Original Article

Anti-inflammatory effect of *Helichrysum oligocephalum* DC extract on acetic acid — Induced acute colitis in rats

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Abstract

Background: *Helichrysum oligocephalum* DC. from Asteraceae family is an endemic plant growing wild in Iran. This study was carried out to investigate the effect of *H. oligocephalum* hydroalcoholic extract (HOHE) on ulcerative colitis (UC) induced by acetic acid (AA) in rats.

Materials and Methods: Rats were grouped ($n = 6$) and fasted for 24 h before colitis induction. Treatments were started 2 h before the induction of colitis and continued for two consecutive days with different doses of HOHE (100, 200, and 400 mg/kg) orally (p.o.) and intraperitoneally (i.p.). The colon tissue was removed and tissue damages were scored after macroscopic and histopathologic assessments.

Results: Among the examined doses of HOHE, 100 mg/kg was the most effective dose that reduced the extent of UC lesions and resulted in significant alleviation. Weight/length ratio as an index of tissue inflammation and extravasation was also diminished in the treatment group administered HOHE at a dose of 100 mg/kg, and the results showed correlation with macroscopic and histopathologic evaluations. These data suggest that HOHE (100 mg/kg) administered either p.o. or i.p. was effective in diminishing inflammation and ulcer indices in this murine model of acute colitis in a non–dose-related manner.

Conclusions: *H. oligocephalum* could be considered as a suitable anticolitis alternative; however, further studies are needed to support this hypothesis for clinical setting.

Key Words: *Helichrysum oligocephalum*, inflammation, rats, ulcerative colitis

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INTRODUCTION

Inflammatory bowel diseases (IBD) are immune-mediated chronic or relapsing disorders of the gastrointestinal (GI) tract and consist mainly of Crohn's disease (CD) and ulcerative colitis (UC).[1]

The etiology of UC is still unknown; however, the most accepted hypothesis currently implicates a combination of one or more of the following factors:[2] increased free radical production and decreased

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antioxidant capacity, immune dysregulation caused by genetic or environmental factors, abnormal GI tract luminal factors such as the GI tract flora, and defects in the GI mucosal barrier that allow luminal factors to penetrate into the mucosa.[3-5]

Furthermore, infiltration of neutrophils, macrophages, lymphocytes and mast cells, ultimately giving rise to mucosal disruption and ulceration.[6]

Because the cause of IBD has not been fully established, current medical therapy is facilitative and supportive rather than curative.[7]

Therapeutic options include mesalamine and other 5-aminosalicylates (5-ASA), antibiotics (including rifaximin and tinidazole), steroids (including topical formulations and budesonide), immunomodulators [including azathioprine, 6-mercaptopurine, methotrexate (MTX), and cyclosporine], and monoclonal antibodies (including infliximab, adalimumab, and natalizumab).[8]

Inadequacies in efficacy and safety, potentially serious complications and side effects, and the costs of medicines provide a strong impetus to seek new approaches to disease management such as alternative and traditional therapies which are vastly considered in the IBD patients for several years.[9-11]

Herbal medicines are generally used in such cases when drugs are to be used for chronic periods.[12] Drugs of herbal origin reduce the offensive factors and have proved to be safe, clinically effective, relatively less expensive, and globally competitive with chemical drugs, and are with better patient tolerance.[13,14]

The genus *Helichrysum* belongs to the Asteraceae family and consists of an estimated 600 species.[15]

Nineteen species of the genus *Helichrysum* are found in Iran, of which eight are endemic. The rate of endemism in the genus *Helichrysum* in Iran is ca. 42%, and *Helichrysum oligocephalum* DC. is an endemic plant growing wild in Iran.[16,17]

The plant is spread over the entire country, but is most abundant in the west of Iran. This plant is currently offered for treatment of GI disease in Isfahan medicinal herbs market as a fraud type of *Artemisia absinthium* whose anti-ulcerative action has been demonstrated previously.[18,19]

Eighty-two components have been detected in the essential oil of *H. oligocephalum*, and thymol and beta-caryophyllene are the most important ones. Moreover, polyphenolic and flavonoids found in the hydroalcoholic extract of *H. oligocephalum* (HOHE) possess high antioxidant capacity with potent anti-inflammatory activity.[20]

Due to the above-mentioned properties, *H. oligocephalum* would be expected to reduce injury and/or improve colitis tissue in models with induced ulcerative colitis. We investigated the effects of this plant on tissue inflammatory activities in an acetic acid (AA)-induced ulcerative colitis model in rats.

