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***Original Article***

# Anti-ulcerogenic effect of ginger (rhizome of *Zingiber officinale* Roscoe) hydroalcoholic extract on acetic acid-induced acute colitis in rats

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## *Abstract*

Spasmolytic, antioxidant, anti-inflammatory and immuno-modulatory properties of ginger (rhizome of *Zingiber officinale* Roscoe) suggest that it may has beneficial effects on inflammatory bowel diseases. In the present study, the effect of this herbal extract on a model of acute colitis was evaluated. Ginger hydroalcoholic extract with doses of 150, 350, 700 mg/kg, prednisolone (2 mg/kg), or vehicle were administered orally to groups of male Wistar rats (n=6) for 5 days. Other four groups received two doses of vehicle, extract (350, 700 mg/kg), or hydrocortisone acetate enema (10 mg/kg) rectally, 15 and 2 h prior to ulcer induction (2 ml of acetic acid 4% was instilled via the anus). All rats were sacrificed 24 h later and the tissue injuries were assessed macroscopically and pathologically. Extracts with all doses used were effective to reduce colon weight/length ratio similar to the reference drugs (corticosteroids). Higher oral doses of extracts (350 and 700 mg/kg) were effective to reduce ulcer severity, area and index as well as mucosal inflammation severity, extent and total colitis index compared to controls. Rectally administered extract, only at high dose (700 mg/kg) was effective to reduce ulcer index and total colitis index. It is concluded that ginger hydroalcoholic extract was effective to protect against experimental colitis, and the efficacy was greater when higher doses of extract were administered orally and in a prolonged period.

***Keywords:*** Acetic acid; Colitis; Ginger; *Zingiber officinale*

## INTRODUCTION

Ginger, the rhizome of *Zingiber officinale* Roscoe, is one of the most widely used species of Zingiberaceae family, which is a common condiment for various foods and beverages. It has a long history of medicinal use dating back 2500 years in China and India for conditions such as nausea and vomiting, diarrhea, dyspepsia, rheumatism, and colds (1). Ginger's total extract contains a number of pungent and active ingredients that for some of them several pharmacologic activities have been identified (2). The major pungent compounds in ginger include potentially active gingerols, which can be converted to shogaols, zingerone, and paradol. 6-gingerol appears to be responsible for characteristic taste of ginger and together with 6-shogaol have been shown to have antipyretic, analgesic, anti-inflammatory, anti-tussive and hypotenssive effects (3).

Ginger extract has also been studied as an alternative to non-steroidal anti-inflammatory drugs (NSAID) therapy for arthritic and osteoarthritis disease conditions (4). *In vitro* studies suggest that ginger may produce anti-inflammatory effects by inhibiting arachidonic acid metabolism in both the cyclooxygenase (COX) and lipoxygenase (LOX) pathways (5). This is a mechanism similar to glucocorticoids and it is assumed that dual inhibitors of COX

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and 5-LOX may have a better therapeutic profile and fewer side effects than NSAID (6). Moreover it has been shown that ginger inhibits the induction of several genes involved in the inflammatory response that include genes encoding cytokines, chemokines and the inducible enzyme nitric oxide synthase (iNOS) and COX-II (5,7).

Ulcerative colitis (UC) and Crohn's disease (CD) are two major categories of inflammatory bowel diseases (IBD). Although the etiology and pathophysiology still remain unclear, immune dysfunction, inflammatory mediators, reactive oxygen species (ROS) and cytokines play crucial roles in its development (8,9). Anti-inflammatory as well as spasmolytic, antioxidant and immunomodulatory properties of ginger suggest that it may have beneficial effects on IBD (10).

The aim of this study was to determine protective effects of ginger total extract using two different routes (oral and rectal) of administration on colonic mucosa against an inflammatory and ulcerative attack induced by acetic acid administration in rats.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (225 ± 25 g) were purchased from Razi Institute (Tehran, Iran). The rats were allowed to adapt to our laboratory environment for one week. They had free access to tap water and normal rat chow and were housed singly in wire-bottomed cages under uniform and controlled conditions of temperature, humidity and light/dark (12/12 h) cycles. All of the experiments were approved by the ethical and research committee of Isfahan University of Medical Sciences, Isfahan, Iran.

