Whole Tart Cherry Improves Disease Activity Index in Rat Model of Dextran Sulfate Solution – Induced Ulcerative Colitis by Downregulating the Janus 1 And Janus 3 Kinases and TNF - Alpha

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WHOLE TART CHERRY IMPROVES DISEASE ACTIVITY INDEX IN RAT MODEL OF DEXTRAN SULFATE SOLUTION – INDUCED ULCERATIVE COLITIS BY DOWNREGULATING THE JANUS 1 AND JANUS 3 KINASES AND TNF - ALPHA

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The School of Nutrition and Food Sciences

by

Johana Alexandra Coronel
B.S., Pan-American Agricultural School Zamorano, 2013
May 2018
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ABSTRACT

Ulcerative colitis (UC) is a disabling inflammatory bowel disease. UC is characterized by chronic, relapsing inflammation of the colon and rectum. Current treatments such as monoclonal antibodies against TNF-α, IL-6, IL-12/p40, adhesion molecules, Janus kinases (JAK) inhibitors have side effects or lose their effects over time. Alternative approaches with fewer side effects for patients are needed. Montmorency tart cherries (*Prunus cerasus*) are a good source of anti-inflammatory flavonoids. We hypothesized that regular consumption whole tart cherry (TC) standardized to its major anthocyanin content, namely cyanidin-3-glucosyl-rutinoside, would be effective in reducing inflammation in UC. The aim of this research was to evaluate the protective effect of TC in a rat model of UC induced by dextran sulfate sodium (DSS). The anthocyanin profile and content of the TC were analyzed by UHPLC-PDA-MS. Cyanidin-3-glucosyl-rutinoside (6.14 ± 0.70 mg/serving size) and cyanidin-3- rutinoside (4.47 ± 0.68 mg/serving size) were the major anthocyanins in TC extracts.

Rats were randomly assigned to one of nine groups (n = 6 each group). UC was induced by adding 4% DSS to the drinking water for 5 days. For the UC prevention group, TC was administered orally in servings equivalent to one or two serving sizes for humans (155 or 310 g of cherries/ 70 kg BW/ day which is translated into 2.21 g/kg BW/day or 4.42 g/kg BW/day for a rat) for 2 weeks prior to DSS administration. For the UC-intervention groups, TC was administered orally in servings of 2.21 g/kg BW/day or 4.42 g/kg BW/day during the DSS administration. For the UC-treatment groups, TC was administered orally in serving of 2.21 g/kg BW/day or 4.42 g/kg BW/day for 2 weeks after the DSS administration.

TC at single or double serving reduced leucocyte infiltration in the colon. TC as a prevention, intervention or treatment significantly (*p < 0.05*) reduced the secretion of
myeloperoxidase (MPO), IL-6, IL-12/p40, IL-17A, TNF-α, and JAK1 and increased the secretion of anti-inflammatory IL-10 and JAK3. IL-1β was not significantly reduced by TC ($p > 0.05$). TC containing cyanidin-3-glucosyl-rutinoside showed promising results in reducing the secretion of inflammatory markers in an experimental rat model of DSS-induced UC.
CHAPTER 1: INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that causes a diffuse inflammation of the mucosa of the colon and rectum (Terzić et al. 2010). UC can occur at any age but is frequently diagnosed in late adolescence or early adulthood (Ordás et al. 2012). The incidence and prevalence of UC has been increasing worldwide, especially in Europe and North America. UC is the most prevalent form of IBD (Null et al. 2016). The pathogenesis of UC is poorly understood, however, the development of chronic inflammation in UC is associated with a combination of environmental, genetic, microbial and immunological factors (Conrad, Roggenbuck, and Laass 2014).

UC patients have to take medication to prevent the development of colectomy or colorectal cancer (Conrad, Roggenbuck, and Laass 2014). Corticosteroids, immunosuppressants, and anti-tumor necrosis factor (TNF-α) are drugs used to treat UC. They inhibit the production of IL-1, IL-2, NF-kB, or TNF-α. (Teixeira, Hosne, and Sobrado 2015). Consumption of these drugs have been associated with nausea, vomiting, headaches, rash, fever, agranulocytosis, pancreatitis, nephritis, hepatitis, male infertility, folic acid deficiency and damage of the immune system (Head, Jurenka, and Ascp 2003; Nasiri, Ellen Kuenzig, and Benchimol 2017). However, twenty five percent of patients diagnosed with UC will require surgery (Da Silva et al. 2014).

Interest in dietary bioactive compounds that inhibit inflammation of the gastrointestinal tract and improve health has increased in the last decade (Ananthakrishnan 2015; Hou, Abraham, and El-Serag 2011). The health benefits of tart cherries including the anti-oxidative and anti-inflammatory activities have been demonstrated in vivo and in vitro (Kirakosyan et al. 2015; Lachin 2014; Losso et al. 2017). However, the potential health benefits of tart cherries on UC
have not been studied. This study evaluated the protective effect of whole tart cherry on inflammation in a rat model of mild UC. Specifically, the aims were to:

1. Determine the anthocyanin profile and content of whole Montmorency tart cherry (*Prunus cerasus*)

2. Determine the disease activity index of Dextran Sulfate Sodium (DSS) induced UC in animals.

3. Determine the macroscopic and microscopic scores of rat colonic tissues.

4. Determine the effect of whole Montmorency tart cherry (*Prunus cerasus*) on myeloperoxidase activity (MPO).

5. Determine the effect of whole Montmorency tart cherry (*Prunus cerasus*) on inflammatory biomarkers in UC including TNF-α, IL-1β, IL-6, IL-12/p40, and IL-17A.

6. Determine the effect of whole Montmorency tart cherry (*Prunus cerasus*) on JAK1 and JAK3 kinases levels.
CHAPTER 2: LITERATURE REVIEW

2.1 Ulcerative colitis

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that causes inflammation of the rectal and colonic mucosa (Terzić et al. 2010). It is considered a T-helper 2 (Th2) mediated disease due to an imbalanced and aberrant immune response to intestinal flora. The imbalance of the immune system activates macrophages, dendritic cells, and other cells that stimulate the production of pro-inflammatory cytokines including TNF-α, IL-12, IL-23, IL-6, and IL1-β in the gut and increases the inflammation of the mucosa (Head, Jurenka, and Ascp, 2003; Z.-J. Liu et al. 2009)

UC can occur at any age but it is frequently diagnosed at ages of 15 to 35 with a median of diagnosis of 34.9 years (Ordás et al. 2012). The incidence and prevalence of UC are high in industrialized countries. Northern Europe has an incidence of 24.3% and a prevalence of 200/100,000 people while North America has an incidence of 19.2% and a prevalence of 150/100,000 people (Ungaro et al. 2017). Approximately 38,000 new cases of UC are diagnosed every year in the United States and 25% of these cases will require surgery (Da Silva et al. 2014). The average annual cost for UC could reach up to $11,477 per patient depending on the severity of the disease (Null et al. 2016).

2.2 Risk factors for ulcerative colitis

The most common risk factors associated with UC are genetic, environmental and immunological (Conrad, Roggenbuck, and Laass 2014). All risk factors can modify the intestinal microbiota, increase the intestinal permeability and could cause a dysregulation of the immune system. The dysregulation of the immune system is created by the imbalance between pro-
inflammatory and anti-inflammatory cytokines, between Th1, Th2, and Th17 responses (Figure 1) (Uranga et al. 2016; Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusho 2008).

Figure 1. Cytokines imbalance between effector and T regulatory cell in IBD

The family medical history is an important risk factor. About 5.7 to 15.5 % of patients with UC have at least one first-degree relative with UC. However, second – degree relatives have a lower risk of development UC than first degree relatives (Orholm et al. 1991). Genetic studies affirmed that family history could have an effect on disease location (pancolitis, left-sided colitis or extensive colitis) (Laharie et al. 2001).

Data from animal models and patients with UC suggested that environmental factors are one of the risk factors for UC (Conrad, Roggenbuck, and Laass 2014). Clinical and experimental observations found a high incidence of UC in gut regions with high concentration of bacteria, which causes leukocyte infiltration and secretion of inflammatory cytokines (Hiatt and Kaufman 1988). In addition, constant gastroenteritis infections with Salmonella, Shigella or Campylobacter and consumption of anti-inflammatory drugs can change the gut microflора and destroy the epithelial barrier of the colon, increasing the probability of developing UC
The summation of all the factors mentioned above increases the risk of developing UC from 29.7 per 100,000 to 77 per 100,000 people/year (Adams and Bornemann 2013).

UC is also related to an aberrant immune response due to a modified Th2 action that increases the production of natural killer T cells (NKT) (Ho, Lees, and Satsangi 2011). Once the NKT increase in the lamina propria of the colon, Th2 T-cells produce cytokines such as IL-4, IL-13, and IL-10 which participate in the development and exacerbation of UC (Sanchez-Muno, Dominguez-Lopez, and Yamamoto-Furusho 2008).

