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Journal of Nutritional Biochemistry 93 (2021) 108616

Journal of Nutritional Biochemistry

Anthocyanin-containing purple potatoes ameliorate DSS-induced colitis in mice

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Received 6 July 2020; received in revised form 19 December 2020; accepted 4 February 2021

Abstract

Ulcerative colitis (UC), a major form of inflammatory bowel disease (IBD), is on the rise worldwide. Approximately three million people suffer from IBD in the United States alone, but the current therapeutic options (e.g., corticosteroids) come with adverse side effects including reduced ability to fight infections. Thus, there is a critical need for developing effective, safe and evidence-based food products with anti-inflammatory activity. This study evaluated the antiinflammatory potential of purple-fleshed potato using a dextran sodium sulfate (DSS) murine model of colitis. Mice were randomly assigned to control (AIN-93G diet), P15 (15% purple-fleshed potato diet) and P25 (25% purple-fleshed potato diet) groups. Colitis was induced by 2% DSS administration in drinking water for six days. The results indicated that purple-fleshed potato supplementation suppressed the DSS-induced reduction in body weight and colon length as well as the increase in spleen and liver weights. P15 and P25 diets suppressed the elevation in the intestinal permeability, colonic MPO activity, mRNA expression and protein levels of pro-inflammatory interleukins IL-6 and IL-17, the relative abundance of specific pathogenic bacteria such as Enterobacteriaceae, *Escherichia coli (E. coli)* and *pks*$^{+}$ *E. coli*, and the increased flagellin levels induced by DSS treatment. P25 alone suppressed the elevated systemic MPO levels in DSS-exposed mice, and elevated the relative abundance of *Akkermansia muciniphila (A. muciniphila)* as well as attenuated colonic mRNA expression level of IL-17 and the protein levels of IL-6 and IL-1β. Therefore, the purple-fleshed potato has the potential to aid in the amelioration of UC symptoms.

Keywords: Anti-inflammation; Purple-fleshed potato; DSS; Colitis.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) represent the two major forms of inflammatory bowel disease (IBD) [1], which are on the rise worldwide [2]. Currently, in the United States alone there are three million IBD sufferers per year [3], but the current therapeutic options (e.g., steroids, immunosuppressants and/or surgery) come with serious negative side effects [4]. Thus, there is an urgent need for developing effective and evidence-based food products with anti-inflammatory activity.

Sufficient research evidence indicated that plant-based diets rich in flavonoids have numerous health benefits including anti-inflammatory, anti-oxidant, anti-obesity, anti-cancer, anti-diabetes, anti-microbial, and immunomodulation properties [5]. Anthocyanins, a sub-class of the flavonoid family, are widely present in fruits, vegetables and grains [6]. Anthocyanin-containing natural foods have been shown to suppress intestinal inflammation and improve intestinal epithelial barrier function [7]. However, most of the previous studies on anti-colitic activity were focused on unacylated anthocyanin-containing berries but not on staple crops like the potato that mainly contain acylated anthocyanins that are resistant to heat-induced degradation [8].

The cultivated potato (*Solanum tuberosum* L.) has been consumed as a food for over 10,000 years [9] and is currently the fourth most common food crop after rice, maize and wheat [10]. The annual per capita consumption of potato (predominantly

Abbreviations: CD, Crohn's disease; DSS, dextran sodium sulfate; HEK, human embryonic kidney; IBD, inflammatory bowel disease; IL, interleukin; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; MUC, mucin; NF-κB, nuclear factor kappa light-chain-enhancer of activated B cells; SCFA, short chain fatty acid; TBHQ, tertiary butylhydroquinone; TJ, tight junction; TLR5, toll-like receptor 5; TNF-α, tumor necrosis factor alpha; UC, ulcerative colitis; ZO-1, zonula occludens-1.

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https://doi.org/10.1016/j.jnutbio.2021.108616
0955-2863/

Table 1
Compositions of experimental diets (g/kg).

Ingredient	AIN-93G	P25	P15
Potato	0	250	150
Corn starch	427.5	208.5	296.0
Casein	200	175.3	185.2
Maltodextrin	132	132	132
Sucrose	100	100	100
Soybean oil	40	40	40
Cellulose	50	43.7	46.2
Mineral Mix[b]	35	35	35
Vitamin Mix[c]	10	10	10
Choline Bitartrate	2.5	2.5	2.5
L-cysteine	3	3	3
TBHQ	0.008	0.008	0.008

[a]potato diets were prepared by supplementing corn starch in AIN-93G diet.
[b] AIN-93G-MX.
[c] AIN-93-VX.

white-fleshed varieties) is over 110 lbs. in the United States [11]. Though the macronutrient composition is similar across many potato cultivars, the phytonutrient content and composition vary significantly depending on the genotype [12]. Studies have shown that purple potatoes have higher anthocyanin concentrations and greater biological activity than less pigmented cultivars [13-16]. Consumption of purple potatoes (eight weeks) can ameliorate high-fat diet-induced obesity in Zucker rats [16]. Besides, the purple-fleshed potato diet suppressed colon tumorigenesis via the elimination of colon cancer stem cells [17]. Purple potato extract (10 μg/mL) significantly strengthened gut barrier function *in vitro* [18]. Emerging evidence suggested that purple-fleshed potatoes can ameliorate colonic inflammation induced by the high-fat diet [19]. However, the role of potato anthocyanins in a whole food matrix against gut barrier function *in vivo* still needs further investigation.

The DSS-induced colitis is a widely used model for translation between mice data and human disease and has been validated using different therapeutic agents for human IBD [20,21]. DSS-induced colitis is an inflammatory disease characterized by weight loss and rectal bleeding [22], higher intestinal permeability [23], shortened colon length, elevated activities of myeloperoxidase (MPO; a pro-oxidant and pro-inflammatory enzyme) [20], and increased expression levels of inflammatory markers [24-26]. We hypothesized that anthocyanin-containing purple-fleshed potatoes will exert anti-colitic activity by suppressing intestinal inflammation, oxidative stress as well as gut permeability in a well-established acute murine colitis model.

2. Materials and methods

2.1. Preparation of purple-fleshed potato powder, diets and experimental design

Purple-fleshed potatoes (*Solanum tuberosum* L. var. Purple Majesty) were grown in San Luis Valley Experiment Station (Center, Colorado, USA). After harvesting and curing, potatoes were washed and baked at 180°C for 55 min, then cooled down and freeze-dried with the VirTis Ultra 35L Pilot Lyophilizer (Warminster, PA, USA). Diets for experimental groups were supplemented with 15% or 25% baked and freeze-dried purple potato powder replacing primarily corn starch according to proximate analysis (Table 1). A control diet (AIN-93G) was purchased from Envigo (Madison, WI, USA).

2.1.1. Identification and quantitation of polyphenols

Samples (100 mg) were extracted with 1 mL 50% methanol containing 0.1% formic acid (v/v). Then, vortexed for 5 min before placing on ice for 30 min. A total of 10 min vortex was followed by centrifugation (11,100 g for 10 min). T The supernatant was collected before adding another 1 mL extraction solvent for second time extraction. Finally combined the two supernatants and the total monomeric anthocyanin content was quantified with the pH-differential method [27].

Identification and quantification of anthocyanins and phenolic acids were achieved with an Agilent 6460 QQQ coupled to an Agilent 1200 Rapid Res LC system. About 30-50 mg purple potato samples were added to 300 μL methanol with 0.1% formic acid and 240 μL H_2O, sonicated 1 sec for 3-5 times. Then added 300 μL of each of the following solvents: $CHCl_3$, H_2O with 0.1% formic acid, $CHCl_3$, vortexed thoroughly after each addition. The aqueous phase of the sample mixture was collected after centrifugation at 2800 g for 30 min for metabolomics analysis. The whole extraction procedure was operated on ice. A Waters XBridge BEH C18 (3.5 μm, 2.1 mm × 100 mm) column was used for the LC separation. The solvent system was composed of (A) acetonitrile:water:formic acid (5:95:0.2, v/v/v) and (B) acetonitrile:formic acid (100:0.2, v/v). The linear gradient was as follows: 0-7 min 100% A, 0% B; 7-10 min, 5% A, 95% B; 10-14 min, 100% A, 0% B. The injection volume was 5 μL and the flow rate was 0.35 mL/min. The autosampler was set to 4°C and in total darkness during the instrument acquisition. Multiple reaction monitoring (MRM) was used for data acquisition. The major MS parameters were: gas temperature, 325°C; gas flow rate, 8 L/min; nebulizer, 45 psi; sheath gas temperature, 250°C; sheath gas flow, 7 L/min; capillary, 3800 V. Data analysis was performed by Agilent Masshunter Quantitative (v6.0).

