ANTI-ULCEROGENIC EFFECT OF GINGER (rhizome of *Zingiber officinale* Roscoe) ON CYSTEMINE INDUCED DUODENAL ULCER IN RATS

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ABSTRACT

Ginger (rhizome of *Zingiber officinale* Roscoe) is a widespread herbal medicine mainly used for the treatment of gastrointestinal (GI) disorders including: dyspepsia, nausea and diarrhea. Aromatic, spasmolytic, carminative and absorbent properties of ginger suggest that it has direct effects on the GI tract and anti-ulcerogenic potential. In the present study, the effects of this herbal remedy on an acute model of experimental duodenal ulcer induced by cysteamine was evaluated.

Hydroalcoholic extract of ginger with doses of 100, 350, 700 mg/kg, ranitidine (50 mg/kg), sucralfate (500 mg/kg) and 5 ml/kg of vehicle were administered orally (p.o.) to separate groups of male Wistar rats. Other groups received vehicle (5 ml/kg), extract (300 mg/kg) and ranitidine (50 mg/kg) intraperitoneally (i.p.). After ulcer induction, the number, scoring, area and finally ulcer index were assessed for each duodenum. Administration of extract by i.p. or at chronic doses (350 mg/kg) and ranitidine (p.o. and i.p.) resulted in significant reduction in mucosal damage for the entire ulcer factors which were assessed. Larger doses of extract given p.o. (350 and 700 mg/kg) were effective to reduce both the ulcer area and index but the lowest dose of extract (100 mg/kg) was not effective. Taken together, we conclude that ginger hydroalcoholic extract was effective to protect against duodenal ulceration and for i.p. injection as well as chronic administration, the efficacy was comparable with ranitidine as reference drug.

Keywords: Ginger (*Zingiber officinale* Roscoe), Duodenal ulcer, Cysteamine, Rats

INTRODUCTION

Herbal medicines are now used by up to 50% of the western population, in a number of instances (~10%) for the treatment or prevention of digestive disorders (1). Considering the morbidity caused by peptic ulcer disease and dyspepsia over the world, cheap and easily available treatments will always be in demand especially for the people of non-industrialized countries (2). Ginger (rhizome of *Zingiber officinale* Roscoe) is among the 20 top- selling herbal supplements in the USA and its retail sales in mainstream of the USA market in 2001 amounted to $ 1.2 million (3). Today, pharmacopoeias of a number of different countries list ginger extract for various digestive diseases (4). Aromatic, spasmolytic, carminative and absorbent properties of ginger are probably responsible for the therapeutic applications in digestive tract ailments (5). Several studies have shown that ginger extract, essential oils and glycolipids possess a number of pharmacological actions, which at least in part for some of them anti-ulcerogenic or ulcer preventive efficacy may be suggested (6). Some of ginger actions are: anti-*Helicobacter pylori* (7), anti- oxidant (8), anxiolytic (9), anti-emetic (10), anti- inflammatory (11), anti-angiogenesis (12), anti- tumor (13), anti-thrombotic (14) and cardiovascular effects (15).

The major compounds found in ginger are the gingerols (*i.e.* 6-gingerol, 8-gingerol, zingerone, and 6-shogaol) (16). The present study was designed to investigate the anti-ulcerogenic effect of ethanolic extract of ginger in an acute animal model of duodenal ulcer.

MATERIAL AND METHODS

Plant material and preparation of extract

Ginger (*Zingiber officinale* Roscoe rhizome), (Zingiberaceae family), was prepared from Food and Drug Committee of Isfahan University of Medical Sciences, Isfahan, Iran as a gift and authenticated both macroscopically and microscopically by Mr. Iraj Mehregan in Herbarium Department of Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. For preparation of hydroalcoholic extract, dried and coarsely powdered rhizome of the plant (400 g) were macerated with 1000 ml of EtOH-H_2O (7:3) for 72 hours. The extract was

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then shaked, filtered and evaporated in a rotatory evaporator under reduced pressure until dryness. Evaporation and removal of the solvent gave a semisolid mass. The yield was 14.8 % (w/w) (16).

Animals

Male Wistar rats, weighting 200-250 g, purchased from the Razi Institute (Tehran- Iran) were employed in this study. The animals were maintained on a standard pelleted diet and water *ad libitum* and were left 48 hours for acclimization to animal room conditions. The food was withdrawn 24 hours before the experiment but animal was allowed to have free access to water. To avoid corpophagy and fighting, the rats were kept singly in wire- bottomed cages.

Grouping

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

1, 2: Sham groups; received vehicle (5 ml/kg, p.o. & i.p.) without ulcer induction.

3, 4: Control groups; received vehicle (5 ml/kg, p.o. & i.p.) 1 hour before ulcer induction.

5, 6, 7: Reference groups; received ranitidine (50 mg/kg p.o. & i.p.) and sucralfate (500 mg/kg) 1 hour before ulcer induction.

