Original Article

Healing Effect of Hydroalcoholic Extract of *Humulus lupulus* L. (Hops) Aerial Parts on Indomethacin-induced Gastric Ulcer in Rats

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Abstract

Background: *Humulus lupulus* L. (Hops) is one of the medicinal plants for which several effects have been reported such as sedative and hypnotic, anti-inflammatory, antioxidant, antibacterial, and anticancer. The fruits of this plant are also used for flavoring and as an aromatizer in the food and beverage industry. This study was done to evaluate the gastric anti-ulcer capacity of this plant in an animal model. **Materials and Methods:** Male Wistar rats were used and the gastric ulcer was induced by oral administration of indomethacin (30 mg/kg, p.o.). The ulcer-bearing rats were orally treated with hydroalcoholic extracts of the leaf (HLE) and fruit (HFE) of hops at similar doses of 50, 100, and 150 mg/kg. Ranitidine (35 mg/kg, p.o.) was used as a reference drug. Gastric acid, pepsin activity, malondialdehyde (MDA), and myeloperoxidase (MPO) were evaluated in gastric tissue, whereas this tissue was examined macroscopically and microscopically. **Results:** The results showed that both extracts (HLE and HFE) at a dose of 150 mg/kg reduced gastric ulcer characteristics such as number and severity, content acidity, pepsin activity, MPO, and MDA values. Also, macroscopic and microscopic images confirmed the effectiveness of the tested extracts in the healing of gastric ulcers. **Conclusion:** It was concluded that leaves and fruits of hops were effective in healing gastric ulcers caused by indomethacin probably by reducing gastric acid and oxidative stress, and this effect was dose-dependent. This effect along with the sedative and anti-*Helicobacter pylori* properties of hops can be useful in introducing this plant as an antigastric ulcer agent under clinical conditions.

Keywords: *Gastric ulcer, Humulus lupulus L. (Hops), indomethacin, plant extract, rats*

Introduction

People all around the world are suffering from gastric ulcers and its associated morbidity and mortality. Gastric ulcers happen when the balance between defensive factors such as mucus, bicarbonate, sub-mucosal blood flow, radical scavenging capacity, and cellular regeneration and aggressive factors including gastric acid and pepsin and reactive oxygen species is upset.[1] This imbalance is mainly caused by *Helicobacter pylori* infection, non-steroidal anti-inflammatory drugs (NSAIDs), emotional and/or physical stress, tobacco smoking, and alcohol consumption, which result in mucosal and sub-mucosal damage.[2] Effective acid reducers such as proton pump inhibitors (PPIs) and H2 blockers as well as anti-*H. pylori* regimens have greatly decreased the incidence, hospitalization, and mortality rates of gastric ulcers.[3,4] However, gastric ulcer disease and its related complications have remained a clinical challenge due to the vast usage of NSAIDs and especially low-dose aspirin.

Many studies have shown that some medicinal herbs can alleviate or prevent peptic and gastric ulcers and their complications. *Glycyrrhiza glabra*, *Matricaria recutita*, *Solanum nigrum*, *Cichorium intybus*, *Ocimum sanctum*, and *Mentha microphylla* are among the best-known plants documented for this indication.[5,6]

Humulus lupulus L. (Hops, Cannabaceae) is a perennial plant wildly grown in Asia, Europe, and North America. It is also cultivated throughout the temperate regions while collected in the late summer. It is used in the food industry to add a bitter flavor and intense aroma to beer, beverages, and some foods.[7] In Iranian traditional medicine, hops flowers have been recommended for the treatment

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of sleep disorders, anxiety, muscle pains, and toothache.[8] In traditional Chinese and Indian medicine, hops flower oil and decoction have been used as a demulcent to remove baldness and prevent hair loss. It has also been reported that the extracts of the leaves and flowers of this plant were used in ancient Europe as a bath oil to relieve stress, pain, joint inflammation, and fever. Hops tea and infusion were also used to relieve fatigue, nervousness, irritability, restlessness, sleep disorders, and headache.[9] Hops are mainly composed of flavonoids, tannins, bitter acids (soft resins), essential oils, and terpenophenolics for which analgesic, anti-inflammatory, antioxidant, antibacterial, cytoprotective, and ulcer-healing properties have been established.[10,11] Due to the widespread use of hops as a flavoring and fragrance agent in food and beverage industries and its beneficial properties in the healing of skin wounds, indigestion, dyspepsia, and gastrointestinal spasmodic disorders, it was chosen in this study.[12] Therefore, the current study aimed to prepare two hops extracts from leaf (HLE) and fruit (female flowers) (HFE) and to evaluate them for anti-ulcerative activity using the indomethacin-induced model of gastric ulcer in rats.

Materials and Methods

Drugs and chemicals

Ranitidine and indomethacin powders were gifted from Chimidarou and Hakim Pharmaceutical Corporations, respectively. Bovine hemoglobin (BH) and Folin–Ciocalteu reagent were purchased from Solarbio Corporation (Beijing, China). The kit for malondialdehyde (MDA) measurement (Cat No.: NS-15022) was prepared by Navand Salamat Corporation (Uremia, Iran). Quercetin, ortodianisidine hydrochloride (ODZ), and hexadecyl-trimethyl ammonium bromide (HDTAB) were purchased from Sigma-Aldrich Company (Germany-Darmstadt).