2.3 Ulcerative colitis symptoms

UC has several symptoms which vary according to the severity of the illness, the location and the duration of the inflammation (Rogler 2014). The major symptoms are diarrhea accompanied by blood or mucus, abdominal cramps with relief after defecation, rectal bleeding, abdominal pain related to the intensity of colon inflammation, and weight loss (Conrad, Roggenbuck, and Laass 2014). Additionally, extra-intestinal conditions such as ophthalmological, hepatobiliary and hematologic disorders may be present after or before the development of UC (Teixeira, Hosne, and Sobrado, 2015).

2.4 Diagnosis and classification

The diagnosis and the severity index (Table 1) of UC can be determined based on clinical symptoms and confirmed by endoscopy, histology, and laboratory findings (Table 2) (Teixeira, Hosne, and Sobrado 2015). Endoscopy and histology are used to determine the extent of UC according to the Montreal Classification. The Montreal Classification was made by the World Congress of Gastroenterology in Montreal to define the extent of the inflammation. UC is classified into proctitis (inflammation of the rectum), left-sided colitis (inflammation of the left
Figure 2. Ulcerative colitis classification by Montreal classification

...
### Table 1. Diagnostic characteristics of ulcerative colitis

<table>
<thead>
<tr>
<th>Test</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td>Gross or occult rectal bleeding</td>
</tr>
<tr>
<td></td>
<td>Abdominal pain</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Intestinal infection (enteric pathogens: <em>Salmonella</em>, <em>Shigella</em>, <em>Campylobacter</em>, <em>Ecoli O157:H7</em>)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Iron deficiency, anemia</td>
</tr>
<tr>
<td></td>
<td>Thrombocytosis</td>
</tr>
<tr>
<td></td>
<td>Hyperalbuminemia</td>
</tr>
<tr>
<td></td>
<td>Autoantibodies (GAB*, atypical or perinuclear ANCA+)</td>
</tr>
<tr>
<td></td>
<td>Elevated fecal calprotectin</td>
</tr>
<tr>
<td>Endoscopy</td>
<td>Diffuse, continuous involvement of the mucosa starting at the rectum and extending continuously proximally to a variable extent</td>
</tr>
<tr>
<td></td>
<td>Loss of visible vascular pattern</td>
</tr>
<tr>
<td></td>
<td>Loss of haustral folds</td>
</tr>
<tr>
<td></td>
<td>Mucosal erosion and mucosal friability (bleeding when touched by endoscope) or spontaneous bleeding and ulceration</td>
</tr>
<tr>
<td></td>
<td>Oedematous, erythematous appearance of the mucosa</td>
</tr>
<tr>
<td></td>
<td>Pseudopolyps with long-standing UC</td>
</tr>
<tr>
<td></td>
<td>Mucopurulent exudates</td>
</tr>
<tr>
<td>Histology</td>
<td>Increased mononuclear inflammation in the lamina propria (plasmacytosis)</td>
</tr>
<tr>
<td></td>
<td>Mucin depletion</td>
</tr>
<tr>
<td></td>
<td>Globet cell depletion</td>
</tr>
<tr>
<td></td>
<td>Crypt distortion, branching, and atrophy</td>
</tr>
<tr>
<td></td>
<td>Crypt abscesses</td>
</tr>
</tbody>
</table>

*Autoantibodies to intestinal goblet cells *Antineutrophil cytoplasmic antibodies
Table 2. Severity index scores for ulcerative colitis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of bowel movements</td>
<td>0 = Normal for the patient</td>
</tr>
<tr>
<td></td>
<td>1 = 1-2 stools/day in addition to the usual</td>
</tr>
<tr>
<td></td>
<td>2 = 3-4 stools/day in addition to the usual</td>
</tr>
<tr>
<td></td>
<td>3 = &gt;5 stools/day beyond the usual</td>
</tr>
<tr>
<td>Rectal bleeding</td>
<td>0 = No blood</td>
</tr>
<tr>
<td></td>
<td>1 = Blood streaks in less than half of evacuations</td>
</tr>
<tr>
<td></td>
<td>2 = Evidence of fresh blood in most of the evacuations</td>
</tr>
<tr>
<td></td>
<td>3 = Bowel movements with fresh blood</td>
</tr>
<tr>
<td>Global medical assessment</td>
<td>0 = Normal</td>
</tr>
<tr>
<td></td>
<td>1 = Mild disease</td>
</tr>
<tr>
<td></td>
<td>2 = Moderate disease</td>
</tr>
<tr>
<td></td>
<td>3 = Severe disease</td>
</tr>
</tbody>
</table>

**Disease Severity Index**

<table>
<thead>
<tr>
<th>Scores</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2</td>
<td>Clinical remission</td>
</tr>
<tr>
<td>3 - 5</td>
<td>Mild activity</td>
</tr>
<tr>
<td>6 - 10</td>
<td>Moderate activity</td>
</tr>
<tr>
<td>11 - 12</td>
<td>Severe activity</td>
</tr>
</tbody>
</table>
2.5 Ulcerative colitis biomarkers

Biomarkers are molecular parameters that can be used to diagnosis, to measure the progress of the disease, and to predict or to monitor the effects of a certain treatment (Burska, Boissinot, and Ponchel 2014). Autoantibodies, cytokine/growth factors, a product of tissue degradation, cell subset, and fecal traces could be used as biomarkers (Lewis 2011). In the context of UC, biomarkers of UC patients provide a probe of an exaggerated Th2 response, elevated tumor necrosis factor (TNF-α), proinflammatory and anti-inflammatory cytokines interleukins IL-1β, IL-6, IL-10, IL-12, IL-17 and signaling pathways such as Janus Kinases 1 and 3 (JAK1, JAK3) (Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusho 2008). Therefore, these biomarkers are useful in UC monitoring.

2.5.1 Tumor necrosis factor (TNF-α)

TNF-α is produced by activated macrophages and T lymphocytes. It is considered as a pro-inflammatory cytokine as IL-1α, IL-1β, IL17 or IL6 (Gottlieb et al. 2005). TNF-α mediates several pro-inflammatory signals including neutrophil recruitment in the site of inflammation, activation of coagulation, and induction of granuloma formation (Kern et al. 2001). Low concentrations of TNF-α augment host defense mechanism against infections. However, high levels of TNF-α not only induces the secretion of IL-6 and IL-1 but also produces mild inflammation in a local area and tumor formation (Strober and Fuss 2011). TNF-α is associated with chronic inflammatory diseases such as UC. TNF-α inhibition is commonly used to help UC patients manage their conditions (Tracey et al. 2008).
2.5.2 Interleukins

IL-1β is a pro-inflammatory cytokine produced by macrophages and it is considered an important mediator of local and systemic inflammatory process (Kaifang et al. 2001; Park et al. 2017). IL-1β induces the proliferation of T cells and secretion of TNF-α and IL-8. IL-1β initiates colonic inflammation by increasing vascular permeability and neutrophil secretion (Balzola et al. 2012). In addition, IL-1β decreases blood pressure and induces fever (Terzić et al. 2010). The elevated body temperature increases leukocyte migration and the release of other pro-inflammatory cytokines such as IL-6, TNF-α, prostaglandins and activation of IL-17 secretion (Garlanda, Dinarello, and Mantovani 2013). IL-1β is considered a sensitive marker of colonic inflammation (Ligumsky et al. 1990).

IL-6 is a potent cytokine produced mainly by monocytes and macrophages at the sites of inflammation during acute inflammation (Naugler and Karin 2008). IL-6 regulates T cell differentiation, activation, and resistance to apoptosis (Kern et al. 2001). It controls the balance between pro-inflammatory and regulatory T cells (Guan and Zhang 2017). IL-6 acts as a mediator of fever and acute phase response modulating intracellular signaling by inducing Janus kinase-signal and activating the transcription of JAK/STAT pathway. IL-6 is considered an accurate biomarker of ongoing inflammation (Galien 2016). Elevated concentrations of IL-6 have been found in patients with active UC (Mańkowska-Wierzbicka et al. 2015).

IL-10 is an anti-inflammatory cytokine produced by activated immune cells including macrophages and T cells (Sabat et al. 2010). IL-10 is an important regulator of inflammation. It inhibits the secretion of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, TNF-α) and reduces the mucosal inflammation by inactivating Th1 cells, natural killer cells (NK) cells, and interferon gamma (INF-γ) (Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusho 2008;
Schreiber, et al., 1995). Kühn et al. (1993), stated that IL-10 is an essential immunoregulatory cytokine in the intestinal tract; the absence of IL-10 produces extensive mucosal hyperplasia, inflammatory reactions and aberrant expression of inflammatory receptors on epithelia cells.

IL-12/p40 is produced by NK cells, T cells, dendritic cells and phagocytes (Kaufman and Dharmadhikari 2017). IL-12/p40 regulates the immune response, including the development, expansion, homeostasis, and differentiation of lymphocytes (Vignali and Kuchroo 2012). In addition, IL-12/p40 determines the type and duration of adaptive immune response during an inflammatory process (Jones and Vignali 2011). IL-12/p40 induces the generation of pro-inflammatory Th1 and Th17 cells and activates JAK3/STAT pathway (Head, Jurenka, and Ascp 2003). The expression of IL-12/p40 is highly correlated with increases in the activity index score and is strongly dependent on the stage of UC (Mańkowska-Wierzbicka et al. 2015).

