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Microscopical Evaluation of Healing Effect of *Falcaria vulgaris* Extract on Aspirin-Induced Gastric Ulcer in Rats

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Abstract

Objectives: Gastroprotective effects of herbal medicine were shown in numerous studies. *Falcaria vulgaris* (Ghazzayaghi) has traditionally been used for the treatment of stomach disorders in the Northwest of Iran. The present study was carried out to evaluate the microscopic healing effect of *F. vulgaris* extract on aspirin-induced gastric damage in rats. **Materials and Methods:** Thirty five young male rats were divided into five groups (7 rats/group). Control (group I) treated with 1 ml/kg distilled water. Four groups of animals had been exposed to starvation for 48 h and then received aspirin (200 mg/kg) in 1 ml carboxymethyl cellulose (CMC) 1% solvent for 3 days. Then, groups II–IV of rats were treated with 150 mg/kg extract (experimental group), 20 mg/kg omeprazole (reference group) and CMC solution (ulcer control group) by gavages for 14 days, respectively. The fifth group only received aspirin for 3 days. Three hours after last treatment rats were sacrificed and the stomachs were fixed in formalin (10%) and sections of 6 µ in diameter were prepared. Ulcer index, curative ratio, and morphological characteristics of the stomach were assessed using hematoxylin and eosin and Van Gieson staining. Data were analyzed using one-way ANOVA and *post hoc* Tukey test. **Results:** The results indicated that *F. vulgaris* extract significantly decreased the ulcer index ($P < 0.01$), increased curative ratio, and enhanced the formation of collagen fibers compare to aspirin and omeprazole groups. **Conclusions:** Hydroalcoholic extract of *F. vulgaris* showed significant microscopic healing effect on the aspirin-induced gastric ulcer.

Keywords: Aspirin, Curative Ratio, *Falcaria vulgaris*, Gastric Ulcer, Hydroalcoholic Extract, Ulcer Index

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1. Introduction

Gastric ulcer is one of the most common diseases affecting mankind, and it is well-known that in this disease there is an imbalance between mucosal damaging mechanisms to mucosal protecting mechanisms (Patel *et al.*, 2012; Watanabe *et al.*, 2002; Metowogo *et al.*, 2011). The etiological factors of this disorder include stress, smoking, acid, bile salts, nutritional deficiencies, proinflammatory cytokines, infections, and frequent use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin (Patel *et al.*, 2012; Watanabe *et al.*, 2002; Metowogo *et al.*, 2011). Aspirin is used for the treatment of rheumatoid arthritis and the prevention of cardiovascular thrombotic diseases, unfortunately, gastric ulcer associated with the use of aspirin is a major problem (Duggan *et al.*, 1999).

The modern drugs used in the treatment of gastric ulcers is based on the inhibition of gastric acid secretion by histamine receptors blockers, proton pump inhibitors such as omeprazole, or enhancing the mucosal production

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like prostaglandin analogous (Patel *et al.,* 2012; Watanabe *et al.,* 2002). Although these drugs have brought about remarkable changes in ulcer therapy, their efficacy is still debatable. Report on the clinical evaluation of these drugs showed that there are incidences of adverse effects and drug interactions during ulcer therapy (Khazaei M and Salehi, 2006; Saha *et al.,* 1993).

Recently, widespread efforts have been launched to identify novel antiulcer drugs from natural resources. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer (Duggan *et al.,* 1999; Mahmood et al., 2010; Alkofahi and Atta, 1999). *Falcaria vulgaris* (Ghazzayaghi) is a plant from *Umbelliferae* family. In the West of Iran, this herb has been used to treat skin ulcer, stomach disorders including peptic ulcer, liver diseases, and stones of kidney and bladder (Azhdari, 2001; Mir-Haidar, 2001). Phytochemical studies have been shown that *F. vulgaris* has tannin and saponin (Fazly Bazaz *et al.,* 1993). It also contains essence, Vitamin C, phytoestrol, protein, and starch (Azhdari, 2001; Mir-Haidar, 2001). In several experimental models of gastric ulcer, tannins have been shown to present antioxidant activity, promote tissue repair, and they are involved in gastrointestinal tract anti-inflammatory processes (Mir-Haidar, 2001; Fazly Bazaz *et al.,* 1993).

