Cyanidin 3-glucoside from Queen Garnet plums and purple carrots attenuates DSS-induced inflammatory bowel disease in rats

Naga K.R. Ghattamaneni, Sunil K. Panchal, Lindsay Brown

Abstract

We have investigated whether dextran sodium sulphate (DSS)-induced inflammatory bowel disease (IBD) in rats can be ameliorated by intervention with cyanidin 3-glucoside (C3G). Rats were given either normal water (C) or 0.5% DSS (D) in drinking water for 12 weeks. C3G 8 mg/kg/day as Queen Garnet plum (Q) juice, purple carrot (P) juice or pure compound was added in food for final 6 weeks to C rats to give CQ, CP and CC groups, and to D rats to give DQ, DP and DC groups. No symptoms of IBD were observed in C, CQ, CP or CC rats. D rats had bloody diarrhoea, ileal and colonic mucosal atrophy, and inflammation. Compared to D rats, DQ, DP and DC rats showed improved stool consistency (P < 0.0001) and bleeding (P < 0.0001), reduced ileum (P < 0.0001) and colon inflammation (P < 0.0001), with no changes in gut microbiota. Thus, supplementation with C3G-containing foods may ameliorate the symptoms of IBD.

Keywords:
Inflammatory bowel disease
Dextran sodium sulphate
Anthocyanins
Cyanidin 3-glucoside
Queen Garnet plum
Purple carrot

1. Introduction

Inflammatory Bowel Disease (IBD) in humans is characterised by chronic and relapsing inflammation of all or part of the digestive tract (Abraham & Cho, 2009). Functional foods and nutraceuticals are now of increasing interest in the treatment of a range of inflammatory diseases, including metabolic syndrome and IBD, as these interventions may be effective, produce fewer adverse effects than conventional drug therapy and reduce costs of treatment (Brown, Poudyal, & Panchal, 2015; Ghattamaneni, Panchal, & Brown, 2018). Marked anti-inflammatory properties have been reported for naturally-occurring anthocyanins, including cyanidin 3-glucoside (C3G) (Farzaei et al., 2018). Thus, anthocyanin-containing foods may improve IBD by inducing anti-inflammatory actions in the intestinal wall.

Bilberry anthocyanins (Piberger et al., 2011) and polyphenol-enriched tomatoes with anthocyanins such as C3G (Scarano et al., 2017) showed anti-inflammatory responses in DSS-induced acute and chronic colitis in mice. Other plant-derived compounds such as curcumin, apple polyphenols, resveratrol and blueberry anthocyanins improved IBD patients or rodents by mechanisms including reduction of anti-oxidative stress, gut bacteria improvement, anti-inflammatory responses by suppressing TNF, immunomodulatory function through p53-dependent apoptosis, and modulation of nitricergic system and cellular signalling pathways such as mitogen-activated protein kinase (MAPK), nuclear factor (NF)-κB and nuclear factor-erythroid 2-related factor-2 (Nrf2) (Farzaei et al., 2016; Ghattamaneni et al., 2018). These possible mechanisms suggest that further anthocyanin sources, especially C3G-containing purple fruit and vegetables, should be investigated as functional foods for IBD. In this study, we used an established chronic IBD rat model (Ghattamaneni, Panchal, & Brown, 2019) to investigate whether chronic IBD in rats is attenuated by C3G-containing Queen Garnet plums and purple carrots as well as C3G. The Queen Garnet plum (Prunus salicina Lindl.) is a Japanese plum variety developed by standard breeding techniques by Department of Agriculture and Fisheries, Queensland Government, Australia to contain C3G up to 272 mg/100 g of fresh fruit, around seven times higher than other anthocyanin-containing plums (Fanning, Topp, Russell, Stanley, & Netzel, 2014). The juice of purple carrots (Daucus carota L. ssp sativus var. atrorubens Alef) contains polyphenols including anthocyanins at 78.06% mainly as cyanidin glycosides, together with phenolic acids at 17.89% and other flavonoids at 4.06% (Smeriglio et al., 2018). Our previous studies reported that C3G from Queen Garnet plum (Bhaswant et al., 2015) or

Abbreviations: C, control rats; C3G, cyanidin 3-glucoside; CC, control rats treated with C3G; CP, control rats treated with purple carrot juice; CQ, control rats treated with Queen Garnet plum juice; D, rats given 0.5% DSS; DC, 0.5% DSS rats treated with C3G; DP, 0.5% DSS rats treated with purple carrots juice; DQ, 0.5% DSS rats treated with Queen Garnet plum juice; DSS, dextran sodium sulphate; IBD, inflammatory bowel disease; MAPK, mitogen-activated protein kinase; NF, nuclear factor; Nrf2, nuclear factor-erythroid 2-related factor-2; P, purple carrots; Q, Queen Garnet plum

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purple carrot (Poudyal, Panchal, & Brown, 2010) reversed the inflammation of the heart and liver as well as other signs of metabolic syndrome in diet-induced obese and hypertensive rats. These responses provided us with a logical reason to investigate the potential therapeutic responses of C3G from these foods in a rat model of IBD as an important chronic inflammatory disorder.