2.1.2. Experimental design and sample collection

Age (four weeks old mice) and gender (male) matched C57BL/6 mice (n=40) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were randomly assigned to the following groups (n=8 per group): control group (AIN-93G diet), DSS group, DSS+P15 (15% purple potato in standard diet) group and DSS+P25 (25% purple potato in standard diet) so that the average weight of animals in each treatment group will be similar (Fig. 1). Mice were maintained in the dedicated pathogen-free animal care facility at Millennium Science Complex, Penn State University (University Park, PA, USA). All animal experimental procedures are approved by the Institutional Animal Care and Use Committee in the American Association for Accreditation of Laboratory Animal Care accredited program. After eight weeks mice were placed on DSS (molecular weight; MW 40 kDa: MP Biomedicals, Soho, OH, USA) at a final concentration of 2% w/v in drinking water for six days [28]. Mice were monitored daily for clinical symptoms of colonic inflammation, including daily activity, bloody stool, diarrhea, and weight loss [29]. Fecal samples were collected weekly during the whole experiment period, stored at -80°C until used. After six days of DSS treatment, mice were euthanized under CO_2 asphyxiation. Blood samples were collected by cardiac puncture. Colons, other tissues and organs were harvested and weighed, then processed or stored immediately for later use.

2.2. Histopathology and intestinal permeability analyses

All mice were fasted for at least four hours before gavaging with 150 μL FITC-dextran/mouse at the concentration of 100 mg/mL. After three hours, blood was collected into BD Microtainer tubes (BD, Franklin Lakes, NJ) via a submandibular vein. Serum FITC-dextran levels were quantified with excitation at 490 nm and emission at 520 nm and the intestinal permeability was calculated with the FITC standard curve.

Colon tissues were collected and fixed in 10% neutral formalin at room temperature for at least 24 hours. The colon tissues were then embedded in paraffin wax. After staining with hematoxylin and eosin (H&E), sections were observed microscopically.

2.3. Serum and colonic Myeloperoxidase (MPO) activity assays

A mouse myeloperoxidase kit from R&D Systems (Minneapolis, MN, USA) was used to quantify the serum MPO levels. For the colonic MPO assay, human neutrophil MPO (Sigma, St. Louis, MO) was used as a standard. Colon tissues (50 mg/mL) were homogenized with 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO) on ice for 10 sec. After three cycles of freezing and thawing, the homogenate was sonicated and centrifuged. The colon tissue homogenization and MPO detection procedure followed the protocol attached to the kit. Dianisidine dihydrochloride (1 mg/mL) and 5×10^{-4}% H_2O_2 were added into sample supernatant, then MPO activity was measured at 450 nm on a kinetics program of 2 min with 10 points reading per sample. One unit of MPO activity was defined as the amount that degraded 1.0 μmol of peroxide/min at room temperature [30]. The total protein content of colon tissue was quantified using a Pierce BCA protein assay kit (Fisher Scientific, Ha Nover Park, IL).

2.4. Cecal flagellin analysis

Human embryonic kidney (HEK)-Blue-mTLR5 cells (San Diego, CA, USA) were used to quantify flagellin in cecal digesta samples. Cecal digesta was diluted with PBS to the final concentration of 100 mg/mL and homogenized for 10 sec using a bullet blender, then centrifuged at 8000 g for 2 min. The collected supernatant was serially diluted to the concentration at 2.5 μg/mL. Purified *E. coli* flagellin standard (Enzo Life Sciences, Farmingdale, NY) and the diluted sample supernatant were

Table 2
Real-time PCR primers of colonic inflammatory cytokines.

Gene	Primers
IL-1β	Forward: 5′-GCCCATCCTCTGTGACTCAT-3′
	Reverse: 5′-AGGCCACAGGTATTTTGTCG-3′
IL-6	Forward: 5′-AGTTGCCTTCTTGGGACTGA-3′
	Reverse: 5′-CAGAATTGCCATTGCACAAC-3′
IL-17	Forward: 5′-TCAGACTACCTCAACCGTTCC-3′
	Reverse: 5′-ATGTGGTGGTCCAGCTTTCC-3′
β-Actin	Forward: 5′-AGCCATGTACGTAGCCATCC-3′
	Reverse: 5′-CTCTCAGCTGTGGTGGTGAA-3′

Genomic DNA of cecal digesta was extracted with the QIAamp DNA Stool Mini Kit (Germantown, MD, USA) according to the handbook. The RT-PCR was performed with the initial denaturation step at 95°C for 10 min, followed by 55 cycles of 95°C for 10 sec annealing temperature with extension step for 60 sec at 55°C. The expression level of the 16S rRNA gene was measured as a reference to normalize the relative abundance. The primers used to quantify the relative microbiota level in the cecum are shown in Table 3. The relative quantitation of gene expression was determined using the $\Delta\Delta$Ct method.

Table 3
Primers of target bacteria.

Bacteria	Primers
Enterobacteriaceae	Forward: 5′- GTGCCAGCMGCCGCGGTAA-3′
	Reverse: 5′- GCCTCAAGGGCACAACCTCCAAG-3′
Akkermansia muciniphila	Forward: 5′- CAGCACGTGAAGGTGGGGAC-3′
	Reverse: 5′- CCTTGCGGTTGGCTTCAGAT-3′
Escherichia coli	Forward: 5′- TACAGGTGACTGCGGGCTTATC-3′
	Reverse: 5′- CTTACCGGGCAATACACTCACTA-3′
pks^{+} *Escherichia coli*	Forward: 5′- GCAACATACTCGCCCAGCT-3′
	Reverse: 5′- TCTCAAGGCGTTGTTGTTTG-3′
16s rRNA/Universal	Forward: 5′-GTGSTGCAYGGYTGTCGTCA-3′
	Reverse: 5′-ACGTCRTCCMCACCTTCCTC-3′

loaded into a 96 well plate, respectively. HEK-Blue detection cell suspension (180 μL) prepared according to the handling instructions was added to each well. After overnight incubation at 37°C, the alkaline phosphatase activity was measured at 635 nm to quantify the flagellin levels.

2.5. Colonic inflammatory cytokines mRNA expression and gut bacteria analyses

Total RNA from frozen colon tissues was isolated with PureLink Mini Kit (Invitrogen, Carlsbad, CA, USA). RNA sample quantification was performed with the BioTek Cytation3 Cell Imaging Reader (Thermo Fisher Scientific Inc., Winooski, VT, USA). RNA Reverse-transcription was achieved with the qScriptcDNA SuperMix (Quantabio, Beverly, MA) synthesis kit according to the manufacturer's instructions. The Real-time PCR amplification and detection were performed on the Roche LightCycler 96 instrument with PerfeCT®SybrGreen FastMix (Quantabio, Beverly, MA). The reaction with an initial denaturation step at 95°C for 10 min, followed by 45 cycles of 95°C for 10 sec annealing temperature with an extension step for 60 sec at 55°C. The mRNA expression was normalized using β-actin. RT-PCR was performed by using respective primers that are indicated in Table 2.

2.6. Colonic inflammatory cytokines analysis

A custom mouse cytokine multiplex ELISA array (RayBiotech, Norcross, GA) was used to determine the protein content of inflammatory cytokines. Colon tissues were lysed in the RayBiotech lysis buffer (RayBiotech, Norcross, GA) with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL) using a bullet blender for 10 min. Centrifuged the lysate at 10000 g for 5 min and collected the supernatant for the following assay. After completely air-drying the slide, slide blocking was performed by adding 100 μL sample diluent to each well and incubate at room temperature for 30 min and decanted buffer from each well. Added 100 μL standards or samples in the well and incubated at room temperature for 2 hours. After washing, the detection antibody cocktail (80 μL) and Cy3 equivalent dye-conjugated streptavidin (80 μL) were applied and incubated for 2 hours, respectively. The slide was completely washed and dried. Slide scanning and data collection were performed by the RayBiotech service group. Data were analyzed using the CUSTOM Q-Analyzer (RayBiotech, Norcross, GA).

2.7. Statistical analysis

The data values were presented as mean±SEM. Statistical analysis was performed using SPSS 26.0 software and Graphpad 8, utilizing One-way ANOVA, Tukey's and Fisher's LSD multiple comparison tests and considered significant if $P<0.05$.

3. Results

3.1. Polyphenol composition of purple-fleshed potato

The quantification of phenolic acids and anthocyanins of freeze-dried potato powder that might contribute to the anti-colitic effect of potato supplementation was done using LC-QQQ (Table 4). Five phenolic acid standards chlorogenic acid, caffeic acid, *trans*-ferulic acid, *p*-coumaric acid and protocatechuic acid were used to quantify different hydroxybenzoic acids in purple potato. Acylated anthocyanins were quantified using respective anthocyanidin standards. The limit of detection (LOD) values were about 1 μg/mL. Chlorogenic acid was the major phenolic acid and accounted for 92.68% of total phenolic acids. Petunidin (75.57%) and malvidin (21.05%) were the predominant anthocyanidins that existed in glycosylated form acylated with either caffeic or *p*-coumaric or ferulic acids. Petunidin 3-caffeoyl rutinoside 5-glucoside was predominant with 1180.80 μg/g dry weight (DW) followed by Malvidin 3-*p*-coumaric-5-glucoside with 323.25 μg/g DW.