In ulcer, wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs, and the cells begin to increase collagen production. Later, the epithelial tissue is regenerated (Saha *et al.,* 1993; Alkofahi and Atta, 1999). To our knowledge, there were no scientific reports available in literature in support of the traditional claims of *F. vulgaris*, and the mechanism of healing effect of this plant against gastric damage is unclear. Therefore, we investigated the efficacy of *F. vulgaris* for its healing effect on an aspirin-induced gastric ulcer in rats.

2. Materials and Methods

2.1 Collection of Plant Materials

F. vulgaris was collected and subsequently analyzed and verified by a botanist (Mr. Caverizadeh, Biology Department, Razi University, Kermanshah, Iran). The leaves and stems of this plant were shade dried for 5 days and pulverized to get course powder (Patel *et al.,* 2012; Khazaei and Salehi, 2006).

2.2 Extract Preparation

About 100 g of powder was extracted with 70% ethanol (Merck) by percolation method. The ethanolic extract was completely dried under vacuum and then weighed and the residue was used in testing (a yield of 37%). The dried extract was dissolved in distilled water before gavages (Patel *et al.,* 2012; Khazaei and Salehi, 2006).

2.3 Acute Toxicity

Acute toxicity study is a study in which the animals were treated with extract at 5 different doses and were kept under observation for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as a toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. The animals were observed for toxic symptoms for 72 h. All the animals remained alive and did not manifest any significant visible of toxicity at 150 mg/kg doses; then we conclude that *F. vulgar* is extract administered to rats was safe and that no drug-related toxicity was detected in this dose (Khazaei *et al.,* 2006; Vinothapooshan and Sundar, 2010).

2.4 Animals

Male rats (Wistar) (Razi Institute of Iran) (weighing 180–230 g) were placed in an animal house and maintained in cages with raised floors of wide wire mesh to prevent. The animals were kept under the conditions of ambient temperature, humidity, and dark-light cycle (12–12 h), and the animals had free access to food and water. Four groups of animals were fasted for 48 h before use of aspirin to ensure an empty stomach. During the fasting period, rats received a nutritive solution of 8% sucrose in 0.2% NaCl to avoid excessive dehydration (Khazaei *et al.,* 2006; Yadegari *et al.,* 2006). The experimental protocol was approved by the Ethics Committee on animal experiments in Kermanshah University of Medical Sciences.

2.5 Experiment Design

The animals were put in five groups, each containing 7 rats. Control (Group I) treated with distilled water. Groups II-IV had been exposed to starvation for 48 h and then received aspirin (200 mg/kg) in 1 ml carboxymethyl cellulose (CMC) 1% solvent for 3 days. Then groups II-IV were treated with extract, omeprazole, and CMC for 2 weeks by gavages, respectively. Group V only received aspirin for 3 days (Table 1).

Table 1. Groups and doses

Groups	Treatment
Group I	Control (1 ml/kg distilled water)
Group II	Aspirin +(150 mg/kg extract) experimental group
Group III	Aspirin +(20 mg/kg omeprazole) reference group
Group IV	Aspirin +(1% CMC solution) ulcer control group
Group V	200 mg/kg aspirin

CMC=Carboxymethyl cellulose

Three hours after the last treatment of aspirin administration all animals were killed. The stomachs were dissected and examined for ulcer and microscopic changes (Khazaei *et al.,* 2006; Vinothapooshan and Sundar, 2010; Zeeyauddin *et al.,* 2011; Mehraein *et al.,* 2014).

2.6 Histological Studies

The stomachs were fixed in 10% neutral buffered formalin for 24 h. Glandular parts of the stomachs were divided into four segments, and the specimens were embedded in paraffin and sectioned. The slides were stained by hematoxylin and eosin and were examined by an experienced pathologist (Dr. Babak Izadi) under a light microscope (Olympus, Japan). Then, ulcer index, curative ratio (% protection), and morphological characteristics of the gastric mucosa was evaluated. A mean microscopic ulcer index and curative ratio were calculated by the formula (Patel *et al.,* 2012; Yadegari *et al.,* 2006; Mehraein *et al.,* 2014).