### Plant material and preparation of extract

Ginger (rhizome of *Z. officinale* Roscoe) was prepared from Goldaru Pharmaceutical Co. (Isfahan, Iran) as a gift and authenticated by Department of Pharmacognosy, Isfahan School of Pharmacy and Pharmaceutical Sciences. For preparation of hydroalcoholic extract, dried and finely powdered rhizome of plant (300 g) was wetted by ethanol:water (70:30) and perculation was undertaken using extra volume of solvent for 48 h to prepare full extraction. The extract was then shook, filtered and evaporated in a rotary evaporator under reduced pressure till a semisolid and gelling nature extract, yielded 17% (w/w) was obtained (11).

### Chemicals

Prednisolone powder and hydrocortisone acetate enema were procured from Iran Hormone Pharmaceutical Co. (Tehran, Iran) and Valeant Pharmaceutical Co. (Saint-Laurent, Canada) respectively. All of organic solvents were of analytical grade and Merck brand (Germany).

### Animal grouping

The animals were randomly divided into following groups of 6 rats.

1 and 2: Sham groups; received vehicle (normal saline) (5 ml/kg, p.o. or i.r.) without colitis induction.

3 and 4: Control groups; received vehicle (5 ml/kg, p.o. or i.r.) bearing colitis induction procedure.

5, 6 and 7: Extract groups; received low, medium, or high doses of extract (150, 350, and 700 mg/kg) p.o. for 5 days and the last dose was administered 2 h before colitis induction.

8 and 9: Extract groups; received medium or high doses of extract (350 and 700 mg/kg) as enema (i.r.) 15 and 2 h before colitis induction.

10 and 11: Reference groups; received prednisolone (2 mg/kg, p.o.) or hydrocortisone acetate (10 mg/kg, i.r.) similar to the respective control and extract groups.

### Experimental protocol

The test samples including solutions or suspensions of drugs or plant extract were freshly prepared. The plant extract was prepared as a suspension in 0.5% v/v tween 80.

Acute colitis was induced by acetic acid using a technique introduced by Mascolo et al. (12). Briefly, rats were fasted for 36 h with access to water *ad libitum* and observed to ensure health before induction of colitis. The rats were lightly anesthetized with ether. A

flexible plastic rubber catheter with an outside diameter of 2 mm was inserted 8 cm into the colon via the anus. Diluted acetic acid 4% (2 ml) was injected into the colon and the rats were maintained in a head-down position for 5 min to prevent solution leakage. In sham-operated groups, normal saline was instilled. After 24 h of colitis induction, rats were sacrificed using ether anesthesia and colonic biopsies were taken for macroscopic scoring and histopathological examination.

**Assessment of colon macroscopic damage**

The tissue of colon, 8 cm in length and 3 cm proximal to the anus was excised, opened longitudinally and washed in saline buffer. The specimens were weighted and wet weight/length ratio was measured for all the rats. A pathologist unaware of treatment conditions recorded macroscopic and histological damage. The criteria of the macroscopic score used a previously validated scoring system from 0-4 according to Morris et al. (13). The scores were: 0 = no ulcer, 1 = mucosal erythema only, 2 = mild mucosal edema, slight bleeding or slight erosion, 3 = moderate edema, bleeding ulcers or erosions, 4 = sever ulceration, erosions, edema and tissue necrosis. Ulcer area was measured using 3M® (USA) scaled surgical transpore tape, which was fixed on a light and transparent sheet. Each cell on the tape was 1 $mm^2$ in area and the number of cells was counted and the ulcer area was determined for each colon. Ulcer index was the last parameter, measured by summing the ulcer score and the ulcer area for each tissue specimen (14).