The total anthocyanin content (Fig. 2) of the control diet (AIN-93G), P15 and P25 were measured using the pH differential method [27]. Anthocyanins were not detected in the control diet. Each gram of P15 diet contained 150 mg of purple potato powder. The anthocyanin content of P15 and P25 diets were 0.23±0.003 and 0.38±0.004 mg cyanidin-3-glucoside equivalents/g DW of diet, respectively.

3.2. Purple-fleshed potato supplementation ameliorates DSS-induced colitis symptoms

Food intake and body weight during the eight week dietary treatment period before colitis induction did not differ between dietary groups (Table 5), which indicated that the mice could tolerate 25% inclusion of freeze-dried purple potato into the diet.

Mice exposed to 2% DSS in drinking water for six days showed a significant reduction in body weight and colon length, a prominent increase in liver hypertrophy (performed as an increase in liver weight) and spleen weight (splenomegaly), typical symptoms of colitis (Fig. 3). Along with the severe colonic epithelial damage (Fig. 3E), intestinal permeability, measured by serum levels of FITC-dextran, was elevated in C57BL/6 wild type mice after DSS exposure. Only P25 supplementation maintained the body weight when challenged by DSS (Fig. 3A). Mice consuming both purple-fleshed potato diets ameliorated the DSS-induced reduction in colon length. With eight-week potato diets consumption, the average colon length of mice in P15 (6.95±0.15 cm) and P25 (7.23±0.12 cm) groups were significantly longer compared with the colon length of mice in the DSS group (5.73±0.17 cm), but shorter than the control group (8.05±0.15 cm). The above results indicate that purple-fleshed potato consumption can suppress the colon length reduction which is induced by DSS administration (Fig. 3B). Furthermore, P25 supplementation significantly reduced the liver hypertrophy (Fig. 3C) and splenomegaly (Fig. 3D); P15 did not protect the spleen and liver from the DSS-induced splenomegaly

Table 4
LC-MS/MS data of major anthocyanins and phenolic acids identified in purple-fleshed potato powder.

Compound[a]	MRM mode	RT (min)	MS (m/z)	MS/MS (m/z)	Quantification (μg/g DW)[b]
Caffeic acid	-	5.45	179	135.1, 89	2.61
Chlorogenic acid	-	3.31	353.1	191.2, 85.1	36.98
Trans-ferulic acid	-	6.07	193	178, 134.1	0.19
p-coumaric acid	-	5.90	163.1	119.2, 93.1	0.03
Protocatechuic acid	-	2.59	153.1	109.2, 53.1	0.09
Cy-glc	+	5.30-6.00	595.2	287.1	1.25
Delp 3-*p*-coum-5-glc	+	5.60	919.2	465, 303	45.84
Pet 3G-chloride	+	5.30	479.1	317, 301.9	10.85
Pet 3-rut	+	5.27	625.1	317	15.50
Pet 3-rut-5-glc	+	4.82	787.2	479.1, 317	141.28
Pet 3-*p*-coum	+	5.86	771.2	479.1, 317	69.25
Pet 3-fer-5-glc	+	5.74	963.3	479.1, 317	30.75
Pet 3-caf-5-glc	+	5.68	933.2	317	1180.80
Pet 3-*p*-coum-5-glc	+	5.68	933.2	771.2, 479.1	60.00
Pel 3-glc	+	5.00-6.00	741.2	741, 271	0.04
Peo 3-5-glc	+	5.00-6.00	917.2	785, 755, 463, 301	20.40
Mal 3-rut	+	5.44	639.1	331	2.86
Mal 3-*p*-coum	+	5.94	785.2	331	14.71
Mal 3-rut-5-glc	+	5.01	801.2	493, 331	44.83
Mal 3-fer-5-glc	+	5.83	977.2	491, 331	33.71
Mal 3-*p*-coum-5-glc	+	5.79	947.2	785.2, 493, 331	323.25

[a] Abbreviations used: Pet, petunidin; Pel, pelargonidin; Mal, malvidin; Cy, cyanidin; Peo, peonidin; Delp, delphinidin; rut, rutinoside; *p*-coum, (*p*-coumaroyl)-rutinoside; fer, feruloyl-rutinoside; caf, caffeoyl-rutinoside; glc, glucoside.
[b] Calculated by each standard (chlorogenic acid, caffeic acid, trans-ferulic acid, p-coumaric acid and protocatechuic acid, petunidin chloride, pelargonidin chloride, malvidin chloride, cyanidin chloride, peonidin chloride and delphinidin chloride) as equivalence.

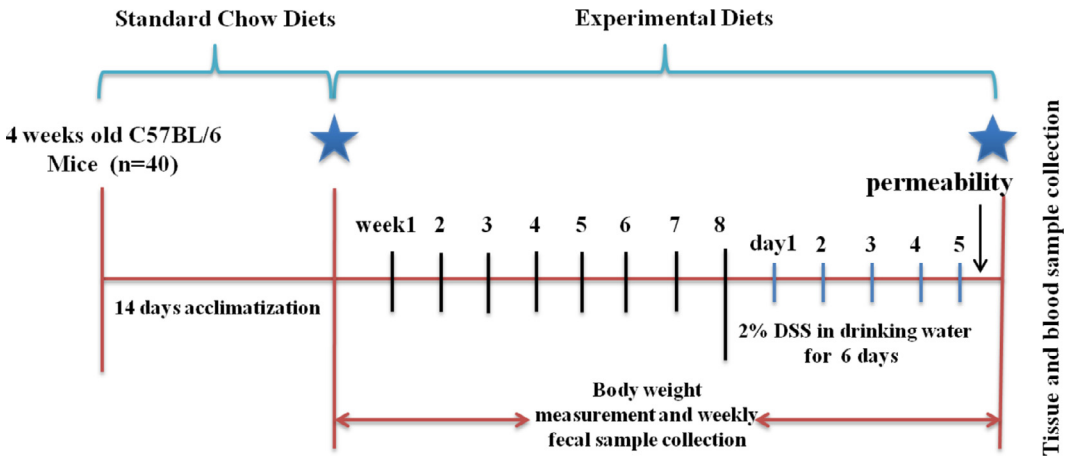

Fig 1. Experimental design. All the mice were maintained on a standard chow diet for first 14 days of acclimatization, then assigned to control, P15 and P25 groups with different diets (AIN-93G, P15 and P25), respectively. After eight weeks, mice were given 2% DSS in drinking water for six days. On day six, intestinal permeability was measured and then all the mice were sacrificed for sample collection.

Table 5
Body weight and weekly intake.

Treatments	Body Weight (g)	Feed Intake (g/mouse/week)
Control	28.00 ± 0.77	22.41 ± 0.46
P15	28.32 ± 1.09	22.84 ± 0.86
P25	26.79 ± 0.60	22.52 ± 0.70

Each value is a mean±SEM; *n*=8 to 13; P15, 15% purple potato in the standard diet; P25, 25% purple potato in the standard diet.

or liver hypertrophy, but the downward trend made the liver weight of animals on 15% purple-fleshed potato diet did not differ from the control group (Fig. 3C). Moreover, mice supplemented with purple-fleshed potatoes were protected from colonic epithelial damage (Fig. 3E), mice with P25 diet alone reversed ($P<.0001$) the DSS-induced elevated FITC level to the control level. Though P15 showed a significant reduction in DSS-induced increase in intestinal permeability, there was still a notable difference between P15 and control groups (Fig. 3F).

3.4. Purple-fleshed potato supplementation suppresses DSS-induced systemic and colonic Myeloperoxidase (MPO) MPO activity

MPO, a well-known pro-oxidative and pro-inflammatory enzyme is mainly released by activated neutrophils. DSS-exposure led to a two-fold elevation in systemic MPO levels (Fig. 4A) and a 2.8-fold elevation of MPO activity in mice distal colon (Fig. 4B). 25%

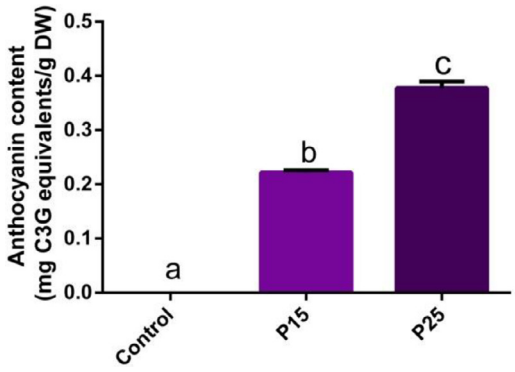

Fig 2. Anthocyanin content in different diets. Different letters on the bars (mean±SEM) indicate differences between the treatments at $P<.05$. P15, 15% purple potato in the diet; P25, 25% purple potato in the diet.

purple-fleshed potato supplementation alone suppressed the elevation of systemic MPO levels (Fig. 4A). Both potato diets significantly ameliorated the elevated MPO activity in colon tissue (Fig. 4B), and this effect did not differ greatly between P15 and P25.

