



## Antiulcerogenic Effect of *Zataria multiflora* Boiss. on Cysteamine Induced Duodenal Ulcer in Rats

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### Abstract

Research advances during recent years offer new insight into therapy and the prevention of gastrointestinal ulcers by using medicinal plants. Flavonoids, tannins, triterpenoids, fatty acids and essential oils are among the most effective herbal constituents that have potential antiulcerogenic properties, and most of them could be found in *Zataria multiflora*. *Z. multiflora* is one of the indigenous plants of Iran, which is readily available and traditionally used to improve gastrointestinal disorders. In a recent trial, we decided to study the potential antiulcerogenic effects of the plant on an animal model of duodenal ulcer. Hydroalcoholic extract of the plant with doses of 200, 400, 800 and 1200 mg/kg, ranitidine (50 mg/kg), sucralfate (2 g/kg) and 1 ml of the vehicle were administered orally to different groups of male Wistar rats. Two other groups received (i.p.) vehicle (1 ml) and extract (800 mg/kg). Duodenum ulcers were induced by cysteamine HCl and the number of ulcers, area, and finally ulcer index were assessed. Ranitidine and sucralfate resulted in significant reduction in the duodenal mucosal damage for the entire ulcer factors assessed. Increasing doses of the extract resulted in a significant reduction in ulcerated area and index in a dose dependent manner. We concluded that *Z. multiflora* extract was effective in protecting against duodenal ulceration, and for the larger doses used, the efficacy was comparable with the reference drugs. The mechanism of action couldn't be clearly proposed for the plant extract, however; the local mucosal enhancement and cytoprotection may be involved.

**Keywords:** Cysteamine; Duodenal ulcer; *Zataria multiflora*.

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### 1. Introduction

Peptic ulcer disease is an ulcerative gastrointestinal (GI) disease which principally

embraces stomach and duodenum and causes a high rate of morbidity especially for the people of non-industrialized countries [1]. Duodenal ulcer is the most common type of peptic ulcer where discontinuity in the gastric mucosa is commonly observed. The pathogenesis of duodenal ulcer is not

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completely understood [2]. It is clear that gastric acid and pepsin secretion are necessary, however, factors related to mucosal resistance particularly the production of gastrointestinal mucus and secretions of bicarbonate are equally important [3]. A principle role for *Helicobacter pylori* in ulcer pathogenesis is now widely accepted [1, 2]. At least 80-95% of patients with duodenal ulcers are infected by *H. pylori* and eradication of this microorganism seems to be curative for the disease [4]. Besides *H. pylori* eradication, common therapies include: Antacids, H<sub>2</sub>-receptor blockers, proton pump inhibitors (PPIs), cytoprotective agents and muscarinic receptors antagonists [3, 4].

Although a range of drugs are available for the treatment of ulcers, many of them do not fulfill all of the requirements, and side effects such as headache, confusion, male hormone disturbances, arrhythmia and potential interference with drug metabolism are common [2, 3]. Thus a search among medicinal plants is still important and might provide a useful source for therapy and prevention of gastroduodenal ulcers or alternatively as simple dietary adjuncts to existing therapies.

*Z. multiflora* (Lamiaceae) is a widely distributed medicinal plant in Iran and the southern neighboring countries. It has similarities to *Thymus vulgaris* and has been traditionally used for controlling pain and some GI disorders (bloating and dyspepsia) [5-7]. The chemical composition and pharmacological evaluation of *Z. multiflora* have been the subject of some studies in the past years. Most of these studies were focused on extracts, fractions and essential oils of the aerial parts and flowers of the plant. In pharmacological and biological tests, *Z. multiflora* is reported to have antinociceptive, anti-inflammatory, spasmolytic, anti-*H. pylori*, antiplasmodic and antifungal activities [6-11]. Phytochemical studies revealed that linalool, linalyl acetate, *p*-cymene and some

other mono and sesquiterpenes, flavonoids e.g. luteolin, hydroxycinnamic acids e.g. rosmarinic acid, triterpenoids e.g. betulinic acid and oleanolic acid were the main components of the aerial parts [8, 10, 12-16]. There is little or no information about the pharmacological effects of *Z. multiflora* on the GI system. In this study, possible protective role of *Z. multiflora* hydroalcoholic extract against cysteamine-induced duodenal ulcer in rats is investigated.