We hypothesised that Queen Garnet plums and purple carrots containing C3G will attenuate intestinal inflammation in a rat model of DSS-induced chronic IBD. We used a reversal protocol to investigate changes in disease severity including stool characteristics, gastrointestinal structure and function of the intestine such as gastric emptying, intestinal transit and permeability, and gut faecal microbiota including C3G will attenuate intestinal inflammation in a rat model of DSS-induced chronic IBD. We used a reversal protocol to investigate changes in disease severity including stool characteristics, gastrointestinal structure and function of the intestine such as gastric emptying, intestinal transit and permeability, and gut faecal microbiota.

The aim of the study was to determine whether C3G from Queen Garnet plums and purple carrots attenuates IBD-induced changes in gastrointestinal structure and function.

2. Materials and methods

2.1. Diets and measurements

All experiments were approved by the University of Southern Queensland Animal Ethics Committee (approval number 14REAA005 valid until July 2017) under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8–9 weeks old, weighing 335 ± 1 g, n = 64) were purchased from the Animal Resource Centre, Murdoch, WA, Australia. All rats were provided with free access to food and water and were individually housed in temperature-controlled (21 ± 2 °C), 12-hour light-dark conditions at the University of Southern Queensland animal house.

Rats were fed with powdered rat food (Specialty Feeds, Glen Forest, WA, Australia). Rats were randomly divided into eight experimental groups (n = 8/group). The first group served as control (C) with normal drinking water and second group (D) was administered 0.5% dextran sodium sulphate (DSS, molecular weight: 40,000–50,000 Da, Thermo Fisher Scientific, Brisbane, QLD, Australia) in drinking water for 12 weeks of the protocol to induce signs of IBD (Ghattamaneni et al., 2019). The remaining six groups of rats were fed with interventions in their diet for the last 6 weeks to provide approximately 8 mg/kg body weight/day of C3G, because this dose allows comparison with our earlier studies in metabolic syndrome (Bhaswant et al., 2015; Bhaswant, Shafie, Mathai, Mouatt, & Brown, 2017). This dose of C3G was provided to three groups of rats as controls with Queen Garnet plum juice (CQ; 65 mL/kg food with C3G 1.9 mg/mL of juice), purple carrots (CP; 11.3 mL/kg food with C3G 10.9 mg/mL of juice) and C3G (CC; 123 mg/kg of food). Similarly, three groups of rats were treated as D rats and given the same interventions and so are defined as DQ, DP and DC. Anthocyanins in the purple carrot juice were analysed by pH differential method (Poudyal et al., 2010). Anthocyanins in the Queen Garnet plum juice were analysed by LC-PDA-MS method (Netzel et al., 2012).

Rats were monitored every morning for body weight, and food and water intakes. Energy intake was calculated from the following values: powdered rat food, 13.8 kJ/g; Queen Garnet plum juice, 3.74 kJ/mL; purple carrots juice, 0.44 kJ/mL. The energy density was calculated based on the energy intake for powdered food diet, 13.8 kJ/g; Queen Garnet plum juice diet, 13.146 kJ/mL; purple carrot juice diet, 13.649 kJ/mL. Daily anthocyanin intake was calculated from the daily food intake. Queen Garnet plum juice contained predominantly C3G, with small amounts of cyanidin 3-rutinoside and queretin glycosides; the purple carrot juice contained predominantly monomeric anthocyanins quantified as C3G, phenolic acids predominantly chlorogenic and caffeic acids, and traces of carotenoids such as β-carotene, lutein and zeaxanthin (Bhaswant et al., 2015; Poudyal et al., 2010). These studies suggested that the anti-inflammatory responses were produced by C3G.

2.2. Gastrointestinal structure and function

Oral glucose tolerance test was performed at the end of protocol on rats that were food-deprived for 12 h, using Medisense Precision Q.I.D glucose meter (Abbott Laboratories, Bedford, USA) (Panchal et al., 2011).

Stool characteristics were examined to assess the disease progression every day for 12 weeks. The stool consistency was scored as 0-formed, 1-mild-soft, 2-very soft, and 3-watery soft (diarrhoea). The stool bleeding was scored as 0-normal colour, 1-brown colour, 2-reddish colour, and 3-bloody red (Vasina et al., 2010). Histological score was calculated as the sum of degree of inflammation (0-absent to 3-severe), extent of inflammation (0-absent to 3-transmural), crypt and epithelial damage (0-absent to 4-severe), and tissue regeneration (0-regenerated or normal to 4-no repair) (Vasina et al., 2010).