3.5. Purple-fleshed potato supplementation suppresses DSS-induced colonic inflammation

To determine the effect of purple-fleshed potato diet on inflammatory gene expression, qPCR analysis was carried out. In DSS-induced acute colitis, the mucosal epithelial barrier disruption enables luminal microorganisms to enter into the mucosa, resulting in an inflammatory response including NF-κB pathway activation and overexpression of proinflammatory cytokines including IL-6, IL-8, IL-12, IL-17, and IL-1β [31-33]. The mRNA expression of IL-6 and IL-17 was increased in DSS-mice (Fig. 5A, B) compared to the control mice. The DSS-induced increase in IL-6 expression was effectively suppressed by P15 and P25 consumption, and there was no significant difference between these two diets. For the colonic IL-17 expression, a similar restriction was only observed in mice supplemented with the P25 diet, but not the P15 diet. However, IL-1β expression levels did not differ between groups. Additionally, the protein content of colonic inflammatory cytokines was detected with a multiplex ELISA kit. Consistent with the gene expression results, the protein levels of IL-6, IL-17, and IL-1β were significantly elevated by DSS treatment. Only P25 supplementation significantly suppressed the elevated protein level of these cytokines (Fig. 5E, F, G).

Flagellin, a compositional protein of flagellar filament in nearly all flagellated bacteria, can be recognized and ligated with Toll-like receptor 5 (TLR5) which in turn activate the NF-κB inflammatory pathway [34,35]. Human embryonic kidney (HEK)-blue-mTLR5 cells were used to quantify the flagellin levels of cecal digesta [36]. Consistent with the elevated mRNA expression of inflammatory cytokines, DSS-treated mice showed high flagellin levels in cecal digesta, which was significantly reduced by P15 and P25 consumption (Fig. 5D). This result was consistent with the suppressed relative abundance of pathogenic bacteria (Fig. 6 A, B, C).

3.6. Purple-fleshed potato supplementation attenuates DSS-induced disruption of gut microbiota

Gut microbiota plays an essential role in maintaining the homeostasis of the host [37]. As reported, dysbiosis of the gut microbiome plays a crucial role in the pathogenesis of IBD. Increased abundance of pathogenic bacteria and reduced beneficial bacteria

Table 6
Correlations of IL-6, IL-17, IL-1β gene expression and protein expression with other inflammatory markers.

Inflammatory markers	Gene expression			Protein expression		
	IL-6	IL-17	IL-1β	IL-6	IL-17	IL-1β
Colonic MPO	0.565*	0.458	0.058	0.277	0.685**	0.434
Systemic MPO	0.604*	0.631**	0.333	0.321	0.543*	0.445
Flagellin	0.255	0.143	-0.171	0.332	0.703**	0.581*
E. coli	0.453	0.447	0.001	0.28	0.778**	0.286
pks$^+$ *E. coli*	0.473	0.457	0.03	0.295	0.810**	0.317
Enterobacteriaceae	0.546*	0.417	0.098	0.238	0.745**	0.369

Correlations are reported as Pearson correlation coefficient.
* Represents correlation $P<.05$.
** Represents correlation $P<.01$.

relative abundance were found in DSS-induced colitis mice [38] as well as colitis patients [39]. Diet can directly modulate the composition of gut microbiota. In this study, the relative abundance of gut bacteria was measured using qPCR. The relative abundance of *A. muciniphila* was elevated four-fold by 25% purple potato diet consumption (Fig. 6 D) compared with control animals treated with DSS, given that decreased *A. muciniphila* levels have been found in colitis mouse models [40] as well as UC patients [41]. Our observation that staple crops like potatoes can elevate beneficial gut bacteria is significant. Additionally, DSS administration significantly elevated the relative abundance of pathogenic gut bacteria including Enterobacteriaceae (37.0-fold increase), the general *Escherichia coli* (*E. coli*, 2.1-fold increase) and *pks*$^+$ *E. coli* (36.4-fold increase) in the mice cecal digesta. This increase was reversed ($P<0.05$) by both 15% and 25% purple-fleshed potato supplementation (Fig. 6 A, B, C).

Pearson correlations were performed to investigate the potential relationship between colonic inflammation, MPO and gut bacteria changes (Table 6). The gene expression levels of IL-6 significantly correlated with the colonic and systemic MPO levels and the relative abundance of Enterobacteriaceae. IL-17 protein levels significantly correlated with other inflammatory markers and MPO, flagellin and three pathogens. These findings indicate that IL-17 is an important marker involved in colonic inflammation and closely links to the modulation of MPO levels and pathogenic bacteria in the gut.

4. Discussion

Purple-fleshed potato is a rich source of health-benefiting anthocyanins with predominant anthocyanin and phenolic acid in Purple Majesty variety were petunidin and chlorogenic acid, respectively. Dietary chlorogenic acid has been demonstrated to possess anti-inflammatory property by suppressing the activation of three inflammatory signaling pathways including AP-1, NF-kB and MAPK [42]. In addition, potato anthocyanins protect colonic barrier function and suppress colon tumorigenesis [17,18]. As a whole food, purple potato has been shown to have a protective effect against DSS-induced colitis during the disease active stage and recovery period [43]. However, the mechanism of the anti-colitic potential of purple-fleshed potato has not been fully elucidated. The present study assessed the extent to which purple potatoes ameliorate DSS-induced colitis via modulation of gut barrier function, intestinal oxidative stress, inflammation as well as gut microbiota composition.

The DSS-induced colitis mouse model is a classic rodent model for UC research, which presents distinct symptoms of body weight loss, colon shortening, crypt distortion, epithelial injury,

