Research in Pharmaceutical Sciences, April 2021; 16(2): 141-152
Received: 03-08-2020
Peer Reviewed: 15-09-2020
Revised: 28-09-2020
Accepted: 21-02-2021
Published: 05-03-2021

School of Pharmacy & Pharmaceutical Sciences
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Original Article

Evaluation of the effect of hydroalcoholic and flavonoid-enriched extracts of *Dracocephalum kotschyi* on indomethacin-induced gastric ulcer in rats

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Abstract

Background and purpose: *Dracocephalum kotschyi* (*Zaringiah*) is a fragrant wild medicinal plant found in Iran. Traditionally, it is used for the treatment of rheumatism, asthma, and gastrointestinal ailments. So far no investigation has been done on the beneficial or side effects of *D. kotschyi* on peptic ulcer. Therefore, this research was performed to find out whether *D. kotschyi* extract would induce peptic ulcer or could alleviate existing peptic ulcer.
Experimental approach: Effect of hydroalcoholic (DKHE) and flavonoid extracts (DKFE) of *D. kotschyi* were determined in normal or indomethacin-induced gastric ulcer rats (n = 6) and compared with the vehicle and ranitidine treated controls. All the treatments were carried out orally and 24 h later the stomach mucus was visually examined for peptic ulcers. A section of the stomach was taken for microscopic histopathological examinations while another section of the stomach was used for measurement of myeloperoxidase (MPO) and malondialdehyde (MDA) activities.
Findings/Results: Oral administration of the DKHE and DKFE alone, did not cause any sign of gastric ulcer induction. The *D. kotschyi* extracts not only didn't aggravate the induced ulcer but also significantly prevented the severity of gastric ulcer induction by indomethacin. In addition, DKHE and DKFE inhibited MPO (up to 58.2%) and MDA (up to 44.2%) activities indicating their anti-inflammatory and antioxidant potential action on the stomach-induced ulcer.
Conclusion and implication: Usage of *D. kotschyi* extracts is not associated with gastric ulcer induction and its co-administration with NSAIDs would be beneficial for controlling both the inflammation and preventing gastric ulcer in diseases such as rheumatism.

Keywords: *Dracocephalum kotschyi*; Gastric ulcer; Indomethacin; Malondialdehyde; Pepsin; Rats.

INTRODUCTION

Peptic ulcer (gastric and duodenal) disease is a worldwide gastrointestinal (GI) disorder that arises when the caustic effects of aggressive factors like acid, pepsin, and bile dominate over the defensive factors within the GI mucosa like mucus, bicarbonate secretion, prostaglandins, blood flow, and antioxidant capacity (1). Its annual incidence is about 0.1-0.19% for outpatient and 0.03-0.17% for hospitalized diagnosed cases (2). More than 90% of gastric ulcers are happened by infection of *Helicobacter pylori* or by use of non-steroidal anti-inflammatory drugs (NSAIDs) (1).

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Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.310521