Normal tissue = 0.

Local damage to gastric pits cells = 1.

Local damage to gastric glands = 2.

Deep damage to gastric glands = 3.

Microscopic ulcer index = (number of lesion 1) + (number of lesion 2) × 2 + (number of lesion3) × 3.

% Protection= (CT/C) × 100.

C = ulcer index in control group.

T = ulcer index in treated group (Patel *et al.,* 2012; Yadegari *et al.,* 2006; Mehraein *et al.,* 2014).

2.6.1 Van Geison Staining

For the better appreciation of collagen deposition and the amount of collagen, Van Geison staining was used which stain the collagen fibers pink. The stomach samples from each group were fixed in formaldehyde, and collagen deposition in sub mucosal layer was determined by using histology method in accordance with routine laboratory procedures (Mehraein *et al.,* 2014). All of the sections were studied by an Olympus light microscopy (Olympus, Japan, at ×10 magnification).

2.7 Statistical Analysis

All values are expressed as the mean ± standard error of the mean. The results were analyzed by one-way ANOVA followed by the *post hoc* Tukey test by SPSS 15.0 for Windows (SPSS Inc, Chicago, IL). $P < 0.01$ was considered statistically significant.

3. Results

3.1 Effect of Falcari vulgaris Extract on Microscopic Ulcer Index and Curative Ratio

The results indicated that *F. vulgaris* extract (150 mg/kg) significantly decreased the ulcer index ($P < 0.01$) and severity. Extract exerted microscopic clear curative ratios compare to aspirin and omeprazole (Figures 1 and 2). Post-treatment with *F. vulgaris* for a period of 14 days revealed a statistically significant improvement in the ulcer healing process.

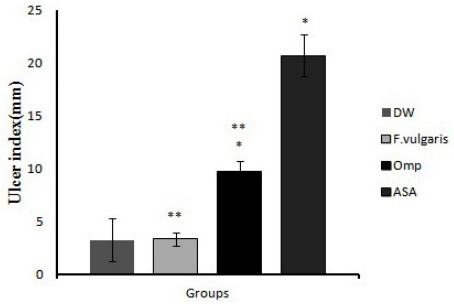


Figure 1. Effect of Falcari vulgaris on microscopic gastric ulcer index. Data expressed as mean±SEM; *P<0.01 compared with control and **P<0.01 compared with ASA.

