Protective effects of myrtle flavored bovine milk against ulcerative colitis in rats: Involvement of oxidative stress

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We aimed in the present to study to evaluate the protective effect of myrtle berries flavored bovine milk in acetic acid-induced ulcerative colitis (UC) model of inflammatory bowel disease in rats. Adult male Wistar rats were used and divided into five groups of ten each: control; UC; UC + bovine milk (BM); UC + myrtle flavored bovine milk (MFBM) (10 ml kg⁻¹, b.w., p.o.); and UC + sulfasalazine (100 mg kg⁻¹, b.w., p.o.). BM or MFBM (10 ml kg⁻¹, b.w., p.o.) effectively suppressed the severity of colon injuries as evidenced by attenuation of histological morphological damages and colon weight/length ratio. BM or MFBM administration attenuated also the colon oxidative stress via reduction of malondialdehyde (MDA) level and restoration of non-enzymatic antioxidant such as thiols groups and reduced glutathione, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).and plasma scavenging activity (PSA). More importantly, BM or MFBM treatment also reversed all acetic acid induced-intracellular mediator perturbations. In conclusion, our data suggests that bovine milk flavored by myrtle berries exerted a potential protective effect against AA-induced colitis in rat owing in part to its antioxidant or by negatively regulating some intracellular mediators implicated in the Fenton reaction.

Key words: flavored bovine milk, colon, oxidative stress, thiols groups, functional beverage.

INTRODUCTION

Ulcerative colitis (UC) is one of the two major forms of chronic inflammatory bowel disease. This is a recurring inflammation of the colonic mucosa with variable extension of the rectum to the cecum (Turner 2009; Arslan et al., 2001). The inflammation in ulcerative colitis is limited to the mucosal layer. It usually begins in the rectum where is the greatest place of inflammation (Müller et al., 2013). The classic symptoms of UC at any age are stomach ache, diarrhea, loss of appetite and weight loss (Loonen et al., 2003). The UC standard treatment begins with 5-aminosalicylic acid. If treatment response is not sufficient, steroids, immunosuppressants or biological products (such as anti- tumor necrosis factor antibodies) can be applied (Martin-de-Carpi et al., 2013).

Milk and dairy cattle have a long tradition in human nutrition (Haug et al., 2007). Besides its well-known calcium and protein content, it may possess many virtues against several diseases including inflammatory diseases (Insel et al., 2004). Besides its nutrient composition necessary for growth and development, bovine milk is rich in cytokines, immunoglobulins, peptides, hormones, enzymes, nucleotides and other bioactive peptides (Haug et
al., 2007; Keenan and Patton 1995). It also contains other anti-inflammatory and antioxidant compounds (Ma et al., 2007; Ruxton et al., 2004). Fruit drinks and dairy products are known to have beneficial health effects because of their richness into biologically active compounds, especially phenolic compounds (Zulueta et al., 2007). Fruits are considered the main dietary sources of bioactive substances, such as phenolic compounds, carotenoids and vitamins, that are capable of reducing the risk of cardiovascular, neurodegenerative and inflammatory diseases as well as some cancer types (Aboul-Enein et al., 2013; Gülçin 2012). On the other hand, milk is also a good source of vitamins, proteins, fats (unsaturated fatty acids), carotenoids and minerals (Claeys et al., 2013). Therefore, fruits and milk have important nutritional values and are good sources of bioactive compounds.

In this context, we therefore investigated the ameliorative effects of myrtle flavored bovine milk on acetic acid-induced ulcerative colitis as well as the mechanisms associated with such protection.

MATERIALS AND METHODS

Chemicals

Sulfasalazine (UPS, 500 mg), 5,5-dithio bis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), acetylcholine iodide, S-butylcholine, butylhydroxytoluene (BHT), methanol, ether, bovine serum albumin (BSA) and NaCl were purchased from Sigma-Aldrich (Steinheim am Albuch, Germany).