IL-17A is a pro-inflammatory cytokine produced by Th17 cells as an inflammatory response (Langrish et al. 2005). It is associated with neutrophil induction and maturation in a response of the acute mechanism in host defense (Miossec 2009). At the same time, IL-17A leads to the secretion of other pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which enhances and amplifies colon inflammation (Balzola, et al., 2012). IL-17A promotes the secretion of pro-inflammatory cytokines and works synergistically with TNF-α to enhance the production of IL-6 (Ruddy et al. 2003). IL-17A is only detected in patients with active UC (Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusho 2008). Therefore, non-expression of IL-17A in a patient may suggest that UC is inactive or in remission.
2.5.3 Fecal biomarkers

Myeloperoxidase (MPO) is an enzyme found in neutrophils. MPO produces a strong oxidant, hypochlorous acid (HOCl), from hydrogen peroxide (H₂O₂) and chloride ion (Cl⁻) (Van-Antwerpen and Zouaoui-Boudjeltia 2015). MPO contributes to tissue damage by oxidation of biomolecules. MPO is related to chronic inflammatory syndromes and diseases when immune cells like neutrophils are involved in the process (Honda et al. 2009). However, a high intracellular concentration of antioxidants protects MPO-containing cells from damage induced by HOCl (Van-Antwerpen and Zouaoui-Boudjeltia 2015).

Matrix metalloproteinases (MMPs) are a calcium-dependent and zinc-containing endopeptidase protein family. MMPs are classified depending on the substrate specificity and membrane types such as collagenases, gelatinases, stromelysins, and matrylsins (Pujada et al. 2016). There are 23 MMPs in humans. MMPs control the activity of inflammatory cytokines, chemokines, serine proteases and the adhesion and migration of leukocytes from blood vessels to the site of inflammation. The secretion of MMPs is important because their secretion facilitate wound healing or tissue repair during UC or IBD. However, overexpression of pro-inflammatory cytokines can increase the expression of MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP12, and MMP14 that accelerate tissue destruction and disrupt subsequent tissue repair (Terzić et al. 2010). Masoodi et al. (2011) reported that MMPs are undetectable in feces when there is no inflammation in the GI tract; nevertheless, MMPs are detectable in the feces of patients that have active UC or have a transition from UC to colon cancer. MMPs play a key role in non-invasive UC biomarkers.
2.6 Treatment of ulcerative colitis

Treatment of UC is principally based on the site and severity of the disease including mild to moderate UC, moderate to severe UC and acute severe UC (Ungaro et al. 2017). The objective of UC treatment is to induce or maintain a remission period to prevent colectomy and colorectal cancer development by the use of drugs that have anti-inflammatory effects. The drugs inhibit the production of IL-1β, IL-2, NF-κB, and/or TNF-α (Teixeira, Hosne, and Sobrado 2015).

2.6.1. Mild to moderate ulcerative colitis

Patients with mild to moderate UC are treated with aminosalicylates (5-ASA), such as mesalamine, balsalazide, and sulfasalazine. Stephen and Podolsky (2016) reported that patients with mild to moderate proctitis are treated with topical 5-ASA at a dosage of 1–4 g per day. Patients with mild-moderate left-sided or extensive colitis are treated with a combination of oral and topical 5-ASA at a dosage of 2–4.8 g per day. The period of time of the dosage varies from 4 to 8 weeks for achieving a symptomatic remission (Ungaro et al. 2017).

2.6.2. Moderate to severe ulcerative colitis

Patients with moderate to severe UC are treated with oral corticosteroids at a dosage of 40–60 mg prednisone per day. Once the remission of UC is achieved after 3-6 months, the treatment is changed to 5-ASA with a continuous monitoring of the remission stage (Ungaro et al. 2017).
2.6.3. Acute severe ulcerative colitis

Patients with acute severe UC are treated with intravenous corticosteroids for 3 to 5 days. If the remission is not achieved, patients are treated with either cyclosporine or infliximab. Patients who do not respond to the treatment and have uncontrolled hemorrhage, perforation, and colorectal carcinoma are redirected to a colectomy surgery (Ungaro et al. 2017).

2.6.4. Side effects of ulcerative drugs

Conventional treatments can be effective in maintaining remission periods of UC. However, a significant number of people with UC fail to respond to even the strongest drugs including corticosteroids (Head, Jurenka, and Ascp 2003). Conventional treatments are not without side effects and nearly 30% of UC patients treated with aminosalicylates could have nausea, vomiting, headaches, rash, fever, agranulocytosis, pancreatitis, nephritis, hepatitis, male infertility, folic acid deficiency and damage of the immune system (Pitcher, Beatty, and Cummings 2000; Bresci et al. 1997). The short-term corticosteroid use could induce fluid retention, weight gain, and mood swings; while the long-term use increases the risk for cataracts, osteoporosis, myopathy, conditions associated with immune suppression, and adrenal insufficiency (Head, Jurenka, and Ascp 2003; Nasiri, Ellen Kuenzig, and Benchimol 2017). Andrianopoulos et al. (1989), found that animals treated with sulfasalazine at similar doses of human doses developed smaller, multiple, flat, sessile and frequently micro-invasive tumors compared to fewer, larger, exophytic tumors observed in animals treated with dimethylhydrazine (DMH). Large doses of sulfasalazine (84 – 337.5 mg/kg) increased the incidence of urinary tract infection, hepatocellular carcinoma and result in the inhibition of folic acid absorption, a deficiency that causes dysplasia or cancer (neoplasia) (Lashner et al. 1989).
A rat model colitis study revealed that the use of low-dose 5-ASA (30mg/kg daily) increased the number or size of tumors, while high-dose 5-ASA (60mg/kg daily) inhibit the tumor size (Bouic et al. 1996).

Because of all the side effects, recent studies have focused on the use of natural products or dietary supplements derived from plants and fruits, which offer alternative and effective anti-inflammatory therapies with low toxicity and minimal or null side effects (Sakthivel and Guruvayoorappan 2013).

2.7 Functional foods and ulcerative colitis

Interests in functional foods in the prevention of chronic degenerative diseases such as gastrointestinal disease, diabetes, cardiovascular disease, age-related diseases, cancer, and celiac disease have increased over the last three decades. Foods and beverages that contain bioactive compounds with potential health benefit activities are considered functional foods (Awaad, El-Meligy, and Soliman 2013; Uranga et al. 2016).

Seeram et al. (2001) suggested that compounds in plants and fruits have the ability to serve as cellular antioxidants by maintaining low levels of reactive oxygen anion or as anti-inflammatory agents by inhibiting prostaglandin synthesis. In vivo and in vitro studies focusing on the benefits of functional foods have demonstrated that consumption of bioactive-rich plants or fruits including apples, blueberries, cherries, strawberries, raspberries, and cocoa is associated with a reduction of UC risk (Saxena et al. 2014).
2.7.1 Tart cherry

Montmorency tart cherries (Prunus cerasus) are also known as sour cherries in the United States (Blando, Gerardi, and Nicoletti 2004). Ou, et al. (2012) reported that more than 75% of Montmorency tart cherries consumed in the United States are produced on small family farms in Michigan, followed by Utah, Washington, New York, Wisconsin and Pennsylvania. Tart cherries are consumed dried, frozen, or as a juice. Tart cherries have a unique sour-sweet flavor that is becoming popular in the United States not only for the flavor but also for the potential benefits of their bioactive compounds (Šarić et al. 2009). In vivo and in vitro studies have demonstrated that tart cherry bioactive compounds like anthocyanins, procyanidins and melatonin are associated with enhanced health including the anti-oxidative and anti-inflammatory activities (Kirakosyan et al. 2015; Lachin 2014; Losso et al. 2017)

Montmorency tart cherries are a rich source of flavonoids, especially anthocyanins. The major anthocyanins present in tart cherries are cyanidin-3-glucosyl-rutinoside and cyanidin-3-
rutinoside (Figure 4) (Ferretti, et al., 2010). Anthocyanins play an antioxidative role by donating electrons or hydrogen atoms from hydroxyl moieties to free radicals. Anthocyanins from cherry extracts reduce inflammation, alleviate inflammatory pain and arthritis in rats (Tall et al. 2004). Tsuda et al., (2003) revealed that cyanidin-3-glucoside isolated from cherries showed strong antioxidative activity in vivo and in vitro.

![Chemical structures](image)

Figure 4. **a**. Chemical structure of cyanidin-3-glucosyl-rutinoside. **b**. Chemical structure of cyanidin-3-rutinoside.