Animals

In this study, 54 male healthy inbred Wister rats weighed 180–220 g and aged 3–4 months were allocated randomly in nine groups of six rats. The animals were prepared from the animal house of Isfahan School of Pharmacy and were housed in suitable polycarbonate cages under standard conditions such as temperature (22 ± 2°C), relative humidity (30–40%), and 12 h of light/dark cycles. Before induction of gastric ulcer, the animals fasted for 12 h while having free access to water. All animal experiments were done in accordance with the National Ethics Committee guidelines for animal care and experiments represented by the Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1399-191).

Preparation of HLE and HFE

The hops (leaf and fruit) were prepared from Essence Giah Company (Golestan, Iran) and identified by a specialist from the Pharmacognosy Department of Isfahan University of Medical Sciences, Isfahan, Iran. A voucher specimen No. 1598 was deposited at the Herbarium Laboratory. Hops leaves and fruits were dried in the shade and then crushed into fine powders by an electric mill (Moulinex®, France).

The hydroalcoholic extracts of dried hops leaf and fruits were prepared by the maceration method. Ethanol/water (70/30) in a volume of 100 mL was applied in three consecutive periods of 24 h for 100 g of fine powdered plant material. The resulting extract was filtered and concentrated using a rotary apparatus (45°C) and subsequently, the solvent remnant was removed by using the freeze dryer.[13] The yield value was finally determined for each extract, and the desired concentrations (50–150 mg/mL) were prepared from the final powdered extract.

Folin–Ciocalteu method for determination of total phenols

Based on this method, polyphenol contents of extracts were measured against a standard phenolic material: gallic acid. Briefly, absorption of solubilized gallic acid (50, 100, 150, 250, and 500 mg/mL) was read in the presence of the Folin–Ciocalteu indicator and sodium bicarbonate at 765 nm wavelength. The standard curve was then depicted and total phenols of extracts were measured in terms of milligrams equal to gallic acid (GAE)/gram of extract. Each experiment was repeated three times and the results were reported as mean ± standard deviation.[14]

Aluminum chloride method for determination of total flavonoids

Total flavonoids were measured by the aluminum chloride method against quercetin as standard. Briefly, dry extract (1 g) of hops leaf and fruit was separately reconstituted in distilled water and mixed with sodium nitrate (5%) and aluminum chloride (10%) solutions. After 10 min of incubation at laboratory temperature, sodium hydroxide (1 M) was added to the samples and made up to the desired volume. The absorbance of the samples was measured in the spectrophotometer (Shimadzu, Japan) at 510 nm wavelength. The standard curve was depicted for quercetin (1, 5, 10, 20, and 50 mg/mL), and total flavonoids were measured in terms of milligram equal to quercetin/gram of extract. Each experiment was repeated three times, and the results were reported as mean ± standard deviation.[15]

Animal groups

The animal grouping was done as mentioned: normal rats treated with normal saline (normal, 5 mL/kg); ulcer-bearing rats treated with normal saline (control, 5 mL/kg); hops leaf extract (HLE, 50, 100, and 200 mg/kg); hops fruit extract (HFE, 50, 100, and 150 mg/kg)[16]; and ranitidine (ranitidine, 35 mg/kg).[17]

Experimental procedure

All the treatments were done orally (p.o.) 1 h before ulcer induction with indomethacin (30 mg/kg, p.o.), after which the animals were kept fasted for 24 h.[18] Six hours later, the

animals were euthanized in a CO_2 chamber, their abdomen was opened, and two pyloric and cardiac sides of their stomachs were ligated, respectively. Then the stomachs were excised and the pH of gastric content was measured by a digital pH meter (phs-3C, China) after centrifugation (1100*g*) and separation of supernatant.[18]

Macroscopic evaluation

The stomachs were cut through the greater curvature and washed with normal saline. Then the tissues were spread upon the working sheet and fixed. Some photographs were taken by a suitable mobile camera, and the pictures were analyzed for the number and severity (scores) of ulcers by Fiji P software offered for image processing and analysis. Gastric injuries were graded as follows: 0: without ulcers; 1: erythema, edema, and thickening of tissue; 2: superficial ulcers and erosions; and 3: deep ulcers and spot bleeding.[18]

Microscopic evaluation

For this purpose, the stomach tissues of all rats were fixed in formalin 10%, sectioned into 4–6 μm-thick slices, and stained with hematoxylin and eosin (H&E). Then histopathological variables such as epithelial layer, edema, inflammation, ulcer, and necrosis were assessed by a pathologist unaware of the treating arrangement.[17,18]

Evaluation of the pepsin activity

The pepsin activity in gastric chymus was determined by using the Anson method.[19] For this purpose, 2 mL of bovine hemoglobin (25 g/1000 mL) was mixed with 0.5 mL of HCl (0.3 M). Next, 0.1 mL of harvested gastric acid in each sample was diluted with 9.9 mL of normal saline. This solution (0.5 mL) was added to tubes containing BH and HCl (0.3 M). In contrast, standard pepsin was used instead of harvested gastric chymus to draw the standard curve for pepsin activity. Five milliliters of trichloroacetic acid was eventually added to end the reaction after 10 min. After filtration, the optical absorption of solubilized hemoglobin byproducts caused by the effect of pepsin on hemoglobin was measured at a wavelength of 280 nm, and the amount of pepsin activity was determined in terms of milligrams after 15 min.