Plant collection and preparation

Berries of Myrtus communis var. italica L. were collected in October, 2014 from Nefza (Northwestern Tunisia). The plants were identified at the taxonomy laboratory of the Faculty of Sciences, Tunis (FST)-Tunisia. A voucher specimen (No. MY01) deposited in our herbarium. Berries were washed and ground using an electric blender, Waring® (Serris France). The resulting juice was then filtered through a colander (0.5 mm mesh size) and centrifuged at 5000 g for 15 min. The juice was then collected and stored at -80°C until use.

Myrtle flavored bovine milk (MFBM) preparation

Samples were prepared by gentle mixing of the myrtle berries juice (10 %, vol/vol) and sugar (2 %, wt/wt) with fresh bovine milk. The samples were then preheated to 40°C, homogenized at 15 MPa (APV homogenizer, Denmark), pasteurized in a bain-marie (75°C for 5 min), cooled to 10°C, and stored at 3°C.

Animals and treatments

Adult male Wistar rats (weighing 220–240 g, 15 weeks old) were purchased from Pasteur Institute of Tunis and used in accordance with the local ethics committee of Tunis University for the use and care of animals with respect to NIH recommendations (1985). They were provided with standard food (ALMES, TN) and water ad libitum, and maintained in animal house at controlled temperature (22 ± 2°C) with a 12-12 h light-dark cycle. Rats were divided into five groups of 10 animals each. Groups 1 and 2 served as controls and received distilled water (5 mL/kg, b.w., p.o.) for seven days. Groups 3 and 4 were orally pre-treated with BM or MFBM (10 mL/kg, b.w.) respectively, while group 5 was pre-treated with sulfasalazine dissolved in distilled water (100 mg/kg, b.w. p.o.) during the same period (seven days) before induction of colitis.

Induction of ulcerative colitis

Ulcerative colitis was induced according to the procedures described by Jabri et al. (2015). Briefly, animals were fasted for 24 h with free access to water and colitis was induced by the infusion of acetic acid (3% v/v, 5 mL/kg b.w.) for 30s using a polyethylene tube, inserted through rectum into the colon up to a distance of 8 cm. Twenty four hours h later, animals were sacrificed; colons were rapidly excised, macroscopically examined and homogenized (-) in phosphate buffer saline (-) - for determining biochemical parameters. The remaining portion of colon was kept in 10% (v/v) Formal-saline for histopathological study.

Assessments of colitis

For each animal, the distal portions of colon was excised and cut longitudinally, cleaned with physiological saline to remove fecal residues and weighed. The colons were photographed and macroscopic damage are assigned based on the clinical features of the colon using an arbitrary scale ranging from 0-4 (Millar et al., 1996), as follows: 0 (no macroscopic changes), 1 (mucosal erythema only), 2 (mild mucosal oedema, slight bleeding or small erosions), 3 (moderate oedema, slight bleeding ulcers or erosions) and 4 (severe ulceration, edema and tissue necrosis).

Histopathological examination

Immediately after sacrifice, colonic segments were harvested and washed with ice cold saline. Tissue fragments were then fixed in a 10% buffered formalin solution (-), embedded in paraffin and used for histopathological examination. Five μm thick sections were cut, deparaffinized, hydrated and stained with hematoxylin and eosin (HE). The sections were examined in blind fashion for all treatments (-), with magnification 40, using a microscope KERN® (Balingen-Germany).

Biochemical estimations

Colon lipid peroxidation

Extent of colonic lipid peroxidation was determined by
measuring the concentrations of MDA as described by (Draper and Hadley 1990). Briefly, aliquots from colon tissue homogenates were mixed with BHT-TCA solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 g for 5 min at 4°C. Supernatant was blended with 0.5 N HCl 120 mM TBA in 26 mM Tris and then heated at 80°C for 10 min. After cooling, absorbance of the resulting chromophore was determined at 532 nm using a UV-visible spectrophotometer (Beckman DU 640B). MDA levels were determined by using an extinction coefficient for MDA-TBA complex of 1.56×10^5 M^-1 cm^-1.