Šarić et al., (2009), found that consumption of tart cherry *Prunus cerasus* might be a good source of phytochemicals, particularly anthocyanins, which could contribute to health-promoting effects by increasing antioxidant and anti-inflammatory status especially for people with digestive ulcers and arthritis. A clinical study examined the efficacy of tart cherry juice in preventing the symptoms of inflammation-associated muscle damage after exercise and found that consumption of tart cherry juice reduced post-exercise pain and loss of muscle strength (Connolly et al., 2006). Kuehl, et al., (2010) assessed the effects of tart cherry juice and a placebo cherry drink on muscle pain in runners. The administration of tart cherry juice for eight
days reduced symptoms of exercise-induced muscle pain among runners in a vigorous endurance event. In addition, a human study based on a short-term supplementation of Montmorency tart cherries powder during an endurance challenge reduced immune an inflammatory stress and improved the redox balance in individuals (Levers et al. 2016).

A rat model of elevated cholesterol, insulin resistance, and prediabetes or metabolic syndrome, fed 1% of the diet with freeze-dried tart cherry showed a significant increase in plasma antioxidant capacity and reduction of plasma inflammatory markers IL-6 and TNF-α (Seymour et al., 2008; Tall et al., 2004). Lachin (2014) proved in an animal model that oral administration of cherry extract at a concentration of 200 mg/kg body weight for 30 days significantly reduced the levels of blood glucose and urinary microalbumin. Jayaprakasam et al., (2005) tested extracts of anthocyanins from tart cherries on pancreatic cells, which produce the hormone insulin in the presence of glucose. Anthocyanin exposed cells increased insulin production by 50% compared to cells not exposed to anthocyanins. Thus, cherries may have a protective effect on diabetes.

Tart cherry intake reduces the expression of pro-inflammatory genes in abdominal fat that is a major source of inflammation in obesity and associated diseases (Seymour et al., 2008). In addition, Tsuda, et al., (2003) reported that high-fat diet supplementation with cherry anthocyanin extract reduced hyperglycemia, hyperinsulinemia, hyperleptinemia, and body weight. A high-fat diet supplemented with 1% freeze-dried, tart cherry powder significantly reduced fasting glucose, insulin, total cholesterol, triglyceride, body weight, and abdominal fat (Seymour et al. 2009). Kim, et al., (2005) demonstrated that tart cherry-derived anthocyanins have a protective effect on neuronal cells from oxidative stress and improve cell survival.

Anthocyanin consumption before ischemic injury induction significantly reduced the brain infarct volume and neuronal damage (Shin, Park, and Kim 2006). In addition, oral
administration of anthocyanins to animals with cerebral ischemic injury (stroke) significantly reduced brain tissue damage, suggesting that the consumption of anthocyanins may have a protective effect against neurological disorders (Kim, et al., 2011).

Howatson et al., (2012) revealed that dietary supplementation with Montmorency tart cherry juice concentrate significantly increased circulating melatonin and improved the time in bed, total sleep time, and sleep efficacy. Tart cherry juice blend administration for two weeks to older adults with insomnia reported sleep enhancement with statistically significant improvement in insomnia severity (Pigeon, et al., 2010). Losso et al. (2017) fed 8 individuals with insomnia with tart cherry juice (240 ml, 2 times/day) and explained that the sleep-enhancing effect of tart cherry juice is associated with the inhibition of the enzyme indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), and increased tryptophan in the plasma.

A mouse model of intestinal cancer revealed that animals fed with anthocyanins from tart cherry extract reduced cecal and colon tumor development by 74% and 17%, respectively compared to untreated animals (Wang and Stoner 2008). Kang et al., (2003) reported that tart cherry anthocyanins and cyanidin could reduce cell growth of human colon cancer cell lines in vitro, suggesting that tart cherry may reduce the risk of colon cancer.
CHAPTER 3: MATERIALS AND METHODS

3.1 Cherries samples and cherry puree preparation

Montmorency whole tart cherries (*Prunus cerasus*) were provided by Cherry Marketing Institute. (Lansing, Mich., U.S.A). Cherries were stored at -80 °C before use. Frozen Montmorency tart cherries were ground in a blender (Magic Bullet ®, Homeland Housewares, Los Angeles, CA. USA.) to develop a puree. The puree was transferred into a 15-ml polypropylene tube and stored at -80 °C before use.

3.2 Chemicals

The dextran sulfate sodium salt (DSS) of MW ~ 40,000, phosphate buffered saline (PBS) pH 7.4, protease inhibitor cocktail, 50 mM potassium phosphate buffer, o-dianisidine hydrochloride, hydrogen peroxide, methanol, hydrochloric acid (HCl), isoflurane, formaldehyde, tris buffer saline solution, tween 20®, hexadecyltrimethylammonium bromide (HTAB) were purchased from Sigma Aldrich (St. Louis, MO., USA). LDS sample buffer, 4-12% bis-tris SDS polyacrylamide gel, west pico substrate, enhanced chemiluminescent HRP-substrate, PVDF membrane (0.4 µm pore size) and the Bicinchoninic Acid (BCA) protein assay were purchased from Invitrogen, Thermo Fisher Scientific (Walthan, MA., USA). The primary antibodies β-actin, JAK1, JAK3 and the conjugated secondary antibody (anti-rabbit) were purchased from Cell Signaling Technology (Danver, MA., USA). Anthocyanin standard were purchased from ChromaDex (Irvine, CA., USA). All reagents were of analytical reagent grade or UHPLC grade, as required.
3.3 HPLC-PDA-MS analysis of anthocyanin content in tart cherry puree

Sample preparation: Fifteen grams of frozen Montmorency tart cherries were ground in a blender (Magic Bullet ®, Homeland Housewares, Los Angeles, CA. USA.) with 15 ml of water until the mix was pureed. Then, 0.5 g of puree was added to 20 ml methanol acidified with 0.1% HCl. For extraction, samples were placed in an orbital shaker for 1 hour at room temperature and centrifuged at 2900 x g for 10 min. The supernatant was filtered using a syringe disposable filter device (25 mm GD/x, PTFE filter media, 0.45 μm), freeze-dried and diluted with 300 μl methanol.

HPLC-PDA-MS analysis of anthocyanins: Anthocyanins were evaluated by reverse phase chromatography using a Shimadzu UHPLC-PDA (Nexera-i LC-2040C 3D system) coupled to a Shimadzu MS (LCMS-8040), a Restek Pinnacle DB C18 (50 × 2.1 mm, 1.9 μm) column and solvents A: 4.5% formic acid acidified water and B: acetonitrile. Separation was achieved with a flow rate of 0.3 mL/min and a gradient of 2% B for 0-1 min and 2-10% B from 1-20 min at 60 °C. Ten μl of each of three-diluted samples was injected for analysis. Spectral data were collected using a photodiode array detector from 250-700 nm. Mass spectrometry was performed under positive ion mode; data were monitored using Q3 total ion scan (SCAN, from m/z 100-1100), and selected ion monitoring was conducted for m/z 271, 287, 301, 303, and 331, representing common anthocyanins (Tian et al. 2005).

3.4 Animals

Adult male Sprague – Dawley rats weighing 250 ± 50 g were provided by Charles Rivers Laboratory. Animals were allowed to acclimate to the laboratory conditions 1 week before the experiment at room temperature (22 ± 1°C). Animals were housed in individual metabolic cages in a temperature-controlled environment (22 ± 1°C) with 55 ± 10% relative humidity and
controlled lighting (12 h light/dark cycle). Animals had access to standard laboratory rodent chow diet (Labdiet® rodent chow 5001) and water *ad libitum*. All experiments were carried out following the guideline of the Institutional Animal Care and Use Committee (IACUC) of Louisiana State University.

3.5 Experimental design

Animals were randomly assigned to one of 9 groups, each group had six rats (n= 6), as described below.

Group 1: Normal diet rats. These rats were fed with normal diet and received saline water by intragastric gavage for 5 days. Animals were euthanized on day 6.

Group 2: DSS colitis induction rats. These rats were induced with 4% Dextran sulfate sodium salt (DSS) in drinking water for 5 days for development of UC. Animals were euthanized on day 6.

Group 3: Tart cherry control rats. These rats were fed with the equivalent of one serving size (2.21g/kg- body weight) of tart cherry puree by intragastric gavage for 14 days. Animals were euthanized on day 15.

Group 4: Prevention group 1. These rats were fed with the equivalent of one serving size (2.21g/kg- body weight) of tart cherry puree by intragastric gavage for 14 days, followed by the induction of UC with 4% Dextran Sodium Sulfate (DSS) for 5 days in drinking water. Animals were euthanized on day 20.

Group 5: Prevention group 2. These rats were fed with the equivalent of two serving sizes (2.21g/kg- body weight) of tart cherry puree by intragastric gavage for 14 days, followed by the induction of UC with 4% Dextran Sodium Sulfate (DSS) for 5 days in drinking water. Animals were euthanized on day 20.
Group 6: Intervention group 1. These rats were fed with the equivalent of one serving size (2.21g/kg- body weight) of tart cherry puree by intragastric gavage and simultaneously were induced with 4% Dextran Sodium Sulfate (DSS) for 5 days in drinking water for development of UC. Animals were euthanized on day 6.

Group 7: Intervention group 2. These rats were fed with the equivalent of two serving sizes (2.21g/kg- body weight) of tart cherry puree by intragastric gavage and simultaneously were induced UC in drinking water with 4% Dextran Sodium Sulfate (DSS) for 5 days. Animals were euthanized on day 6.

