The Healing Effect of *Berberis vulgaris* in Acetic Acid-Induced Ulcerative Colitis in Rat

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**Abstract:** Several anti inflammatory, immunomodulatory and antioxidant effects were reported for anthocyanins noticed in berberry fruits. So they may have beneficial effects in inflammatory bowel diseases (IBD). This study was undertaken to determine the healing effect of *Berberis vulgaris* in acetic acid-induced ulcerative colitis in rat model. In summer 2010, seventy male Sprague Dawley rats were divided into seven equal groups. Group I received 1 ml of 25% intra-colonic gel form of *B. vulgaris* extract daily; Group II received 1 ml of 50% intracolonic gel form of *B. vulgaris* extract daily; Group III received 1 ml of 2000 mg/ml *B. vulgaris* extract orally per day; Group IV received 1 ml of 1000 mg/ml *B. vulgaris* extract orally per day; Group V received just intra-colonic gel base; Group VI group received 0.5 ml/kg intracolonic normal saline after induction of colitis as the negative control; and Group VII received 2 ml of asacol intracolonic (0.5 ml asacol that dissolved in 9.5 ml normal saline) as the positive control group. All animals were evaluated for histological investigation and malondialdehyde (MDA) activity. The acetic acid induced inflammation was characterized by neutrophil infiltration, fibrin deposition, submucosal neutrophil margination, submucosal edema, epithelial necrosis and epithelial ulceration. *B. vulgaris* extract in both forms of intracolonic and oral administration caused a significant decrease in inflammation in acetic acid-induced colonic tissue and significant reduction in MDA activity. So *B. vulgaris* extract can be a good choice for treatment of UC and can broaden the current treatment options as an easily available and in-expensive herbal medicine.

**Key words:** Acetic Acid · Healing · Ulcerative Colitis · *Berberis Vulgaris* · Rat

**INTRODUCTION**

Ulcerative colitis (UC) and Crohn's disease (CD) are the two important inflammatory bowel diseases (IBD) defined as relapsing, chronic and remitting inflammatory diseases of the large intestine. They are multi-factorial and complex diseases with unknown etiology caused by many patho-physiological mechanisms and have several clinical manifestations such as abdominal pain, diarrhea, blood in the stool and weight loss [1]. Ulcerative colitis (UC) can involve the mucosa of distal parts of the colon and rectum leading to symptoms such as bloody diarrhea, abdominal pain, weight loss, chronic pain and increased risk of colon cancer [2].

The incidence of UC is identical in both sexes and is more in the 3rd and 4th decades of life [3] and its prevalence was shown to be about 10-20/1,000,000 per year in Western countries [4]. A persistent UC can raise the risk for development of colorectal cancer about 10-fold [5-7]. Immunologically, the mediated inflammatory process in the mucosa of colon and rectum due to excessive production of oxygen-derived free radicals by neutrophils and monocytes is considered as a key role in mucosal damage in UC [6]. Treatment of the disease is complicated due to its complex etiology but various medications such as 5-aminosalisylic acid derivatives, sulphasulphapyridine, corticosteroids, immune modulating agents, etc. have been introduced in treatment...
of UC even many side effects were reported after their long-term use and with a high relapse rate [7].

During the past 20 years, various animal models of colitis were introduced as indispensable tools to study on underlying mechanisms of IBD pathogenesis as well as to investigate on several potential therapeutics [8-13]. Various chemical compounds such as dextran sodium sulfate, 2,4,6-trinitrobenzene sulfonic acid, oxazolone, acetic acid and indomethacin have been applied for induction of experimental colitis in animal models among them, acetic acid induced colitis models are identical to human ulcerative colitis in terms of histopathological changes and various herbal drugs were studied in treatment of experimental models of UC [14].

Herbal extracts have also been used in therapy of IBD and the beneficial effects of these medicinal plants were shown to be due to their antioxidant properties [9-13, 15]. *Berberis vulgaris* is a shrub and a member of *Berberidaceae* family with protoberberines and bisbenzylisoquinoline alkaloids responsible for its anti-inflammatory properties [16]. Berberine as a major isoquinoline alkaloid ingredient of *B. vulgaris* was shown to be effective in treatment of diarrhea and gastrointestinal disorders [17,18]. The antioxidant and cytoprotective activity of *B. vulgaris* were also demonstrated in several studies [19, 20]. Also, other medicinal effects for *B. vulgaris* have been shown including antimicrobial, anti-pyretic, anti-colitic, anticholinergic and antihistaminergic properties [21-24]. This study was performed to determine the healing effect of *B. vulgaris* extract on acetic acid-induced ulcerative colitis in rat as an animal model.