Intestinal permeability was measured at 12 weeks on rats by withdrawing food for 4 h, and then rats were gavaged with 2 mL of a solution containing 0.04 g/mL mannitol, 0.5 g/mL sucrose, 0.06 g/mL lactulose, and 0.03 g/mL sucralose (Sigma-Aldrich Australia, Sydney, NSW, Australia) (Meddings & Gibbons, 1998). For the next 3 h, rats were placed in a metabolic chamber and deprived of food and water. The urine was collected in 100 µL solution of 10% thymol in isopropanol at regular intervals for 24 h after oral gavage. Measurements of sugars in rat urine samples were performed at the Central Analytical Research Facility (CARF) of the Queensland University of Technology, Gardens Point, Brisbane. The samples were derivatised and sugar analysis was performed using GCMS Shimadzu TQ8040 (Shimadzu, Rydalmere, Australia) (Ghattamaneni et al., 2019). Cumulative percent recovery of each sugar was measured for 24 h as a measure of gut permeability (Farhadi et al., 2008).

Gastrointestinal transit was measured by the charcoal method for the Queen Garnet plum study (Ghattamaneni et al., 2019). Gastric emptying and gastrointestinal transit experiments were performed by the phenol red method in purple carrot and C3G groups. Phenol red method was not used for Queen Garnet plum study as this method was available only after the termination of rats in this treatment. After 12 h of food deprivation, rats were gavaged with 3 mL of 0.05% phenol red solution (Sigma-Aldrich, Sydney, Australia). After 20 min, rats were euthanased and the stomach was ligated at the pyloric and cardiac ends and removed. The total intestinal length was then recorded and it was cut along its length longitudinally. A few drops of 0.5 M NaOH were added to the lumen of the intestine from duodenum to ileum and the end point of the change to the pink colour was recorded. The length of intestine with pink colour divided by the total small intestinal length was expressed as percentage of gastrointestinal transit. To measure gastric emptying, the stomach was placed in 100 mL 0.1 M NaOH and was homogenised in a tissue homogeniser for 2 min at moderate speed. The homogenised mixture was kept aside for 1 h. To 5 mL of the supernatant, 1 mL of 33% trichloroacetic acid was added and centrifuged at 2500g for 20 min. The supernatant was mixed with 0.5 M NaOH and the absorbance was recorded at 565 nm to determine the intensity of the colour that correlated to the intensity of the compound that remained in the stomach (Fu et al., 2014). Gastric emptying (%) was calculated as (1 – absorbance of test sample/absorbance of baseline control) × 100.

2.3. Measurements after euthanasia

Rats were euthanased using intraperitoneal injection of pentobarbital sodium (Lethabarb, 100 mg/kg, Virbac, Milperra, Australia). Heparin (~ 200 IU; Sigma-Aldrich, Sydney, Australia) was administered into the right femoral vein. The rats were dissected and the left ventricle along with septum and the right ventricle, kidney, spleen, liver and abdominal fat pads were collected, blotted dry and weighed from each rat. The organ weights were normalised to the tibial length at the time of organ isolation and expressed as mg/mm (Panchal et al., 2011).
Gut contractility and histology were determined on isolated ileum and colon. The lengths of the small intestine and large intestine were measured in all rats by gently stretching the intestine on a flat platform soon after euthanasia and using a measuring tape. Distal ileum and distal colon (∼1.5 cm) were collected and washed in Tyrode’s buffer for organ bath studies and histological examination. The tissue was placed in an organ bath chamber filled with Tyrode’s buffer and concentration-response curves were recorded to acetylcholine (Sigma-Aldrich, Sydney, Australia) using pressure transducers and Chart software on a MacLab system (ADI Instruments, Bella Vista, NSW, Australia) (Panchal et al., 2011). The isolated ileum and colon sections for histology were fixed in 10% neutral buffered formalin for three days. Thereafter, the tissues were processed, blockaded in paraffin wax and 5 μm sections were cut and placed on slides. The sections were stained with haematoxylin and eosin stains and were observed by using 20× objective lens EVOS FL Colour Imaging System (v 1.4 (Rev 26059); Advanced Microscopy Group, Bothell, WA, USA) to determine the infiltration of inflammatory cells and damage to the intestinal tissue (Panchal et al., 2011).

Gut microbiota diversity profiling was performed on rat faecal samples of C, CC, D and DC rats collected at euthanasia and stored at −80 °C, by Australian Genome Research Facility, Brisbane, Australia, where the gDNA extraction of faecal samples and sequencing were performed (Wanyonyi, du Preez, Brown, Paul, & Panchal, 2017). The PCR amplification of the gDNA was performed with primers specific for V3–V4 region of 16S rRNA gene. The primers used were EF1F′ (5′-CCTAYGGGRBGCASCAG-3′) and R806 (5′-GGACTACNNGGGTATC TAAT-3′) (Wanyonyi et al., 2017).

2.4. Statistics

All data are expressed as mean ± SEM. Results from all groups were analysed by two-way analysis of variance. When interaction and/or the main effects were significant, means were compared using Newman-Keuls multiple comparison post hoc test. *P* < 0.05 was considered as significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (San Diego, CA, USA).