**Plasma scavenging activity**

The free radical scavenging activity of plasma samples was measured using the DPPH radical method according to Brand-Williams et al. (1995). Briefly, 100 µL of each plasma sample was added to 2 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol solution (100 mM). After incubation at 37 °C for 30 min, 1 mL of chloroform was added and the solution centrifuged at 1000 g for 5 min at 4°C. Supernatant was collected, and absorbance of the sample was determined at 517 nm using a UV-visible spectrophotometer. DPPH solution was used as control and the plasma scavenging activity (PSA), expressed in percentage, was calculated according to the following equation: PSA (%) = 100 × (A_517(control) - A_517(sample))/A_517(control)

**Antioxidant enzymes activities assays**

The activity of SOD was determined using modified epinephrine assays (Misra and Fridovich 1972). At alkaline pH, superoxide anion O2•- causes the autoxidation of epinephrine to adenochrome; while competing with this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of the extract that inhibits the rate of adenochrome formation by 50%. Enzyme extract was added to 2 mL reaction mixture containing 10 µL of bovine catalase (0.4 U/µL), 20 µL of epinephrine (5 mg/mL) and 62.5 mM of sodium carbonate/bicarbonate buffer pH 10.2. Changes in absorbance were recorded at 562 nm. CAT activity was assayed by measuring the initial rate of H2O2 disappearance at 240 nm (Aebi 1984). The reaction mixture contained 33 mM H2O2 in 50 mM phosphate buffer pH 7.0 and CAT activity was calculated using the extinction coefficient of 40 mM^-1 cm^-1 for H2O2.

The activity of GPx was quantified following the procedure of Flohé and Günzler (1984). Briefly, 1 mL of reaction mixture containing 0.2 mL of colonic supernatant, 0.2 mL of phosphate buffer 0.1 M pH 7.4, 0.2 mL of GSH (4 mM) and 0.4 mL of H2O2 (5 mM) was incubated at 37°C for 1 min and the reaction was stopped by the addition of 0.5 mL TCA (5%, w/v). After centrifugation at 1500 g for 5 min, aliquot (0.2 mL) from supernatant was combined with 0.5 mL of phosphate buffer 0.1 M pH 7.4 and 0.5 mL DTNB (10 mM) and absorbance was read at 412 nm. The activity of GPx was expressed as nmol of GSH consumed/min/mg protein.

**Non-enzymatic antioxidants measurement**

The total concentration of thiol groups (-SH) was performed according to the method of Ellman (1959). Briefly, homogenates of colonic tissue were mixed with 800 µL of 0.25 M phosphate buffer (pH 8.2) and 100 µL of 20 mM EDTA, and the optical density was measured at 412 nm (A1). Then, 100 µL of 10 mM DTNB were added, incubated during 15 min and the absorbance of the sample was quantified at 412 nm (A2). The thiol groups concentration was calculated from A1 to A0 subtraction using a molar extinction coefficient of 13.6×10^3 M^-1 cm^-1. The results were expressed as µmo of thiol groups per mg of protein.

GSH was estimated in colonic tissue by the method of Sedlak and Lindsay (1968). Briefly, 500 µL of tissue homogenate prepared in 20 mM EDTA, (pH 4.7) were mixed with 400µL of cold distilled water and 100 µL of 50% TCA. The samples were shaken using vortex mixer and centrifuged at 1200×g during 15 min. Following centrifugation, 2 mL of supernatant were mixed with 400 µL of 400 mM Tris-buffer (pH 8.9) and 10 µL of 10 mM DTNB. The absorbance was read at 412 nm against blank tube without homogenate.

**H2O2 measurement**

Colonic H2O2 level was performed according to Dingeon et al. (1975). Briefly, in the presence of peroxidase, the hydrogen peroxide reacts with p-hydroxybenzoic acid and 4-aminoantipyrine leading to a quantitative formation of a quinoneimine which has a pink color detected at 505 nm.

**Iron determination**

The non haem iron was measured colorimetrically using ferrozine as described by Leardi et al. (1998). Briefly, iron is dissociated from transferrin-iron complex by a solution of guanidine acetate and reduced by ascorbic acid, which then reacts with ferrozine to give a pink complex measured at 562 nm.