**MATERIALS AND METHODS**

*B. vulgaris* fresh fruits were prepared from Shiraz, Iran and its species was determined in Department of Botany of Shiraz University. To prepare hydroalcoholic extract, the provided fruits were dried for five days in the room temperature and were then powdered, using percolation method while 1000 gr of the powdered form of fruit was transferred in ethanol:water (70:30) solution for 48 hour. The semisolid and gel form of the extract (37.7% w/w) were provided after filtration and evaporation under reduced pressure in the rotary evaporator [25].

Seventy male Sprague Dawley rats weighing 200-250 g were provided from Laboratory Animal Center of Shiraz University of Medical Sciences and were randomly divided into seven equal groups (10 rats). Group I received 1 ml of 25% intra-colonic gel form of *B. vulgaris* extract daily; Group II received 1 ml of 50% intracolonic gel form of *B. vulgaris* extract orally daily; Group III received 1 ml of 2000 mg/ml *B. vulgaris* extract orally per day; Group IV received 1 ml of 1000 mg/ml *B. vulgaris* extract orally per day; Group V received just intra-colonic gel base; Group VI group received 0.5 ml/kg intracolonic normal saline after induction of colitis as the negative control; and Group VII received 2 ml of asacol intracolonic (0.5 ml asacol that dissolved in 9.5 ml normal saline) as the positive control group.

Each rat was housed individually in a single cage in a 65-70% relative humidity and an ambient temperature of 21±2°C. The animals received a balanced diet and had access to water ad libitum. All animals were fasted overnight and their bowels were cleaned before induction of colitis. A polyurethane cannula (Diameter=2 mm) was used for rectal introduction of acetic acid and the tip was inserted up to 8 cm proximal to the anus verge. Two milliliters of 3% acetic acid was transferred intracolonic in the colon using a cannula during a 30 seconds period for induction of UC under ketamin and xylazine anesthesia. To evaluate the induction of UC, All animals were euthanized after one week. For all animals, the distal 10-cm portion of the colon was transferred into formalin buffer for histological evaluation. They were embedded in paraffin and cut into 5 μ sections and stained with hematoxylin and eosin (H and E) and studied under light microscope. The degree of inflammation of the colon was graded as described before (Table 1) [26].

<table>
<thead>
<tr>
<th>Neutrophil infiltrate</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Slight increase</td>
<td>1</td>
</tr>
<tr>
<td>Marked increase</td>
<td>2</td>
</tr>
<tr>
<td>Epithelium</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Muscularis mucosa</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Muscularis propria</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Serosa</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Fibrin deposition</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Mucoa</td>
<td>(0-1)</td>
</tr>
<tr>
<td>Submucoa</td>
<td>(0-1)</td>
</tr>
<tr>
<td>Submucosal neutrophil margination</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Submucosal oedema</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>Patchy</td>
<td>1</td>
</tr>
<tr>
<td>Confluent</td>
<td>2</td>
</tr>
<tr>
<td>Epithelial necrosis</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>Localised</td>
<td>1</td>
</tr>
<tr>
<td>Extensive</td>
<td>2</td>
</tr>
<tr>
<td>Epithelial ulceration</td>
<td>(0-1)</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Maximum score</td>
<td>20</td>
</tr>
</tbody>
</table>
Tissue specimens were provided and stored at -80°C for subsequent evaluation of the tissue MDA activity. MDA production were determined in the tissues [27]. MDA resulted into formation of a colored complex in the presence of TBA and HCL which was later detectable by spectrophotometer measurement at absorbance of 532 nm (Shimadzu uv-160). 1,1',3,3'-tetraethoxypropane was used as the standard solution and the findings were presented as µmol/g of protein.

The results were presented as mean±SD. Differences among groups were determined using one-way ANOVA and Tukey HSD post-hoc test. Non-parametric data were analyzed by Mann-Whitney U test. All statistical analyses were performed by SPSS software (Version 11.5, Chicago, IL, USA). Significance was considered at p<0.05.

RESULTS

In comparison to Control group while no epithelial ulceration and infiltration of PMN's leukocytes were visible (Figure 1), moderate PMN infiltration, aggregation of submucosal lymphoid, epithelial necrosis with focal surface ulceration were noticed 24 hours after intracolonic administration of acetic acid (Figure 2). After 7 days when colonic tissues were removed and examined histologically, severe neutrophil infiltration, submucosal neutrophil margination, submucosal edema, epithelial necrosis, fibrin deposition and epithelial ulceration as severe inflammatory features were noticed (Figure 3). Macroscopically, bloody diarrhea and abdominal distension were seen in all rats too. Figure 4 indicates the healing process and absence of epithelial ulceration and PMN infiltration after oral administration of 2000 mg/ml of B. vulgaris and Figure 5 denotes to healing process and absence of epithelial ulceration and mild PMN infiltration after intracolonic administration of 50% gel form of B. vulgaris.