3. Results

3.1. Control and DSS rats

C rats showed normal stool consistency and no blood in stools whereas D rats had loose stools with bleeding indicated by the increased scores of stool consistency and scores for blood in stools (Fig. 1A and B). Compared to C rats, the ileum and colon of D rats showed inflammation with inflammatory cells, epithelial disruption, crypt distortion, crypt atrophy and mucosal atrophy, therefore showing a higher histological score (C: Fig. 2A and I; D: Fig. 2B and J; Fig. 2Q for comparison). There were no significant differences between C and D rats for results in Table 1 except for decreased colon length in D rats.

3.2. QG plum juice, PC juice and C3G

CQ, CP and CC rats showed normal stool consistency and no blood in stools while DQ, DP and DC rats showed decreased scores in stool consistency and bleeding compared to D rats (Fig. 1A and B). The small intestinal and colon lengths, and gastrointestinal transit of CQ, CP and CC rats and DQ, DP and DC rats were unchanged compared to C and D rats, respectively (Table 1). Histology of ileum and colon of CQ, CP and CC rats was similar to C rats (Fig. 2C, E, G, K, M, O and Q). The ileum of DQ, DP and DC rats showed regeneration of crypt and epithelial membranes with fewer inflammatory cells compared to D rats (Fig. 2D, F and H). The colon of DQ, DP and DC rats improved when compared to the D rats with reduced inflammation, and healthy crypts and mucosa (Fig. 2L, N and P). The histological score of ileum and colon for DQ, DP and DC rats decreased compared to D as a measure of improvement (Fig. 2Q). The maximal forces of contraction of the isolated ileum and colon preparations in response to acetylcholine were not different between C and CQ, CP or CC rats (Table 1). Similarly, D and DQ, DP or DC rats did not differ in the ileum and colon maximal forces of contraction produced by acetylcholine (Table 1). The cumulative percent urinary recovery of sucrose and lactulose did not differ between groups of rats at any time intervals (Fig. 3A and B). The cumulative percent urinary recovery of mannitol and sucralose increased at 21 h and 24 h in CQ, CP, CC, DQ, DP and DC rats (Fig. 3C and D). The lactulose/mannitol ratio, an indicator of small intestine permeability, and sucralose/mannitol ratio, an indicator of whole gut permeability, did not differ between groups (Fig. 3E).

The energy intake and feed efficiency did not differ among C, CQ, CP and CC rats, the final body weight of CQ rats and their water intake were lower than C rats, CP and CC were similar to C rats (Table 1). DSS inclusion did not change water or food intakes compared to C rats and none of the interventions changed water or food treatment in DSS-treated rats (Table 1). In comparison to D rats, DQ, DP and DC rats did not show any difference in final body weight, energy intake, or feed efficiency (Table 1). The anthocyanin (C3G) intakes in CQ and DQ or CP and DP or CC and DC rats were similar, and quercetin intake was similar in CQ and DQ rats (Table 1). Fasting blood glucose and area under

Fig. 1. Effect of Queen Garnet plum juice, purple carrot juice and cyanidin 3-glucoside on stool consistency (A) and blood in stools (B) in rats given normal water (CQ, CP and CC) or 0.5% DSS water (DQ, DP and DC) in comparison to control (C) and 0.5% DSS (D) rats. Values are mean ± SEM, n = 8. Endpoint means with a different alphabet differ, *P* < 0.05. DSS, dextran sodium sulphate; C, normal drinking water; CQ, normal drinking water + Queen Garnet plum juice; CP, normal drinking water + purple carrot juice; CC, normal drinking water + cyanidin 3-glucoside; D, 0.5% DSS in drinking water; DQ, 0.5% DSS in drinking water + Queen Garnet plum juice; DP, 0.5% DSS in drinking water + purple carrot juice; DC, 0.5% DSS in drinking water + cyanidin 3-glucoside. Stool consistency scores: 0-formed, 1-mild-soft, 2-very soft, and 3-watery soft (diarrhoea). Stool bleeding scores: 0-normal colour, 1-brown colour, 2-reddish colour, and 3-bloody red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
the curve were similar among all groups (Table 1). The wet weights of abdominal fat pads, liver, kidney, spleen, left ventricle with septum, and right ventricle are given in Table 1.

The relative abundances of bacterial phyla in the colon are presented in Fig. 4. There were individual variations in relative abundance within the groups (Fig. 4A). C3G treatment did not change abundance of bacterial phyla in CC and DC rats compared to C and D rats, respectively. C3G treatment in CC and DC rats did not change gut microbiota at genus level (Fig. 4B and C). The ratio of Firmicutes and Bacteroidetes and the Shannon diversity index, an indicator of alpha diversity, did not differ among groups (Firmicutes/Bacteroidetes ratio: C, 4.96 ± 1.45; CC, 6.97 ± 0.68; D, 5.78 ± 0.61; DC, 3.89 ± 0.40; Shannon diversity index (H): C, 2.3 ± 0.1; CC, 2.4 ± 0.1; D, 2.4 ± 0.1; DC, 2.6 ± 0.1). Fig. 5 represents the heat map showing diversity of gut microbiota among C, CC, D and DC rats.

