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The Modulatory Effects of Aqueous Extract of the Plant Biebersteinia Multifida on the Gastric Acid Level and Intestinal Cytokines in Ulcerative Colitis Model

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ABSTRACT

BACKGROUND AND OBJECTIVE: Inflammatory bowel disease is a chronic disease of the gastrointestinal tract with unknown etiology, which includes crohn's disease and ulcerative colitis (UC). Because an effective treatment has not been found so far, the present study was designed to investigate the effects of aqueous extracts of Biebersteinia multifida plant on inflammatory changes of the colon following acetic acid- induced ulcerative colitis in male rats.

METHODS: In this experimental study, 32 male wistar rats weighing 200-250 g were used in vivo. Animals were randomly assigned into four groups (each group consisting of 8 rats): 1- Intact; 2- Acetic acid-induced ulcerative colitis; 3- Biebersteinia multifida aqueous extract (200 mg /Kg); 4- Sulfasalazine treatment group (500 Mg /kg). In each group, the amount of gastric acid and intestinal TNF-α and IL-10 cytokines were measured at 8 days after of ulcerative colitis induction.

FINDINGS: The concentration of gastric acid in the induction group of colitis was 2/4533 ± 0.95 mEq/15 min, which in the treatment group with Biebersteinia multifida extract decreased the level of gastric acid to 0.4400 ± 0.17 (p = 0.000). Also, TNF-α changes in the colitis and extract group were 279.68±42.71 and 160.35±28.79, respectively, which was significant (p=0.000). The concentration of IL-10 in the colitis group was 349.75±31.89, which increased to 353.75±66.46 after injection of the extract (p=0.015).

CONCLUSION: The results showed that aqueous extract of Biebersteinia multifida in a dose of 200 mg/kg has an effect on colon inflammation and it can be improved.

KEY WORDS: *Biebersteinia Multifida, Ulcerative Colitis, Cytokine, Rat.*

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Introduction

The inflammatory bowel disease is a chronic disease of the digestive tract, including Crohn's disease and ulcerative colitis, which is more common in Iran (2, 1). ulcerative colitis is characterized by inflammation limited to the mucous membrane of the large intestine, which almost always includes rectum and may include other parts of the large intestine. The ulcerative colitis is more common in young adults; therefore, patients manage their illness for a long time. The most important signs and symptoms include diarrhea, bleeding, and severe symptoms such as anorexia, weight loss, fever and tachycardia (4, 3). Diagnosis is based on endoscopic findings and pathologic confirmation (5). Gastric ulcer is caused due to the lack of coordination between offensive and defensive factors. offensive factors include acid and pepsin and mucus and bicarbonate can be mentioned as defensive factors. Knowing the pattern of recurrence of the disease plays an important role in deciding on the treatment and follow up of patients. Although there are several treatments for inflammatory bowel disease, it has not yet been found effective in these conditions. Among the treatments include amino salicylates, antibiotics, corticosteroids and immunosupressives, which are associated with extensive side effects (8-6). Therefore, newer, cheaper and more effective treatments are needed. On the other hand, studies have found that people with inflammatory bowel disease have gastric ulcers, which have been done through gastric acid (9). TNF-α is a potent pro-inflammatory cytokine, and it seems to play a key role in the pathogenesis of inflammatory bowel disease. The significance of TNF-α in the pathogenesis of ulcerative colitis is characterized by significant results from TNF-α clinical trials. On the other hand, TNF-α levels are correlated with the severity of the disease (12-10). Clinical beneficial effects following immunotherapy leading to reduced or inhibited TNF-α production have been reported in intestinal inflammation (13). In recent years, the use of medicinal plants has given special attention to itself. One of the plants that has a widespread effect is Biebersteinia multifida. The Geraniaceae family in Iran has five genera and one of them is Biebersteinia. Biebersteinia has four species in the world. B. multifida DC is the only species that grows in Iran. This plant is found in many provinces of Iran and is also found in the Caucasus, Central Asia, Afghanistan, Iraq and Lebanon (15,14). B. multifida has yellow flowers and thick root and a height of 20 -70cm (16). In traditional medicine, the roots of B. multifida are used to relieve muscle pain, which may be attributed to the anti-inflammatory and analgesic properties of this plant (17). It is also used for treatment of fear and anxiety and also used to treat children's nocturnal enuresis (18). It has been shown that B. multifida is effective in the reconstruction of bone fractures and reduction of catatonic severity after treatment with anti-anxiety drugs (19). Previous data indicate the presence of polysaccharides, peptides, alkaloids such as vascinons and flavonoids, including 7-glucosides of apigenin, luteolin and thrysteine, as well as 7-retinosides of apigenin and luteolin in this plant (21, 20). There are no reports of B. multifida apoptotic activity. Therefore, considering that ulcerative colitis is in fact a complex disorder that can affect many tissues, including the stomach, the purpose of this study was to investigate the effects of aqueous extract of the plant on the secretion of gastric acid and colon inflammatory changes following ulcerative colitis induced by acetic acid in male rats.

