Objective: The aim of the present study was to investigate the protective effect of WB against acetic acid-induced colitis in rats.

Method: The rats were divided into four groups with eight rats in each group: Control group, Wolf Berry (WB) group, Colitis group and WB + Colitis group. Distal colitis was induced in rats by intracolonic instillation of 4% acetic acid. WB + Colitis group received 100 mg/kg of WB extract dissolved in saline through the intraperitoneal route for seven days. Acute colitis was created on the 8th day and the rats were sacrificed 48 hours later. Colonic damage was assessed by macroscopic and histological criteria as well as biochemical markers.

Results: Mean TAS, TOS, TNF-α, IL-1β, and IL-6 levels were significantly higher in the colitis group compared to the control and WB groups (p < 0.05). The WB + colitis group had significantly lower TAS, TOS, TNF-α, IL-1β and IL-6 levels compared to the colitis group (p < 0.05). The analysis of the histopathological findings indicated that the colitis group had a significantly higher histopathological damage score (HDS) than the control group (3.12 ± 0.45, 0 ± 0.00, respectively; p < 0.05). Histopathological damage score was significantly higher in the WB + colitis group than the control group and significantly lower than the colitis group (1.62 ± 0.44, 0 ± 0.00, respectively; 3.12 ± 0.45, respectively; p < 0.05 for both comparisons).

Conclusion: Wolf Berry extract is an agent that is effective for preventing acetic acid-induced colitis in rats.

Keywords: Acute colitis, antioxidant, Wolf berry
INTRODUCTION

Inflammatory bowel diseases have ill-defined aetiopathogenesis and may exhibit non-specific inflammatory and extraintestinal signs. Impaired balance of mucosal protective factors, bacterial overgrowth and alterations in cytokine and inflammatory mediator synthesis have been implicated in their pathogenesis (1). Cytokines have a major role in immune system regulation and inflammatory response. Some cytokines activate inflammatory cells, while some others mediate the growth, differentiation and activation of hematopoietic cells (2).

Proinflammatory cytokines are thought to be upregulated and take part in inflammation in inflammatory bowel disease. Tumour necrosis factor (TNF-α) is a proinflammatory cytokine playing a role in regulation of mucosal immune response as a part of the pathogenesis of the inflammatory bowel disease. Interleukin-1 (IL–1) and TNF-α activate macrophages, polymorphonuclear leucocytes, fibroblasts, and endothelial cells to synthesize and release other cytokines, arachidonic acid metabolites and proteases. In addition, IL-1 and TNF-α mediate the release of adhesion molecules that enable inflammatory cells to bind to and migrate from the vessel wall (3).

Wolf berry (WB), also colloquially named goji berry or Lycium barbarum, is an herb that belongs to the Solanaceae family, which has been historically used as an herbal product in the traditional Chinese medicine and as a “super food” among the population. It has recently drawn scientific attention over its antioxidant effects and benefits as a nutrient, which are brought about by its polysaccharide complex content. It has recently been shown that WB extract and its polysaccharide content have certain anti-ageing, neuroprotective, anti-fatigue/endurance-increasing, and anti-oxidant biological effects with anti-tumour activity and cytoprotection; they also exert beneficial effects on body metabolism, diabetes, glaucoma and immune functions (4, 5).
MATERIALS AND METHODS

Chemicals

Acetic acid was purchased from Erkim Medikal, Turkey.

Cytokine, TAS, TOS assays

Tumour necrosis factor-alpha (TNF-α), IL-6 and IL-1β were purchased from eBioscience (Austria), and TAS and TOS from the Rel Assay Diagnostics (Gaziantep, Turkey). The analyses were based on the instructions of the manufacturer.

Preparation of the Wolf Berry Extract

The WB extract was prepared by soaking 50 g of shade-dried, ground WB in 500 mL of ethanol for seven days at room temperature. The mixture was then treated by a filtering process and 2 g of the extract was obtained by desiccating the resulting mass (6). A high concentration of polysaccharides in the extract was achieved by ethanol-mediated extraction process of WB (7, 8).