MATERIALS AND METHODS

Plant material and preparation of extract

H. oligocephalum was purchased from a local medicinal herbs market in Isfahan and authenticated by Department of Pharmacognosy, Isfahan School of Pharmacy and Pharmaceutical Sciences. For preparation of HOHE, dried and finely powdered aerial parts of the plant (100 g) were wetted by ethanol:water mixture (70:30) and percolation was undertaken using extra volume of solvent for 48 h for full extraction. The extract was then shaken, filtered, and evaporated in a rotary evaporator under reduced pressure till an extract of semi-solid and gelling nature of yield 11% (w/w) was obtained.[21]

Chemicals

Dexamethasone was purchased from Iran Hormone Pharmaceutical Co. (Tehran, Iran). Hexadecyltrimethylammonium bromide (HTAB), aprotinin A, bovine serum albumin, phenylmethylsulfonyl fluoride, benzethonium chloride, ethylenediaminetetraacetic acid (EDTA), and Tween-20 were all purchased from Sigma Chemical Company (St. Louis, MO, USA). Tumor necrosis factor-alpha (TNF-α) (ALPCO, Windham, USA) kit was used for measurement of TNF-α variables. All the organic solvents and AA used were of analytical grade and procured from Merck (Darmstadt, Germany).

Animals

Seventy adult male Wistar rats (180–220 g) were used for this work (supplied by the Animal House, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences). The animals were maintained under standard and constant conditions of light, humidity, and temperature. All rats were maintained on standard pelleted rat chow and water *ad libitum*. All experiments were conducted according to the local ethics guidelines for research on animals and approved by the Research Committee of Isfahan University of Medical Sciences.

Animal groups

Animals were randomly assigned to sham, control, test, and reference groups of six rats in each as follows:

Sham group: Treated with vehicle (distilled water) orally (p.o.) (1 ml) and intraperitoneally (i.p.) (0.5 ml) without colitis induction (normal group)

Control group: Treated with vehicle p.o. (1 ml) and i.p. (0.5 ml), respectively, after induction of colitis

Extract groups: Treated with HOHE at doses of 100, 200, and 400 mg/kg (1 ml p.o. and 0.5 ml i.p.)

Reference group: Treated with dexamethasone (2 mg/kg, p.o. and 1 mg/kg, i.p.)

The doses were given once daily starting 2 h before the induction of colitis and continued for two consecutive days. The test plant samples were freshly prepared prior to administration.

Induction of experimental colitis in rats

The animals were fasted for 24 h with free access to water before induction of colitis. Induction of colitis was performed using a modification of the method described by Mascolo *et al*. (1995).[22] Colitis was stimulated in the rats by AA-induced colonic inflammation under light ether anesthesia, administering 2 ml of 4% (v/v) AA intrarectally with a soft 6-Fr pediatric catheter. The catheter was inserted into the anus up to a length of 8 cm, and then AA was administered.

On the third day after induction, all rats were sacrificed. The last 8 cm of the colon was excised, opened longitudinally, and rinsed with normal saline solution. Then, the distal colon was weighed and the mucosal lesions were scored macroscopically. Tissue samples were taken for macroscopic scoring, histopathologic examination, and biochemical studies.

Assessment of colitis

After washing the mucosa with saline (0.9%) solution, the macroscopic appearance of the colonic mucosa was scored by an independent observer according to a scale ranging from 0 to 3 as follows:

0 = No ulcer
1 = Inflammation, edema, thickness, and superficial ulcer
2 = Bleeding and deep ulcer
3 = Necrosis and/or perforation

After macroscopic evaluations, the tissues were cut into three equal parts. One part of the sample of colonic tissue was preserved in 10% formalin for histologic examination and the other two colonic samples were frozen on liquid nitrogen and stored immediately at –70ºC until analysis.