4. Discussion

Functional foods are foods that can prevent or reverse disease states in addition to providing nutrition, and may be useful to treat signs of metabolic syndrome such as obesity (Brown et al., 2015). Anthocyanins, a major class of flavonoids abundant in fruits and vegetables, have shown anti-inflammatory activity in vitro, in vivo and in clinical studies (Farzai et al., 2018; Lee et al., 2017). One of the most abundant anthocyanins, C3G, showed anti-inflammatory effects in human intestinal cell lines and thus could have a major potential role in the treatment of IBD, a chronic inflammatory disease, as a functional food (Jing et al., 2008). In this study, we showed that the C3G-containing foods, Queen Garnet plum and purple carrot, as well as pure C3G, reduced inflammation and induced regeneration of epithelial membranes, crypts and mucosal architecture in the ileum and colon of rats chronically dosed with 0.5% DSS. The improvements in inflammation, ileal and colonic structure were associated with...
Table 1

Physical, dietary, metabolic and gastrointestinal parameters in rats treated with Queen Garnet plum juice, purple carrot juice and cyanidin 3-glucoside.

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CQ</th>
<th>CP</th>
<th>CC</th>
<th>D</th>
<th>DQ</th>
<th>DP</th>
<th>DC</th>
<th>P value</th>
<th>Treatment</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>632 ± 19b</td>
<td>552 ± 15b</td>
<td>584 ± 12ab</td>
<td>597 ± 14b</td>
<td>612 ± 13ab</td>
<td>558 ± 17b</td>
<td>551 ± 18b</td>
<td>571 ± 15b</td>
<td>0.10</td>
<td>0.0004</td>
<td>0.62</td>
</tr>
<tr>
<td>Water intake (mL/d)</td>
<td>59.9 ± 5.9a</td>
<td>40.4 ± 2.1b</td>
<td>57.0 ± 4.7ab</td>
<td>50.3 ± 4.2b</td>
<td>53.1 ± 3.2b</td>
<td>51.0 ± 3.0b</td>
<td>55.4 ± 4.4b</td>
<td>57.5 ± 5.4b</td>
<td>0.44</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>37.0 ± 2.2</td>
<td>37.0 ± 1.5</td>
<td>37.0 ± 1.9</td>
<td>33.9 ± 1.6</td>
<td>36.5 ± 2.6</td>
<td>40.4 ± 2.1</td>
<td>36.5 ± 2.1</td>
<td>34.3 ± 1.0</td>
<td>0.61</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>42.0 ± 0.1</td>
<td>43.0 ± 0.1</td>
<td>41.0 ± 0.1</td>
<td>41.0 ± 0.2</td>
<td>45.0 ± 0.1</td>
<td>40.0 ± 0.2</td>
<td>40.0 ± 0.2</td>
<td>43.0 ± 0.2</td>
<td>0.43</td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Feed efficiency (g/kJ)</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.17</td>
<td>0.16</td>
<td>0.74</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>0.31</td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>AUC (mmol/L × minutes)</td>
<td>727 ± 19ab</td>
<td>768 ± 30ab</td>
<td>680 ± 32ab</td>
<td>702 ± 25ab</td>
<td>740 ± 9ab</td>
<td>689 ± 28ab</td>
<td>709 ± 34ab</td>
<td>0.91</td>
<td>0.09</td>
<td>0.75</td>
<td></td>
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<tr>
<td>Abdominal fat pads (mg/mm)</td>
<td>942 ± 66a</td>
<td>768 ± 70ab</td>
<td>759 ± 52ab</td>
<td>775 ± 44ab</td>
<td>711 ± 56b</td>
<td>661 ± 74a</td>
<td>743 ± 86b</td>
<td>0.19</td>
<td>0.04</td>
<td>0.34</td>
<td></td>
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<tr>
<td>LV + septum (mg/mm)</td>
<td>23.5 ± 0.9a</td>
<td>20.2 ± 0.9ab</td>
<td>20.4 ± 0.6ab</td>
<td>22.4 ± 0.7ab</td>
<td>21.9 ± 0.8ab</td>
<td>20.3 ± 0.5ab</td>
<td>21.3 ± 0.6ab</td>
<td>0.36</td>
<td>0.008</td>
<td>0.23</td>
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<tr>
<td>RV wet weight (mg/mm)</td>
<td>6.5 ± 0.6a</td>
<td>4.85 ± 0.31a</td>
<td>4.68 ± 0.26b</td>
<td>5.36 ± 0.41b</td>
<td>4.30 ± 0.36b</td>
<td>3.91 ± 0.30b</td>
<td>4.02 ± 0.40b</td>
<td>0.03</td>
