ORIGINAL ARTICLE

Protective Effect of Wolfberry Extract on Acetic Acid-induced Colitis in Rats

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ABSTRACT

Objective: *To investigate the protective effect of wolfberry (WB) against acetic acid-induced colitis in rats.*
Methods: *The rats were divided into four groups with eight rats in each group: the control group, WB group, colitis group and WB + colitis group. Distal colitis was induced in rats by intracolonic instillation of 4% acetic acid. Wolfberry + colitis group received 100 mg/kg of WB extract dissolved in saline through the intraperitoneal route for 7 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 total antioxidant capacity (TAC), total oxidant status (TOS), tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 levels were significantly higher in the colitis group compared with the control and WB groups (*$p < 0.05$*). The WB + colitis group had significantly lower TAC, TOS, TNF-α, IL-1β and IL-6 levels compared with the colitis group (*$p < 0.05$*). The analyses of the histopathological findings indicated that the colitis group had a significantly higher histopathological damage score 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 in the control group and statistically 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: *Wolfberry extract is an agent that is effective for preventing acetic acid-induced colitis in rats.*

Keywords: Acute colitis, antioxidant, wolfberry.

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 had 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 haematopoietic 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 the regulation of mucosal immune response as a part of the pathogenesis of the inflammatory bowel disease. Interleukin (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).

Wolfberry (WB), also colloquially named goji berry or *Lycium barbarum*, is a herb that belongs to the Solanaceae family, which has been historically used as a herbal product in the traditional Chinese medicine and as a ‘super food” among the population. It has recently

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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 Arkim Medikal (Sokak, Turkey).

Cytokine, TAC and TOS assays

Tumour necrosis factor-α, IL-6 and IL-1β were purchased from eBioscience (Vienna, Austria), and total antioxidant capacity (TAC) and total oxidant status (TOS) from the Rel Assay Diagnostics (Gaziantep, Turkey). The analyses were based on the instructions of the manufacturer.

Preparation of the WB extract

The WB extract was prepared by soaking 50 g of shade dried, ground WB in 500 ml of ethanol for 7 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

Thirty two Wistar albino rats with a weight of 250–300 g from the Dicle University Health Sciences and Research Centre (Diyarbakir, Turkey) were randomized into four groups for the experiment. During the experiment, all the animals were treated 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 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, 10) of WB extract dissolved in saline through the intraperitoneal route for 7 days. The rats were sacrificed on the 8th day; colitis group: acute colitis was created 1 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 7 days. Acute colitis was created on the 8th day and the rats were sacrificed 48 hours later.

The rats in all the study's groups were anaesthesized 24 hours after the administration of ketamin 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 the 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 exsanguination method at the end of the procedure, and the bowel samples were taken thereafter. Following the centrifuge of the homogenates at 3000 rpm for 10 minutes at 4°C, the supernatants were removed and stored at −80°C until the biochemical analyses for TAC, TOS, TNF-α, IL-1β and IL-6.

BIOCHEMICAL ANALYSES

Measurement of TNF- α, IL-6 and IL-1β

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

Measurement of TOS

Tissue TOS levels were measured with a commercially available kit, developed by Erel (11). This assay contained certain antioxidant molecules that performed the oxidation of the ferrous ion-o-dianisidine complex to ferric ion. This reaction was augmented by the glycerol molecules that were abundant in the reaction medium. The product of this reaction formed a coloured complex

with xylenol orange in an acidic medium. The spectrophotometrically measured colour intensity of the resulting mixture reflects the total quantity of oxidant molecules in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed as µmol H2O2 equivalent/L.

Measurement of total antioxidant status

The rats' tissue total antioxidant status (TAS) levels were measured using a commercially available kit developed by Erel (11). This assay reflected the antioxidative effect of the sample against the potent free radical reactions, which was started by the produced hydroxyl radical. The assay had been reported to have excellent precision values (< 3%). The results were presented as mmol Trolox equivalent.

Macroscopic and microscopic assessment of colitis

The animals were sacrificed using ether 24 hours after the induction of colitis. The 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 hyperaemia; 3 = generalized patchy hyperaemic regions; 4 = diffuse hyperaemia and haemorrhage (12). The microscopic evaluation of the colonic samples was carried out after fixing them in 10% formalin in phosphate buffer saline, embedding in paraffin, slicing into 5-mm thick sections, staining with haematoxylin and eosin, and randomly examining under light microscopy (five or more sections per colon). The scoring was based on the following scale: 0 = intact epithelium, leucocytes or haemorrhage absent; 1 = < 25% disrupted epithelium, focal leucocyte infiltrates and focal haemorrhage; 2 = 25% disrupted epithelium, focal leucocyte infiltrates and focal haemorrhage; 3 = 50% disrupted epithelium, widespread leucocytes and haemorrhage; 4 = > 50% disrupted epithelium, extensive leucocyte infiltration and haemorrhage (13).

