in the microbiota.

A search for new sources of substrate (prebiotics) for the selective growth of probiotics is underway.[6,20,21] The consumption of a prebiotic enzyme-treated rice fiber (ERF) showed no differences in bifidobacteria and lactobacilli compared to untreated group; however, decreases in *Clostridium* and *Eubacterium* were observed, were increases in acetic and butyric acids in the feces of male Sprague-Dawley rats.[6] Histological analysis showed significant decreases in mucosal damage scores of rats that consumed ERF, which could be related to the changes in SCFA production. Increases in bifidobacteria and lactobacilli were observed in HLA-B27 transgenic rats after seven weeks of intake of a prebiotic containing a mixture of chicory-derived long-chain inulin-type fructans and short-chain inulin fraction oligofructose (1:1), without modification of SCFA levels compared with the untreated group.[20] Female Wistar rats receiving lactulose at 2.5% in their drinking water for three weeks showed higher counts of bifidobacteria and lactobacilli in their colonic contents compared to the control colitis group.[19] A mixture of 60% kale leaves plus 40% papaya pulp administrated orally for two weeks in female Wistar rats, resulted in a significant increase in lactobacilli and a decrease in aerobic bacteria and enterobacteria in the colon luminal content; changes that improve the ratio of beneficial/pathogen bacterias in the feces.[21] However, intake of green dwarf banana flour, a rich source of resistant starch and amylase, did not promote changes on microbiota.[22]

Intestinal protective mechanisms of PFF are still relatively unknown. Our data show that PFF intake exhibit effect in modulating the intestinal microbiota. In the

treatment protocol, the increases on butyric acid could be directly related to decreases in TBARS levels; and in the prevention protocol, the consumption of PFF improves bifidobacterias and lactobacillus in cecal content. Thus, more studies need to be developed to evaluate the effects of PFF intake in the maintaining the intestinal barrier and in the immune response.

In conclusion, PFF consumption provided benefits in both protocols; however, the consumption of PFF in the prevention protocol showed that long-term intake could have more benefits compared to short-term consumption. Long-term intake of PFF improved antioxidant status and modulated the microbiota in TNBS-induced colitis. However, an improvement in the mucosal damage score was not observed. PFF could be incorporated into the diet as a source of fiber and polyphenols, which could prevent oxidative stress in liver and colon tissue and improve serum antioxidant status.

Author contributions: CBBC, ALF, RLZ and MRMJ participated in the design of this study; all of the authors participated in the interpretation of the results, analysis of the data, and review of the manuscript; CBBC, JKS, TCC, and AGB conducted the experiments; CBBC, CAV, and LRM performed the histological analyses; SBJ, KF, and FA performed the chromatographic analyses; CBBC and MRMJ wrote the manuscript.

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