<td>&lt;0.0001</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Liver wet weight (mg/mm)</td>
<td>403 ± 19a</td>
<td>335 ± 9a</td>
<td>330 ± 16b</td>
<td>379 ± 12b</td>
<td>372 ± 17b</td>
<td>326 ± 10b</td>
<td>348 ± 9b</td>
<td>0.41</td>
<td>0.0001</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>Kidney wet weight (mg/mm)</td>
<td>71.4 ± 1.4ab</td>
<td>62.9 ± 16ab</td>
<td>70.9 ± 19ab</td>
<td>73.2 ± 2.6ab</td>
<td>69.0 ± 2.1ab</td>
<td>67.0 ± 2.7ab</td>
<td>70.8 ± 2.8ab</td>
<td>0.37</td>
<td>0.043</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Spleen wet weight (mg/mm)</td>
<td>27.3 ± 1.3</td>
<td>22.6 ± 1.4</td>
<td>25.7 ± 1.1</td>
<td>22.9 ± 1.4</td>
<td>25.5 ± 1.3</td>
<td>23.2 ± 1.2</td>
<td>24.4 ± 2.5</td>
<td>25.6 ± 2.1</td>
<td>0.97</td>
<td>0.18</td>
<td>0.49</td>
</tr>
<tr>
<td>Small intestinal length (cm)</td>
<td>114 ± 3</td>
<td>114 ± 7</td>
<td>120 ± 1</td>
<td>121 ± 2</td>
<td>120 ± 2</td>
<td>120 ± 3</td>
<td>118 ± 2</td>
<td>119 ± 4</td>
<td>0.42</td>
<td>0.77</td>
<td>0.45</td>
</tr>
<tr>
<td>Colon length (cm)</td>
<td>22.6 ± 1.4a</td>
<td>21.6 ± 1.3a</td>
<td>21.2 ± 0.5a</td>
<td>22.3 ± 0.6a</td>
<td>17.9 ± 0.6a</td>
<td>19.8 ± 0.6a</td>
<td>17.3 ± 0.9b</td>
<td>19.2 ± 0.7ab</td>
<td>&lt;0.0001</td>
<td>0.31</td>
<td>0.39</td>
</tr>
<tr>
<td>Ileum force of contraction with acetylcholine (mN)</td>
<td>27.3 ± 4.9</td>
<td>39.1 ± 1.6</td>
<td>16.7 ± 3.5</td>
<td>13.1 ± 3.0</td>
<td>19.9 ± 4.1</td>
<td>32.4 ± 4.6</td>
<td>16.0 ± 4.2</td>
<td>14.0 ± 3.0</td>
<td>0.20</td>
<td>&lt;0.0001</td>
<td>0.61</td>
</tr>
<tr>
<td>Colon force of contraction with acetylcholine (mN)</td>
<td>64.8 ± 8.8</td>
<td>61.9 ± 5.9</td>
<td>45.4 ± 10.3</td>
<td>64.5 ± 6.0</td>
<td>68.2 ± 7.8</td>
<td>80.2 ± 12.3</td>
<td>58.4 ± 8.6</td>
<td>37.3 ± 5.8</td>
<td>0.19</td>
<td>0.003</td>
<td>0.45</td>
</tr>
<tr>
<td>Gastrintestinal transit - charcoal (%)</td>
<td>76.8 ± 5.2</td>
<td>84.4 ± 3.0</td>
<td>-</td>
<td>-</td>
<td>86.6 ± 3.2</td>
<td>86.0 ± 3.2</td>
<td>-</td>
<td>-</td>
<td>0.35</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>Gastrintestinal transit - phenol red (%)</td>
<td>71.4 ± 3.9</td>
<td>-</td>
<td>79.0 ± 3.0</td>
<td>74.0 ± 4.9</td>
<td>69.6 ± 2.5</td>
<td>-</td>
<td>75.6 ± 1.4</td>
<td>74.2 ± 4.7</td>
<td>0.72</td>
<td>0.58</td>
<td>0.65</td>
</tr>
<tr>
<td>Gastric emptying (%)</td>
<td>46.8 ± 5.9</td>
<td>-</td>
<td>48.5 ± 6.7</td>
<td>50.8 ± 7.2</td>
<td>56.7 ± 6.6</td>
<td>-</td>
<td>51.0 ± 7.7</td>
<td>49.8 ± 8.2</td>
<td>0.42</td>
<td>0.89</td>
<td>0.74</td>
</tr>
<tr>
<td>Anthocyanin intake (mg/kg/day)</td>
<td>8.92 ± 0.32b</td>
<td>8.33 ± 0.51b</td>
<td>7.43 ± 0.36c</td>
<td>-</td>
<td>9.61 ± 0.33b</td>
<td>8.62 ± 0.34bc</td>
<td>7.83 ± 0.19bc</td>
<td>0.12</td>
<td>0.0002</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Quercetin intake (mg/kg/day)</td>
<td>1.41 ± 0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.52 ± 0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n = 7–8 (except for gastrointestinal transit – charcoal where n = 4). Mean values within a row with a different superscript are significantly different, P < 0.05. DSS, dextran sodium sulphate; C, control group with normal drinking water; CQ, normal drinking water + Queen Garnet plum juice; CP, normal drinking water + purple carrot juice; CC, normal drinking water + cyanidin 3-glucoside; D, 0.5% DSS in drinking water; DQ, 0.5% DSS in drinking water + Queen Garnet plum juice; DP, 0.5% DSS in drinking water + purple carrot juice; DC, 0.5% DSS in drinking water + cyanidin 3-glucoside; AUC, area under the curve, LV, left ventricle; RV, right ventricle.
improved clinical symptoms of IBD.