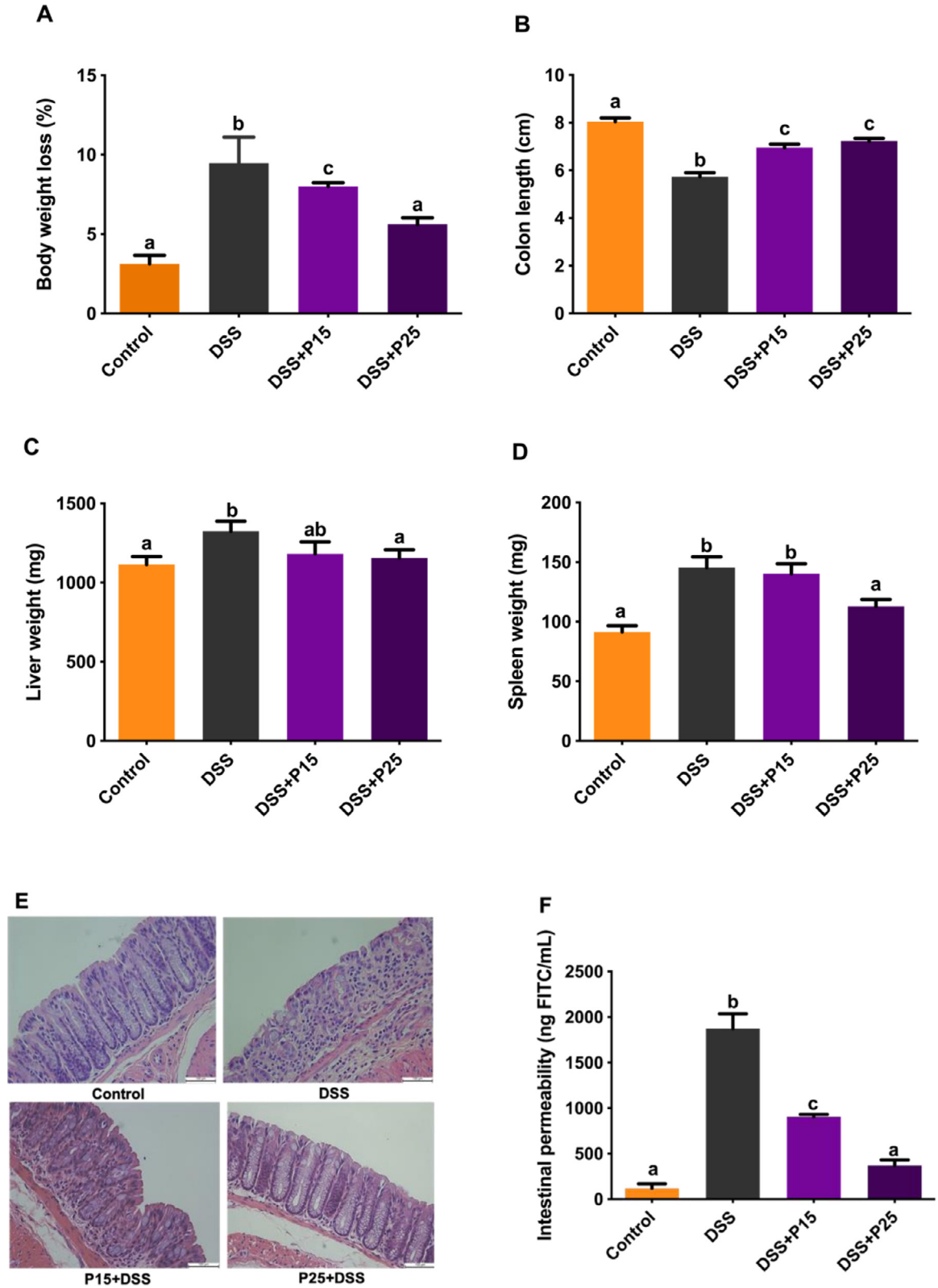

Fig 3. Purple-fleshed potato supplementation ameliorates DSS-induced colitis symptoms, epithelial damage and intestinal permeability. Body weight loss (A) is presented as the percentage of weight reduction induced by six days of DSS administration. Colon length (B), liver weight (C) and spleen weight (D) were measured immediately after excising the tissues. Representative images (E) of H&E stained colon sections (magnification, 100x). Intestinal permeability (F) was measured by the FITC-dextran level of serum. Different letters on the bars (mean±SEM; n=6 to 8) indicate significant differences between the treatments at $P<0.05$. P15, 15% purple potato in the diet; P25, 25% purple potato in the diet.

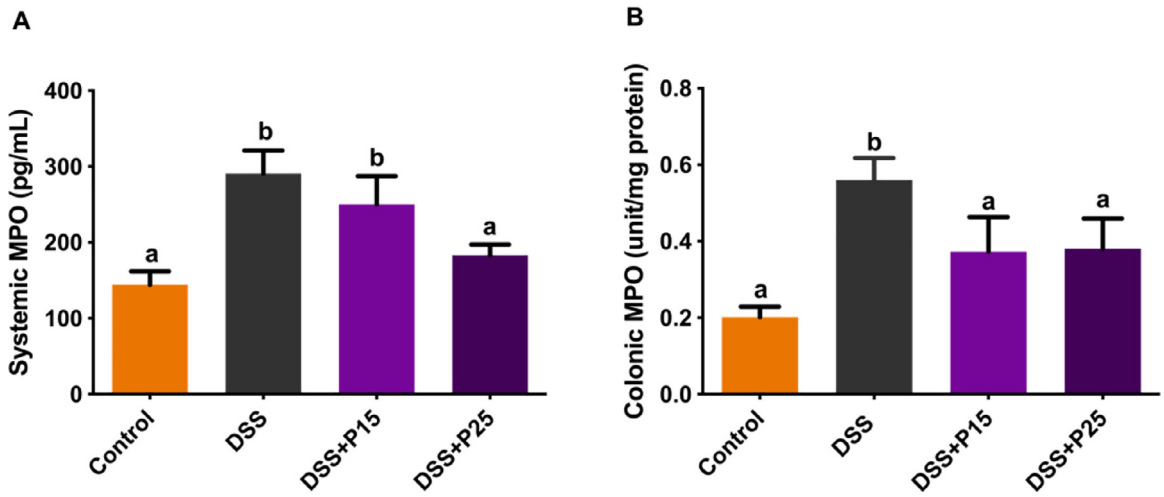

Fig 4. Purple-fleshed potato supplementation reduces DSS-induced systemic myeloperoxidase (MPO) (A), colonic MPO activity (B). Different letters on the bars (mean±SEM; n=6 to 8) indicate significant differences between the treatments at $P<0.07$. P15, 15% purple potato in the diet; P25, 25% purple potato in the diet.

inflammatory cell infiltration, mucosal ulceration, diarrhea and bloody feces [20,44]. The results of the present study demonstrate that many of these symptoms were effectively alleviated by the aisupplementation of purple-fleshed potatoes in a dose-dependent manner. The higher dose (25%) of the purple potato diet reversed ($P<0.05$) the DSS-induced body weight loss, colon length reduction and protected the intestinal mucosal structure. In addition, enlargement of the spleen and liver also characterizes a systemic inflammatory reaction as it occurs in acute DSS-colitis, which has been correlated with disease severity [45-47]. Purple-fleshed potato supplementation (25%) attenuated splenomegaly and liver hypertrophy significantly.

Intestinal permeability is a hallmark of intestinal barrier function [48]. Altered intestinal permeability can lead to gut homeostasis disorder, a failure of the mucosal barrier to regulate the selective passage of intestinal luminal contents [49]. Alteration of gut permeability has been linked to IBD [50]. As reported, mice administered DSS via drinking water for a week showed mucosal damage and increased intestinal permeability [51,52]. Combined with the histological and morphological analysis results, this study indicated that the purple-fleshed potato diet exhibits a protective effect to the intestinal mucosa and remarkably ameliorates elevation in gut permeability. Especially in the higher dose (25%) group, the FITC concentration of serum was the closest to the control group. Furthermore, previous research has shown that deregulating the pro-inflammatory cytokines IL-6 and TNF-α by using their specific monoclonal antibody can attenuate the intestinal permeability in DSS-induced colitis [51]. In our study, the mRNA expression level of IL-6 was decreased by P15 and P25 diets administration, which can further contribute to the reversal of DSS-induced intestinal permeability.

NF-κB signaling pathway has been considered as a prototypical inflammation pathway. Inhibiting the pro-inflammatory cytokine expression is one effective way to block the activation of the NF-κB signaling pathway, which may explain the anti-inflammatory mechanism by the natural bioactive compounds to alleviate colitis [53-55]. A large number of pro-inflammatory cytokines and chemokines are essential mediators involved in intestinal inflammation, mucosal immunity and barrier function [1,56]. Increased expression levels of serum and colonic pro-inflammatory markers such as IL-6, IL-1β, IL-17 and TNF-α are treated as signature features of colitis [57]. Anthocyanin-rich foods such as black rice, purple carrots, grapes, etc. efficiently suppressed the secretion of IL-6, IL-17, and IL-1β which were highly elevated by DSS treatment [26,58-60]. These results are in line with our study, where we found 25% purple potato diet significantly decreased the mRNA expression levels of IL-6 and IL-17 in colon tissue, and remarkably suppressed the protein level of IL-6, IL-17, and IL-1β. This effect was found to be dose-dependent.

MPO is an acceptable marker to evaluate the inflammation in the colonic mucosa since the MPO activity is linearly correlated to neutrophils infiltration [59,61]. Previous studies showed that MPO level was elevated by DSS treatment [62,63]. In this study, both purple potato diets effectively suppressed the MPO activity in colon tissue, indicating 15% purple potato supplementation is sufficient to suppress the excessive infiltration of neutrophils into the intestine. However, only 25% purple potato diet significantly decreased the serum MPO level but not 15% purple potato diet indicating that the systemic effect of purple potato diets was dose-dependent, and the bioavailability of anti-inflammatory components in purple potato such as anthocyanins may play a role in the anti-colitic activity. As potato anthocyanins are poorly bioavailable, their concentration is higher in the colon compared to the serum. This may explain the differential effect of purple potato diets on colon vs. serum MPO levels. Another study also reported that daily supplementation of black rice anthocyanin-containing extract (100 mg/kg of diet) suppressed the colonic MPO activity induced by DSS in mice [64].

Flagellin, a globular protein, is the major composition of flagellar filament [65,66]. Flagella are essential portions of flagellated bacteria, which provide the motility, virulence and adhesive ability to pathogenic bacteria [67]. In recent years, several studies demonstrated that flagellin is an important pro-inflammatory factor and highly involved in the UC-associated inflammatory process [68,69]. Upregulated flagellin level has been applied as a typical detective index for DSS-induced colitis [70]. This study revealed that purple potato administration can effectively reduce the pathogenic bacteria which was elevated by DSS. These results were further confirmed by gut microbiota analysis (Fig. 6). Thus, anthocyanin-containing purple-fleshed potatoes can help suppress pathogenic gut bacteria and maintain healthy intestinal bacteria [71,72].