8, 9, 10, 11: Extract groups; received hydroalcoholic extract of ginger (100, 350, 700 mg/kg p.o., and 300 mg/kg i.p.) 1 hour before ulcer induction.

12: Chronic extraction group; received hydroalcoholic extract of ginger (350 mg/kg) for 5 consecutive days before ulcer induction. The last dose was administered 1 hour prior to cysteamine administration.

Experimental procedure

The test samples including solutions or suspensions of drugs and extracts were freshly prepared and were administered to animals in 5 ml/kg. The plant extract was prepared as a suspension in 0.5% tween 80/saline. After 24 hours of fasting, duodenal ulceration was induced by oral cysteamine hydrochloride (450 mg/kg) according to the previously described method (17). After 24 hours, the animals were killed by an over dose of ether and the stomachs as well as duodenum (5cm in length) were removed after a clamping at the esophagus and duodenum. The stomach and adjacent duodenum were opened and tissues were rinsed with saline and examined by 5-fold binocular magnifier to assess the ulcer formation. Two observers including a pathologist unaware of the experimental protocol assessed the lesions.

The number of ulcers was counted and scoring was undertaken according to the reported method (18). The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, and 3 = perforation. Ulcer area was assessed by using 3M® scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was 1 mm^2 in area, so the number of cells was counted and the ulcer area was measured for each duodenum (19). Ulcer index was measured by the following formula (20) using the factor of ulcer area instead of ulcer prevalence.

$U_I = U_N + U_S + U_A \times 10^{-1}$ which U_I = ulcer index, U_N = ulcer number, U_S = ulcer score, and U_A = ulcer surface area for each duodenum.

Statistical analysis

The data were analyzed by a one-way ANOVA, following by Post Hoc Tukey HSD test. For ulcer scores, non-parametric Mann-Witney U test was used. The results are expressed as mean ± SEM.

RESULTS

Results of the study are shown in tables 1 and 2 and figure1. No ulcer or erosions were observed in rats of Sham-operated groups indicating that handling and surgical procedure had no interference with experimental outputs. In the control groups, administration of cysteamine for 24 hours invariably resulted in the production of both duodenal and gastric lesions mainly in the proximal segments of duodenum. From significant ($p<0.01$) reduction in different parameters it appears that pretreatment with ranitidine (p.o. & i.p.) reduced duodenal mucosal damage. For all parameters, sucralfate was less effective than ranitidine suggesting a principle role for acid secretory inhibition rather than cytoprotection. Sucralfate didn't reduce the number of ulcers and ulcer scores compared to the control groups ($p>0.05$) (table 1). Pretreatment along with increase in single oral dose of extract resulted in significant reduction in ulcerated area and ulcer indices (table 1) but the efficacy was not dose-dependent for the dose greater than 350 mg/kg. The lowest dose of the extract (100 mg/kg) was not effective in reduction of the ulcer index, number and scoring. Results also indicated that parenteral administration (300 mg/kg) of extract was more effective than equal and even higher oral dose of plant extract, suggesting a more effectiveness for systemic mechanisms. The efficacy of the extract was meaningful and comparable with ranitidine. Chronic pretreatment with an average dose of extract (350 mg/kg) caused a significant reduction in all parameters, which were assessed for ulcer evaluation. This method of treatment was quite more effective than corresponding single oral dose of extract and had comparable efficacy with ranitidine treatment (Tables 1and 2, Figure 1).

Table 1. Effects of *Zingiber officinale* Roscoe hydroalcoholic extract against cysteamine-induced duodenal ulcer in rats (Data are means ± S.E.M, n= 6)

Treatment	Dose (mg/kg)	Number	Scoring	Area (mm^2)
Vehicle	5 (ml/kg)	4.5 ± 0.43	2.0 ± 0.25	58.33 ± 6.85
Extract	100	3.5 ± 0.50	2.0 ± 0.25	27.0 ± 4.5**
Extract	350	2.8 ± 0.65	1.33 ± 0.33	17.3 ± 4.6***
Extract	700	2.8 ± 0.60	0.83 ± 0.17*	20.6 ± 4.9***
Extract (chr.)	350	1.16 ± 0.47**	0.66 ± 0.21*	13.7 ± 4.6***
Ranitidine	50	1.5 ± 0.5**	0.66 ± 0.21*	9.0 ± 4.4***
Sucralfate	500	2.8 ± 0.3	1.33 ± 0.33	23.3 ± 4.6***

The extracts and reference drugs were administered (p.o.) 1 hour prior to ulcer induction. Chronic (chr.) treatment with extract was undertaken for five consecutive days before ulcer induction. Tissue assessment was done 24 hours after ulcer induction. * P<0.05, ** P<0.01, *** P<0.001, significant difference from control group (Tukey HSD test)

Table2. Effects of *Zingiber officinale* Roscoe hydroalcoholic extract against cysteamine-induced duodenal ulcer in rats (Data are means ± S.E.M, n= 6)