Statistical analyses

The study's data were expressed as means ± SD and analysed by a one-way analysis of variance, followed by Tukey post hoc test for multiple comparisons. A non-parametric test (Kruskal–Wallis test) with Dunn post hoc analysis was used for the macroscopic and histopathological data. A *p*-value of less than 0.05 was considered to be statistically significant.

RESULTS

The biochemical parameters were studied in all the groups. Mean TAC, 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 TAC, TOS, TNF- α, IL-1β and IL-6 levels compared with the colitis group ($p < 0.05$). The Table presents the statistical comparisons of the mean biochemical parameters across the study's groups.

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

DISCUSSION

Our study demonstrated the effectiveness of WB extract in an experimental model of colitis in rats. The effect of

Table: Statistical comparisons of the mean biochemical parameters and pathology results across the study's groups

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

TAC = total antioxidant capacity (µmol H_2O_2 equivalent/L); TOS = total oxidant status (mmol Trolox Equiv./L); TNF-α = tumour necrosis factor-alpha (pg/ml); IL-1β = interleukin-1 beta (pg/ml); MDS = macroscopic damage score; HDS = histologic damage score; IL-6 = interleukin-6 (pg/ml); WB = wolfberry.

[a]$p < 0.05$ *vs* group 1.

[b]$p < 0.05$ *vs* group 2.

The results were presented as means ± SD.

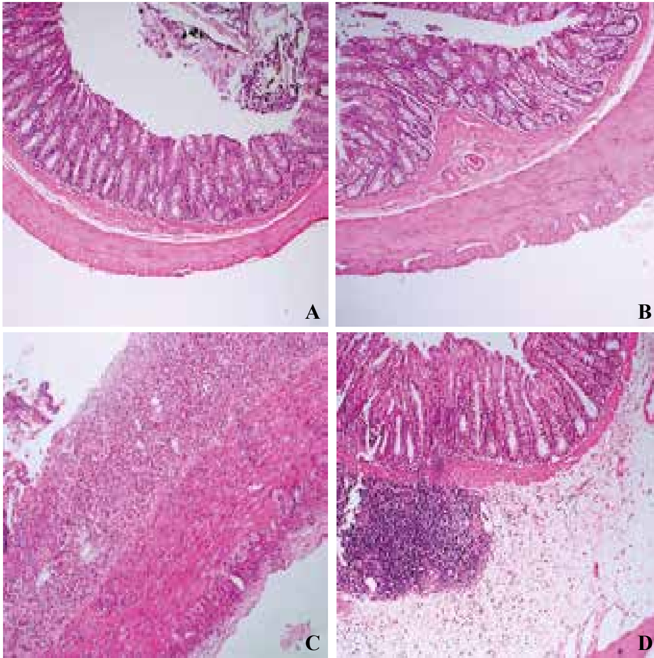

Figure: The histopathological changes in the study's groups. (A) Colonic tissues section of the control group (H&E ×100): intact epithelium, no leucocyte or haemorrhage. (B) Colonic tissues section of the wolfberry group (H&E ×100): intact epithelium, no leucocyte or haemorrhage. (C) Colonic tissues section of the colitis group (H&E ×100): intense inflammation and ulceration of the epithelial surface and thickened colonic muscle layer. (D) Colonic tissues section of the colitis + wolfberry group (H&E ×100): mild inflammation in the epithelium, submucosal severe oedema and moderate inflammatory cell infiltration of mixed type.

WB extract was evident in terms of the inflammatory markers such as TAC, 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 the 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 the standardized models of acute colitis. Certain factors are required to create and maintain colitis, including increased vasopermeability, 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 anti-oxidative properties of some natural herbal extracts on organ disorders of different origins had been investigated. Some examples of such extracts were the epigallocatechin gallate in green tea (20), the coprinus comatus fungus (21) and ginsan obtained from panax ginseng (22). Likewise, many studies had explored the effects of WB extract at the biochemical level and reported that it acts as an anti-oxidant, anti-ageing, anti-tumour and immune-stimulant agent. These effects had been linked to different organic and inorganic elements including beta carotene, riboflavin, ascorbic acid, thiamine cerebroside, and betaine (23–27). Wolfberry extract had also been studied in the cellular injury of the liver, eyes, small intestine and the kidney (28–31).

Previous work had 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 the 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 TAC and TOS.

Tumour necrosis factor-α, IL-1β and IL-6 are the main proinflammatory cytokines synthesized and released in response to oxidative stress and inflammation (38). Gastrointestinal host defence is an important area where cytokines is beneficial to the human body, although their excessive production may lead to bowel inflammation and impaired bowel motility (39). Tumour necrosis factor-α 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) favourably responded to antibodies by avoiding 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 WB + colitis group and the colitis group with respect to their mean cytokine levels. This result was consistent with the previous literature data.

CONCLUSION

Wolfberry extract is an agent that is effective for preventing acetic acid-induced colitis in rats. The introduction of this agent into clinical practice as a protective and therapeutic agent requires further clinical studies in both animals and humans.

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