## 2. Materials and methods

### 2.1. Plant material and preparation of extract

The aerial parts of *Z. multiflora* were collected in June 2002 at the flowering stage, on the Kolehghazi mountainous area near Isfahan, Iran. The plant material was identified by I. Mehregan and a voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Isfahan University of Medical Sciences.

For preparation of hydroalcoholic extract, air-dried and finely powdered aerial parts of the plant (100 g) were macerated with 500 ml of EtOH-H<sub>2</sub>O (7:3) for 24 hours. The extract was then shaken, filtered and evaporated in a rotary evaporator under reduced pressure until dryness [8]. Evaporation and solvent removal of the hydroalcoholic extract of the plant gave semi-solid and syrup mass with a yield of 15.46% (w/w).

### 2.2. Animals

Male Wistar rats, weighing 200-250 g, purchased from the Razi institute (Tehran-Iran) were used in pharmacologic studies. The animals were maintained on a standard chaw diet and water *ad libitum*, and were left 48 h for acclimation to the animal room conditions. The food but not water was withdrawn 24 h before the experiment. To avoid cannibalism and fighting, the rats were kept singly in wire-bottomed cages.

### 2.3. Grouping

Eleven groups of rats with 7 rat per group were used. Sham groups (1 and 2) received vehicle (1 ml, p.o. or i.p.) without ulcer induction. Control groups (3 and 4) received vehicle (1 ml, p.o. or i.p.) 0.5 h before the induction of ulcers. Reference groups (5 and 6) received ranitidine (50 mg/kg, p.o.) and sucralfate (2 g/kg, p.o.), 0.5 h before the induction of ulcers. Groups 7 to 11 received different doses of the hydroalcoholic extract of *Z. multiflora* (200, 400, 800, 1200 mg/kg, p.o., or 800 mg/kg, i.p.), 0.5 h before the induction of ulcers.

### 2.4. Experimental procedure

The test samples were administered to animals in 5 ml/kg as a suspension in 0.5% tween 80/saline. After 24 h of fasting, duodenal ulceration was induced by oral cysteamine hydrochloride (450 mg/kg) according to the method described by Szabo [17]. Twenty four hours later, the animals were killed by an over dose of ether and the stomach and duodenum (5 cm in length) were removed after clamping the esophagus and duodenum. After 30 minutes, the stomach and adjacent duodenum were opened and the tissues were rinsed with saline and examined by 5-fold binocular magnifier to assess the formation of ulcers. Two observers, unaware of the experimental protocol, assessed the

lesions. The number of ulcers was counted. Ulcer scoring was undertaken according to Desai *et al.* [18]. The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer and 3 = perforation. Ulcer area was assessed by using 3 M scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was 1 mm<sup>2</sup> in area, so the number of cells was counted and the ulcer area was measured for each duodenum [19]. Ulcer index was measured by using the following formula offered by Vogel [20], after incorporating the factor of ulcer area instead of ulcer prevalence:

$$U_I = U_N + U_S + U_A \times 10^{-1}$$

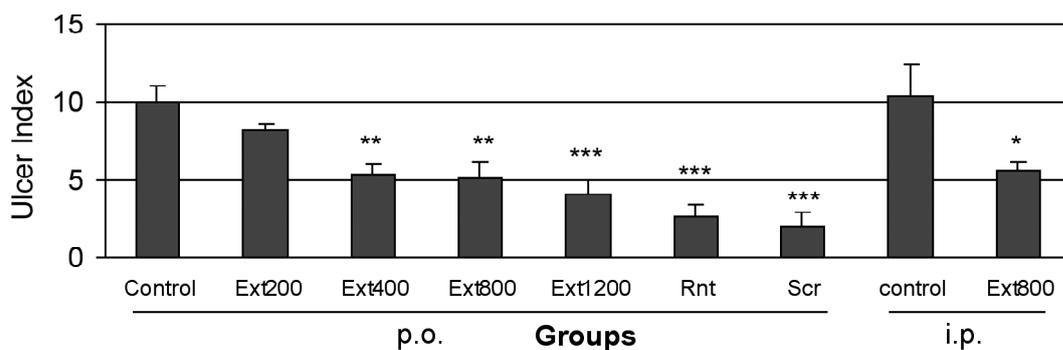
in which  $U_I$  is ulcer index,  $U_N$  is ulcer number,  $U_S$  is ulcer score, and  $U_A$  is ulcer surface area for each duodenum.