Evaluation of myeloperoxidase (MPO) activity

Gastric tissue (0.1 g) was chopped and homogenized in a phosphate buffer solution (5 mL) containing 0.5% of HDTAB. After four 45-s cycles, the resultant homogenate was sonicated and centrifuged in a refrigerator (3200*g*) for 15 min. The supernatant (0.1 mL) was mixed with 2.9 mL of phosphate buffer containing 0.005% of H_2O_2, and ODZ (0.167 mg/mL). The absorption density was read by a spectrophotometer after 0 and 3 min at 450 nm wavelength and was changed to MPO activity by using the following formula[20]:

A=10×change in absorbance per minute/volume of supernatant,

MPO activity (U/g) = A/weight of the tissue.

Evaluation of MDA

MDA value was analyzed by the lipid peroxidation (MDA) assay kit, according to the package insert protocol that was reported previously. Briefly, 1 mL of potassium chloride 1.15% w/v was added to 0.1 g of chopped gastric tissues, whereas the tissues were homogenized and centrifuged (1100*g* for 10 min) in a refrigerated centrifuge. The supernatant was separated, centrifuged again (2200 g for 15 min), and the absorbance density was measured at 532 nm.[17]

Statistical analysis

Data were reported as mean ± standard error of the mean (SEM). The results were analyzed by using one-way analysis of variance followed by Tukey's *post-hoc* test (SPSS software version 16.0). Scoring data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney *U*-test. $P < 0.05$ was considered significant.

Results

Yield value, total phenolic content, and total flavonoids of hops extracts

According to the final dry extract, the yield values were 8.2 ± 0.2% and 8.0 ± 0.2% for HLE and HFE, respectively.

Based on the standard curve, polyphenolic contents equivalent to gallic acid (GAE/g) were 309.9 ± 3.8 and 281.7 ± 3.1 mg for HLE and HFE, respectively.

Total flavonoids equivalent to quercetin (QE/g) were 41.1 ± 2.6 and 36.7 ± 2.4 mg for HLE and HFE, respectively.

Effect of hops extracts on gastric acidity (pH)

The effect of hops extracts on gastric acidity was determined by measuring pH. The results showed that both leaf and flower extracts of hops were effective in reducing gastric acidity and increasing pH, especially when the doses of extracts were increased to 150 mg/kg [Figure 1]. This effect was more pronounced with HLE ($P < 0.01$); however, the difference between the two extracts was not significant. Ranitidine as expected was effective ($P < 0.001$) in reducing gastric acidity [Figure 1].

Effect of hops extracts on pepsin activity

The effect of hops extracts on gastric pepsin activity has represented in Figure 2. Both HLE and HFE at a dose of 150 mg/kg could significantly ($P < 0.05$) reduce gastric pepsin activity. Ranitidine was effective in reducing pepsin activity ($P < 0.01$), and this effect was consistent with its effect on gastric acidity.

Effect of hops extracts on ulcer score

The severity of ulcers in gastric mucosa has presented in Figure 3. The results indicated that both extracts of hops at a dose of 150 mg/kg were effective ($P < 0.01$) in reducing the severity of ulcers in stomachs. Ranitidine was also effective ($P < 0.01$) in this regard. Lower doses of both

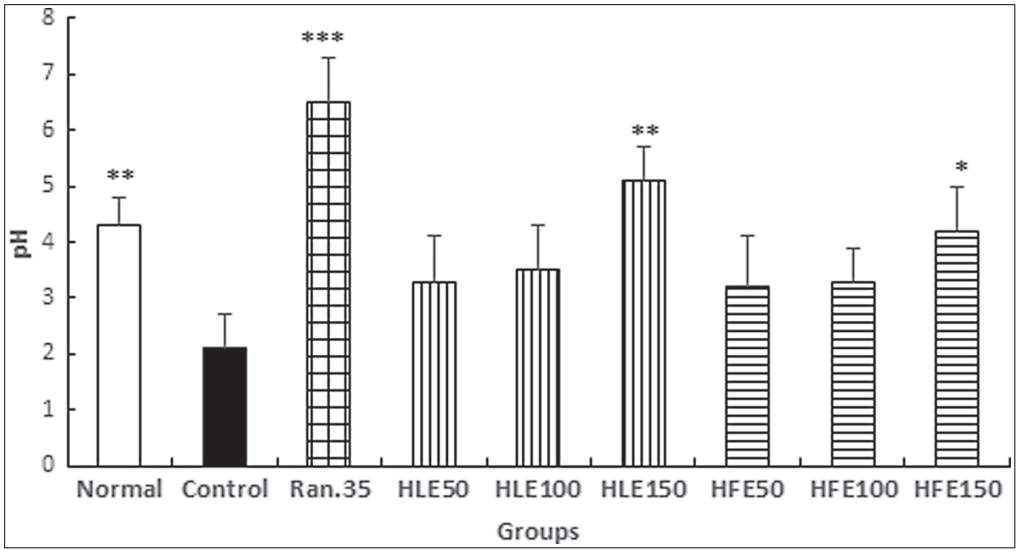

Figure 1: Effects of hops leaf and fruit extracts on pH of gastric chymus in rats. Normal rats received normal saline (5 mL/kg/day), control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: hops leaf extract (50, 100, 150 mg/kg), HFE: hops fruit extract (50, 100, 150 mg/kg), Ran: ranitidine (35 mg/kg). Data are presented as mean ±SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 show a significant difference vs. the control group