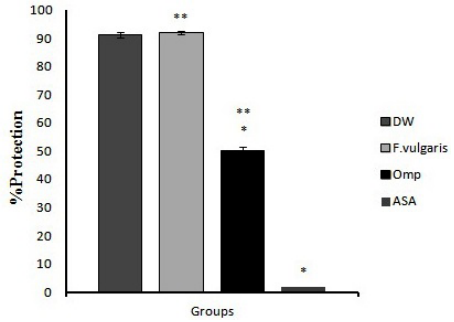
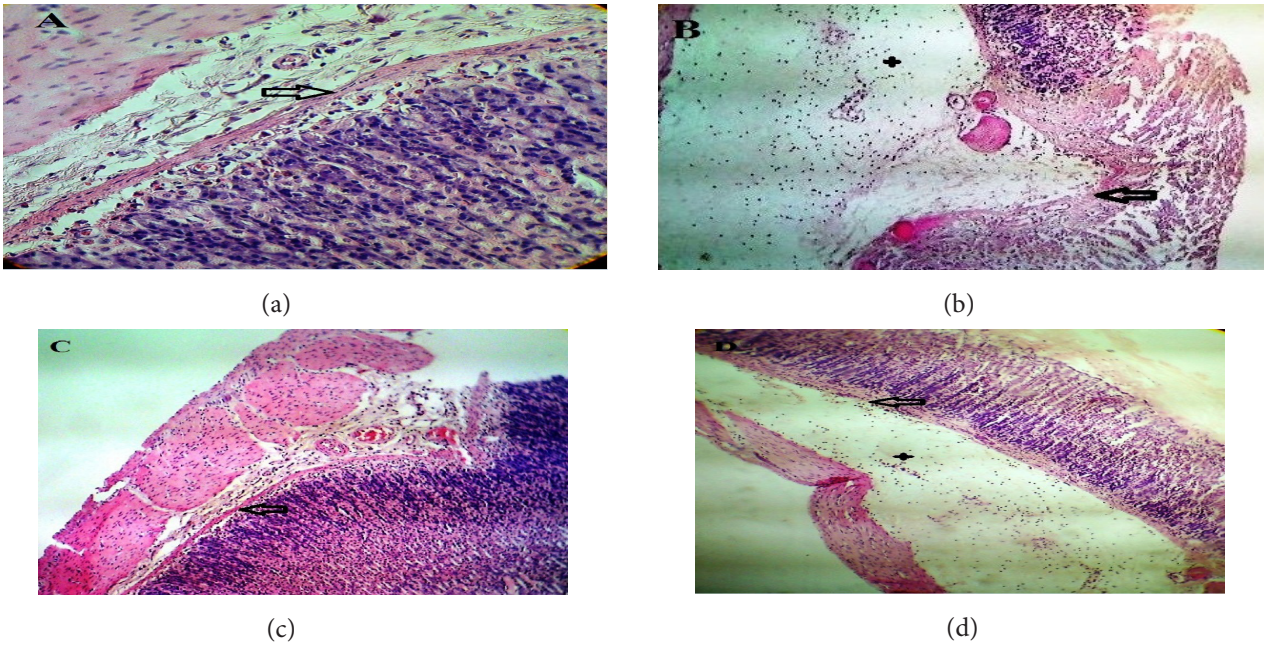


Figure 2. % Protection (microscopic) of Falcari vulgaris on aspirin induced gastric ulcer. Data expressed as mean±SEM; *P<0.01 compared with control and **P<0.01 compared with ASA.

3.2 Histopathological Findings of Gastric Tissue

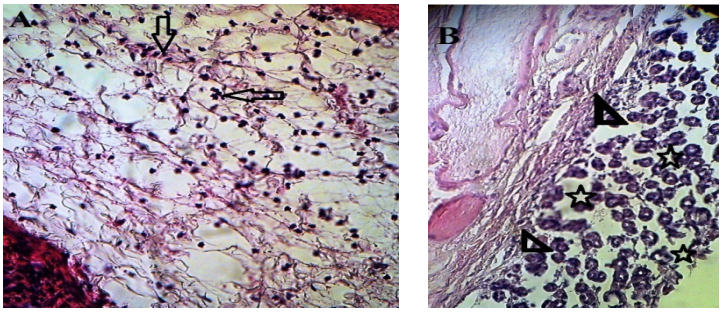
The histopathological findings of the gastric specimens are shown in Figures 3 and 4. The gastric mucosa of control (Group 1) showed normal tissue and structure (Figure 3(a)). In the stomachs of the aspirin-treated rats, extensive damage to the gastric mucosa, extensive edema, and leukocytes infiltration of the submucosal layer are present. In this group, ulcer formation and discontinuity in muscularis mucosal were also seen (Figures 3(b), 4(a), and (b)). Using of *F. vulgaris* extract resulted in the maintenance of glandular organization and the structure of the muscularis mucosa with no pathological changes. The extract had comparatively better protection of the gastric mucosa as seen by absent of ulcer formation, absent of submucosal edema, and leukocytes infiltration when compared with the aspirin and omeprazole groups (Figure 3(c)). In rats treated with omeprazole, injuries to the gastric mucosa were very milder compared to aspirin group. Mild disruption of the surface epithelium mucosa was present, but there was clear edema and leukocytes infiltration in the submucosal layer (Figure 3(d)).