**Protein determination**

Protein concentration was determined according to the method of Hartree (1972), which is a slight modification of the Lowry method. Serum albumin was used as standard.

**Statistical analysis**

Data were analyzed by one-way analysis of variance (ANOVA) and are expressed as means ± standard error of the mean (SEM). Data are representative of four independent experiments. All statistical tests were two-tailed, and a p value of 0.05 or less was considered.
RESULTS

Effect of BM and MFBM on macroscopic evaluation of the colonic lesions and colon wet weight

Anal AA administration triggered severe colon macroscopic edematous inflammation (Figure 1), as assessed by the high score of colonic injuries with increased colon wet weight (Table 1). However, pretreatment with BM and MFBM significantly attenuated these macroscopic perturbations compared to the control group. Likewise, the standard sulfasalazine (100 mg/kg b.w.) showed better effects in all these parameters.

Effect of BM and MFBM on colonic histological changes

As shown in Figure 2, AA-induced ulcerative colitis has caused a mucosal and submucosal congestion, necrotic lesion and edematous area as well as diffuses a mixed inflammatory cells infiltration, particularly neutrophils. BM and MFBM pretreatment significantly protected the colonic cells. Sulfasalazine, used as a standard drug, also attenuated the histological injuries induced by acetic acid.

Effect of BM and MFBM on colonic lipid peroxidation

Rats intoxicated with acetic acid showed marked higher colonic MDA levels, a lipid peroxidation marker than all groups (Table 2). BM and MFBM groups revealed significant decrease of MDA levels as compared with UC groups. Sulfasalazine also significantly decreased the colonic lipid peroxidation.

Effect of BM and MFBM on the plasma scavenging activity (PSA)

As shown in Figure 3, BM, MFBM and sulfasalazine pretreatment significantly increased the plasma scavenging activity, which has been altered after 24 h of anal acetic acid.
Table 1. Effect of bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine on acetic acid (AA)-induced changes in the gross lesion score and colon weight to length ratio. Animals were treated with BM or MFBM (10 ml kg⁻¹, b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg⁻¹, b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg⁻¹, b.w., p.o.) (3%, v/v, 5 mL kg⁻¹, b.w.) or NaCl 0.9% for 24 hours. The data are expressed as means ± S.E.M. (n=10). *: p < 0.05 compared to control group and #: p < 0.05 compared to AA group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gross lesion score</th>
<th>Wet colon weight/length (mg/cm)</th>
<th>(% protection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>80.66 ± 6.43</td>
<td>--</td>
</tr>
<tr>
<td>UC + BM</td>
<td>4.8 ± 0.64*</td>
<td>154.88 ± 11.73*</td>
<td>--</td>
</tr>
<tr>
<td>UC + MFBM</td>
<td>1.7 ± 0.53*</td>
<td>114.26 ± 5.17*</td>
<td>64.58</td>
</tr>
<tr>
<td>UC + Sulfasalazine</td>
<td>1.2 ± 0.62*</td>
<td>96.44 ± 6.50*</td>
<td>75.00</td>
</tr>
<tr>
<td>UC + Sulfasalazine</td>
<td>0.9 ± 0.74*</td>
<td>88.39 ± 4.68*</td>
<td>81.25</td>
</tr>
</tbody>
</table>

Figure 2: Bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine mitigates the histological damage in colon tissues induced by acetic acid (AA). Animals were treated with BM or MFBM (10 ml kg⁻¹, b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg⁻¹, b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg⁻¹, b.w., p.o.) (3%, v/v, 5 mL kg⁻¹, b.w.) or NaCl 0.9% for 24 hours. (A): H₂O + NaCl, (B): AA + H₂O, (C): AA + BM (10 ml kg⁻¹, b.w., p.o.), (D): AA + MFBM (10 ml kg⁻¹, b.w., p.o.), (E): AA + Sulfasalazine (100 mg kg⁻¹, b.w., p.o.).