### 2.5. Statistical analysis

The data were analyzed by a one-way ANOVA, followed by Post Hoc Tukey HSD test. The results are expressed as mean $\pm$ SEM.

## 3. Results

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 control groups, cysteamine for 24 h invariably resulted in the production of both duodenal



**Figure 1.** Effects of *Zataria multiflora* Boiss. hydroalcoholic extract on cysteamine-induced duodenal ulcer in rats. Control (1 ml of vehicle), Ext (extract with doses of 200, 400, 800, 1200 mg/kg), Rnt (ranitidine 50 mg/kg), and Scr (sucralfate 2 g/kg). Treatments carried out 30 min prior to ulcer induction. Data are mean  $\pm$  S.E.M, n=7. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, significantly different from control group (Tukey HSD test).

**Table 1.** Effects of *Zataria multiflora* Boiss. hydroalcoholic extract given orally against cysteamine-induced duodenal ulcer in rats.

Treatment	Dose	Number	Scoring	Area (mm <sup>2</sup> )
Vehicle	1 ml	3.28±0.52	2.00±0.21	47.8±6.23
Extract	200 mg/kg	3.42±0.20	1.57±0.20	32.6±3.17 <sup>a</sup>
Extract	400 mg/kg	2.00±0.31	1.71±0.18	17.0±1.89 <sup>c</sup>
Extract	800 mg/kg	2.57±0.57	1.42±0.30	11.2±2.11 <sup>c</sup>
Extract	1200 mg/kg	2.00±0.49	1.14±0.26	10.0±2.66 <sup>c</sup>
Ranitidine	50 mg/kg	1.42±0.42 <sup>a</sup>	0.71±0.18 <sup>b</sup>	6.0±1.85 <sup>c</sup>
Sucralfate	2000 mg/kg	0.85±0.34 <sup>b</sup>	0.57±0.20 <sup>c</sup>	5.4±2.21 <sup>c</sup>

The extracts and reference drugs were administered (p.o.) 30 min prior to ulcer induction. Tissue assessment was done 24 h after ulcer induction. Data are means±SEM, n= 7. SEM: Standard error of mean.

<sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.01$ , <sup>c</sup>  $p < 0.001$ , significant difference from control group (Tukey HSD test).

and gastric lesions mainly in the proximal segments of duodenum. Pretreatment with ranitidine and sucralfate at doses studied, resulted in significant reduction in the duodenal mucosal damage (Table 1). Sucralfate, for all of the parameters, was more effective than ranitidine suggesting a principle role for cytoprotection rather than acid secretory inhibition. Pretreatment with increasing doses of the extract resulted in a significant reduction in ulcerated area and ulcer indices in a dose dependent manner (Table 1 and Figure 1). The exception was for the lowest dose of the extract (200 mg/kg) which only decreased the ulcer area (Table 1). Results also indicated that parenteral administration (800 mg/kg) was less effective than oral administration with equal doses, suggesting a local effect (Tables 1 and 2). Pretreatment with increasing doses of the extract did not result in significant reduction in the number of ulcers and scores compared to the related control groups (Tables 1 and 2).

#### 4. Discussion

To study the protective effects of *Z. multiflora* on experimentally induced duodenal lesions, method of cysteamine was used. The ulcerogenic effect of cysteamine is both rapid and constant thus providing a particularly reliable model for investigating the mechanism of duodenal ulcerogenesis and possible means for its prevention [21,

22]. Our results confirmed the suitability of the method, so acute and almost invariably prominent duodenal ulcers were developed in rats received cysteamine HCl.