Methods

Animals: In this experimental study after approval by ethics committee of Bojnourd University of Medical Sciences with code IR. NKUMSREC.1396.62. was performed on 32 male Wistar rats weighing 250-200 grams. The animals were kept at temperature of 20 to 22 ° C and a 12-hour lighting-darkness period, and water and food were freely provided to them. Animals were randomly divided into four groups of eight, each group was evaluated for gastric acid values and cytokine changes in their intestinal tissue 8 days after induction of ulcerative colitis. The study groups included the following:

1. Intact: Male healthy rats, the above mentioned indexes were studied.

2. Ulcerative colitis was induced in animals using acetic acid, and then the indices were evaluated eight days after induction of colitis.

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3) The treatment group received orally aqueous extract of Biebersteinia multifida at a dose (200 mg/kg) for 8 days after induction of ulcer, and the indices were evaluated on the eighth day after the induction of ulcer.

4. The sulfasalazine treatment group received standard sulfasalazine at a dose (500 mg/kg), which was started orally two days before the induction of colitis, and for 5 days after induction of ulcer and indices were evaluated after treatment.

The method of inducing acute ulcerative colitis: animals were deprived of food for 24 hours before the test, but there was free access to water. Anesthetizing of each animal was done with ether, then was placed in the back position and a 2 mm outer diameter polyethylene tube was inserted through a rectum up to 8 cm. 2 ml of acetic acid (3% v/v in saline 0.9%) was entered into the colon. Acetic acid was stored in the colon for 30 seconds and then allowed to leave. At the end of the experiment (on the eighth day after the induction of ulcerative colitis), the animals were killed, then colon biopsies were carried out for 10 centimeters at the end.

Cytokine Level Measurement Method: An intestinal tissue sample was collected immediately after the animal was killed and kept at -70 ° C for a week until further analyzes. After preparing the homogenous tissue, the level of interleukin 10 and tumor necrosis factor was determined by ELISA kit according to the manufacturer's instructions. Results were calculated according to the standard curve.

Measurement of gastric acid level: 24 hours before the experiment, the animals were deprived of food, but there was free access to water. To remove the effect of daytime rhythms, the experiment began at 8 am. In this study, thiopental sodium (50 mg/kg, ip) was used for anesthetizing. After anesthetizing the animal, the tracheostomy and simultaneously esophagus were blocked to prevent the oral discharge from entering the trachea. Then the animal was laparotomized, and by making a hole in the duodenum, a cannula entered the duodenum and moved to the stomach. To measure the concentration of gastric acid, the Washout method was used for 15 minutes. In the washout method, for each concentration, two samples were taken at a distance of 15 minutes to minimize the error of the test. Titration was carried out in the laboratory immediately after sampling and about 0.5 ml of gastric secretion was sampled and normalized to 0.01. Using the formula N1V1 = N2V2, normality of gastric secretion was obtained, indicating concentration of the gastric acid. To measure the total amount of titratable acid, a titration device with a precision of 0.2 ml was used.

Preparation and administration of the extract: A certain amount of dried Biebersteinia multifida plant is poured into an electric mill to powder. Soak the dried powder for up to 48 hours and dissolve in water and stir it for several times during this time so that the water can easily evaporate. Lay off and apply the aqueous extract in the test tube and put it in a centrifuge machine at a speed of 4500 rpm for 8 minutes to separate the suspended particles. After centrifugation, the resulting liquid was placed in an oven at 70 ° C to maintain a concentrated extract. Dried precipitates were weighed to calculate the weight of undissolved material and subtracted from the initial value of the dissolved mass. The extract was then given orally to the animal.

Ethical considerations: In order to observe ethical considerations and prevent animal distress, all of them were anesthetized before surgery and tried to observe all the principles of animal research ethics during work with animals. At the end of the work, the animal was killed by intracardiac injection of KCl to maintain the working ethics.

Data analysis: The results of the cytokine values were expressed as mean ± standard error (SEM ± Mean), and the ANOVA(Tukey) test was used to compare the quantitative variables between the two groups, and $p < 0.05$ was considered significant.