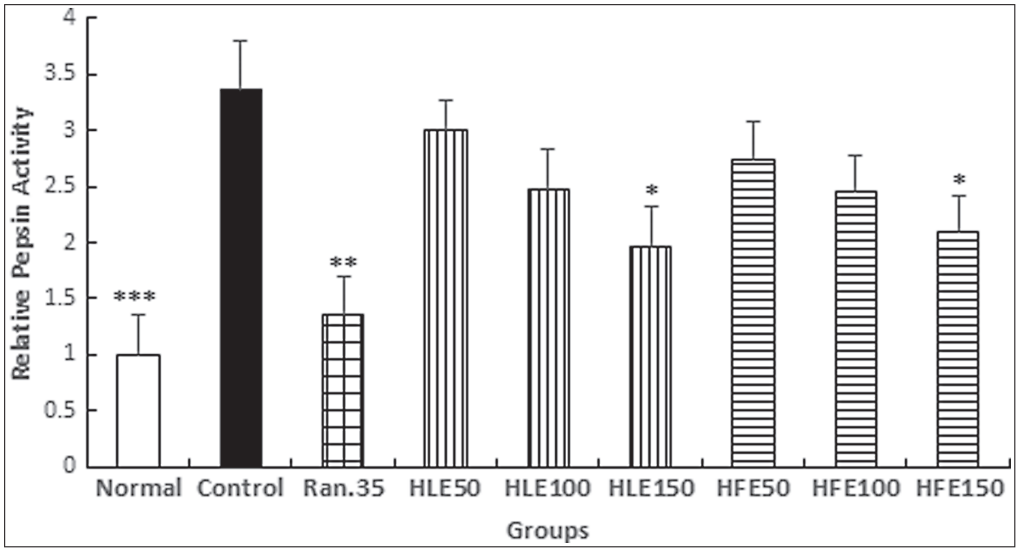

Figure 2: Effects of hops leaf and fruit extracts on pepsin activity of gastric chymus in rats. Normal rats received normal saline (5 mL/kg/day), control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: hops leaf extract (50, 100, 150 mg/kg), HFE: hops fruit extract (50, 100, 150 mg/kg), Ran: ranitidine (35 mg/kg). Data are presented as mean ±SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 show a significant difference vs. the control group

extracts (50 and 100 mg/kg) could not diminish ulcer score significantly ($P > 0.05$).

Effect of hops extract on ulcer number

The number of ulcers was counted for each sample and has presented in Figure 4. HLE and HFE at the doses of 100 and 150 mg/kg were able to reduce the number of ulcers significantly (at least $P < 0.05$). Ranitidine was also significantly effective ($P < 0.001$) in reducing the number of ulcers in gastric tissue. Regarding the number and severity of ulcers, it was concluded that greater doses of extracts were effective in ulcer healing. This result was confirmed by examining macroscopic and microscopic images of gastric tissue, as represented in Figures 5 and 6.

Effect of hops extracts on microscopic parameters

The results of the microscopic examination showed ulcer healing in the groups treated with hops extracts (150 mg/kg) and ranitidine. Microscopic images showed that the size of the ulcerative zone and the severity of inflammation and/or edema were diminished while the epithelium became regenerated [Figure 6].

Effect of hops extract on myeloperoxidase activity

Hop extracts altered the activity of MPO in gastric tissue [Figure 7]; however, this effect was only effective at the dose

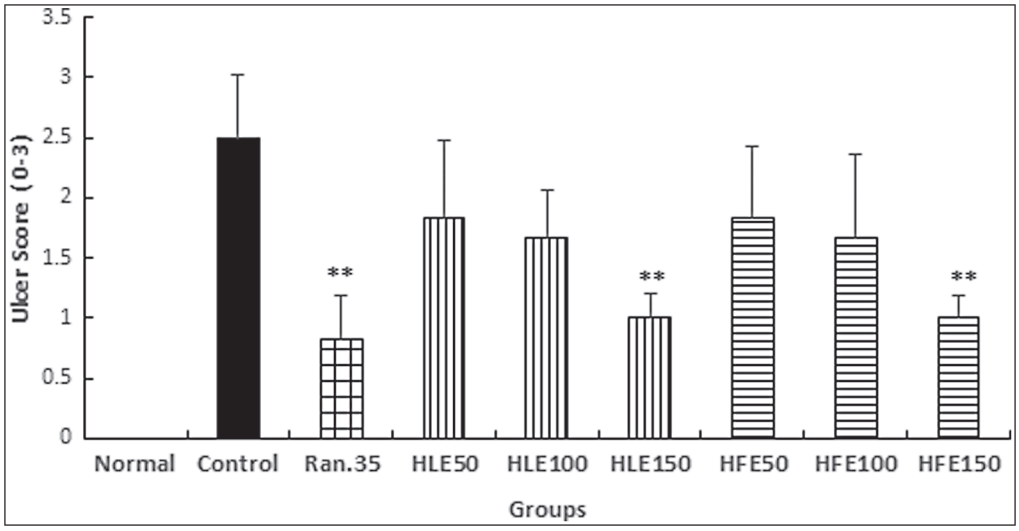

Figure 3: Effects of hops leaf and fruit extracts on ulcer score of gastric tissue in rats. Normal rats received normal saline (5 mL/kg/day), control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: hops leaf extract (50, 100, 150 mg/kg), HFE: hops fruit extract (50, 100, 150 mg/kg), Ran: ranitidine (35 mg/kg). Data are presented as mean ±SEM (n = 6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ show a significant difference vs. the control group