The exact mechanism of pathogenesis in the cysteamine-induced duodenal ulcer model is not fully known but hypersecretion of gastric acid, deterioration of 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 the mechanisms that are probably involved in ulcer pathogenesis. Results showed an effective protection for both of the reference drugs, however, the effectiveness was more significant for sucralfate indicating an important role for cytoprotective mechanisms [26, 27]. Broad spectrum of sucralfate activity on GI may explain the greater protective effects against cysteamine induced duodenal ulcers. Results also indicated that *Z. multiflora* extract was effective with doses used in our study. For the main parameters including ulcer area and ulcer index, the effect of larger doses of the extract was comparable with the reference tested drugs. The exact mechanism of action could not be clearly delineated but the candidate plant contains active materials which for most of them ulcer protective properties have been suggested. Luteolin is one of the principal flavonoid components that is found in the plant [14]. Although several

**Table 2.** Effects of *Zataria multiflora* Boiss. hydroalcoholic extract given i.p. against cysteamine-induced duodenal ulcer in rats.

Treatment	Dose	Number	Scoring	Area (mm <sup>2</sup> )
Vehicle	1 ml	3.4±0.32	2.00±0.14	50.2±6.2
Extract	800 mg/kg	2.0±0.22	1.71±0.18	19.1±2.2 <sup>a</sup>

The extract and vehicle were administered (i.p.) 30 min prior to ulcer induction. Tissue assessment was done 24 hours after ulcer induction. Data are means±SEM, n= 7. SEM: Standard error of mean.

<sup>a</sup>  $p < 0.001$ , significant difference from control group (Tukey HSD test).

mechanisms have been proposed to explain the gastroprotective effects of flavonoids [28, 29], luteolin is among those, which inhibit the growth of *H. pylori* by inhibition of arylamine N-acetyltransferase activity [30]. Other main mechanisms accounting for flavonoids include increase of mucosal prostaglandine contents, decrease of histamine secretion and scavenging of free radicals [31, 32]. Rosmarinic acid is an ester of caffeic and 3,4-dihydroxyphenyllactic acids occurred naturally in the plant and could be readily obtained from the cell culture of *Z. multiflora* [15]. A number of interesting biological activities like antiviral, antibacterial, anti-inflammatory and antioxidant are attributed to this compound and a review on its pharmacology indicating a wide therapeutic potential including treating or preventing bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory disease and atherosclerosis [33, 34]. In addition, antiulcer drugs of plant origin show that triterpenoids, because of their ability to strengthen defensive factors such as stimulation of mucus synthesis or maintenance of the prostaglandines level are potential compounds with antiulcer activity [35]. Oleanolic acid is a triterpenoid fatty acid could be found in significant amounts in *Z. multiflora*. Astadillo *et al.* [36] showed the gastroprotective effect of triterpene oleanolic acid and two related metabolites on various experimentally induced gastric lesions in rats and mice. In addition, by doing an acute toxicity test at doses up to 600 mg/kg in mice, the safety of oleanolic acid was confirmed. It seems that luteolin, rosmarinic acid, linoleic acid and even

essential oils such as linalool and linalyl acetate [37] have been partly associated in pharmacological findings in our study, although it is not so clear that whether and how much these natural compounds are involved.

The ulcer number and the scores were other factors didn't reduce significantly by the extract doses studied. A number of reasons are likely to be involved; ulcer number alone cannot present an accurate and sensitive index, because the number of ulcers may increase while their dimensions actually became smaller. In addition, there are several investigations for which ulcer scoring is used as a reliable and relevant parameter to assess ulcer severity. Respected results in our study showed that a mean score reduction was occurred in treatment groups especially for greater doses of the extract but the difference was not significant from the control groups. Lack of histopathologic evaluation, scoring assessment by two independent observers and limited ulcer scoring range could be likely accounted for unusually scoring results. By attention to ulcer area, it is evident that ulcer protective efficacy of treatments particularly for plant has been grossly reflected in this parameter. Several studies [38, 39] including ours, support the view that ulcer area is one of the most reliable and accurate factors that could determine the mucosal injury properties so we included this factor to the equation measured the ulcer index. Regarding the results obtained in our study, we conclude that hydroalcoholic extract of *Z. multiflora* was effective in protecting against ulcer formation in duodenum and the efficacy was

greater for higher doses and when it was used after oral administration. This may suggest a rational basis for folk and traditional uses of this herb in Iran for some gastrointestinal ailments.

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