Other studies have shown improvements with anthocyanins in acute and preventive protocols in acute rodent models of IBD (Farzaei et al., 2018). Anthocyanins from red grape skin and metabolically engineered tomatoes improved the pathological symptoms of IBD in a prevention study of chronic colitis in mice induced by 1% DSS for 2 weeks (Scarrano et al., 2017). Our study has extended these findings with a reversal study of chronic DSS-induced IBD following a low dose of 0.5% DSS for 12 weeks and testing pure anthocyanin C3G; interventions were added after 6 weeks when the disease state was relatively moderate. We selected a C3G dose of 8 mg/kg/day for comparison with our previous studies (Bhaswant et al., 2015, 2017) and this dose reduced the intestinal inflammation in this study. QG plums also contain quercetin, which improved IBD symptoms in the acetic acid-induced colitis model at a dose of 50 or 100 mg/kg (Dodda, Chhajed, & Mishra, 2014). However, as the concentration of quercetin glycosides in Queen Garnet plum juice was 0.3 mg/mL (Bhaswant et al., 2015) to provide a dose of ~1.4 mg/kg/day, quercetin is unlikely to be responsible for the reversal of IBD symptoms at such a low dose in our study. Therefore, C3G, the main bioactive compound in Queen Garnet plum, probably improves the gut structure and function.

Our previous studies on purple carrots at a C3G dose of 15 mg/kg/day showed improvement in the structural and functional changes of heart and liver in rats with diet-induced metabolic syndrome, a model of chronic low-grade inflammation (Poudyal et al., 2010). We have also reported that pure C3G and the juice of Queen Garnet plums at a C3G dose of 8 mg/kg/day reversed the symptoms of metabolic syndrome in obese rats, improving the structure and function of heart and liver (Bhaswant et al., 2015). Further, anthocyanins in purple maize (Zea mays) and black chokeberry (Aronia melanocarpa) at 8 mg/kg/day prevented heart and liver damage in diet-induced obese rats (Bhaswant et al., 2017). In these studies, C3G prevented heart and liver damage by inhibiting the infiltration of inflammatory cells. This current study suggests that C3G is an effective anti-inflammatory agent with similar effects in this IBD model.

Dietary interventions with anthocyanin-containing foods have been widely reported as beneficial for human health because of anti-inflammatory and anti-oxidant responses (Lee et al., 2017; Speciale et al., 2018). The improved intestinal histology in C3G-treated rats suggests that C3G in foods increases the renewal of healthy cells against oxidative stress-induced apoptosis as in inflammatory conditions (Jiang, Tang, Zhang, Liu, & Guo, 2014). The oxidative colonic DNA damage was inhibited by the antioxidant activity of the anthocyanins from purple carrots, quenching reactive oxygen species in human colonic mucosa cells (Olejnik et al., 2016). This could explain the increase in intestinal epithelial layer thickness with C3G and C3G-containing foods in the current study.

Anthocyanins such as C3G taken up by hexose transporters into cells may modulate inflammation by inhibiting NF-κB and MAPK-mediated inflammatory cell signalling pathways as observed in an inflamed Caco-2 BBe1/THP-1 co-culture cell model (Zhang et al., 2017). Anthocyanins including C3G promoted nuclear factor erythroid-2 related factor 2/ heme oxygenase-1 (Nrf2/HO-1) signalling pathway and inhibited oxidative stress and inflammation (Wang et al., 2016). Thereby, they may reduce the concentrations of pro-inflammatory cytokines such as TNF-α and IL-8, and attenuate inflammation in the tissues. Anthocyanins did not change gut permeability in our study which was unchanged between healthy and DSS-induced IBD rats (Ghattamaneni et al., 2019). This may have been due to the lower dose of DSS not causing severe damage to the intestine. Gut microbial degradation of anthocyanins...
Fig. 4. Effect of cyanidin 3-glucoside on gut microbiota at phylum level (A) and genus level (B and C). Values are presented as means for each phyla or genus in (A) and as means ± SEM (n = 6/group) in (B) and (C). Endpoint means with a different letter differ, P < 0.05. DSS, dextran sodium sulphate; C, control group with normal drinking water; CC, normal drinking water + cyanidin 3-glucoside; D, 0.5% DSS in drinking water; DC, 0.5% DSS in drinking water + cyanidin 3-glucoside.
including C3G resulted in phenolic acids, which are demethylated to the hydroxyl groups that impart the antioxidant property (Hanske et al., 2013). Many of the metabolites derived from microbial breakdown of C3G have synergistic or additive effects with increased HO-1 protein expression (Warner, Rodriguez-Ramiro, O’Connell, & Kay, 2018). These findings further strengthen the concept of health benefits following C3G uptake into gastrointestinal cells in IBD. Other anthocyanins tested in DSS-induced colitis in rodents showed similar efficacy by reducing intestinal inflammation as C3G in our study. Pomegranate anthocyanins (delphinidin 3-glucoside, delphinidin 3,5-diglucoside, cyanidin and pelargonidin) inhibited the mTOR downstream pathway, purple potatoes containing anthocyanins including peonidin, petunidin, delphinidin and malvidin derivatives, and high anthocyanin tomatoes inhibited p38-MAPK inflammatory pathway, and black raspberries anthocyanins inhibited 1k-B phosphorylation and mitigated colonic inflammation (Farzaei et al., 2018).