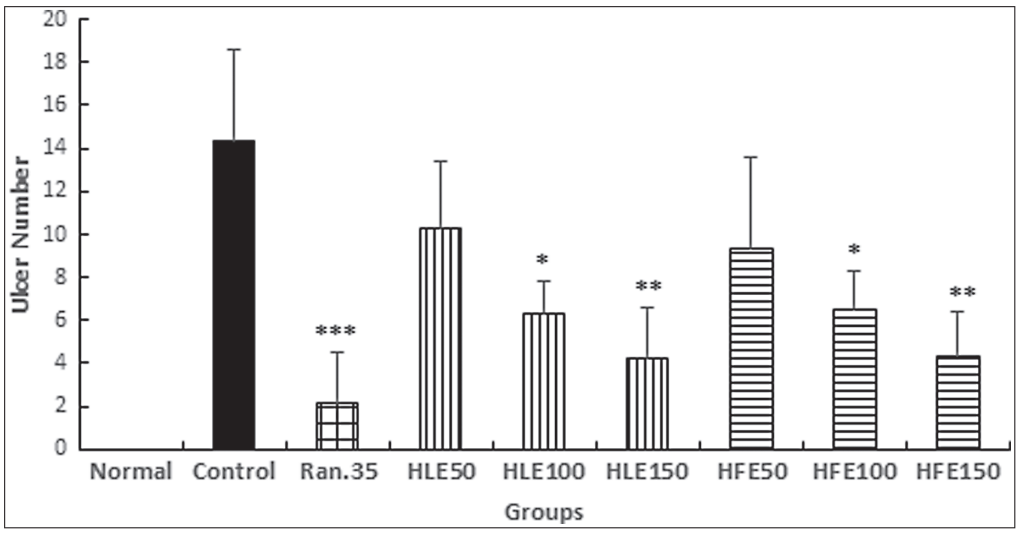

Figure 4: Effects of hops leaf and fruit extracts on ulcer number of gastric tissue in rats. Normal rats received normal saline (5mL/kg/day), Control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: Hops leaf extract (50,100,150 mg/kg), HFE: Hops fruit extract (50,100,150 mg/kg), Ran: Ranitidine (35 mg/kg). Data are presented as mean ±SEM (n = 6). *$P < 0.05$ **$P < 0.01$, ***$P < 0.001$ shows significant difference versus control group

of 150 mg/kg ($P < 0.01$). Ranitidine was similarly able to diminish MPO activity ($P < 0.01$) [Figure 7].

Effect of hops extracts on malondialdehyde value

HLE and HFE had a great reducing effect on the amount of MDA in gastric tissue. Both extracts at the doses of 100 and 150 mg/kg were effective in diminishing MDA value, indicating greater antioxidant activity for hops extracts (at least $P < 0.01$). Ranitidine was also effective in reducing this parameter ($P < 0.01$) [Figure 8].

Discussion

This study was performed to explore the gastric anti-ulcer effect of hops that had not been studied yet. The results indicated that indomethacin easily induced ulcers in gastric tissue at a single dose. This damage was also accompanied by an increase in gastric acid levels, a decrease in pH, and an increase in pepsin activity. Moreover, indomethacin increased the level of oxidative stress and leukocyte migration by increasing the level of MDA and MPO markers, respectively, in damaged gastric tissue.[16,20]

In contrast, the results showed that HLE and HFE similarly altered the pathogenesis and biochemical indices of gastric tissue and improved indomethacin-induced ulcers in rats. Ranitidine was also effective, indicating that gastric acid and pepsin play an important role in this model.[21]