**Assessment of colon histological damage**

Colon tissue was fixed in 10% formalin, and dehydrated, paraffin embedded, processed, sectioned in 4 μm thick sections, and stained with haematoxylin and eosin (H&E). Inflammation and crypt damage were assessed on H&E-stained, coded sections using a modification of a validated scoring scheme described by Cooper et al. (15) and Dieleman et al. (16). Total colitis score was the sum of the 3 subscores (inflammation severity, inflammation extent, and crypt damage). Histologic evaluation and scoring was performed using a Zeiss® microscope equipped with a Sony® color video camera for digital imaging.

**Statistical analysis**

Statistical analysis was performed using SPSS 10.0 statistical software. Differences among groups were examined using parametric one-way analysis of variance (ANOVA) with Tukey HSD as post hoc test. Non-parametric data were analyzed by Kruskal–Wallis followed by Mann-Whitney U test. Results are expressed as the mean ± SEM. The minimal level of significance was identified at $P<0.05$.

## RESULTS

**Macroscopic presentation**

Macroscopic damage parameters of the colon after acetic acid treatment revealed colonic mucosal hyperemia, edema, erosion, and ulceration in control groups. No changes were observed in sham groups suggesting that handling and surgical procedure had no interference with experimental outputs. Pretreatment with prednisolone and hydro-cortisone acetate enema as reference drugs, reduced the intensity of scores ($P<0.01$), ulcer area ($P<0.05$), ulcer index ($P<0.01$) and wet weigh/length ratio ($P<0.001$) (Table 1 and Fig. 1). Pretreatment with ginger extract, orally or as enema, reduced the severity of gross lesion scores, ulcer area and indices in such a manner which the highest dose (700 mg/kg) was significantly effective while low or medium doses (150 and 350 mg/kg) had no significant effect or had a lower efficacy (Table 1). All of the pretreatments with plant's extract and reference drugs were effective to lower weight/length ratio in colon specimens compared with control groups ($P<0.001$) (Table 1).

**Histological evaluation**

No histological damage was seen in sham groups. Rats with acetic acid-induced colitis and vehicle pretreatment (control groups) showed necrotic destruction of epithelium hemorrhage, edema, inflammatory cellular infiltration, crypt damage and ulceration at

**Table 1.** Effects of *Zingiber officinale* Roscoe hydroalcoholic extract on the macroscopic parameters of colitis induced by acetic acid in rats.

| Groups | Route of Administration | Score 0-4 | Ulcer Area ($Cm^2$) | Ulcer Index | W/L Ratio |
|---|---|---|---|---|---|
| Sham | p.o. | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 67.6 ± 5.6 |
| Control | p.o. | 3.8 ± 0.4 | 5.3 ± 0.6 | 9.6 ± 1.0 | 151.4 ± 15.6 |
| Pred. | p.o. | 1.4 ± 0.5** | 3.0 ± 0.8* | 6.6 ± 0.5** | 86.4 ± 8.6*** |
| Ext150 | p.o. | 2.7 ± 1.1 | 5.7 ± 0.5 | 8.2 ± 1.4 | 87.4 ± 5.1*** |
| Ext.350 | p.o. | 1.9 ± 0.6** | 4.4 ± 0.8 | 6.1 ± 0.8* | 78.4 ± 12.1*** |
| Ext.700 | p.o. | 1.2 ± 0.4** | 3.4 ± 0.5* | 4.6 ± 0.5** | 86.4 ± 5.5*** |
| Sham | i.r. | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 65.4 ± 6.1 |
| Control | i.r. | 3.6 ± 0.5 | 5.0 ± 0.5 | 9.0 ± 1.0 | 142.8 ± 10.1 |
| Hydroc. | i.r. | 1.4 ± 0.5** | 2.7 ± 1.0 | 3.9 ± 0.5** | 82.2 ± 6.5*** |
| Ext.350 | i.r. | 2.3 ± 1.0 | 5.4 ± 0.5 | 7.4 ± 0.8 | 67.2 ± 6.7*** |
| Ext.700 | i.r. | 2.0 ± 0.8* | 4.4 ± 0.9 | 6.5 ± 0.5* | 62.2 ± 8.4*** |

p.o. = Oral, i.r. = Intra-rectal, W/L = Weight/Length, Pred. = Prednisolone (2 mg/kg), Ext. = Extract of ginger (150, 350, 700 mg/kg), Hydroc. = Hydrocortisone acetate enema (10 mg/kg). The results were expressed as means ± SEM, (n=6). *$P$<0.05, **$P$<0.01, ***$P$<0.001 denote significant difference vs. control groups.