Animals and the study protocol

Forty-eight Wistar Albino rats with a weight range between 250 and 300 grams from the Dicle University Health Sciences and Research Center (Diyarbakir, Turkey) were randomized into six groups for the experiment. During the experiment, all animals were behaved humanely and fed with standard rat chow and water ad libitum while being kept in wooden cages with a size of 24 × 20 × 18 cm, which were located in an air-conditioned room with a constant temperature of 21 °C and alternating 12-hour light/dark cycles. The rats were not allowed to eat or drink before the experiment. The local ethics committee for animal studies at Dicle University (Diyarbakir, Turkey, 2013/41) approved the study. In our study a model of acute colitis was created with acetic
Effect of Wolf Berry Extract on Colitis in Rats

acid. A soft paediatric catheter was placed 6 cm into the rectum of the rats under mild ether anaesthesia. The animals were then put in the Trendelenburg position and a 4% acetic acid solution (1 mL, pH:2.5) was applied into the rectum through the intrarectal catheter. The rats were held in the Trendelenburg position for 30 seconds. The experimental groups were designed as follows:

- The rats were divided into four groups with eight rats in each group:
  - Control group: Underwent the cannulation procedure without colitis induction, receiving normal saline instead of acetic acid.
  - WB group: These rats were administered 100 mg/kg (9) of WB extract dissolved in saline through the intraperitoneal route for seven days. The rats were sacrificed on the 8th day.
  - Colitis group: Acute colitis was created one-hour after intraperitoneal administration of 1 mL saline; the rats were sacrificed 48 hours later.
  - WB + Colitis group: The rats in this group received 100 mg/kg of WB extract dissolved in saline through the intraperitoneal route for seven days. Acute colitis was created on the 8th day and the rats were sacrificed 48 hours later.

Rats in all study groups were anaesthesized 24 hours after administration of ketamine hydrochloride (50 mg/mL, 10 mg/kg, Ketalar; Bayer, Leverkusen, Germany) and intramuscular xylazine (2%, 0.1 mL/kg, Rompun; Bayer) 50 mg/kg in a solution of 2 mL/kg.

All rats were put in the supine position for the surgical procedure and the laparotomy was done with a midline incision. The rats were sacrificed with the exsanguinuation method at the end of the procedure and the bowel samples were taken there after. Following centrifuge of the homogenates at 3000 rpm for 10 minutes at 4 °C, the supernatants were removed and stored at −80 °C until biochemical analyses for total antioxidant capacity (TAS), total oxidant capacity (TOS), tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6).
Biochemical analysis

Measurement of TNF-α, IL-6 and IL-1β

Tumour necrosis factor-alpha, IL-6 and IL-1β were measured with commercially available rat enzyme-linked immunosorbent assay (ELISA) kits in compliance with the manufacturer's instructions.

Measurement of total oxidant status

Tissue total oxidant status (TOS) levels were measured with a commercially available kit, developed by Erel (10). This assay contains certain antioxidant molecules that perform oxidation of the ferrous ion-o-dianisidine complex to ferric ion. This reaction is augmented by glycerol molecules that are abundant in the reaction medium. The product of this reaction forms a colored complex with xylenol orange in an acidic medium. The spectrophotometrically measured color intensity of the resulting mixture reflects the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as μmol H₂O₂ equivalent/L.

Measurement of total antioxidant status

Tissue total antioxidant status (TAS) levels were measured using a commercially available kit developed by Erel (11). This assay reflects the antioxidative effect of the sample against the potent free radical reactions, which is started by the produced hydroxyl radical. The assay has been reported to have excellent precision values (< 3 %). The results are presented as mmoL Trolox equivalent.
Macroscopic and microscopic assessment of colitis

The animals were sacrificed using ether 24 hours after induction of colitis. Distal colon of each animal was removed, incised in a longitudinal direction and washed with normal saline.

An independent observer using a magnifying glass scored macroscopic damage, based on the criteria below: 1. intact, non-damaged epithelium; 2. patchy superficial hyperemia; 3. generalized patchy hyperemic regions; 4. diffuse hyperemia and haemorrhage (12).

Microscopic evaluation of the colonic samples was carried out after fixing them in 10% formaline in phosphate buffer saline, embedding in paraffine, slicing into 5 mm thick sections, staining with haematoxylin and eosin, and randomly examining under light microscopy (5 or more sections per colon). Scoring was based on the following scale: 0. intact epithelium, leukocytes or haemorrhage absent; 1. < 25% disrupted epithelium, focal leukocyte infiltrates and focal haemorrhage; 2. 25% disrupted epithelium, focal leukocyte infiltrates and focal haemorrhage; 3. 50% disrupted epithelium, widespread leukocytes and haemorrhage; 4. > 50% disrupted epithelium, extensive leukocyte infiltration and haemorrhage (13).