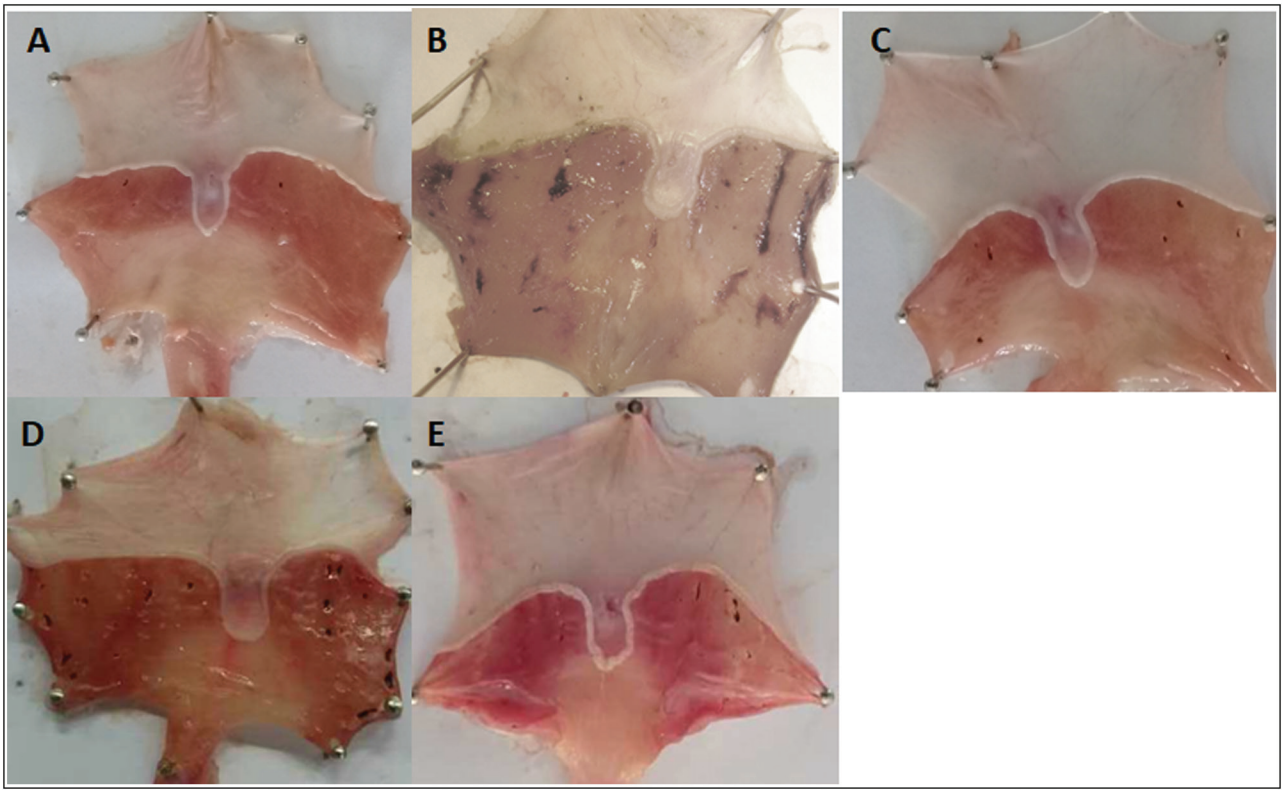

Figure 5: Macroscopic images of gastric ulcers in rats. (a): Normal tissue received normal saline (5 mL/kg), (b): ulcerated gastric tissue received indomethacin (30 mg/kg) and treated with normal saline, (c): ulcerated tissue treated with ranitidine (35 mg/kg), (d): ulcerated tissue treated with hops leaf extract (HLE, 150 mg/kg), (e): ulcerated tissue treated with hops fruit extract (HFE, 150 mg/kg)

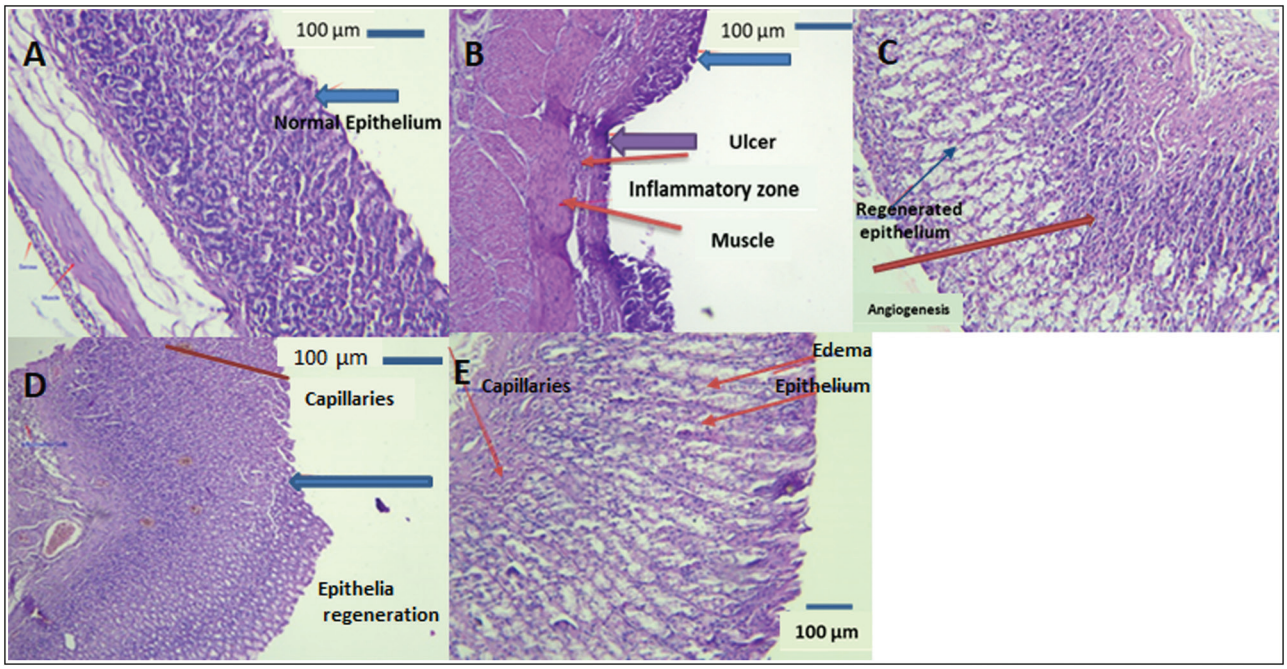

Figure 6: Microscopic images of gastric ulcer tissue in rats. (a): Normal tissue received normal saline (5 mL/kg), (b): ulcerated gastric tissue received indomethacin (30 mg/kg) and treated with normal saline, (c): ulcerated tissue treated with ranitidine (35 mg/kg), (d): ulcerated tissue treated with hops leaf extract (HLE, 150 mg/kg), (e): ulcerated tissue treated with hops fruit extract (HFE, 150 mg/kg). Tissue samples were stained with H&E and magnified ×40

The extracts of hops were also effective orally, which could be due to the local effects on the surface of the gastric mucosa and/or their systemic effects after absorption into the bloodstream. Also, the beneficial effects were dose-dependent, so that they became significant with increasing doses (150 mg/kg), whereas at lower doses tested, they were generally not significant.