Gut microbiota plays an essential role in the health of the gastrointestinal tract [73]. Thousands of species of bacteria act as a “metabolizing organ” and are heavily involved in the host’s metabolism [74,75]. The presence of gut bacteria is essential to humans and animals due to its direct impact on the absorption

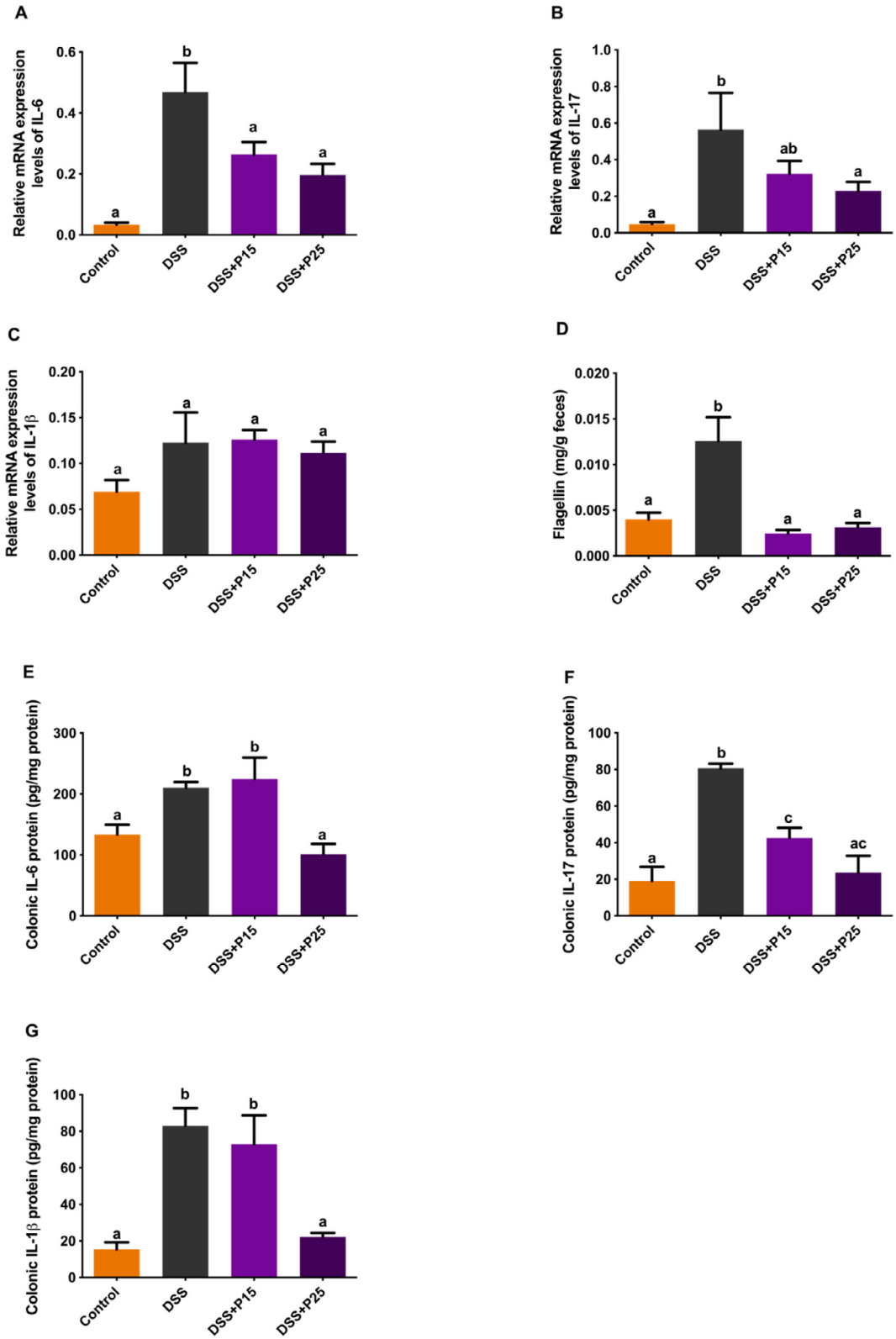

Fig 5. Purple-fleshed potato supplementation suppresses DSS-induced colonic inflammation. (A & B & C) Relative mRNA expression levels of IL-6, IL-17, and IL-1β; (D) Cecal flagellin level; (E & F & G) Protein content of colonic inflammatory cytokines. Different letters on the bars (mean±SEM; n=6 to 8) indicate significant differences between the treatments at P<0.05. P15, 15% purple potato in the diet; P25, 25% purple potato in the diet.

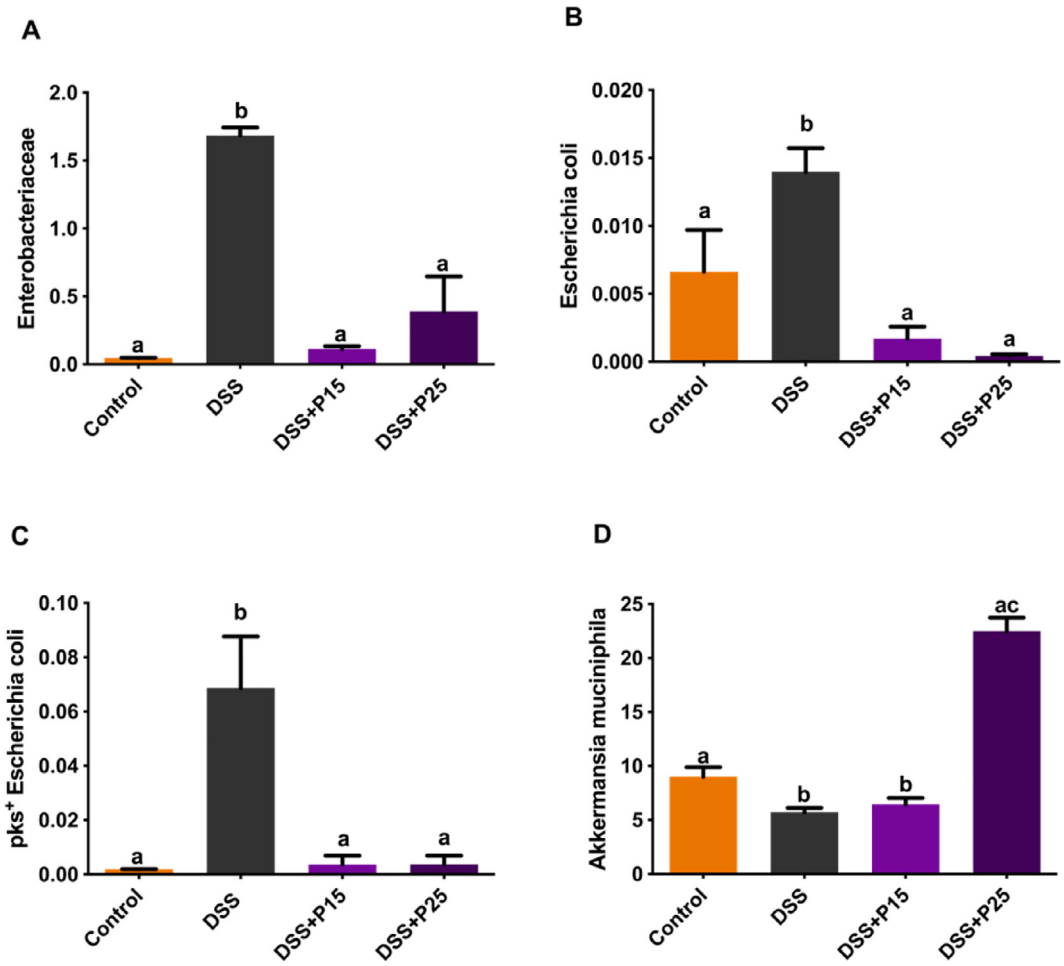

Fig 6. Purple-fleshed potato supplementation attenuates DSS-induced disruption of gut microbiota. (A & B & C & D) Relative mRNA expression levels of Enterobacteriaceae, *Escherichia coli, pks*$^{+}$ *Escherichia coli* and *Akkermansia muciniphila.* Different letters on the bars (mean±SEM; n=6 to 8) indicate significant differences between the treatments at $P<0.05$. P15 and P25, 15% and 25% purple potato in the diet, respectively.

of nutrients and bioactive compounds such as anthocyanins. Over 100 trillion microbes have been detected in the human gut, including both beneficial bacteria and pathogens [76]. Probiotics are beneficial to human health, whereas elevation of pathogenic bacteria is a risk factor in the onset of many chronic inflammation-driven diseases, such as colitis and Crohn's disease [76]. Increased pathogenic and decreased beneficial bacteria relative abundance were observed in UC patients [77-79]. In this study, DSS administration led to the dysbiosis of the gut microbiome, representing the dramatic increase in the abundance of pathogenic bacteria including Enterobacteriaceae, *E. coli* as well as *pks*$^{+}$ *E. coli,* which are known to produce enterotoxins. Pathogenic bacteria produced enterotoxins are known to elevate intestinal permeability. Both 15% and 25% purple potato diets consumption significantly reversed the dysbiosis, and this result was also further confirmed by the deregulated flagellin level in both purple potato groups. P25 also elevated the relative abundance of *A. muciniphila* which is beneficial for gut health. Supporting our findings, malvidin-3-glucoside, a predominant anthocyanin in the human diet, has been demonstrated to elevate the abundance of *Bifidobacterium spp.* and *Lactobacillus spp.* which can improve gut health [71]. Additionally, gallic acid, one of the anthocyanin metabolites, exerted antimicrobial properties against pathogenic bacteria such as *E. coli, Clostridium* and *Staphylococcus aureus* without influencing the growth of beneficial bacteria [80]. Collectively, anthocyanin-containing purple potatoes have the potential to suppress the bacterial dysbiosis induced by DSS treatment and maintain gut health.