Group 8: Treatment group 1. These rats were induced with 4% Dextran Sodium Sulfate (DSS) for 5 days in drinking water for development of UC, followed by the feeding the equivalent of one serving size (2.21g/ 70 kg- BW) of tart cherry puree by intragastric gavage for 14 days. Animals were euthanized on day 20.

Group 9: Treatment 2. These rats were induced with 4% Dextran Sodium Sulfate (DSS) for 5 days in drinking water for development of UC, followed by the feeding the equivalent of two serving sizes (2.21g/kg- body weight) of tart cherry puree by intragastric gavage for 14 days. Animals were euthanized on day 20.

3.6 Determination of disease activity index

The severity of DSS-induced UC was assessed by a disease activity index (DAI) including weight loss, stool consistency, and colorectal bleeding on each day and by each group throughout the 5 days of UC induction. DAI was scored from - (healthy) to ++++ (maximal colitis activity), as shown in Table 1 (Murthy et al. 1993). The presence of blood in feces was determined using a commercial test (Hemoccult®, Fecal Occult Blood Test, Beckman Coulter, Brea, CA.).
Table 3. Criteria for scoring disease activity index*

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight loss (%)</th>
<th>Stool consistency[1]</th>
<th>Occult blood or gross bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>None</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td>+</td>
<td>1 - 5</td>
<td>Loose stool</td>
<td>Negative</td>
</tr>
<tr>
<td>++</td>
<td>5 - 10</td>
<td>Loose stool</td>
<td>Hemoccult positive</td>
</tr>
<tr>
<td>+++</td>
<td>10 - 15</td>
<td>Diarrhea</td>
<td>Hemoccult positive</td>
</tr>
<tr>
<td>++++</td>
<td>&gt;15</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
</tbody>
</table>

*Disease activity index = (combined score of weight loss, stool consistency, and bleeding)

\[1\] Normal stools = well-formed pellets; loose stool = pasty stool that does not stick to the anus; and diarrhea = liquid stools that stick to the anus.

3.7 Tissue collection

At the end of the experiments, animals were humanely euthanized. Rats were anesthetized with isoflurane and euthanized by cardiac exsanguination. The colon was opened longitudinally and cleaned with PBS buffer (Morampudi et al. 2014). The weight and length of the colon were recorded.

Macroscopic damage was assessed using an established scoring system as follows: -, no ulcer and no inflammation; +, local hyperemia without ulceration; ++, ulceration without hyperemia; ++++, ulceration and inflammation at one site only; +++++, two or more sites of ulceration and inflammation; ++++++, ulceration extending more than 2 cm.

The colon was divided into 5 sections of approximately 3 cm. Four sections were transferred to Eppendorf tubes for the snap freeze to be later stored at -80°C for assays for cytokine amounts (IL-1β, IL-6, IL-10, IL-12, IL-17), tumor necrosis factor-α (TNF-α), Janus kinases (JAK1 and JAK 3), and myeloperoxidase (MPO) activity. The last section of the distal colon was fixed in 10% formaldehyde buffer for histological analysis.
3.8 Histological examination of the colon

The fixed colon piece was sectioned and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. Histological changes were graded as follows: -, no sign of inflammation; +, very low level of leucocyte infiltration; ++, low level of leucocyte infiltration; ++++, high level of leucocyte infiltration, high vascular density, and thickening of the colon wall; +++++, transmural infiltration, loss of goblet cells, high vascular density, and thickening of the colon wall.

3.9 Determination of cytokines IL-1ß, IL-6, IL-8, IL-12/p40, IL-17A or TNF-α

Fifty milligrams of colonic tissue was homogenized using a tissue grinder (FastPrep®-24, MP Biomedical, Solon, OH.) and suspended in PBS (pH 7.4) containing protease inhibitor cocktail. The homogenized solution was centrifuged at 10,000 x g for 10 min at 4°C.

Total protein in the supernatant was determined by the Bicinchoninic Acid (BCA) protein assay. After BCA assay, the supernatant was used to determine the levels of TNF-α and interleukins by The Bio-Plex® multiplex system (Bio-Rad, Hercules, CA). The Bio-Plex is built upon three core elements of Xmap technology: fluorescence dyed microspheres, flow cytometry-based, and a high-speed digital signal processor. The equipment used the Bio-Plex Pro™ kit containing a 96-well plate, diluent components, reagents, magnetic beads and detection antibodies. The assay followed the protocol provided by Bio-Rad (Figure 5). The results were expressed as picogram per milligram of protein tissue.
**3.10 Determination of Janus kinases JAK 1 and JAK3 levels by western blot**

One hundred milligrams of colonic tissue was homogenized using a tissue grinder (FastPrep®-24, MP Biomedicals, Solon, OH.) and suspended in CelLytic containing protease inhibitor cocktail. The supernatant was stored at -80°C until use. Total protein in the supernatant was determined by the Bicinchoninic Acid (BCA) protein assay. Equal amounts of protein (725 μg) of colon protein were mixed with LDS sample buffer boiled for 5 min and vortexed at a high setting. Twenty-five microliters of each sample were loaded to each lane of a 4-12% Bis-Tris...
SDS polyacrylamide gel. Separation was performed using the Mini cell (XCell SureLock®, Novex by Life Technologies, Carlsbad, Ca., USA) at voltage (v): 30, (m.A.): 170 for 1 h and 10 min.

Proteins of interest were transferred to a polyvinylidene fluoride (PVDF) membrane (0.4 μm pore size) and blocked in 0.2% I-BLOCK in Tris buffered saline with 0.1% Tween-20® (TBST) for 1 h. The primary antibody was prepared in 0.2% I-BLOCK and incubated with the membrane overnight at 4°C on a shaker. The membrane was washed three times for 5 minutes using TBST, incubated for one hour with secondary antibody and washes were repeated. Visualization of the bound antibody was done in a dark room using West Pico Substrate, an enhanced chemiluminescent HRP-substrate and a BioRad ChemiDoc MP System (Hercules, CA). After analyzing band density, membranes were stripped and reprobed with β-actin to serve as a loading control. Results were reported as a ratio of the density of each band to its β-actin.

3.11 Determination of rat colonic myeloperoxidase activity (MPO)

Fifty milligrams of colonic tissue was homogenized using a tissue grinder (FastPrep®-24, MP Biomedicals, Solon, OH) and suspended in CelLytic containing myeloperoxidase homogenization buffer (0.5% hexadecyl trimethyl-ammonium bromide (HTAB) in 50 mM potassium phosphate buffer at pH 6.0). The homogenized solution was centrifuged at 10,000 x g, for 10 min at 4 °C. One hundred microlites of supernatant was mixed with 2.9 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.17mg/ml o-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The changes in absorbance were measured at 450 nm using a DX800 spectrophotometer (Beckman Coulter, Pasadena, CA, USA).
3.12 Statistical analysis

Eight single degrees of freedom contrasts were performed (PRV1+PRV2 vs DSS; INT1+INT2 vs DSS; TRT1+TRT2 vs DSS; NC vs DSS; INT1 + INT2 vs PRV1 + PRV2; INT1 + INT2 vs TRT1+TRT2; PRV1+PRV2 vs TRT1+TRT2; PRV1+INT1+TRT1 vs PRV2+INT2+TRT2) that were within the degrees of freedom. The differences were considered significant at the $p < 0.05$. Additionally, six post hoc single degree of freedom contrasts (PRV1 vs DSS; PRV2 vs DSS; INT1 vs DSS; INT2 vs DSS; TRT1 vs DSS; TRT2 vs DSS), were performed and adjusted using Bonferroni correction ($\alpha < 0.05/6$ for new $\alpha < 0.00833$). The data were expressed as the mean ± SEM. Statistical analysis was conducted using the Statistical Analysis Software (SAS) version 9.4.
CHAPTER 4: RESULTS AND DISCUSSION

4.1 UHPLC analysis of Montmorency tart cherry puree

To determine the anthocyanin composition and quantity of Montmorency tart cherry UHPLC-PDA-MS analysis of cherry was done. The UHPLC chromatogram is illustrated in Figure 6. Samples were injected following the extraction method of Ou et al. 2012a, however, the resolution was very poor, likely due to the solvent carrier of methanol. Subsequent samples were diluted with 0.01% HCl acidified water (10 µl sample with 30 µl), resulting in highly improved chromatographic results.

Figure 6. UHPLC chromatogram of cherry extracts. The UHPLC analysis was performed with Nexera-i LC-2040C 3D system. Detection at 520 nm.