Statistical analysis

Study data were expressed as mean ± SD and analysed by one-way (ANOVA), followed by TUKEY post hoc test for multiple comparisons. A non-parametric test (Kruskal-Wallis test) with Dunn post hoc analysis was used for macroscopic and histopathological data. A p-value of less than 0.05 was considered significantly.
RESULTS

Biochemical parameters were studied in all groups. Mean TAS, TOS, TNF-α, IL-1β, and IL-6 levels were significantly higher in the colitis group compared to the control and WB groups \((p < 0.05)\). The WB+ colitis group had significantly lower TAS, TOS, TNF-α, IL-1β and IL-6 levels compared to the colitis group \((p < 0.05)\). Table 1 presents the statistical comparisons of the mean biochemical parameters across the study groups.

Table 1. Statistical comparison of the mean biochemical parameters and pathology results across the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TAS</th>
<th>TOS</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>MDS</th>
<th>HDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.1 ± 0.7</td>
<td>182.5 ± 51.2</td>
<td>668.4 ± 139.1</td>
<td>755.4 ± 109.6</td>
<td>720.5 ± 49.9a</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
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<tr>
<td>Group 2</td>
<td>0.3 ± 0.1a</td>
<td>325.6 ± 22.5a</td>
<td>2455.3 ± 26.6a</td>
<td>1015.2 ± 345.6a</td>
<td>1480.1 ± 540.9a</td>
<td>3.7 ± 0.32a</td>
<td>3.12 ± 0.45a</td>
</tr>
<tr>
<td>(Colitis)</td>
<td></td>
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<tr>
<td>Group 3</td>
<td>1.1 ± 0.3b</td>
<td>188.5 ± 32.4b</td>
<td>755.7 ± 284.4b</td>
<td>645.0 ± 58.5b</td>
<td>804.7 ± 47.6b</td>
<td>0 ± 0.00b</td>
<td>0 ± 0.00b</td>
</tr>
<tr>
<td>(WB)</td>
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<tr>
<td>Group 4</td>
<td>0.6 ± 0.1ab</td>
<td>255.9 ± 29.5ab</td>
<td>1286.4 ± 155.7ab</td>
<td>844.6 ± 64.2b</td>
<td>1012 ± 12.2ab</td>
<td>1.71 ± 0.68ab</td>
<td>1.62 ± 0.44ab</td>
</tr>
<tr>
<td>(WB+ Colitis)</td>
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</tbody>
</table>

TAS: total antioxidant capacity (μmol H2O2 equivalent/L), TOS: total oxidant capacity (mmol Trolox Equiv./L), TNF-α: tumour necrosis factor-alpha (pg/mL), IL-1β: interleukin-1beta (pg/mL), MDS: macroskopik damage score, HDS: Histologic damage score, IL-6: interleukin-6 (pg/mL), WB: wolf berry, \(^a\): \(p < 0.05\) vs Group 1, \(^b\): \(p < 0.05\) vs Group 2. The results were presented as mean ± SD.

The analysis of the histopathological findings indicated that the colitis group had a significantly higher histopathological damage score (HDS) than the control group \((3.12 ± 0.45, 0 ± 0.00, \text{ respectively}; p < 0.05)\). HDS was significantly higher in the WB+ colitis group than the control group and significantly lower than the colitis group \((1.62 ± 0.44, 0 ± 0.00, \text{ respectively}; 3.12 ± 0.45, \text{ respectively}; p < 0.05 \text{ for both comparisons})\). The statistical comparisons of all groups with respect to the mean macroscopic damage score (MDS) and HDS were presented in Table 1.
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Figure 1 shows the histopathological changes in the study groups.