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Fig. 1: No epithelial ulceration and infiltration of PMN's leukocytes (H and E, x100).

Fig. 2: Moderate PMN inflammatory cell infiltration with focal surface ulceration 24 hours after induction of UC (H and E, x200).

Fig. 3: Severe PMN infiltration and severe epithelial ulceration with fibrin deposition and necrosis 7 days after induction of UC (H and E, x400).

Fig. 4: Healing and absence of epithelial ulceration and moderate PMN infiltration (H and E, x200).
Fig. 5: Healing and any absence of epithelial ulceration and mild PMN infiltration (H and E, x200).

Fig. 6: Histopathologic evaluation of colon tissue of acetic acid induced ulcerative colitis rats after 7 days.
Fig. 7: MDA activity in colitis induced colonic tissue of rats after 7 days of study. Different superscript letters show significant differences between different groups. Superscript letters show statistically significant differences between groups (P<0.05).

Regarding scoring of all inflammatory features, a significant decrease was observed in groups I-IV and VII when compared with groups V and VI; whereas this decrease was significantly more visible in groups II and III. Considering neutrophil infiltration, this decrease was statistically significant in groups II, III and VII. Regarding fibrin deposition, a statistically significant decrease was visible in groups I-III and VII. Considering submucosal margination and epithelial ulcer, a statistically significant decline was seen in groups I-V and VII and for submucosal edema, this decrease was not statistically significant. For epithelial necrosis, a statistically significant decrease was observed in groups I-III and VII (Figure 6A-G).

*B. vulgaris* extract was shown to have a dose dependent healing effect while the 50% gel intracolonie administration resulted into a better healing effect in comparison to the 25% gel form. In oral administration, the 2000 mg/ml dosage resulted into more healing effect in comparison to the 1000 mg/ml dose of the extract. This healing effect was identical to asacol especially in 50% intracolonial gel form and 2000 mg/ml oral administration, which shows a significant healing effect on acetic acid-induced colitis in rats.

The results of tissue-associated malondialdehyde (MDA) activity were shown in Figure 7. There was a significant decrease in MDA activity in groups I-IV and VII in comparison with groups V and VI. This decrease was statistically more significant in groups II, III and VII.

**DISCUSSION**

Intacolonie administration of acetic acid was shown to induce rapid development of severe inflammation, infiltration of neutrophils and lymphocytes, presence of cryptic abscesses and ulceration in the colonic tissue together with diarrhea, weight loss and hematochezia.

Drugs such as salazosulphapyridine, mesalazine, corticosteroids, azathioprine, 6-mercaptopurine, methotrexate and cyclosporine as anti-inflammatory and immune-modulating agents are current methods of treatment for IBD [28].

There was a significant decrease for MDA activity after administration of *B. vulgaris* extract and there was a significant healing effect after therapy by intracolonie and oral administration of the extract. This process is due to granulation tissue formation and deposition of connective tissue, proliferations of fibroblasts and angiogenesis for distribution of the oxygen and nutrients to the inflamed area after injuries in bowel tissue [29] leading to re-epithelialization and healing of mucosal tissue [30].

Black raspberry powder was also demonstrated to have healing effects in UC which are probably due to its antioxidant and anti-inflammatory bioactive ingredients [31]. *Gymnema sylvestre* leaf is the other extract to significantly improve experimentally acetic acid induced colitis due to its anti-inflammatory and antioxidative properties too [32]. *B. vulgaris* has been administrated for a long period of time as a traditional medicine in Iran [33] while its consumption has been approved by FDA [34] because of its great amount of phenol contents such as anthocyanins [35] and its antioxidant and cytoprotective activities [36].

Our findings showed that the *B. vulgaris* extract in different doses and in the two forms can have healing effects in experimental acetic acid induced UC. The protective effects of this fruit lead to a decrease in MDA concentration as the index for lipid peroxidation and the ability to diminish oxygen free radicals by its polyphenolic contents.
Regarding the clinical and histological findings, it was evident that B. vulgaris extract had a dose dependent healing effect while the 50% gel intracolonic administration resulted into a better healing effect in comparison to the 25% gel form. In oral administration, the 2000 mg/ml dosage resulted into more healing effect in comparison to the 1000 mg/ml dose of the extract. This healing effect was identical to asacol especially in 50% intracolonic gel form and 2000 mg/ml oral administration, which shows a significant healing effect on acetic acid-induced colitis in rats. So B. vulgaris extract may be considered as a treatment option for UC and may broaden the current therapy choices for inflammatory bowel diseases.

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REFERENCES