Five major peaks were separated from the cherry extracts, Figure 6. Peak 1 (cyanidin-3-sophoroside) and peak 2 (cyanidin-3-glucoside) were minor peaks accounting for 5.1% and 8.4% of the area under the curve (520nm), respectively. Peak 3 (cyanidin-3-glucosyl-rutinoside) was the major anthocyanin in the cherry extracts, accounting for 49% of AUC (520 nm).
Peak 4 (cyanidin-3- rutinoside) also accounted for a large portion of the anthocyanins (35.4%) identified in the cherry extracts. Peak 5, a peonidin derivative (3-rutinoside) was also identified as a minor anthocyanin (2.2%). Anthocyanins peaks were identified based on order of elution, spectral characteristics, and m/z data from mass spectrometry. The anthocyanin composition of Montmorency tart cherries found in this study is consistent with previous reports (Tian et al. 2005, Chaovanalikit and Wrolstad 2004, Bonerz et al. 2007, Ou et al. 2012a). Qualitative data of the peak areas are described in Table 4.

Table 4. Characterization of anthocyanins of cherry extracts samples, n=3

<table>
<thead>
<tr>
<th>Peak</th>
<th>Identity*</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>m/z</th>
<th>%AUC**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyanidin-3-sophoroside</td>
<td>514</td>
<td>611 (287)</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>Cyanidin-3-glucoside</td>
<td>514</td>
<td>449 (271)</td>
<td>8.4 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>Cyanidin-3-glucosyl-rutinoside</td>
<td>517</td>
<td>757 (287)</td>
<td>48.9 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>Cyanidin-3-rutinoside</td>
<td>515</td>
<td>595 (287)</td>
<td>35.4 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>Peonidin-3-rutinoside</td>
<td>517</td>
<td>609 (301)</td>
<td>2.2 ± 0.0</td>
</tr>
</tbody>
</table>

*Tentative identified based on order of elution, spectral characteristics, m/z data form mass spectrometry, and comparison to literature.

**%AUC: area under the curve in the 520 nm chromatogram.

Quantitative values of anthocyanins in the extracts were calculated based on the standard curve of cyanidin-3-glucoside built as a reference material. The curve showed a good fit by linear regression \((R^2 = 0.9991)\) with injections of cyanidin-3-sophoroside amounts of 0.05 – 1.25 \(\mu\)g; which means a high confidence to determine the concentration of the anthocyanins as a function to the peak area (AUC*min) integrated into the chromatogram. Anthocyanin concentrations of the extracts were determined to be \(5.45 \pm 0.87\) mg /serving size cyanidin-3-glucoside equivalents, as determined by UHPLC calibration curve (520 nm; Table 5).
The most prevalent anthocyanin of the cherry extracts was cyanidin-3-glucosyl-rutinoside (2.65 ± 0.30 mg/serving size, accounting for the majority of the total anthocyanins. Cherry extract 1 contained the highest content of anthocyanins while cherry extract 2 contained the lowest content of anthocyanins.

Table 5. Anthocyanin content (mg/serving size) in Montmorency tart cherries

<table>
<thead>
<tr>
<th>Peak</th>
<th>Identity</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyanidin-3-sophoroside</td>
<td>0.65 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>Cyanidin-3-glucoside</td>
<td>1.06 ± 0.26</td>
</tr>
<tr>
<td>3</td>
<td>Cyanidin-3-glucosyl-rutinoside</td>
<td>6.14 ± 0.70</td>
</tr>
<tr>
<td>4</td>
<td>Cyanidin-3-rutinoside</td>
<td>4.47 ± 0.68</td>
</tr>
<tr>
<td>5</td>
<td>Peonidin-3-rutinoside</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12.60 ± 1.81</td>
</tr>
</tbody>
</table>

One serving of frozen cherries or 155 g.

4.2 Induction and course of colitis

The use of DSS in drinking water may promote the development of acute or chronic colitis in animal models. The severity and response to DSS, i.e. induction of colitis may vary according to DSS concentration, duration of DSS supplementation, DSS molecular weight, genetics of the animal, intestinal flora, and stress (Perše and Cerar 2012, Melgar et al. 2008, Vowinkel et al. 2004).

The Disease Activity Index (DAI) is a score that has been used to determine the severity of DSS-induced colitis throughout an induction period. The induction period for this study was 5 days at 4% DSS in drinking water. The minimum possible result of DAI is - (Healthy) and the maximum possible result is ++++ (Maximal colitis activity) (Murthy et al. 1993).
Results of DAI in Table 6 show that treatment 1 (TRT1; ++++) followed by prevention 1 (PRV1; +++), dextran sodium sulfate group (DSS; ++++) and intervention 1 (INT 1; ++) had a DAI higher or equal than ++. These animals showed 10 – 15% of weight loss, diarrhea, and occult blood during the DSS induction period. Consequently, TRT1, PRV1, DSS, INT1 developed moderate to severe UC (Okayasu et al. 1990). Perše and Cerar (2012), used 40 kDa DSS in an animal model and induced moderate to severe colitis in the rats. This finding is comparable to the results of this study because the molecular weight of the DSS used in the study was (~40 kDa). However, prevention 2 (PRV2; +) and intervention 2 (INT 2; +) developed mild UC even when they received the same dose (4%) of DSS. They showed 1-10% of weight loss, normal or loose stool, and negative occult blood during the DSS induction period (Okayasu et al. 1990). According to previous research (Bibi et al. 2018), changes on the type of induced UC is due to the presence of an external agent such as bioactive compounds, and in this case PRV2 and INT2 groups were treated with a daily double serving (4.42 g/kg/day) of cherries a day prior to and during the DSS colitis induction respectively. Tart cherries and sweet cherries are good source of anthocyanins, quercetin, hydroxycinnamates, and melatonin that support the potential disease preventive health benefits of cherry intake in relation to cancer, cardiovascular diseases, and inflammatory diseases such as UC (Lachin 2014). Tall et al. (2004), used an animal model to produce inflammation with carrageenan and showed that the supplementation with tart cherry anthocyanins (400 mg/kg/day) reduced the induced inflammation in rats. Procyanidin, cyanidin, and anthocyanidin extracts from grapes and plants significantly improved UC symptoms by downregulating some mediators involved in the intestinal inflammatory response (Awaad, El-Meligy, and Soliman 2013). Therefore, we could say that cherry supplementation as a daily
double serving (4.42 g/kg/day) might help to develop only a mild form of UC instead of a severe UC in PRV2 and INT2 groups.

Table 6. Disease activity index (DAI) during five days of colitis induction

<table>
<thead>
<tr>
<th>DSS</th>
<th>Prevention</th>
<th>Intervention</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

DSS untreated UC group; NC negative control; TCH tart cherry for 14 days; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction, and TRT2 UC group treated orally with 4.42g/kg/day tart cherries after UC induction.

4.3 Effect of tart cherries on DSS-induced morphological colonic changes

Macroscopic and microscopic analyses of rat tissues were performed in this study. The damage of macroscopic changes was scored from - (no ulcer, no inflammation) to ++++ (ulceration extending more than 2 cm) and microscopic changes from - (no inflammation) to ++++ (transmural infiltration, loss of goblet cells, high vascular density, thickening of the colon).

For microscopic analysis, results in Table 7 show that DSS (+++), followed by INT2 (++), and TRT1 (+++) had a score of ++ or higher than ++. These scores suggest that, animals in DSS, INT2, and TRT1 groups presented a low and high level of leucocyte infiltration, high vascular density, and thickening of the colon wall; signs that can be seen in Figure 7 C, G and H.

Rats in group TRT2 (+) followed by PRV1 (+), INT1(+), PRV2 (+) had a score less than ++. Figure 7 I, D, F, E. shown that these animals presented very low or low levels of leucocyte infiltration. This might suggest that TRT2, PRV1, INT1, PRV2 groups compared to DSS group shown an improvement in the colon due to the cherry supplementation either at single or double serving. In addition, Figure 7 A and B shown the negative control group (NC) and tart cherry
supplementation only (TCH) respectively and these groups had a negative score (-) which means that these animals presented no inflammation and no ulceration in the colon.

Geboes (2003) said that microscopic analysis is considered a tool for measuring the disease activity of UC. The results of this study agree with Geobes because our microscopic scores for DSS, INT2 and TRT1 reflected the disease activity that our DAI score did.

For the macroscopic analyses, DSS (+) followed by PRV1 (+), PRV2 (+), INT1 (+), INT2 (+), TRT1(+), and TRT2 (+) had a score equal to +. Animals showed local hyperemia without ulceration or ulceration without hyperemia. NC (-) and TCH (-) had a negative score. Animals showed no inflammation and no ulceration.

Table 7. Microscopic and macroscopic alteration score into the different groups

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<td><strong>Intervention</strong></td>
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DSS untreated UC group; NC negative control; TCH tart cherry for 14 days; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction.
Figure 7. Representative colonic images of rats (5x); (A) NC, (B) TCH (C) DSS, (D) PRV1, (E) PRV2, (F) INT1, (G) INT2, (H) TR1, (I) TRT2.
4.4 Effects of tart cherries on inflammatory biomarkers

4.4.1 Effect of tart cherries on myeloperoxidase activity (MPO)

Myeloperoxidase (MPO) is secreted by activated neutrophils and it is considered a marker of neutrophil infiltration into the colon and a specific biomarker of acute inflammation (Pervin et al. 2016). MPO is released to catalyze the formation of potent oxidants. Consequently, MPO-derived oxidants contribute to tissue damage and the initiation of acute and chronic vascular inflammatory disease such as UC (Liu et al. 2011). In this study, the results in Figure 7 show that MPO activity significantly increased in the DSS group compared to the negative control (NC). MPO activities in INT2 and TRT1 groups were not significantly different from DSS. The levels of MPO activities in DSS, INT2, and TRT1 groups suggest that these rats presented neutrophil infiltration in the colon (Pervin et al. 2016). This affirmation is in agreement with the results obtained on the MPO levels and on microscopic scores in these groups (Table 7). These results suggest that these animals showed leucocyte infiltration in the colon and could explain why MPO levels of INT2 and TRT1 groups were not significantly different from DSS (p > 0.05).