(a) (b)

(c) (d)

Figure 3. Histology evaluation of gastric mucosal lesions of the rats in different groups. Hematoxylin and eosin ×100. **(a)** In control group, normal structures of stomach tissue were seen. **(b)** Aspirin-induced gastric ulcer formation, discontinuity in muscularis mucosal, and leukocytes infiltration in the submucosal layer. **(c)** Rats treated with extract reduced the formation of gastric lesions and intact mocusa and muscularis mucosal were seen. **(d)** In omeprazole group, mild disruption of the surface epithelium mucosa was present. Leukocytes infiltration of the submucosal layer and muscularis mucosal was shown by * and arrow, respectively).



(a) (b)

Figure 4. Microscopic view of aspirin-induced ulcer (limited area), × 400. **(a)** and **(b)** Leukocytes infiltration of the submucosal layer, muscularis mucosal discontinuity and distorted gastric glands, a damaged mucosal epithelium were shown by arrow, arrow head and * respectively.

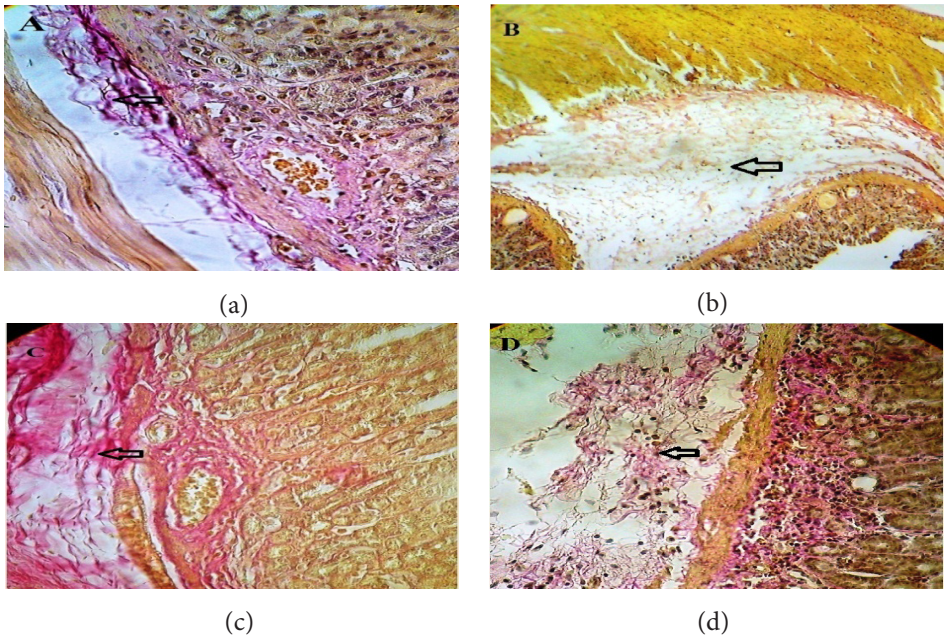


Figure 5. Histology evaluation of submucosal deposition collagen scale. Van Geison ×100. **(a)** Showed normal collagen amount in control group. **(b)** in aspirin treated group, there are less and irregular collagen compared with other groups. **(c)** collagen fibers in the extract group are more compact and regular compared with aspirin and omeprazole groups. **(d)** showed moderate deposition collagen in omeprazole group. Collagen deposition in sub mucosal layer was shown by arrow.

3.3 Effect of Falcari vulgaris Extract on Ulcer Healing

The histological study of the tissue of the control group showed a normal amount of collagen deposition in the sub mucosal layer (Figure 5(a)). A*spirin* treated animals showed a larger number of inflammatory cells and lesser and irregular collagen (Figure 5(b)). The tissue from the extract treated group showed the presence of a lesser number of inflammatory cells and increased collagen formation compared to aspirin group (Figure 5(c)). Omeprazole also enhanced collagen formation, but it was lesser compared to the extract group (Figure 5(d)).