Treatment	Dose (mg/kg)	Number	Scoring	Area (mm^2)
Vehicle	5 (ml/kg)	4.4 ± 0.32	2.00 ± 0.18	55.2 ± 5.51
Extract	300	0.83 ± 0.30***	0.83 ± 0.30*	12.8 ± 4.66***
Ranitidine	50	1.33 ± 0.61**	0.50 ± 0.22**	9.6 ± 4.99***

The extract and vehicle were administered (i.p.) 1 hour prior to ulcer induction. Tissue assessment was done 24 hours after ulcer induction. *P<0.05, **P<0.01, ***P<0.001significant difference from control group (Tukey HSD test)

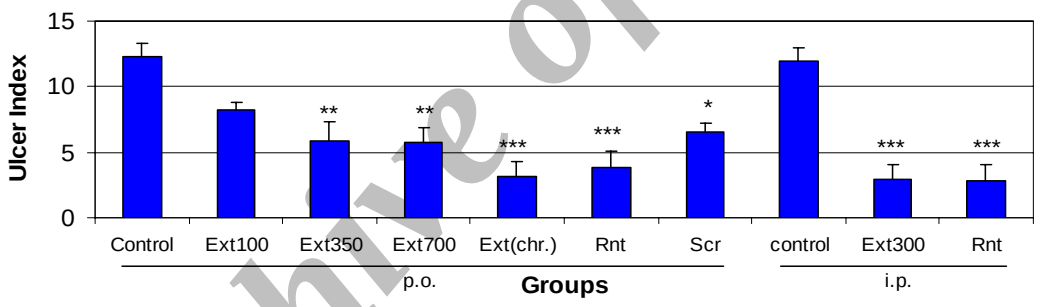

Figure1. Effects of *Zingiber officinale* Roscoe hydroalcoholic extract on cysteamine-induced duodenal ulcer in rats (Data are mean ± S.E.M, n=6).
Control (5ml/kg of vehicle), Ext (Extract with doses of 100, 300, 350, 700mg/kg), Ext (chr.) (Chronic administration of extract with dose of 350 mg/kg for 5 consecutive days), Rnt (ranitidine 50 mg/kg) and Scr (sucralfate 0.5 g/kg). Treatments carried out 1hour prior to ulcer induction.
*P<0.05, **P<0.01, ***P<0.001, significant difference from control group (Tukey HSD test)

DISCUSSION

The results confirmed the suitability of the method since acute and almost invariably prominent duodenal ulcers developed in rats treated with cysteamine HCl (21, 22). The exact mechanism of pathogenesis in the cysteamine induced duodenal ulcer model has not been fully known, but hypersecretion of gastric acid, deterioration of the mucosal resistance and promotion of gastric emptying are among the possible mechanisms (23-25). In this study, ranitidine and sucralfate were used as reference drugs to delineate in part the mechanism(s), which are probably involved in ulcer pathogenesis. The results obtained in reference (positive control) groups indicate that ginger extract possesses its anti-ulcerative properties through a mechanism mainly related to acid and pepsin inhibition. Our results are in accordance with previous report (26) in which water and methanolic extract of eight Zingiberaceae herbs caused a significant decrease in gastric secretion in un-anesthetized rabbits and the effect of water extract was very similar to that of cimetidine.

Results also indicated that i.p. injection of plant extract as well as its chronic administration with average dose were more effective in prevention of lesion formation and the effectiveness was

comparable with ranitidine therapy. It is suggested that parenteral administration has a higher absorption and systemic availability. The same explanation could be applied for results obtained after chronic administration. Moreover counter-acting the active oxidant radicals, decreasing mucosal cell shedding and thickening the mucus membrane are among the mechanisms which probably are involved after chronic treatments (27). Results of one study (28), for effects of three herbal medicines including *Z. officinalis* L. on gastric ulceration and secretion in rats indicated a significant protection against gastric ulcers induced by cold restraint stress, aspirin and pylorus ligation. The proposed anti-ulcerogenic effects were augmentation of mucin secretion and decrease in cell shedding. In another study (29), roasted ginger decoctions had an obvious inhibiting tendency on three gastric ulcer models except for the indomethacin induced model. While the exact mechanism of action has not been clearly delineated, the plant contains active materials which for some of them at least, ulcer protective properties have been identified. 6-Gingersulfonic acid (30) and three monoacyldigalactosyl glycerols including gingerglycolipids A, B, and C (31) have been isolated from dried rhizomes of *Z. officinale* which are potent anti-ulcer constituents. 6-Gingerol and 6-shogaol are two other anti-ulcer components that are less potent but are mainly responsible for ginger pungency (32, 33).

A knowledge of the chemical composition of a given plant extract is required in order to extrapolate the proposed mechanism of actions to its possible *in vivo* efficacy (or safety). This will depend on variety of factors including amount of individual constituents in the extract, interaction between individual constituents, and their pharmaco-kinetics, which by itself require further studies (34). Taken together it is concluded that hydroalcoholic extract of ginger is able to prevent cysteamine induced duodenal ulceration in rats and may suggest a rational basis for therapeutic uses of this herb for some other gastrointestinal ailments.

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