Results

Changes of gastric acid concentration: The concentration of gastric acid in the healthy group was 1.52±0.19 mEq/15 min, which increased to 2.4533±0.95 after induction of colitis (p=0.006). Treatment with Biebersteinia multifida extract reduced the amount of gastric acid to 0.44 ± 0.17 (p=0.000). Also, this amount in sulfasalazine group reached 1.262 ± 0.18, which was also significant (p = 0.013).

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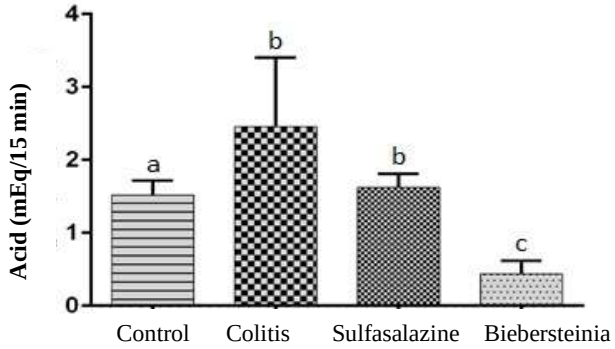

Figure 1. Comparison of the mean concentration of gastric acid (mEq/15 min) in the 1) control group 2- group of acetic acid-induced ulcerative colitis, 3- sulfasalazine treatment group, 4- treatment group with Biebersteinia multifida extract for 8 days.

TNF-α changes: TNF-α in the healthy group was 176.06±8.53 µg/ml and after induction of colitis, this increased to 279.68±42.71 (p=0.000), in addition in Sulfasalazine and Biebersteinia multifida extracts decreased to 162.99±9.16 and 160.35±28.79 (p=0.000), which was significant in all of these groups, but in Biebersteinia multifida extract group was further reduced.

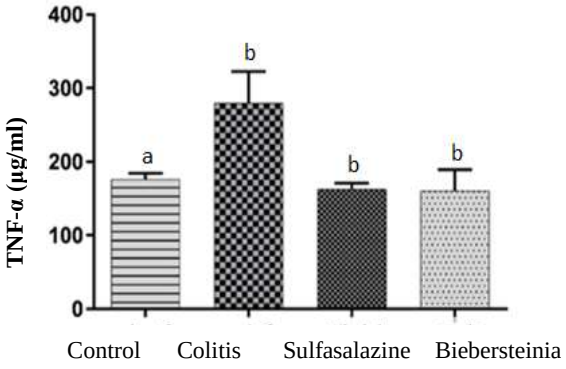

Figure 2. Comparison of mean TNF-α (µg/ml) in the 1) control group, 2. Acetic acid-induced ulcerative colitis, 3- Sulfasalazine treatment group, 4- Treatment group with Biebersteinia multifida extract for 8 days.

IL-10 changes: IL-10 concentration in the healthy group was 406.25±60.35 µg/ml, which decreased to 349.75±21.89 after induction of colitis (p=0.071), and these values in sulfasalazine group, it was 378.5±93.84 (p=0.001) and in with Biebersteinia multifida extract was 353.75 66.66 (p=0.015). The increase in both groups was significant.

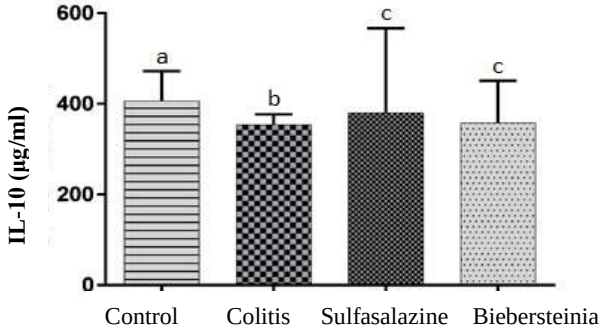

Figure 3. Comparison of mean IL-10 (µg/ml) in the 1) control group, 2. Acetic acid-induced ulcerative colitis, 3- Sulfasalazine treatment group, 4- Treatment group Biebersteinia multifida extract for 8 days.

Macroscopic evaluation of colitis: intra-rectum administration of acetic acid produced macroscopic colitis symptoms. The macroscopic score in the control group was 1.0±0.01, in the colitis group was 3±0.3 and in the sulfasalazine and Biebersteinia multifida extract group was 2.2±0.2 and 2.25±0.3, respectively. The macroscopic values of the Biebersteinia multifida extract group (25 mg/kg) and the sulfasalazine-treated groups were significantly lower than the colitis group ($p < 0.05$).