Purple-fleshed potatoes as whole food contain anthocyanins, resistant and nonresistant starches, proteins, vitamins, and dietary fibers [81]. The health beneficial properties of purple potatoes are possibly due to the synergistic action of the whole matrix. Anthocyanins, one of the major anti-inflammatory compounds in purple potato, were highlighted in the present study. However, in order to dissect the contribution source, other bioactive components of purple potato also need further investigation along with their synergistic effects. This information will aid in the identification of potato cultivars with greater anti-colitic activity.

In summary, the findings in this study demonstrated that purple-fleshed potato exerts anti-colitic activity via modulation of the gut microbiome as well as oxidative stress and inflammatory markers in a well-established acute murine colitis model. Most of these beneficial effects are dose-dependent. Therefore, purple-fleshed potatoes and other anthocyanin-containing staple crops can be potentially used to counter the UC globally.

CRediT authorship contribution statement

Shiyu Li: Investigation, Data curation, Writing – original draft, Formal analysis, Visualization. **Tianmin Wang:** Investigation, Data curation. **Binning Wu:** Investigation. **Wenyi Fu:** Formal analy-

sis. **Baojun Xu:** Investigation. **Ramachandra Reddy Pamuru:** Investigation. **Mary Kennett:** Investigation. **Jairam K.P. Vanamala:** Writing – review & editing. **Lavanya Reddivari:** Conceptualization, Funding acquisition, Supervision.

Acknowledgments

This study was supported by the USDA National Institute of Food and Agriculture foundation awards 2016-67017-29285 and 2019-67017-29258 to Dr. Lavanya Reddivari. We gratefully acknowledge Vijaya Indukuri for his assistance with animal management and tissue collection. Dr. P. Ramachandra Reddy is grateful to University Grants Commission (UGC), New Delhi, India for financial support (F. No. 5-85/2016 (IC) Dt: 10.06.2016) under Raman Fellowship for Postdoctoral Research in USA.

References

[1] Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. The Lancet 2007;369:1627–40.
[2] Molodecky NA, Soon S, Rabi DM, Ghali WA, Ferris M, Chernoff G. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46–54 e42.
[3] Dahlhamer JM. Prevalence of inflammatory bowel disease among adults aged≥ 18 years—United States. MMWR Morb Mortal Wkly Rep 2015;65:1166–9.
[4] Morrison G, Headon B, Gibson P. Update in inflammatory bowel disease. Aust Fam Physician 2009;38:956.
[5] Pérez-Gregorio RM, García-Falcón MS, Simal-Gándara J, Rodrigues AS, Almeida DP. Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. J Food Compos Anal 2010;23:592–8.
[6] Sui X, Zhang Y, Zhou W. Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: Its quality attributes and in vitro digestibility. Food Chem 2016;196:910–16.
[7] Li S, Wu B, Fu W, Reddivari L. The Anti-inflammatory Effects of Dietary Anthocyanins against Ulcerative Colitis. Int J Mol Sci 2019;20:2588.
[8] B'kowska-Barczak A. Acylated anthocyanins as stable, natural food colorants–a review. Pol J Food Nutr Sci 2005;14:55.
[9] Engel F. Exploration of the Chilca canyon, Peru. Current Anthropology 1970;11:55–8.
[10] Secor GA, Rivera-Varas VV. Emerging diseases of cultivated potato and their impact on Latin America. Revista Latinoamericana de la Papa (Suplemento) 2004;1:1–8.
[11] Osteen C, Gottlieb J, Vasavada U. Agricultural resources and environmental indicators. USDA-ERS Economic Information Bulletin 2012.
[12] Reddivari L, Hale AL, Miller JC Jr. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. J Agric Food Chem 2007;55:8073–9.
[13] Ezekiel R, Singh N, Sharma S, Kaur A. Beneficial phytochemicals in potato—a review. Food Res Int 2013;50:487–96.
[14] Lachman J, Hamouz K. Red and purple coloured potatoes as a significant antioxidant source in human nutrition-a review. Plant Soil Environ 2005;51:477.
[15] Radhakrishnan S, Kim SW, Reddivari L, Vanamala J. Processed purple-fleshed potato prevents and protects against high-fat diet elevated oxidative stress and inflammation markers in vivo in a pig model. FASEB J 2013;2013(862):21.
[16] Ayoub HM, McDonald MR, Sullivan JA, Tsao R, Platt M, Simpson J. The effect of anthocyanin-rich purple vegetable diets on metabolic syndrome in obese zucker rats. J Med Food 2017;20:1240–9.
[17] Charepalli V, Reddivari L, Radhakrishnan S, Vadde R, Agarwal R, Vanamala JK. Anthocyanin-containing purple-fleshed potatoes suppress colon tumorigenesis via elimination of colon cancer stem cells. J Nutr Biochem 2015;26:1641–9.
[18] Sun X, Du M, Navarre DA, Zhu MJ. Purple potato extract promotes intestinal epithelial differentiation and barrier function by activating AMP-activated protein kinase. Mol Nutr Food Res 2018;62:1700536.
[19] Radhakrishnan S, Kim S, Reddivari L, Vanamala J. Purple-fleshed potato, even after processing, prevents and reverses high-fat diet elevated colonic-mesenteric fat—systemic inflammation cascade in pig model (1045.47). The FASEB Journal 2014;28:1045–7.
[20] Jeengar MK, Thummuri D, Magnusson M, Naidu V, Uppugunduri S. Uridine ameliorates dextran sulfate sodium (DSS)-induced colitis in mice. Sci Rep 2017;7:3924.
[21] Melgar S, Karlsson L, Rehnström E, Karlsson A, Utkovic H, Jansson L. Validation of murine dextran sulfate sodium-induced colitis using four therapeutic agents for human inflammatory bowel disease. Int Immunopharmacol 2008;8:836–44.
[22] Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. The Lancet 2007;369:1641–57.
[23] Gäbele E, Dostert K, Hofmann C, Wiest R, Schölmerich J, Hellerbrand C. DSS induced colitis increases portal LPS levels and enhances hepatic inflammation and fibrogenesis in experimental NASH. J Hepatol 2011;55:1391–9.
[24] Fitzpatrick LR. Inhibition of IL-17 as a pharmacological approach for IBD. Int Rev Immunol 2013;32:544–55.
[25] Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 2011;140:1756–67.
[26] Zhang J, Dou W, Zhang E, Sun A, Ding L, Wei X. Paeoniflorin abrogates DSS-induced colitis via a TLR4-dependent pathway. Am J Physiol Gastrointest Liver Physiol 2013;306:27–36.
[27] Madiwale GP, Reddivari L, Stone M, Holm DG, Vanamala J. Combined effects of storage and processing on the bioactive compounds and pro-apoptotic properties of color-fleshed potatoes in human colon cancer cells. J Agric Food Chem 2012;60:11088–96.
[28] Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol 2014;104:15–25.
[29] Zielińska M, Lewandowska U, Podsędek A, Cygankiewicz AI, Jacenik D, Sałaga M. Orally available extract from Brassica oleracea var. capitata rubra attenuates experimental colitis in mouse models of inflammatory bowel diseases. J Funct Foods 2015;17:587–99.
[30] Castaneda FE, Walia B, Vijay–Kumar M, Patel NR, Roser S, Kolachala VL. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. Gastroenterology 2005;129:1991–2008.
[31] Egger B, Bajaj-Elliott M, MacDonald TT, Inglin R, Eysselein VE, Büchler MW. Characterisation of acute murine dextran sodium sulphate colitis: cytokine profile and dose dependency. Digestion 2000;62:240–8.
[32] Melgar S, Karlsson A, Michaëlsson E. Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Am J Physiol Gastrointest Liver Physiol 2005;288:1328–38.
[33] Ito R, Shin-Ya M, Kishida T, Urano A, Takada R, Sakagami J. Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. Clin Exp Immunol 2006;146:330–8.
[34] Yu Y, Sitaraman S, Gewirtz AT. Intestinal epithelial cell regulation of mucosal inflammation. Immunol Res 2004;29:55–67.
[35] Vijay-Kumar M, Gewirtz A. Flagellin: key target of mucosal innate immunity. Mucosal Immunol 2009;2:197.
[36] Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 2015;519:92.
[37] Moreno II. Benefits of the beer polyphenols on the gut microbiota. Nutr Hosp 2017;34:41–4.
[38] Zhang Q, Wu Y, Wang J, Wu G, Long W, Xue Z. Accelerated dysbiosis of gut microbiota during aggravation of DSS-induced colitis by a butyrate-producing bacterium. Sci Rep 2016;6:27572.
[39] Shen Z-H, Zhu C-X, Quan Y-S, Yang Z-Y, Wu S, Luo W-W. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J Gastroenterol 2018;24:5.
[40] Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. World J Gastroenterol 2017;23:4548.
[41] Shang Q, Sun W, Shan X, Jiang H, Cai C, Hao J. Carrageenan-induced colitis is associated with decreased population of anti-inflammatory bacterium, Akkermansia muciniphila, in the gut microbiota of C57BL/6J mice. Toxicol Lett 2017;279:87–95.
[42] Shan J, Fu J, Zhao Z, Kong X, Huang H, Luo L. Chlorogenic acid inhibits lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 cells through suppressing NF-κB and JNK/AP-1 activation. Int Immunopharmacol 2009;9:1042–8.
[43] Bibi S. Beneficial effects of dietary red raspberry and purple potato on colitis. Experimental Mice Models 2017.
[44] Wirtz S, Neurath MF. Mouse models of inflammatory bowel disease. Adv Drug Delivery Rev 2007;59:1073–83.
[45] Axelsson LG, Landström E, Bylund-Fellenius AC. Experimental colitis induced by dextran sulphate sodium in mice: beneficial effects of sulphasalazine and olsalazine. Aliment Pharmacol Ther 1998;12:925–34.
[46] Morteau O, Morham SG, Sellon R, Dieleman LA, Langenbach R, Smithies O. Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. J Clin Invest 2000;105:469–78.
[47] Balato N, Napolitano M, Ayala F, Patruno C, Megna M, Tarantino G. Nonalcoholic fatty liver disease, spleen and psoriasis: new aspects of low-grade chronic inflammation. World J Gastroenterol 2015;21:6892.
[48] Galipeau H, Verdu E. The complex task of measuring intestinal permeability in basic and clinical science. Neurogastroenterol Motil 2016;28:957–65.
[49] Julio-Pieper M., Bravo J., Intestinal barrier and behavior. Int Rev Neurobiol: Elsevier; 2016. p. 127-41.
[50] Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol 2013;11:1075–83.
[51] Xiao Y-T, Yan W-H, Cao Y, Yan J-K, Cai W. Neutralization of IL-6 and TNF-α ameliorates intestinal permeability in DSS-induced colitis. Cytokine 2016;83:189–92.
[52] Llewellyn SR, Britton GJ, Contijoch EJ, Vennaro OH, Mortha A, Colombel J-F. Interactions between diet and the intestinal microbiota alter intestinal permeability and colitis severity in mice. Gastroenterology 2018;154:1037–46 e2.
[53] Sun A, Ren G, Deng C, Zhang J, Luo X, Wu X. C-glycosyl flavonoid orientin improves chemically induced inflammatory bowel disease in mice. J Funct Foods 2016;21:418–30.