Determination of myeloperoxidase (MPO) activity

MPO activity has been used as an index of leukocyte adhesion and accumulation in several tissues including the intestine. The principle of the method depends on the release of MPO enzyme in the homogenate of the colonic tissue used. MPO activity was determined by the method of Motavallian *et al*.[23]

The tissue was weighed and then each sample was finely chopped in homogenizing tube with 2 ml potassium phosphate buffer (pH 6.0) containing 0.5% HTAB. After sonication, the homogenates were kept in an ice bath for 3 × 10 s, the suspensions were centrifuged at 15,000 rpm for 15 min at 4°C, and then the supernatant was decanted for analysis. Assay mixture was placed in a 1-cm path length cuvette that contained 0.1 ml of colonic supernatant and 2.9 ml of freshly prepared 50 mM phosphate buffer (pH 6.0) containing 0.005% H_2O_2 and *o*-dianisidine dihydrochloride (0.167 mg/ml). The supernatant was added last, and the change in absorbance at 450 nm was followed for 5 min. MPO activity was expressed in units (U) per gram tissue weight of wet tissue.

Measurement of TNF-α

TNF-α was determined according to the method of Motavallian *et al*.[23] The tissue samples were weighed, snap frozen on liquid nitrogen, and stored at –70°C to be processed for TNF-α determination. Colon samples were homogenized in phosphate-buffered saline (PBS; pH = 7.4) containing 0.4 M NaCl, 0.05% Tween-20, 0.5% bovine serum albumin, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, aprotinin A 20 KI, and 10 mM EDTA. They were then centrifuged at 12,000 ×*g* for 30 min at 4°C, and then enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of TNF-α in the supernatants.

Statistical analysis

Data analysis was performed using the SPSS statistical package (version 20.0) and the results were reported as mean ± standard error of mean (SEM). The differences between groups were tested by one-way analysis of variance (ANOVA) with Tukey's *post-hoc* test. Non-parametric data were analyzed by Mann–Whitney U test. *P*-value <0.05 was considered significant.

RESULTS

Macroscopic results

In the control group, severe macroscopic damage and hemorrhagic or ulcerated mucosa in the colon were

found after rectal administration, as assessed by the colonic damage score scale.

Dexamethasone significantly decreased ulcer severity ($P < 0.01$ for p.o. and $P < 0.05$ for i.p.) and weight/length ratio ($P < 0.05$), compared to control group [Table 1].

On the other hand, regardless of the mode of administration, *H. oligocephalum* (100 mg/kg) significantly reduced the ulcer severity in a non–dose-related manner ($P < 0.05$ in comparison to the control group) as shown in Table 1.

We found that ulcer area and ulcer index were decreased in dexamethasone and *H. oligocephalum* groups (100 mg/kg), compared to control group ($P < 0.05$) [Table 1].

Microscopic results

In the normal group, histopathologic assessment and microscopic images of the colon tissue revealed regular colonic mucosa with intact epithelium, crypts, and submucosa.

However, it was found that the AA-induced colitis group exhibited transmural necrosis, edema, and submucosal inflammatory cells' infiltration and distortion of crypt architecture [Figure 1a].

Rats treated with dexamethasone [Figure 1b] or HOHE (100 mg/kg) [Figure 1c] significantly diminished neutrophil infiltration, submucosal edema, and the extent or severity of tissue damage, as reflected in total colitis index parameter [Table 1].

MPO activity

Colonic levels of MPO in different groups were assessed and compared with the level in control group. Colonic MPO activity in the control group was significantly higher than that in the normal group ($P < 0.001$), indicating increased leukocyte infiltration.

Treatment with either dexamethasone or HOHE (100 mg/kg) had similar effect on MPO activity in such a manner that both of them were effective in reducing the MPO level significantly as compared to that of the control group ($P < 0.1$) [Figure 2].

TNF-α levels

We found that TNF-α activity was significantly enhanced in the inflamed colon of control rats in comparison with that of sham group rats ($P < 0.001$).

Administration of HOHE (100 mg/kg), on the other hand, resulted in a significant reduction in colonic TNF-α level.

Greater doses of HOHE (200 and 400 mg/kg) did not show significant changes in macroscopic, histopathology, MPO activity, and TNF-α levels, as compared to those of control group [Table 1, Figures 1-3].

DISCUSSION

The present investigation demonstrated that intrarectal administration of 4% AA caused extensive macroscopic damage and formed hemorrhagic and ulcerated colonic mucosa.

Additionally, the present study showed a significant increase in MPO activity and in the level of some proinflammatory cytokines such as TNF-α in the control group.

The results obtained in the reference (dexamethasone) group, which showed an effective protection considering the macroscopic and microscopic outputs, in comparison to the control group indicated the consistency, effectiveness, and suitability of the method.