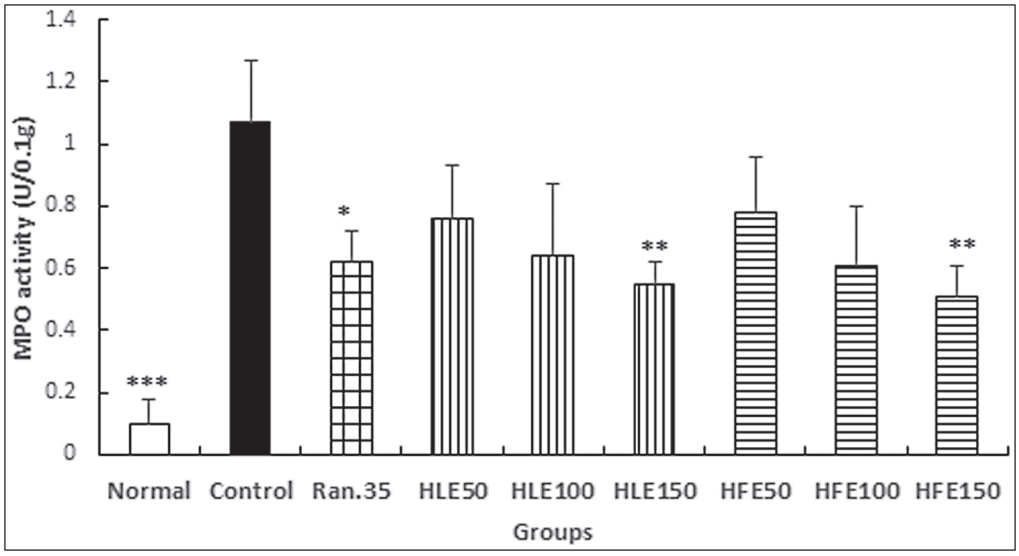

Figure 7: Effects of hops leaf and fruit extracts on MPO activity in rats gastric tissue. Normal rats received normal saline (5 mL/kg/day), control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: hops leaf extract (50, 100, 150 mg/kg), HFE: hops flower extract (50, 100, 150 mg/kg), Ran: ranitidine (35 mg/kg). MPO: myeloperoxidase. Data are presented as mean ±SEM (n = 6). *$P < 0.05$, $P < 0.01$, ***$P < 0.001$ show a significant difference vs. the control group**

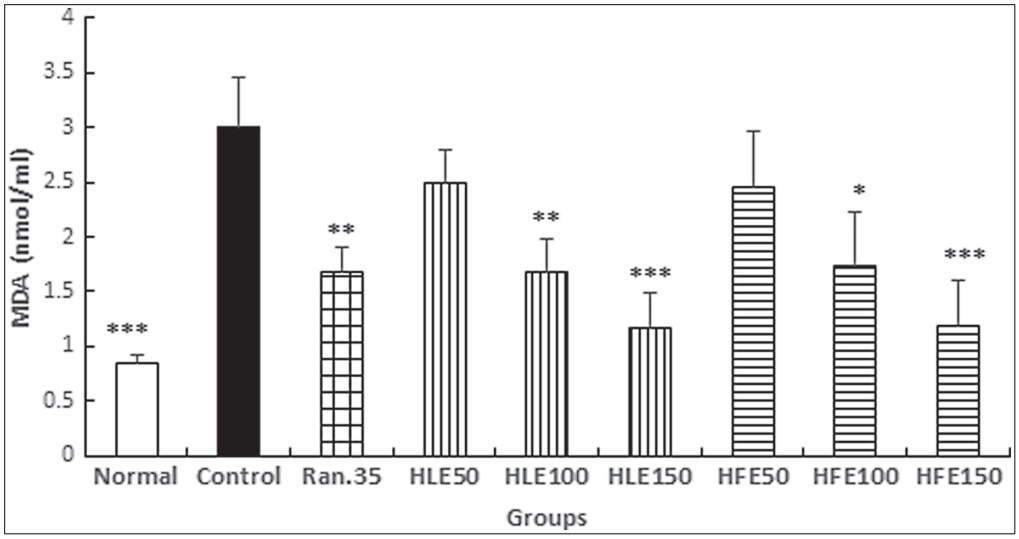

Figure 8: Effects of hops leaf and fruit extracts on MDA value in rats gastric tissue. Normal rats received normal saline (5 mL/kg/day), control: control rats with gastric ulcer induced by indomethacin (30 mg/kg, p.o.) received normal saline. HLE: hops leaf extract (50, 100, 150 mg/kg), HFE: hops fruit extract (50, 100,150 mg/kg), Ran: ranitidine (35 mg/kg). MDA: malondialdehyde. Data are presented as mean ±SEM ($n = 6$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ show a significant difference vs. the control group