Discussion

In this study, it was shown that oral administration of Biebersteinia multifida extract reduced the damage induced by intra-colon injection of acetic acid, and also reduced acid secretion. The results showed that the aqueous extract Biebersteinia multifida at a dose of 200 mg/kg affect colon inflammation and improve it. In the case of cytokines, this study also found that the Biebersteinia multifida extract increased insignificantly IL-10 which decreased in colitis induction, and significantly reduced the TNF-α cytokine that increased in colitis induction. IL-10 cytokine is an immunosuppressive cytokine that is produced by various types of leukocytes and non-blood cells and plays an important role in regulating intestinal mucosal homeostasis and intestinal inflammation. However, according to published articles, IL-10 levels increase in the inflammatory bowel tissue (22-24). TNF-α is a potent pro-inflammatory cytokine, and it seems to play

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a key role in the pathogenesis of inflammatory bowel disease. The significance of TNF-α in the pathogenesis of ulcerative colitis is characterized by significant results from TNF-α clinical trials. On the other hand, TNF-α levels are correlated with the severity of the disease (12-10).

Effective clinical effects following immunotherapy leading to a decrease or inhibition of TNF-α production have been reported in intestinal inflammatory disease (13), which in the present study Biebersteinia multifida extract has significantly reduced the TNF-α level in ulcerative colitis induced by acetic acid. A coherent study following use of Biebersteinia multifida extract was not found in the colitis model. On the other hand, studies regarding Biebersteinia multifida plant, Golshan and colleagues showed that Biebersteinia multifida extract has a cytotoxic effect on malignant cells and normal HEK293 cells in dose-dependent manner and significantly reduces cell viability (IC50 between 199/2 to 9.2 µg/ml) (15). Nabavi et al showed that the root had high phenol content (1/80 ± 1/3 mg/ml) (-1) and had the highest activity in DPPH radical activity (9/95 +/- 2.3 µg/ml -1). It also showed better power loss than other parts. As a result, this study showed that the Biebersteinia multifida extract showed different levels of antioxidant and antifungal activity in all tested models (25). Farsam et al. observed similar activity between Biebersteinia multifida root extract (10 mg/ kg; i.p.) and indomethacin (4 mg/kg; i.p.). The results of formalin test showed that analgesic activity of root extract (50 mg/kg/day) with morphine (10 mg/kg) was comparable in the first stage of formalin test (17). Monsef-Esfahani and colleagues in their study showed that root extract had anti-diazepam effects with longer duration. The sustained effects of crude extracts were stable for 90 minutes and after administration of 45 mg/kg, the effect of diazepam was reduced by 90 minutes. For the first time, the biological classification of B. multifida root extract shows stable anxiolytic effects that result in the isolation of the three coumarin derivatives with known inhibitory and anti-anxiety effects. These data help the traditional evidence-based use of B. multifida root for anxiety disorders (18). Roth et al., in a study entitled Blueberry Effects in the expression of cytokines in the intestines of patients with ulcerative colitis, found that patients treated with Blueberry successfully increased the level of specific cells of the Th17-specific cell line of IL-22 cytokine and immune cells regulating IL-10 cytokine, as well as a decrease in serum levels of TNF-α and MCP-1. The results showed that the treatment with blueberries in patients with Ulcerative colitis caused anti-inflammatory effects and the modulation of T cell cytokines and inhibition of IFN-γ transmission (26). Park et al. reported that the aqueous extract of the Bentham Pogostemon Cabin (PCW) stopped colon inflammation. Treatment with PCW effectively inhibited TNF-α, in addition, PCW reduced the expression of IL-8, MCP-1 and IL-6-induced TNBS in the mouse colon. Taken together, the results of this study showed that PCW has suppressed colon inflammation that these results are mechanically consistent with our study (27).

Regarding gastric acid, Niazmand et al., in a study entitled the effect of aqueous extract of Teucrium Polium L on gastric acid secretion under baseline, vagotomy and vagal nerve stimulation, the results showed that the different concentrations of aqueous extract Teucrium Polium L in three conditions did not make a significant difference in the level of gastric acid secretion (8), which, unlike in our study, had a significant effect on the secretion of gastric acid. Due to the numerous effects of this plant, as well as the effect of eliminating colon and stomach inflammatory problems and reducing gastric acid and its effects on cytokines, this plant can be used in gastrointestinal disorders and ulcerative colitis. It is also suggested that other properties of this plant be evaluated in order to be able to replace the chemical in the future as a drug.

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