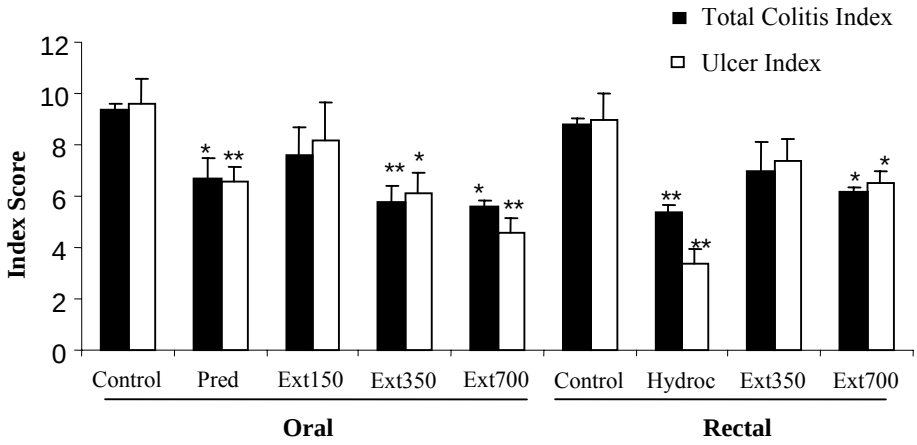


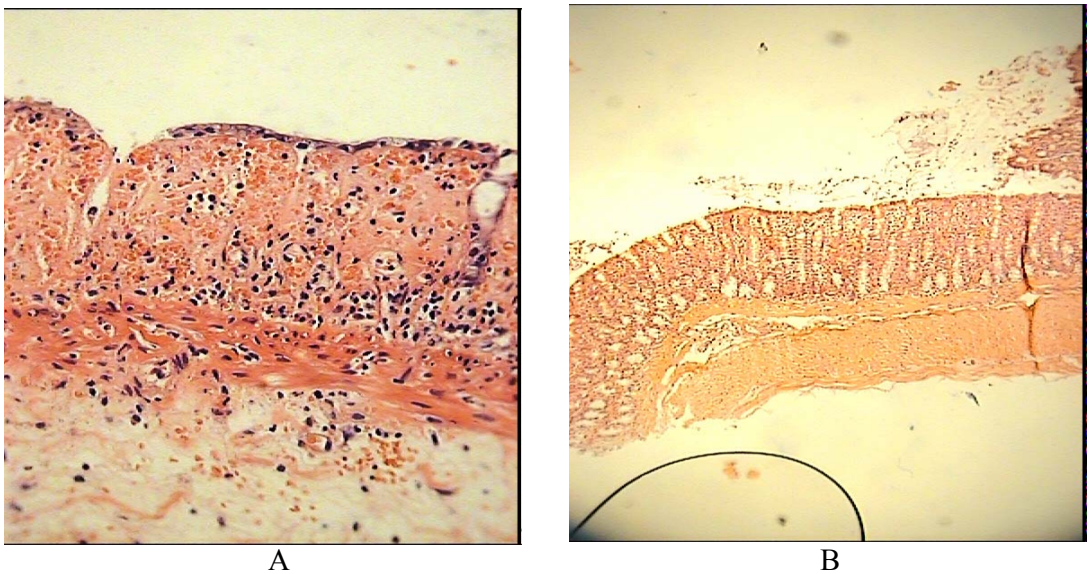

**Fig. 1.** Effect of *Zingiber officinale* Roscoe hydroalcoholic extract on total colitis index and ulcer index of colon tissue damage induced by actic acid in rats. Extract of ginger (Ext) with doses of 150, 350, 700 mg/kg, prednisolone (Pred, 2 mg/kg) or hydrocortisone acetate enema (Hydroc, 10 mg/kg) were administered orally or rectally respectively prior to ulcer induction by acetic acid enema. The results were expressed as means ± SEM, (n=6). *$P$<0.05, **$P$<0.01 denote significant difference vs. control groups.