[54] Lawrence T. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harb Perspect Biol 2009:a001651 a001651. doi:10.1101/cshperspect.a001651.
[55] Medicherla K, Sahu BD, Kuncha M, Kumar JM, Sudhakar G, Sistla R. Oral administration of geraniol ameliorates acute experimental murine colitis by inhibiting pro-inflammatory cytokines and NF-κB signaling. Food & function 2015;6:2984–95.
[56] Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003;3:521–33.
[57] Műzes G, Molnár B, Tulassay Z, Sipos F. Changes of the cytokine profile in inflammatory bowel diseases. World J Gastroenterol 2012;18:5848.
[58] Ito R, Kita M, Shin-Ya M, Kishida T, Urano A, Takada R. Involvement of IL-17A in the pathogenesis of DSS-induced colitis in mice. Biochem Biophys Res Commun 2008;377:12–16.
[59] Boussenna A, Cholet J, Goncalves-Mendes N, Joubert-Zakeyh J, Fraisse D, Vasson MP. Polyphenol-rich grape pomace extracts protect against dextran sulfate sodium-induced colitis in rats. J Sci Food Agric 2016;96:1260–8.
[60] Kim Y-J, Ju J, Song J-L, S-g Yang, Park K-Y. Anti-colitic effect of purple carrot on dextran sulfate sodium (dss)-induced colitis in C57BL/6J mice. Prev Nutr Food Sci 2018;23:77.
[61] Itzkowitz SH. Molecular biology of dysplasia and cancer in inflammatory bowel disease. Gastroenterology Clini 2006;35:553–71.
[62] Solomon L, Mansor S, Mallon P, Donnelly E, Hoper M, Loughrey M. The dextran sulphate sodium (DSS) model of colitis: an overview. Comparative clini pathol 2010;19:235–9.
[63] Chen L, Zhou Z, Yang Y, Chen N, Xiang H. Therapeutic effect of imiquimod on dextran sulfate sodium-induced ulcerative colitis in mice. PLoS ONE 2017;12 e0186138.
[64] Zhao L, Zhang Y, Liu G, Hao S, Wang C, Wang Y. Black rice anthocyanin-rich extract and rosmarinic acid, alone and in combination, protect against DSS-induced colitis in mice. Food & function 2018;9:2796–808.
[65] Macnab RM. How bacteria assemble flagella. Annual Reviews in Microbiology 2003;57:77–100.
[66] Yonekura K, Maki-Yonekura S, Namba K. Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. Nature 2003;424:643.
[67] Yang X, Yang F, Wang W, Lin G, Hu Z, Han Z. Structural basis for specific flagellin recognition by the NLR protein NAIP5. Cell Res 2018;28:35.
[68] Duan Q, Zhou M, Zhu X, Bao W, Wu S, Ruan X. The flagella of F18ab Escherichia coli is a virulence factor that contributes to infection in a IPEC-J2 cell model in vitro. Vet Microbiol 2012;160:132–40.
[69] Magalhaes D, Soares-da-Silva P, Magro F. The effect of PRR ligands on the membrane potential of intestinal epithelial cells. Pharm Rep 2017;69:978–84.
[70] Luo S, Deng X, Liu Q, Pan Z, Zhao Z, Zhou L. Emodin ameliorates ulcerative colitis by the flagellin-TLR5 dependent pathway in mice. Int Immunopharmacol 2018;59:269–75.
[71] Hidalgo M, Oruna-Concha MJ, Kolida S, Walton GE, Kallithraka S, Spencer JP. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. J Agric Food Chem 2012;60:3882–90.
[72] Ramos HC, Rumbo M, Sirard J-C. Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. Trends Microbiol 2004;12:509–17.
[73] Faria A, Fernandes I, Norberto S, Mateus N, Calhau C. Interplay between anthocyanins and gut microbiota. J Agric Food Chem 2014;62:6898–902.
[74] Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006;124:837–48.
[75] Pan P, Lam V, Salzman N, Huang Y-W, Yu J, Zhang J. Black raspberries and their anthocyanin and fiber fractions alter the composition and diversity of gut microbiota in F-344 rats. Nutr Cancer 2017;69:943–51.
[76] Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci 2007;104:13780–5.
[77] Matsuoka K, Uemura Y, Kanai T, Kunisaki R, Suzuki Y, Yokoyama K. Efficacy of bifidobacterium breve fermented milk in maintaining remission of ulcerative colitis. Dig Dis Sci 2018;63:1910–19.
[78] Zocco M, Dal Verme LZ, Cremonini F, Piscaglia A, Nista E, Candelli M. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 2006;23:1567–74.
[79] Sokol H, Lepage P, Seksik P, Dore J, Marteau P. Temperature gradient gel electrophoresis of fecal 16S rRNA reveals active Escherichia coli in the microbiota of patients with ulcerative colitis. J Clin Microbiol 2006;44:3172–7.
[80] Lee HC, Jenner AM, Low CS, Lee YK. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res Microbiol 2006;157:876–84.
[81] Camire ME, Kubow S, Donnelly DJ. Potatoes and human health. Crit Rev Food Sci Nutr 2009;49:823–40.