Figure 1. The histopathological changes in the study groups

(A) Colonic tissues section of control group (H&E ×100): intact epithelium, no leukocyte or haemorrhage. (B) Colonic tissues section of wolf berry group (H&E ×100): intact epithelium, no leukocyte or haemorrhage. (C) Colonic tissues section of colitis group (H&E ×100): intense inflammation and ulceration of the epithelial surface and thickened colonic muscle layer. (D) Colonic tissues section of colitis+ wolf berry group (H&E ×100): mild inflammation in the epithelium, submucosal severe edema and moderate inflammatory cell infiltration of mixed type.

DISCUSSION

Our study demonstrated the effectiveness of WB extract in an experimental model of colitis in rats. The effect of WB extract was evident in terms of the inflammatory markers such as TAS, TOS, TNF-α, IL-1β and IL-6 and also the repair of histopathological damage.
Inflammatory bowel disease is a disorder with ill-defined aetiology, for which search for an effective therapy continues (14). Mounting evidence suggests that signal molecules and pathways play an important role in its onset and course. There is evidence that upregulated inflammatory mediators such as cytokines, chemokines and adhesion molecules have a basic role in both humans and experimental models of colitis (15, 16).

Experimental colitis created with the use of acetic acid is among standardized models of acute colitis. Certain factors are required to create and maintain colitis, including increased vasoperme ability, prolonged neutrophilic infiltration and increased level of inflammatory mediators (17). Our study indicated that acetic acid-induced acute colitis caused some macroscopic, microscopic and biochemical alterations. Acetic acid, when instilled into the rectum of the rats, caused severe localized mucosal erosion, as well as inflammation and bleeding (18, 19).

In the last decade, the protective effects of the antioxidative properties of some natural herbal extracts on organ disorders of different origins have been investigated. Some examples of such extracts are the epigallocatechin gallate in green tea (20), the coprinus comatus fungus (21), and ginsan obtained from panax ginseng (22). Likewise, many studies have explored the effects of WB extract at biochemical level and reported that it acts as an anti-oxidant, anti-aging, anti-tumour and immune-stimulant agent. These effects have been linked to different organic and inorganic elements including beta carotene, riboflavin, ascorbic acid, thiamine cerebroside and betaine (23–27). WB extract has also been studied in cellular injury of liver, eyes, small intestine, and kidney (28–31).

Previous work has shown the efficacy of antioxidant therapy in experimental models of colitis (32–35). Ghatule et al (36) reported an increase in antioxidant levels and a decrease in antioxidative capacity with Azadirachta indica leaves extract in acetic acid-induced colitis in rats.
Yıldız et al (37) showed macroscopic and microscopic recovery with the TNF-α inhibitor pentoxifylline in an experimental model of acetic acid-induced acute and chronic colitis in rats. El-Medany et al (32) demonstrated that cyclooxygenase inhibitors induced recovery by reducing oxidative capacity and increasing antioxidative capacity in an experimental model of colitis. Our study indicated that WB reduced oxidative capacity and augmented antioxidative capacity in an experimental model of acetic acid-induced acute experimental colitis, as evidenced by the change in the levels of TAS and TOS.

Tumour necrosis factor-alpha, IL-1β, and IL-6 are the main proinflammatory cytokines synthesized and released in response to oxidative stress and inflammation (38). Gastrointestinal host defense is an important area where cytokines is beneficial to human body, although their excessive production may lead to bowel inflammation and impaired bowel motility (39). Tumour necrosis factor-alpha is a pivotal protein for the bowel inflammation that is responsible for most of the clinical features of inflammatory bowel diseases (16, 40). The production and release of some other cytokines such as adhesion molecules and arachidonic acid metabolites are enhanced by TNF-α; it also activates immune and non-immune cells. It was reported that inflammatory bowel disease in rats (41) and humans (42, 43) favorably responded to antibodies to avian TNF. The present study indicated that acetic acid instillation caused an increase in the production of the proinflammatory cytokine TNF-α. In addition, we detected a significant increase in the tissue levels of the cytokines including IL-1β and IL-6 in addition to TNF-α in rats with experimental colitis. There was a significant difference between the WF+ colitis group and the colitis group with respect to mean cytokine levels. This result was consistent with and supportive of the literature data.
In conclusion, Wolf Berry extract is an agent that is effective for preventing acetic acid-induced colitis in rats. Introduction of this agent into clinical practice as a protective and therapeutic agent requires further clinical studies in both animals and humans.

Conflict of interest statement

We declare that we have no conflict of interest.
REFERENCES