MPO activity was significantly reduced in PRV2 group compared to the DSS group. Reduced MPO activity (3.56 ± 0.28), DAI score (1.41 ± 1.12) and microscopic score (1.00 ± 0.89) in PRV2 group compared to DSS group suggested that tart cherry supplementation had an anti-inflammatory effect on UC induction. Our findings are comparable to other studies showing the preventive and therapeutic effect of oral administration of bioactive compounds such as cyanidins and anthocyanins in DSS-induced colitis (Bibi et al. 2018). Therefore, tart cherry supplementation serving of 4.42 g/kg/day prior to the DSS treatment could have led to less neutrophil infiltration and tissue damage compared to the other groups.
Figure 8. Effect of whole tart cherries on myeloperoxidase activities in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).

4.4.2 Effect of tart cherry on cytokine levels

Cytokines are produced by activated lymphocytes, macrophages, dendritic cells, endothelial cells, epithelial cells, and connective tissue cells that mediate and regulate immune and inflammatory reactions (Burska, Boissinot, and Ponchel 2014). Interleukin - 1 beta (IL-1-β) is produced by blood monocytes, tissue macrophages, and skin dendritic cells in response to an inflammatory process (Garlanda, Dinarello, and Mantovani 2013). IL-1-β is an important mediator of intestinal inflammation (Dinarello 2009). In this study, the results in Figure 9 showed that IL-1-β did not have any significant difference compared to DSS group and between groups.
These results differ from an animal model study where rats were fed red raspberries after UC induction and the researchers found lower levels of IL1-β in groups treated with red raspberries than positive control groups (Bibi et al. 2018). However, Leal et al. (2008) found high levels of IL-1-β in healthy and sick tissues of UC patients and they concluded that even under non-inflammatory conditions, patients present some inflammatory activity. Nevertheless, high values of SE of this marker might suggest that IL1-β was not a good biomarker for UC.

![Figure 9. Effect of whole tart cherries on IL-1-β in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).]
IL-6 is a proinflammatory cytokine that is considered an indicator of the inflammatory state and is used as a biomarker in inflammatory diseases as the same as IL-1β and TNF-α (Burska, Boissinot, and Ponchel 2014; Zhou, Nair, and Claycombe 2012). IL-6 was measured in this study and results in Figure 10 show a significant increase in the level of IL-6 in DSS group compared to NC group. Literature suggests that elevated levels of IL-6 reflect the degree and the presence of the disease, which means that DSS administration significantly increased the level of IL-6 and the rats accurately developed signs of UC. These results were also seen in DAI score shown in Table 7 (Kishimoto 2005; Mankowska-Wierzbicka et al. 2015). Significantly reduced levels of IL-6 were found in PRV1, PRV2, INT1, INT2, TRT1, and TRT2 groups compared to DSS group. These results suggest that tart cherry supplementation in a single serving (2.21g/kg/ day) or double serving (4.42g/kg/ day) worked as an anti-inflammatory agent in reducing IL-6 expression. However, double serving (4.42g/kg/ day) (Figure 11) had a significantly lower level of IL-6 compared to a single serving (2.21g/kg/ day). Our results are comparable to Zhou, Nair, and Claycombe (2012), who found that a mixture of tart cherry anthocyanins and cyanidin-3-glucoside used in culture mouse adipose stem cells reduced the secretion of IL-6. Consumption of tart cherry juice (16 oz of commercial juice) after a marathon improved muscle recovery by reducing inflammatory markers such as IL-6 (G Howatson et al. 2010). All previous and our current results are in agreement with the anti-inflammatory function of tart cherry.
Figure 10. Figure 4.5 Effect of whole tart cherries on IL-6 in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group).

Figure 11. Serving effect of whole tart cherry on IL-6 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM. DSS untreated UC; Single serving represents UC groups treated orally with 2.21g/kg/day tart cherries prior, during, and after UC induction; Double serving represents UC groups treated orally with 4.42g/kg/day tart cherries prior, during, and after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).
TNF-α is produced by macrophages, lymphocytes and natural killer cells and plays an important role in the inflammatory process (Murch et al. 1991; Tracey et al. 2008). High levels of TNF-α have been found in blood, stool and intestinal tissues of UC patients (Braegger, Nicholls, and Murch 1992). Results in Figure 12 show a significant increase in the level of TNF-α in the DSS group compared to NC group. Similar results have been reported by others (Braegger, Nicholls, and Murch 1992). TNF-α activates monocytes and vascular endothelial cells to secret other pro-inflammatory cytokines, such as IL-6 and IL-1β. This effect is known as the cascade effect that results in an inflamed lesion of tissue (Murthy et al. 1993).

TNF-α produces mild inflammation marked by neutrophil accumulation (Tursi et al. 2011). Figure 7 showed that DSS animals in this study had inflamed tissue, vascularization, and infiltration. Myers et al. (2003), suggested that is necessary to inhibit, prevent or alleviate the expression of TNF-α in the early stage of DSS colitis. Results in Figure 12 show that PRV1, PRV2, INT1, INT2, TRT1, and TRT2 groups had significantly lower levels of TNF-α than the DSS group when they received a tart cherry supplementation either at single serving (2.21/kg/day) or double serving (4.42g/kg/day). Thus, tart cherry consumption inhibits, prevent or alleviate the expression of TNF-α in a DSS colitis animal model. The double serving (4.42g/kg/day) had a better effect on TNF-α than a single serving (2.21/kg/day). Down-regulation of TNF-α in UC patients was associated with histological and clinical improvement (Tursi et al. 2011). Figure 7 shows histological improvement compared to the DSS group.

In this study, down-regulation of TNF-α was associated with histological improvement in animals that receive tart cherry supplementation as part of the experimental model. Our results compare well to results from animal models of ischemic stroke that received 300 mg/kg/day of anthocyanin and showed a significant reduction on TNF-α (Shin, Park, and Kim 2006).
Tart cherry extracts reduced inflammation, oxidative stress and production of TNF-α in microglial cells (Shukitt-Hale et al. 2016). The results of this study and previous studies that use tart cherries support the hypothesis that tart cherries inhibit inflammation by down-regulating TNF-α.

Figure 12. Effect of whole tart cherries on TNF-α levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group), a P < 0.008 (vs. DSS group).
Figure 13. Serving effect of whole tart cherries on TNF-α levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM. DSS untreated UC; Single serving represents UC groups treated orally with 2.21g/kg/day tart cherries prior, during, and after UC induction; Double serving represents UC groups treated orally with 4.42g/kg/day tart cherries prior, during, and after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).

IL-12 is produced by NK cells, T cells, dendritic cells, and phagocytes. It regulates the immune response and can increase the production of Th1 and Th17 cytokines (Kaufman and Dharmadhikari 2017). IL-12 levels were measured and were higher in the DSS group compared to the NC group (Figure 14). Tart cherry supplementation in a single serving (2.21g/kg/ day) or double serving (4.42g/kg/ day) significantly reduced the levels of IL-12 compared to DSS group. There was not a significant difference between prevention, intervention, and treatment. However, there was a serving effect (Figure 15), where the double serving (4.42g/kg/ day) significantly reduced IL-12 levels compared to the DSS group and also had a significantly lower level of IL-12 compared to the single serving (2.21g/kg/ day). In rheumatoid arthritis patients, high levels of IL-12 were positively correlated with high levels of IL-6 and TNF-α but inversely correlated with IL-10. (Kim et al. 2000). The similarity between our study and the human study by Kim et al. (2000) lies in the trend. This rat study also reports high levels of IL-6, TNF-α and low levels
of IL-10. Both studies were on autoimmune diseases, and both demonstrated that IL-12 inhibition can help manage the disease.

Figure 14. Effect of whole tart cherries on IL-12 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SE of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group).

Figure 15. Serving effect of whole tart cherries on IL-12 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM. DSS untreated UC; Single serving represents UC groups treated orally with 2.21g/kg/day tart cherries prior, during, and after UC induction; Double serving represents UC groups treated orally with 4.42g/kg/day tart cherries prior, during, and after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).
IL-17 is secreted by Th17 cells and plays an important role in inflammatory responses including localization and amplification of inflammation (Mangan et al. 2006; Strober and Fuss 2011). IL-17 enhances the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1-β. Consequently, high levels of IL-17 activates an inflammatory cascade that causes damage in the mucosal barrier (Ruddy et al. 2003). Results in Figure 16 show a significant increase in the level of IL-17 production in the DSS group compared to the NC group suggesting that DSS administration activated Th 17 cells in the rats. In addition, significantly reduced levels of IL-17 were found in INT1, PRV2 and TRT2 groups (Figure 16). These groups had tart cherry supplementation at a single serving (2.21/kg/day) and a double serving respectively (4.42/kg/day). However, a serving effect was seen as double serving (4.42g/kg/day) significantly reduced IL-17 levels compared to the single serving (2.21g/kg/day) and DSS group.