Table 1: Effect of *H. oligocephalum* hydroalcoholic extract (HOHE) on macroscopic and pathologic parameters and indices of colitis in rats

Group/dose (mg/kg)	Route	W/L ratio (mg/cm)	Ulcer area, cm²	Ulcer severity (0-3)	Ulcer index	Total colitis index (0-10)
Sham	p.o.	84.0±3.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control	p.o.	133.0±6.7	6.67±1.04	2.66±0.21	9.3±0.6	9.33±0.49
HOHE100	p.o.	97.0±3.1*	3.02±0.32*	2.00±0.36	5.0±0.4*	4.16±0.65**
HOHE200	p.o.	130.0±8.4	4.11±0.84	2.00±0.36	6.1±0.6	8.83±0.47
HOHE400	p.o.	124.0±9.1	5.85±1.2	2.00±0.36	7.9±0.9	9.16±0.54
Dexa 1	p.o.	91.0±4.1*	2.03±0.6*	1.33±0.21**	3.4±0.3**	4.00±1.33**
Control	i.p.	123.1±8.2	6.1±1.3	2.7±0.4	8.8±0.5	8.5±0.6
HOHE100	i.p.	88.0±6.2*	2.66±0.47*	1.5±0.22*	4.2±0.5*	6.00±1.23*
HOHE200	i.p	126.0±7.3	6.00±1.36	2.00±0.36	8.0±0.6	8.83±0.74
HOHE400	i.p.	111.0±6.1	6.04±0.85	2.00±0.36	8.0±0.4	8.16±0.65
Dexa 2	i.p.	91.0±9.9*	2.54±0.76*	1.33±0.33**	4.0±0.4**	3.33±0.91**

Data are represented as mean ± SEM; Sham and control groups were treated with distilled water; Dexa: dexamethasone; p.o., oral; i.p., intraperitoneal; *$P < 0.05$; **$P < 0.01$ compared to related control group; ANOVA for parametric data and Mann–Whitney U test for non-parametric data

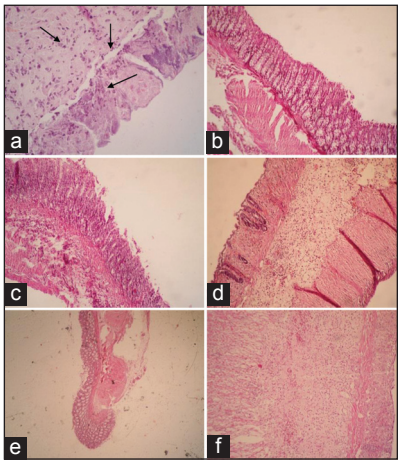

Figure 1: Microscopic presentation of acetic acid–induced colitis in rats. (a) Distortion of crypt architecture, loss of epithelial cells, ulceration, and neutrophil infiltration were observed in colitis rats. (b and c) Normal colonic tissue architecture was seen in dexamethasone (oral or parenteral) treated group. (d) *H. oligocephalum* (100 mg/kg) treated rats showed mild to moderate mucosal and submucosal inflammation and mucosal inflammatory cell infiltrates. (e and f) *H. oligocephalum* treated rats (200 and 400 mg/kg) showed destruction of mucosal architecture and infiltration of neutrophils

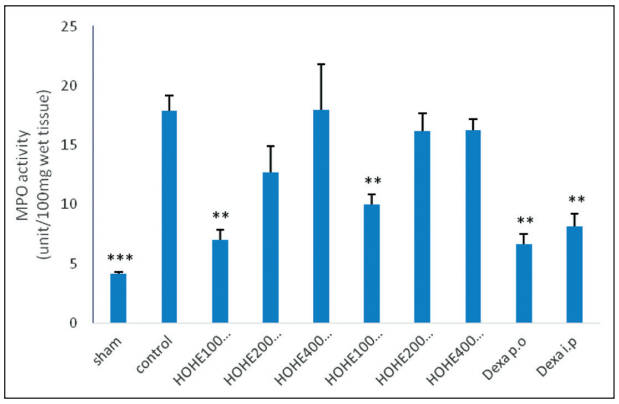

Figure 2: Effects of different doses of *H. oligocephalum* on colonic myeloperoxidase (MPO) levels in acetic acid–induced colitis in rats [HOHE, *H. oligocephalum* hydroalcholic extract (100, 200, 400 mg/kg); Dexa, dexamethasone (1 and 2 mg/kg); p.o., oral; i.p., intraperitoneal]