Effect of BM and MFBM on the sulfhydryl groups and reduced glutathione levels

The level of non-enzymatic antioxidants, including −SH groups and GSH, in UC group was significantly decreased compared with the control group. However, administration of BM and MFBM significantly increased the acetic acid mediated decrease in these non-enzymatic antioxidants. A similar effect was observed in sulfasalazine treated group (Table 2).

Effect of BM and MFBM on antioxidant enzyme activities

The effects of BM and MFBM on colonic antioxidant enzymes (SOD, CAT and GPx) were studied and the results were summarized in Table 2. The administration of AA to rats strongly inhibited the antioxidant enzyme activities compared to the control group. This inhibition was significantly improved by BM or MFBM treatments as well.
Table 2. Effect of bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine on acetic acid (AA)-induced colon oxidative stress. Animals were treated with BM or MFBM (10 ml kg⁻¹, b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg⁻¹, b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg⁻¹, b.w., p.o.) (3%, v/v, 5 mL kg⁻¹, b.w.) or NaCl 0.9% for 24 hours. MDA, nmol of MDA/mg protein; SOD, units/mg protein (one unit of the SOD activity is the amount of enzyme required to give 50% inhibition of epinephrine auto oxidation); CAT, μmol of H₂O₂ consumed/min/mg protein; GPx, nmol GSH oxidized/min/mg protein; -SH groups, μmol/mg protein; GSH, nmol of GSH/mg protein. The data are expressed as means ± S.E.M. (n=10). * : p < 0.05 compared to control group and # : p < 0.05 compared to AA group

<table>
<thead>
<tr>
<th></th>
<th>MDA</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>-SH groups</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.22 ± 0.72</td>
<td>13.45 ± 0.62</td>
<td>21.33 ± 1.27</td>
<td>22.66 ± 0.93</td>
<td>93.44 ± 8.41</td>
<td>32.58 ± 2.30</td>
</tr>
<tr>
<td>UC</td>
<td>5.41 ± 0.51*</td>
<td>6.37 ± 0.85*</td>
<td>9.38 ± 0.89*</td>
<td>10.41 ± 0.53*</td>
<td>44.20 ± 4.83*</td>
<td>14.66 ± 1.12*</td>
</tr>
<tr>
<td>UC + BM</td>
<td>2.26 ± 0.31*</td>
<td>10.44 ± 0.54*</td>
<td>14.38 ± 0.74*</td>
<td>15.92 ± 0.41*</td>
<td>72.85 ± 3.64*</td>
<td>22.21 ± 0.82*</td>
</tr>
<tr>
<td>UC + MFBM</td>
<td>1.98 ± 0.44*</td>
<td>11.33 ± 0.82*</td>
<td>16.21 ± 0.61*</td>
<td>19.08 ± 0.70*</td>
<td>81.81 ± 6.32*</td>
<td>25.50 ± 0.76*</td>
</tr>
<tr>
<td>UC + Sulfasalazine</td>
<td>1.72 ± 0.68*</td>
<td>13.00 ± 0.68*</td>
<td>18.41 ± 1.02*</td>
<td>20.68 ± 0.72*</td>
<td>85.71 ± 5.68*</td>
<td>29.78 ± 1.58*</td>
</tr>
</tbody>
</table>

Figure 3: Effect of bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine on acetic acid (AA)-induced changes in plasma scavenging activity (PSA). Animals were treated with BM or MFBM (10 ml kg⁻¹, b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg⁻¹, b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg⁻¹, b.w., p.o.) (3%, v/v, 5 mL kg⁻¹, b.w.) or NaCl 0.9% for 24 hours. The data are expressed as means ± S.E.M. (n=10). * : p < 0.05 compared to control group and # : p < 0.05 compared to AA group

as by sulfasalazine.

Effect of BM and MFBM on intracellular mediators levels

As expected, acetic acid intoxication caused a colonic free iron (Figure 4) and H₂O₂ (Figure 5) overload. The BM, MFBM and sulfasalazine pretreatment significantly protected against acetic acid-induced intracellular mediators deregulation.