The results of this study are comparable to findings of a clinical study that reported significantly higher IL-17 expression in UC patients compared to healthy controls (Fujino 2003). Result in Figure 4.11 show a similar pattern in INT1, PRV2, and TRT2 groups that had lower expression of IL-17 compared to the control group (NC). IL-17 inhibition in UC - induced models are seldom investigated. However, a study on a Th17 knockout animal model, found that mice developed mild or no colitis compared to control animals that developed severe colitis with DSS treatment (Oppmann et al. 2000; Yang et al. 2008; Karow et al. 2006). These findings suggest that the presence of Th17 cells and the secretion of IL-17 might mediate inflammation to a severe form of UC. Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusbo (2008), as part of potential biological therapies in inflammatory diseases, suggested that controlling the expression of IL-23 and IL-17 would allow developing a treatment strategy with anti-
inflammatory efficacy and with suppressive effects on host defenses. Results from this study suggest the potential of tart cherries as inhibitors of IL-17.

Figure 16. Effect of whole tart cherries on IL-17 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group).

Figure 17. Serving effect of whole tart cherries on IL-17 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the means ± SE. DSS untreated UC; Single serving represents UC groups treated orally with 2.21g/kg/day tart cherries prior, during, and after UC induction; Double serving represents UC groups treated orally with 4.42g/kg/day tart cherries prior, during, and after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).
IL-10 is an anti-inflammatory cytokine mainly secreted by Th 2 cells, T cells, and B cells (Radford-Smith and Jewell 1996). IL-10 inhibits antigen presentation to cells (APC), macrophages, and subsequent secretion of pro-inflammatory cytokines such as IL-1β, IL-1α, IL-6, IL-8, TNF-α (Jahr et al. 1975). IL-10 and other regulatory cytokines play a key role in downregulating the secretion of pro-inflammatory cytokines due to an aberrant immune response caused by diseases like rheumatoid arthritis, asthma, or UC (Germann and Rüde 1995). Results in Figure 18 show that DSS administration to the animals significantly reduced the IL-10 level in the DSS group compared to the NC group. Melgar et al. (2003) showed that, low levels of IL-10 are correlated with granuloma presence in inflamed tissues. Results from this study show the presence of granuloma formation in cross-sectional colon slides from DSS rats (Figure 4.2). Results from this study also show no serving effect in IL-10 suggesting that either the single serving (2.21/kg/ day) or the double serving (4.42g/kg/ day) similarly enhanced the anti-inflammatory function of IL-10. Tart cherry supplementation servings significantly increased IL-10 levels compared to DSS group (Figure 18). The elevated levels of IL-10 could be explained by the ability of tart cherries to downregulate the secretion of pro-inflammatory cytokines and up-regulated the secretion of anti-inflammatory IL-10, in the DSS-induced UC model (Germann and Rüde 1995).

IL-10 inactivates Th1 cells, NK cells, and IN-γ and reduces the secretion of IL-1α, IL-1β, IL-6, IL-8, TNF-α, and IL-12 (Sanchez-Munoz, Dominguez-Lopez, and Yamamoto-Furusho 2008; Schreiber, et al., 1995). Tart cherry supplementation either at single (2.21/kg/ day) or double serving (4.42g/kg/ day) significantly increased IL-10 levels and reduced IL-12, IL17, and TNF-α levels.
Figure 18. Effect of whole tart cherries on IL-10 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group).

Figure 19. Serving effect of whole tart cherries on IL-17 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the means ± SE. DSS untreated UC; Single serving represents UC groups treated orally with 2.21g/kg/day tart cherries prior, during, and after UC induction; Double serving represents UC groups treated orally with 4.42g/kg/day tart cherries prior, during, and after UC induction. All animals were fasted for 24 hours before euthanasia. P < 0.05 (vs. DSS group).
4.4.4 Effect of tart cherries on JAK1 and JAK3 expressions

JAK1 is an essential tyrosine kinase protein for signaling Th1 and Th2 cytokines. The inhibition of JAK1 attenuates the proliferation of pro-inflammatory cytokines including IL-6 and interferon-gamma (Coskun et al. 2013). IL-6 and JAK1/STAT3 pathway had been identified as the major actors of the initiation and development of inflammation in IBD, like UC. Therefore, there is the potential of JAK1 inhibition for the treatment of UC (Galien 2016). This study evaluated the expression of JAK1 in the colon of rats by Western blotting. The results (Figure 20) showed a significantly increased expression of JAK1 in the DSS group compared to the NC group. The results suggest that elevated levels of IL-6 in the DSS group activated the JAK1/STAT 3 pathway (Coskun et al. 2013). However, a significantly reduced expression of JAK3 was seen in PRV1, INT2, TRT1, and TRT2 groups. These results might support the protective effect of tart cherry supplementation in a DSS-colitis animal model. These animals were treated with a single serving (2.21/kg/ day) or a double serving (4.42/kg/ day) of tart cherry, prior, during and after DSS-colitis induction.
Figure 20. Effect of whole tart cherries on JAK1 levels in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group), a P < 0.008 (vs. DSS group).

JAK3 is a nonreceptor tyrosine kinase expressed in hematopoietic and nonhematopoietic cells. JAK3 mediates signals initiated by IL-12, IL-5, IL-7, IL-9, and IL-15 cytokines receptors (Takahashi and Shirasawa 1994). It plays an important role in cytoskeletal remodeling, wound repair and mucosal homeostasis (Mishra and Kumar 2014). JAK 3 is expressed in intestines and kidney and it is associated with IBD. This study evaluated the expression of JAK3 in the colon of rats by Western blotting. The results (Figure 21) showed that JAK3 expression significantly increased in prevention, intervention and treatment groups compared to DSS group.
Mishra et al. (2013), reported that JAK3 plays a protective role against predisposition to colitis by promoting mucosal differentiation.

Mishra found that deficiency in JAK3 during a DSS- colitis induction enhanced the secretion of IL-6, IL-17, increased MPO activity level, and the severity of experimental UC. These findings compared with our results may suggest that the increased expression of JAK3 in PRV, INT, and TRT was due to the protective effect of JAK3 in mucosal differentiation. In addition, this may also suggest that due to the increased levels of JAK-3 the secretion of IL-6, IL-17 and MPO activity was significantly reduced in animals that had tart cherry supplementation either at single serving (2.21/kg/day) or a double serving (4.42/kg/day). As a result, consumption of tart cherry may have a protective effect on rat colonic mucosa during DSS- colitis induction.

![JAK3 and β-actin](image)

**Figure 21.** Effect of whole tart cherries on JAK3 level in DSS-induced ulcerative colitis male Sprague-Dawley rats. The values represent the mean ± SEM of six rats in each group. DSS untreated UC group; NC negative control; PRV1 UC group treated orally with 2.21g/kg/day tart cherries prior to UC induction; PRV2 UC group treated orally with 4.42 g/kg/day tart cherries prior to UC induction; INT1 UC group treated orally with 2.21g/kg/day tart cherries during UC induction; INT2 UC group treated orally with 4.42g/kg/day tart cherries during UC induction; TRT1 UC group treated orally with 2.21g/kg/day tart cherries after UC induction. All animals were fasted for 24 hours before euthanasia. aa P < 0.05 (vs. DSS group), a P < 0.008 (vs. DSS group).
CHAPTER 5: SUMMARY AND CONCLUSIONS

Whole tart cherry *Prunus cerasus* standardized to its major anthocyanin content namely Cyanidin-3-glucosyl-rutinoside was effective in reducing the expression of inflammatory markers in an experimental rat model of dextran sulfate sodium (DSS)-induced ulcerative colitis. However, further investigation is required to determine the effects of tart cherries on pro- and anti-inflammatory gene expression. A comparison to drugs used for UC management is warranted.

The major finding of this study is that tart cherries containing Cyanidin-3-glucosyl-rutinoside as a major anthocyanin, reduced the severity of DSS-induced UC in prevention and treatment at single and double serving. Also, tart cherry cherries at double serving (4.42 g/kg/day) significantly reduced the MPO activity and the secretion of pro-inflammatory cytokines (IL-6, IL-12, IL17, and TNF-α). In addition, tart cherry either at single serving (2.21 g/kg/day) or double serving (4.42 g/kg/day) significantly increased the secretion of an anti-inflammatory cytokine, IL-10 and JAK3.

The anti-inflammatory effects of tart cherries have been demonstrated in an animal model of UC. Studying these effects in a clinical trial will further support the potential anti-inflammatory effects of tart cherries.
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VITA

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