Several medicinal plants have been studied for their potential healing effects on peptic ulcer disease among them *Glycyrrhiza glabra, Ocimum basilicum, Aloe vera, and Myrthus communis* are the most popular (3). Safety, availability, reasonable efficacy and cost are among important factors that have drawn popular attention to herbal medicines as alternative therapies for peptic ulcer disease (4). *Dracocephalum kotschyi* Boiss is the scientific name for an Iranian native plant known as Zaringiah (5). *D. kotschyi* belongs to Lamiaceae family and is a wild plant grown in cold and high-altitude mountainous areas in northern and central parts of Iran (6). Although native people living in Kouhrang region (Charamahalo-Bakhtiari Province) traditionally use the boiled extract of *D. kotschyi* for the treatment of rheumatism, medicinal uses of this plant have not been well documented in validated Iranian traditional sources. Despite scarce formal information about the traditional beneficial use of *D. kotschyi*, it is used widely by indigenous people for various ailments including rheumatism, asthma, and GI disorders (4,7). In recent years a wide range of investigations has been done on the pharmacological properties of this medicinal plant. For instance, it has been suggested that its extracts have anti-hyperlipidemic (8), antidiabetic (9), anti-inflammatory, anti-allergy (10), anticancer (11), antioxidant (12), and spasmolytic activities (13). However, the most prominent pharmacological effect of *D. kotschyi* is based on its anti-inflammatory activity. For example, it has been reported that the essential oil of Zaringiah in the animal model has subsided both pain and inflammatory edema (14,15). Furthermore, an extract of *D. kotschyi* markedly inhibited both innate and adaptive immunity reactions in an animal model suggesting its immunomodulatory effect *in vivo* (16). In the ulcerative colitis model, pretreatment with *D. kotschyi* extract significantly prevented the induction of colitis, further emphasizing on anti-inflammatory and anti-ulcerative properties of this herbal plant (17). *D. kotschyi* is enriched with active substances that could explain why its beneficial uses have been suggested for numerous ailments. Most of the anti-inflammatory effect of *D. kotschyi* is attributed to its flavonoid's contents including apigenin, luteolin, isokaempferide, xanthomicrol, and calycopterin (18-20). Hydroalcoholic extract of *D. kotschyi* has also potent anti-spasmodic activity on tracheal smooth muscle as well as on ileum, uterus, and bladder smooth muscles indicating its useful potential for the treatment of asthma, premature uterine contractions, and bladder inconsistency (21-23).

We know that corticosteroids have strong anti-inflammatory properties and are used for the treatment of many inflammatory and immune-based disorders. However, one of their disadvantages is that they cause and/or aggravate peptic ulcers (24). NSAIDs are also used for the treatment of rheumatism and chronic inflammatory skeletal diseases and might similarly cause peptic ulcers and/or accentuate them (25).

So far, there is no report on induction and/or aggravation of peptic ulcer due to consumption of *D. kotschyi* while several evidences suggest its potential for GI disorders such as peptic ulcer. Therefore, the current research was aimed to investigate whether oral administration of *D. kotschyi* extracts can induce gastric ulcer per se or would aggravate or alleviate existing gastric ulcer induced in the animal model.

MATERIAL AND METHODS

Plant extracts preparation

Arial parts of *D. kotschyi* were procured from a Pertican Rahnama Kesht farm in Fereydun-shahr (Isfahan province, Iran) in June 2019 and authenticated by Mohammad Asfa, a botanist from Isfahan Natural Resources Organization. A voucher specimen (No. 1519) was deposited at the herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences, Isfahan, Iran. *D. kotschyi* hydroalcoholic extract (DKHE) was prepared with ethanol/water (70/30) as the solvent and by maceration method, as described before (26). The ratio of plant powder to solvent for hydroalcoholic and flavonoid enriched extracts (DKFE) were 1:10 and 1:8, respectively. The yields of hydroalcoholic and flavonoid enriched

extracts were 28% and 40% (w/w), respectively. The total flavonoid content of DKHE and DKFE was determined by the aluminum chloride method and resulted in 0.36% and 1.12% respectively (20). DKFE was prepared from the hydroalcoholic extract by stepwise solvent/solvent partitioning technique using chloroform (2000 mL) and ethyl acetate (1/1) (27). DKHE and DKFE were prepared as 80 and 40 mg/mL stock solutions in distilled water, respectively.

Drugs and solutions

Indomethacin and ranitidine were prepared as 30 and 150 mg/mL stock solutions in distilled water, respectively. All the stock solutions were emulsified by adding 0.1% tween 80. Indomethacin and ranitidine were purchased from Darou-Pakhsh and Kimi-Darou Corporations (Iran), respectively.