4. Discussion

In this study, antiulcer activity and healing effect of hydroalcoholic extract of *F. vulgaris* have been studied. The finding of the present study demonstrated that *F. vulgaris* extract (150 mg/kg) significantly reduced gastric mucosal damage induced by aspirin. It is remarkable that this extract decreased microscopic ulcer index and increased curative ratio or percent protection and significantly increased collagen fiber deposition in sub mucosal layer, compare to aspirin and omeprazole groups. In our previous studies, *F. vulgaris* showed clear protective effect on gastric ulcer induced by aspirin (Khazaei *et al.*, 2006) and showed the significant healing effect on gross gastric lesions (Yadegari *et al.*, 2006).

In the current study, *F. vulgaris* decreased the microscopic ulcer index and increased curative ratio more than Khazaei and Salehi (2006) that induced gastric mucosal damage by ethanol 50%, because they used different methods for the detection of induce a gastric ulcer. In addition, the other possible explanation for this discrepancy might be because of the duration of incubation with extract 14 days in our study compared with 3 days in their research.

In the present study, histological evaluation of the gastric tissue showed that aspirin-induced significant lesions in gastric of rats. These changes consist of sever gastric damage, ulcer, discontinuity in muscularis mucosal, hemorrhage, edema, leukocytes infiltrate, loss of epithelial cells, and collagen content in sub mucosal layer. An ulcer is a lesion that penetrates the muscularis mucosa. There is no detectable ulcer in extract treated group and continuity of muscularis mucosal were seen. Therefore, repairing effect of the extract might be due to the preservation of muscularis mucosal. The result of the present study proved the wound healing property of the *F. vulgaris* extract in the company of inhibition of leukocytes infiltration to preserve the gastric wall. In addition, *F.*

vulgaris restored collagen formation in submucosal layer. Enhancement in the collagen content deposition provides stabilization and contributes to wound strength, which in turn, helps to repair wound tissue (Mehraein *et al.,* 2014).

It is generally accepted that peptic ulcer is developed because of the imbalance between aggressive factors and defensive factors (mucous, bicarbonate, blood flow, epithelial cell restoration, and prostaglandins) (Pradhan *et al.,* 2013; Guth *et al.,* 1984). NSAID's such as aspirin can cause damage to the gastroduodenal mucosa via several mechanisms, including the topical irritant effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow, and interference with the repair of superficial injury. The exact etiology of gastric ulcer that induced by aspirin is unclear (Nafeeza *et al.,* 2002; Wallace, 2000).

Drugs and some of plants extract can decrease gastric ulcer with a decrease in gastric mucus lesion by decrease production of free and lipid peroxidation, inhibition acid secretion (Azhdari, 2001; Mir-Haidar, 2001; Bazaz *et al.,* 1993). *F. vulgaris* is a member of *Umbelliferae* family which in traditional medicine has been used for gastric disorders. Phytochemical studies showed the presence of tannin, saponin, essence, Vitamin C, phytoestrol, protein, and starch (Azhdari, 2001; Mir-Haidar, 2001; Bazaz *et al.,* 1993; Khazaei, 2006).

Previous studies showed that all of the plant extracts that have gastroprotective effects contained tannins and/or flavonoids (Alkofahi and Atta, 1999; Morton, 1999; Verma, 2012; Ismail, 2012). Ramirez and Roa (2003) also showed that tannins extracted from *Syzygium cumini* have significant gastroprotective or repairing property. Then, healing effect of *F. vulgaris* extract could be due partly to the presence of tannins. In the other study, Pihan *et al.* (1987) reported that the ulcer induced by aspirin and ethanol is because of increase in free radicals. Therefore, because *F. vulgaris* extract has an extensive amount of Vitamin C wound healing effect of this extract might be due to this component. The vasoconstrictive, repairing effects and antioxidant property excreted by Vitamin C can reduce free radical effects by scavenging them.

The findings of our studies indicate that this extract displays both ulcer preventive (Khazaei *et al.,* 2006) and macroscopic ulcer healing effect (Yadegari *et al.,* 2006). Further studies are required to isolate the active principals and to elucidate the exact mechanism of healing effects of *F. vulgaris*.

5. Conclusion

To our knowledge, this study provided for the first time evidence that showed the microscopic healing effect of *F. vulgaris* against aspirin-induced ulcers which correlated with the folk medicinal use of this herb in the west of Iran.

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