By examining the effects of both extracts on pH and pepsin activity, it was concluded that the decrease in pepsin function was mainly due to the increase in gastric pH, and the direct interaction of the content of the extract with pepsin function, as shown in some previous studies, was ruled out.[17] In one study conducted by Hejazian *et al.*[22] on the antispasmodic effect of hydroalcoholic extract of hops *ex vivo*, it was found that higher concentrations of the extract (0.5 mg/mL) could inhibit ACh-induced smooth muscle contraction of the rat's ilium. This effect suggested that hops might have an anticholinergic effect similar to pirenzepine, and this might explain the reducing effect of gastric acid secretion by hops.[4] In contrast, Kurasawa *et al.*[23] showed that consumption of hops' bitter acids can increase gastric secretion but had no effect on gastric acidity. The researchers concluded that this effect was due to a kind of cephalic effect of bitter acids and the resulting taste, which helps in better digestion of foods without causing stomach ulcers. In another study carried out by Xin *et al.*,[24] xanthohumol as one of the main prenylated flavonoids of hops inhibited thrombosis induced by carotid artery injury without increasing the risk of bleeding. The researchers attributed this effect to the antioxidant, antiplatelet, and radical scavenging properties of xanthohumol. They concluded that flavonoids-enriched fraction of hops could

be used as antithrombotic in hemorrhagic disorders, such as peptic ulcers, without increasing the risk of bleeding.

Other studies on the sedative and hypnotic effects of hops also confirmed the anticholinergic effects of this plant on the brain.[22,25] The role of the anticholinergic effect of hops on its anti-ulcer effect needs further study; however, the calming and anti-stress effects of medicinal herbs have long been considered by researchers in the healing of stress-related gastric ulcers and functional dyspepsia. The use of infusions or decoctions of some sedative plants such as Chamomile, Rosemary, Borage, and Valerian could be considered in this regard.[26]

The primary effects of indomethacin and other NSAIDs are reductions in the synthesis and function of prostaglandins (PGs) as protective factors in the gastrointestinal tract, and this inhibitory effect will increase gastric acid and pepsin levels. Therefore, in addition, to inhibit acid secretion or pepsin function, the beneficial effect of hops in this study might be an increase in the level of PGs or mucus secretion in the gastric mucosa, which requires further study.[27]

The effect of tested extracts on MPO and MDA parameters as markers of oxidative stress, leukocyte migration, and proinflammatory factors emphasized that hops were effective in reducing these markers. This effect was more pronounced on the MDA parameter, indicating a greater antioxidant effect of hops in gastric tissue.[28,29]

Reviewing the literature about the active ingredients of hops, it seems that most of the active ingredients are polyphenols (flavonols and flavonoids), tannins, resins, alpha- and beta-acids (bitter acids), xanthamol, and volatile oils, which might be involved in creating the effects observed in this study.[30] The identity of individual flavonol constituents was unequivocally validated through a combination of ultraviolet–visible (UV–Vis) absorption spectra and mass spectrometry (MS/MS) analysis. Furthermore, the ultra-performance liquid chromatography method with diode array detection and mass spectrometry was used to analyze and determine individual alpha- and beta-acids, flavonols, and prenylchalcone compounds from dried extracts of hops fruits and leaves.[31] Alpha-acids have been mentioned to be the major component of hops flower, which enhance the pentobarbital effect and exhibit an antidepressant effect.[32] Other pharmacologically active components of hops are alpha- and beta-bitter acids. The main component of alpha-bitter acids is humulone and for beta-bitter acids, the main components are lupulone, colupulone, and adlupulone.[8,22] Also, in the liquor and beverage industry, the bitter acids of hops are used for aromatizing and flavoring.[22] Owing to the fact that the consumption of alcoholic beverages and beer, to a large extent, is involved in the occurrence of gastric ulcers, this study showed that hops extract could be effective in healing or at least preventing the aggravation of gastric ulcers.[33]

A review by Weiskirchen *et al.* on the hepatoprotective effects of hops showed that xanthomols in hops prevented or improved acute and/or chronic hepatotoxicity caused by carbon tetrachloride, alcohol, fatty liver, and liver cancer in animal models. This property was attributed to the anti-inflammatory, cytoprotective, antioxidant, and anti-cancer effects of hops.[34]

The anti-*H. pylori* effect of fresh hops was also investigated and confirmed by Cermak *et al.*[35] In that study, which was performed on 27 clinical specimens containing *H. pylori* cultures, a mixture of fresh hops mixed with hops extract was able to have a significant inhibitory effect on most specimens. These effects were mainly attributed to the alpha- and beta-organic acids (bitter acids) of hops, although polyphenols and xanthamols were also involved. Extensive antifungal, antiprotozoal, and antibacterial effects have also been reported for the active components of the hops plant.[36]

Therefore, considering that hops have potential anti-*H. pylori* effect, it seems to be more effective and useful in the treatment or prevention of gastric ulcers. Taken together, it is concluded that fruits and leaf extracts of hops can be effective in preventing or treating gastric ulcers, at least caused by NSAIDs. More experiments at both the preclinical and clinical levels are recommended to introduce this plant as a stomach ulcer remedy.

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Authors' contribution

Mohsen Minaiyan presented the idea of research, designed, and supervised all of the parts related to grouping of animals, determining the doses of drugs, arrangement of interventions, induction of gastric ulcer and statistical analysis of data. Hamidreza Razzaghi executed all of the experiments and interventions under supervision of professors. Afsaneh Yegdaneh designed and supervised all of the experiments related to identification, preparation, and evaluation of herbal materials and extracts. Ardeshir Talebi designed and supervised all of the experiments related to the sampling, preparing, and evaluation of tissues for histopathologic analysis. All the authors contributed in writing and preparation of manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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