DISCUSSION

Chronic inflammatory bowel disease (IBD) represents a group of diseases characterized by a chronic inflammation of a part of digestive tract wall. This inflammation can be intermittent or continuous, and frequently leads to ulceration of the bowel wall (Frei et al., 2011). In the present study, we examined the ameliorative effects of myrtle flavored bovine milk on acetic acid-induced ulcerative colitis as well as the mechanisms associated
Figure 4: Effect of bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine on acetic acid (AA)-induced colon free iron overload. Animals were treated with BM or MFBM (10 ml kg\(^{-1}\), b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg\(^{-1}\), b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg\(^{-1}\), b.w., p.o.) (3%, v/v, 5 mL kg\(^{-1}\), b.w.) or NaCl 0.9% for 24 hours. The data are expressed as means ± S.E.M. (n=10). * : p < 0.05 compared to control group and # : p < 0.05 compared to AA group

Figure 5: Effect of bovine milk (BM), myrtle flavored bovine milk (MFBM) and sulfasalazine on acetic acid (AA)-induced colon H\(_2\)O\(_2\) overload. Animals were treated with BM or MFBM (10 ml kg\(^{-1}\), b.w., p.o.), the reference molecule (sulfasalazine 100 mg kg\(^{-1}\), b.w., p.o.) or bidistilled water, and challenged with a single anal administration of AA (300 mg kg\(^{-1}\), b.w., p.o.) (3%, v/v, 5 mL kg\(^{-1}\), b.w.) or NaCl 0.9% for 24 hours. The data are expressed as means ± S.E.M. (n=10). * : p < 0.05 compared to control group and # : p < 0.05 compared to AA group
with such protection.

We firstly demonstrated that a single anal administration of acetic acid (3% v/v, 10 ml /kg, b.w) caused severe ulcerations in the colon mucosa, manifested by macroscopic edematous inflammation and increase of colon wet weight in comparison with control groups. In fact, in our study colitis appears rapidly after acetic acid administration and often leads to severe lesions by alteration of the intestinal barrier that contacting the flora and intestinal immunity subjacent (Strober et al., 2002). Activation of the immune intestinal system and recruitment of inflammatory cells in the intestine contribute to maintain inflammation and intestinal lesions (Robinson 2008; Strober et al., 2002). Histologically, we have shown that acetic acid intoxication induces an alteration of colon surface coating as well as epithelial and vascular cells. We also showed necrosis in the colonic mucosa with submucosal inflammation, whose lymphocytes and neutrophils being the predominant infiltrating inflammatory cells. Indeed the transepithelial resistance is reduced in the destroyed mucosa in ulcerative colitis, even in areas with no obvious disruption of the mucosa (Gitter et al., 2001). The decrease of the transepithelial resistance was attributed to increase of cell apoptosis and dysfunction of the tight junction (Iwamoto et al., 1996; Schulzke et al., 2006). The release of proteases is a determining factor contributing to the tissue destruction and necrosis, which induces epithelial cells apoptosis by disrupting their anchorage to the basement membrane (Faurschou and Borregaard, 2003). The bovine milk per se and the myrtle flavored bovine milk significantly mitigated all the colon morphological and histological injuries induced by anal acetic acid administration. In fact bovine milk is rich in proteins including antibodies and immune stimulants. 80% of proteins consist of caseins and 20% of whey, which possess numerous biological activities (Severin and Xia 2005). Transforming growth factor-ß2 (TGF- ß2) from bovine milk is considered responsible for anti-inflammatory effects of Modulen® IBD, an enteral polymeric diet developed by Nestlé® to provide complete nutrition for IBD patients (Beattie et al., 1994; De Jong et al., 2007). Similarly, lactoferrin, a whey protein has been shown to provide anti-inflammatory effects against colitis induced by dextran sulphate sodium (DSS) in mice (Haversen et al., 2003). More importantly, supplementation myrtle berries to milk improves its anti-inflammatory properties, seen the wealth of myrtle berries of chemical compounds known by their anti-ulcer and anti-inflammatory effects (Jabri et al., 2015; Messaoud and Boussaid 2011; Sumbul et al., 2010). In this context several studies have shown the importance of fruit supplementation to dairy products in their improvement much as functional beverages (Zulueta et al., 2007; Cilla et al., 2012; Zulueta et al., 2007).