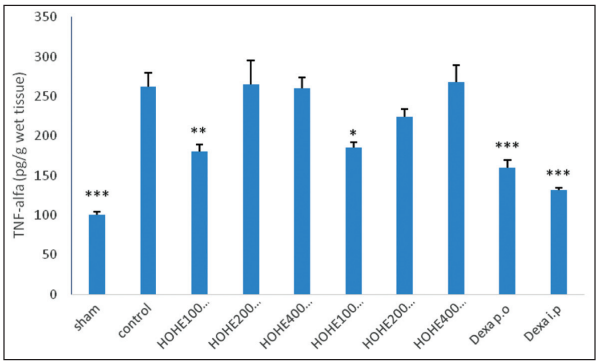

Figure 3: Effects of different doses of *H. oligocephalum* on colonic TNF-α levels in acetic acid–induced colitis in rats [TNF-alfa, tumor necrosis factor-alpha; HOHE, *H. oligocephalum* hydroalcholic extract (100, 200, 400 mg/kg); Dexa, dexamethasone (1 and 2 mg/kg); p.o., oral; i.p., intraperitoneal]. Values are expressed as mean ± SEM of six rats per group

The effects of different traditional medicines have been studied on AA-induced colitis, including *Matricaria aurea*,[24] *Zingiber officinale*,[25] *Ginkgo biloba*,[26] *Boswellia serrata*,[27] and *Calendula officinalis*.[28]

The present study is the first to demonstrate the effect of *H. oligocephalum* on UC. Regarding the macroscopic (ulcer index) and histologic (total colitis index) results, it was evident that treating rats with HOHE (100 mg/kg) reduced the mucosal damage and subsequently attenuated MPO activity in colonic tissues.

Moreover, our study established that *H. oligocephalum* decreased the production of TNF-α in the colonic tissue. The most effective dose was 100 mg/kg, which shows that the active ingredients are potent. Higher doses (200, 400 mg/kg) administered either p.o. or i.p. had no significant therapeutic effect on inflammation and ulcerative indices in rat colons, suggesting that there is no relationship between dose and efficacy. It is notable that these higher doses of HOHE did not aggravate the colitis.

It could be attributed to some active components of extract which probably oppose with the above-mentioned therapeutic actions, and became more accentuated by using higher doses of HOHE. This is in accordance with the results reported by Bairy *et al*. They revealed that *Amaranthus spinosus* Linn. aqueous extract possesses diuretic property which is observed predominantly at 500 mg/kg dose and there is no dose–response relationship.[29]

Beta-thujone, one of the active ingredients of HOHE, has been shown to be toxic in greater amounts in rats.[30]

Preliminary phytochemical analysis of the studied extract revealed that flavonoides, saponins, and tannins are the main constituents. Thymol, on the other hand, is found in the greatest amount in the essential oil of *H. oligocephalum*, for which antioxidant,[31] anti-inflammatory,[32] and local anesthetic and analgesic[33] activities have been demonstrated, which can inhibit edema, inflammation, neutrophil migration, and TNF-α expression.[32,34]

Flavonoids have been reported to act in the GI tract, and have antispasmodic,[35] anti-secretory, antidiarrheal,[36] antiulcer, and antioxidant properties.[37]

The role of oxidative stress has been confirmed in IBD. These species are cytotoxic agents, causing cellular dysfunction and damage.[38]

Flavonoids react with the free radicals to form more stable radicals with lower toxicity. Besides, they can chelate Fe^{2+}, which results in inhibiting the effects of free radicals.

Flavonoids can also inhibit the secretion of inflammatory mediators such as nitric oxide, interferon (INF)-γ, interleukin (IL)-12, and TNF-α, of which the latter has a principal role in IBD pathology.[39, 40]

Saponins, which have anti-inflammatory, antioxidant, and anti-cancer effects, are the other ingredients found in significant amounts in HOHE.[41]

Tannins can protect intestinal mucosal layers by precipitating their microproteins, and also protect the layers against chemical injuries and proteolytic enzymes.[42]

CONCLUSION

In conclusion, this study suggests that *H. oligocephalum* possesses anti-inflammatory activity and improves ulcerative colitis at low doses and that it may be a promising therapeutic option for ulcerative colitis. More studies are strongly recommended to establish the mechanisms involved and the active constituents that are responsible for its beneficial pharmacologic actions.

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