The low plasma concentrations of the anthocyanins following oral dosage is a major factor to consider when determining the anti-inflammatory activity in the tissues, especially the intestinal lining (Talavera et al., 2004). C3G is rapidly and efficiently absorbed into cells in the jejunum and ileum (Talavera et al., 2004) and we observed the anti-inflammatory effects in the ileum. It was observed that the digested purple carrot extract had less potency to quench reactive oxygen species than the extract before digestion (Olejnik et al., 2016). This suggests that sufficient anthocyanins were absorbed by the intestinal cells from the gastrointestinal fluids to produce local anti-inflammatory and antioxidant activities, and improve the gut structure and function in our treated rats, so these properties may not be correlated with the plasma concentrations of anthocyanins.

The gut microbiota is crucial to maintain human gut health as a metabolising “organ” with a pivotal role in the uptake of carbohydrates, lipids and xenobiotics (Faria, Fernandes, Norberto, Mateus, & Calhau, 2014). Dysbiosis involved in IBD could be either the causative factor or the consequence of gut inflammation (Celiberto et al., 2018). The relatively mild changes in the gut microbiome in our study could correlate with the chronic and moderate gastrointestinal symptoms produced by relatively low-dose DSS, compared to literature studies with higher doses. This suggests that bacterial changes occur as a consequence of the changes in gastrointestinal function, including inflammation and cellular damage, in mild IBD. Anthocyanins can both modulate bacterial growth in the intestine and undergo colonic metabolism by the bacteria by the cleavage of glycosidic linkages to give anthocyanidins and simple phenolics with potential direct health benefits to the host (Faria et al., 2014). DSS (4% w/v in drinking water for 7 days) induced imbalance of gut microbiota and mimicked human gut microbiota changes in IBD but with no change in alpha diversity (Hakansson et al., 2015) as in our study. C3G was converted to 3,4-dihydroxybenzoic acid (protocatechuc acid) and 2,4,6-trihydroxybenzoic acid by human intestinal bacteria in human microbiota-associated rats; these C3G metabolites could produce the beneficial effects in the gut (Hanske et al., 2013).

In colitic mice following DSS dosage at 5% for 5 days, gut commensal bacterial families Lachnospiraceae (butyrate-producing) and S24-7 were decreased (Osaka et al., 2017), and they were increased in mice with remission of colitis during treatment phase, respectively (Cheng et al., 2016; Rooks et al., 2014). However, extensive studies on anthocyanins and their effects in vivo or in clinical studies are lacking in the literature. In our study, we found no differences in the microbiota between the control and IBD rats with 0.5% for 12 weeks, and C3G was unable to induce modulation of the profile.

The limitations of our study include that only one dose of C3G was used for chronic treatment, thus no concentration-response curve was
generated, and we did not analyse anthocyanins and their metabolites in intestinal, plasma or urine samples. The concentrations of C3G in the intestinal cells rather than in plasma could give us a better understanding of the active ingredients of the foods available for the cell uptake and metabolism in vivo to deliver its pharmacological effect. Further, faecal samples were collected from the colon, rather than the intestinal fluid, so we cannot define the concentrations of C3G and its metabolites that were in contact with colonicocytes. Molecular markers of inflammation such as cytokines and cell adhesion molecules were not examined in this study; these markers could have suggested the molecular targets of C3G in attenuating IBD in the absence of changes in gut microbiota.

5. Conclusion

The current study supports the role of C3G in attenuation of IBD symptoms. This study emphasises the relevance of functional foods in treating chronic diseases such as IBD, which could become useful clinical therapy following prospective clinical trials on these C3G-containing foods.

Ethics statement

All experiments were approved by the University of Southern Queensland Animal Ethics Committee (approval number 14REA005 valid until July 2017) under the guidelines of the National Health and Medical Research Council of Australia.

Author contributions

N.K.R.G., S.K.P. and L.B. developed the original study aims. N.K.R.G. conducted the experiments. N.K.R.G., S.K.P. and L.B. analysed and interpreted the data; N.K.R.G. and S.K.P. prepared manuscript drafts. L.B. has been the corresponding author throughout the writing process. All authors read and approved the final manuscript.

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