Acetic acid poisoning has also influenced the colonic redox balance by inducing lipid peroxidation which is manifested by an increase in MDA levels, deleterious effects on the antioxidant enzymes activity such as superoxide dismutase, catalase and glutathione peroxidase and a decrease in non-enzymatic antioxidants levels such as reduced glutathione and sulphydryl groups as well as plasma scavenging activity. This is consistent with previous data observed in rats (Dogan et al., 2014) and mice (Somani et al., 2014). Indeed, during intestinal inflammation, among the mechanisms used by phagocytes is the production of reactive oxygen species starting from superoxide anions (O2−) (Gougerot-Pocidalo et al., 2002). The superoxide anion is a precursor of several other forms of highly ROS and more toxic such as hydrogen peroxide (H2O2), hydroxyl radical (OH-) and singlet oxygen (1O2) (Hurtado-Nedeleca et al., 2014). If these products are released in an exaggerated manner in the extracellular medium, they can lead to tissues damage due to lipid peroxidation, causing a membrane disruption, or also an alteration of proteins and nucleic acids (Gougerot-Pocidalo et al., 2002). Subacute bovine milk pretreatment during one week corrected all colic redox status disturbances modified by the anal acetic acid administration. These results are consistent with many previous studies showing the antioxidant and anti-inflammatory properties of bovine milk (Lindmark-Mansson and Akesson 2000), camel milk (Arab et al., 2014) and oligosaccharides goat milk (Lara-Villoslada et al., 2006). Indeed bovine milk contains several minerals, vitamins and antioxidants molecules, whose most important are selenium as well as vitamins E and A (Lara-Villoslada et al., 2006). BM is also a good source of glutathione, a tripeptide formed by the condensation of glutamic acid, cysteine and glycine. Glutathione regulates the ROS level in the cells; it acts as a growth factor, an anti-apoptotic factor in leukocytes and is involved in the cytokine secretion regulation (Sprietas 1999). The addition of myrtle in bovine milk improved its antioxidant power, in fact a broad range of phenolic compounds has been identified in the myrtle berries (Messaoud and Boussaid 2011) and seeds (Jabri et al., 2015). Polyphenols are the most abundant antioxidants in our diets. They reinforce our natural defenses against oxidative stress and thus would prevent various chronic diseases such as cancer, IBD and cardiovascular diseases (Nève 2002).

On another hand, we demonstrated that ulcerative colitis induced by acetic acid has been accompanied by an increase in colic hydrogen peroxide and free iron levels, and BM per se or flavored with myrtle protect against this increase and bring the values to baseline levels. Indeed, hydrogen peroxide is a very important ROS because of its ability to cross biological membranes (Miranda-Vilela et al., 2010). However, it may be more toxic then its conversion into hydroxyl radical, whose its production largely depends of free iron which accumulates in the tissues and catalyzes via the Fenton pathway the hydroxyl radical production who is involved in lipid peroxidation (Gulcin et al., 2003) and DNA oxidation (Khan and Sultana 2005). The BM free iron chelation and H2O2 scavenging activities can be attributed to its richness in antioxidants molecules and the improving of these activities by myrtle adding is may be due to the phenolic compounds allowing to H2O2 reduction to water (Rice-Evans et al., 1997). The same mechanism
was previously described in the small intestines (Jabri et al., 2016) and in the colon (Jabri et al., 2015).

**Conclusion**

In conclusion, the results of the present study demonstrate the pharmacological credence of myrtle berries flavored bovine milk in the pathogenesis of IBD. This is owing, in part to the antioxidant properties of this functional beverage, and to its opposing effect on some intracellular mediators such as hydrogen peroxide and free iron on other part.

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**Declaration of interest**

The authors alone are responsible for the content of this paper.

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