mucus and sub-mucosal layers (Table 2, Fig. 1 and 2). Pretreatment with prednisolone and hydrocortisone enema was effective to reduce histopathological scores particularly inflammation severity ($P$<0.05) and inflammatory extent ($P$<0.05). The reference drugs were also effective to diminish total colitis index after oral ($P$<0.05) and rectal administration. Administration of plant's extract by oral route was invariably effective to reduce histopathologic scores including inflammatory severity ($P$<0.05), inflammatory extent (0.05) and total colitis index ($P$<0.01). Additionally, oral administration of extracts at higher doses (350 and 700 mg/kg) were the only treatment effective to reduce crypt damage ($P$<0.05) (Fig. 2). Plant's extract enema was not effective to reduce histologic damage scores ($P$>0.05) and inflammatory parameters (Table 2).

**Table 2.** Effects of *Zingiber officinale Roscoe* hydroalcoholic extract on the histopathologic parameters of colitis induced by acetic acid in rats

| Groups | Route of Administration | Inflam. Severity | Inflam. Extent | Crypt Damage | Total Colitis |
|---|---|---|---|---|---|
| Sham | p.o. | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Control | p.o. | 2.6 ± 0.4 | 2.8 ± 0.4 | 3.6 ± 0.8 | 9.4 ± 0.2 |
| Pred. | p.o. | 1.9 ± 0.4* | 1.7 ± 0.5* | 3.9 ± 0.5 | 6.7 ± 0.8* |
| Ext.150 | p.o. | 2.4 ± 0.5 | 2.4 ± 0.5 | 2.8 ± 1.4 | 9.6 ± 1.1 |
| Ext.350 | p.o. | 1.8 ± 0.4* | 1.8 ± 0.4* | 2.2 ± 0.8* | 5.8 ± 0.6** |
| Ext.700 | p.o. | 1.5 ± 0.6* | 1.6 ± 0.4* | 2.3 ± 0.5* | 5.6 ± 0.2** |
| Sham | i.r. | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Control | i.r. | 2.3 ± 0.6 | 2.6 ± 0.5 | 3.7 ± 1.0 | 8.8 ± 0.2 |
| Hydroc. | i.r. | 1.3 ± 0.6* | 1.6 ± 0.4* | 2.2 ± 0.5 | 5.4 ± 0.3** |
| Ext.350 | i.r. | 2.5 ± 0.9 | 2.8 ± 0.9 | 2.1 ± 0.5 | 7.0 ± 1.1 |
| Ext.700 | i.r. | 1.8 ± 0.8 | 2.1 ± 0.8 | 2.4 ± 0.8 | 6.2 ± 0.2* |

p.o. = Oral, i.r. = Intra-rectal, Inflam. = Inflammation, Pred. = Prednisolone (2 mg/kg), Ext = Extract of ginger (150, 350, 700 mg/kg), Hydroc. = Hydrocortisone acetate enema (10 mg/kg). The results were expressed as means ± SEM, (n=6). * $P$<0.05, ** $P$<0.01 denote significant difference vs. control groups.



A B

**Fig. 2**. Microscopic illustration of rat colon tissue**.** A: Acetic acid induced colitis in rats treated with vehicle: there is erosion of surface epithelium, crypt loss, hemorrhage, edema and acute inflammation of the wall of the colon (H&E section, high power). B: Ginger extract (700 mg/kg) treated colon shows absence of acute inflammation, crypt damage and surface epithelial loss (H&E section, low power).

## DISCUSSION

In the present study, method of administrating acetic acid that is both rapid and reproducible was used for producing diffuse colonic inflammation which resembles many histological characteristics of human UC (17). Our results confirmed the suitability of this method since an acute and invariably characteristic colitis was developed in experimental rats. In our study, oral prednisolone and hydrocortisone acetate enema were used as reference drugs to delineate the efficacy of ginger extract as well as the role of administration route and the probable involved mechanisms. Results showed an effective protection for both the reference drugs considering the macroscopic and microscopic outputs. The exception was for the ulcerated area and extent of crypt damage which the enema medication was not effective to reduce the mean values. It is assumed that oral

pretreatment with prednisolone for a period of 5 days caused a better condition for absorption and systemic availability of active drug. The same results were obtained when medium and high oral doses of ginger extract were used in comparison to the same doses of extract enema. The above explanation can be used in this case too. Moreover, it is likely that prolonged treatment with tested drugs provided an opportunity for delayed protective mechanisms such as scavenging the oxido-radicals and/or counteracting their unpleasant effects. It has been shown that both ginger crude extract (18) and a number of its components such as [6]-gingerol (19), [6]-paradol (20), and zingerone (21) have superoxide-desmotase (SOD) like activity. They could counteract ROS; scavenging directly the superoxide anions, hydroxyl and peroxyl radicals and nitric oxide as well.

Regarding to the macroscopic (ulcer index) and histologic (total colitis index) results, it was evident that ginger hydroalcoholic (total) extract possessed anti-ulcerogenic effect in such a manner that was somewhat dependent to the dosage. The lowest doses of oral extract similar to the medium dose of extract enema were not effective to reduce ulcer indices while higher doses were effective. This conclusion needs further studies to examine higher doses of extract both orally and rectally. It can be concluded that for compensating lower efficacy of rectal mucosal absorption, higher doses are needed when extract enema is used. Wet weight / length ratio, as an indirect index of inflammation and extravasation, reduced significantly after oral and rectal pretreatments irrespecting to the used doses. Our results are in accordance with El-Abhar et al. which all of ginger extract doses were effective to reduce colon weight/length values in a non-dose dependent manner (10). Similarly the lowest test dose of ginger extract (100 mg/kg) had failed to affect the lesion scores (10). This is an accent for the sensitivity of this parameter however; it is not a suitable marker for the efficacy of treatments. The exact mechanism of action has not been clearly delineated but ginger contains a number of active constituents, which for some of them anti-inflammatory (22), antioxidant (23) and immunomodulatory (24) properties have been elucidated. Ginger also suppresses prostaglandin biosynthesis through an inhibitory effect on COX-I and COX-II enzymes (5,24).

An extension of these findings was the observation that ginger also suppresses leukotriene biosynthesis; a mechanism which shares pharmacological property with glucocorticoids and may play a key role for their usefulness in human IBD (6). Moreover, it has been shown that ginger inhibits the induction of several genes involved in the inflammatory response (5). These include genes encoding cytokines, chemokines, iNOS and COX-II (7,24). El-Abhar et al. found that ginger was effective to block several cytokines including tumor necrosis factor-α (TNF-α) production (10). This effect may be mediated either by blocking TNF-α activation and transcriptional regulation (25,26) and/or inhibiting its secretion from macrophages (27).

It is probable that additional mechanisms such as mast cells stabilization, inhibiting myeloperoxidase and γ-glutamyl transe-peptidase activity (γGTP), inhibition of platelet aggregation, and anti-spasmodic activities have a role in protective effects of ginger (28,29). Results of this animal experiment reveal that *Z. officinale* rhizome extract possesses anti-ulcerogenic properties compared to those reported by glucocorticoides. These findings offer more pharmacological support to folkloric, ethnopharmacological uses of ginger in IBD management.

In conclusion we suggest that ginger, alone or in combination with other drugs may be a promising agent for the treatment of UC or may be used as a functional food in dietary supplements for prevention of disease recurrence. Further sufficient preclinical and clinical studies should be conducted to clarify this fact.

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