Gastric ulcer induction

Gastric ulcer was induced in male Wistar rats (180-220 g) by oral administration of indomethacin using a feeding tube (28). This research project was approved by the Isfahan University of Medical Sciences Ethics Committee for the handling of laboratory animals and specified by the national ethics code: IR.MUI.Research.Rec.1398.329. Rats were purchased from the School of Pharmacy and Pharmaceutical Sciences (Isfahan University of Medical Sciences, Iran) animal house and acclimated with the laboratory environment one week before the start of the experiment. They were fasted for 24 h prior to gastric ulcer induction with free access to water. Extracts, drugs, or vehicle were given orally using a feeding tube (p.o.) to a group of 6 rats and an hour later indomethacin (30 mg/kg) was administered orally (p.o.). Altogether, eleven groups of rats were used in this study. The first group received the vehicle (distilled water/tween 80) without indomethacin. The second group was treated with the vehicle followed by administration of indomethacin. Third, fourth, and fifth groups were treated with DKHE (20, 40, 80 mg/kg) followed by indomethacin. Groups 6 to 8 were treated with DKFE (10, 20, 40 mg/kg) followed by indomethacin. The ninth group received ranitidine (30 mg/kg, i.p.) followed by indomethacin as the reference drug. Groups 10 and 11 were given vehicle followed by a single oral dose of DKHE (80 mg/kg) and DKFE (40 mg/kg), respectively instead of indomethacin to assess their possible ulcerogenic activity.

Twenty-four hour later, animals were sacrificed and the abdominal cavity was opened and both sides of the stomach were ligatured with threads before the whole stomach was dissected out.

Measurement of pH and pepsin activity

The stomach fluid content was collected for pH measurement and pepsin activity. Values of pH were determined by digital desktop pH meter after diluting the stomach content by 10-fold with distilled water and accounting for this dilution factor as 1 unit when pH was subsequently measured (29). Pepsin activity, on the other hand, was determined by the Anson method in gastric secretions of experimental rats.

Two mL of hemoglobin (25 g/1000 mL) was mixed with 0.5 mL of HCl (0.3 M). Then, 0.1 mL of harvested gastric acid was diluted with 9.9 mL of normal saline. Standard pepsin was used to draw the standard curve for pepsin activity while, 10 min later, 5 mL of trichloroacetic acid was added to terminate the reaction. After filtration, optical absorbance of amino acids produced by the reaction of pepsin with hemoglobin was measured at the wavelength of 280 nm, and pepsin activity was determined in terms of mg after 15 min (30).

Macroscopic and microscopic evaluations

The stomach was then cut into two longitudinal sections for further examination. One section was placed into formalin solution (10%) and sent for pathological examination to assess inflammation severity, hemorrhagic spot, sub-mucosal edema, and superficial mucosal ulcers (30). The other section was used for the measurement of myeloperoxidase (MPO) activity and malondialdehyde (MDA) value.

The macroscopic examination was performed on the photos taken from the stomach using Fiji P computer program (Image Analysis Program, V.2) for number and ulcer area. The microscopic examinations were scored according to Table 1.

Table 1. Pathological scores and descriptions for gastric tissue.

Featured score	Score	Description
Inflammation severity	0	None
	1	Slight
	2	Moderate
	3	Severe
Hemorrhagic spot	0	None
	1	Slight
	2	Moderate
	3	Severe
Edema (sub-mucosal)	0	None
	1	Slight
	2	Moderate
	3	Severe
Superficial mucosal ulcers	0	None
	1	Slight
	2	Moderate
	3	Severe

Assessment of MPO and MDA values

MPO activity was assessed by the method previously set up in our laboratory (31). MDA activity was analyzed by lipid peroxidation (MDA) assay kit (Navand-Salamat Lab Kit, Iran) according to package insert protocol was carried out previously (29,30).

Statistical analysis

All the data were expressed as mean ± standard error of the mean (SEM). Results of each group were compared with its corresponding control group. For histological results, non-parametric test (Mann-Whitney U-test) was used and the data were presented as median (range) as needed. For other data, Student's t-test and one-way ANOVA with Tukey's post hoc test were used as appropriate.

RESULTS

Effect of DKHE and DKFE on pH values

Results of pH values suggested that indomethacin can induce acid secretion in the stomach and subsequently cause a significant decline in pH ($P < 0.01$) for about two units (Fig. 1). None of the treatments with herbal extracts could alter the pH significantly except for DKHE at 10 mg/kg ($P < 0.01$) against the control group. Ranitidine as a standard acid reducer was significantly effective to enhance pH up to 7 ($P < 0.001$). DKHE and DKFE alone were not effective to enhance the pH of gastric juice significantly when compared with the normal group ($P > 0.05$) (Fig. 1).

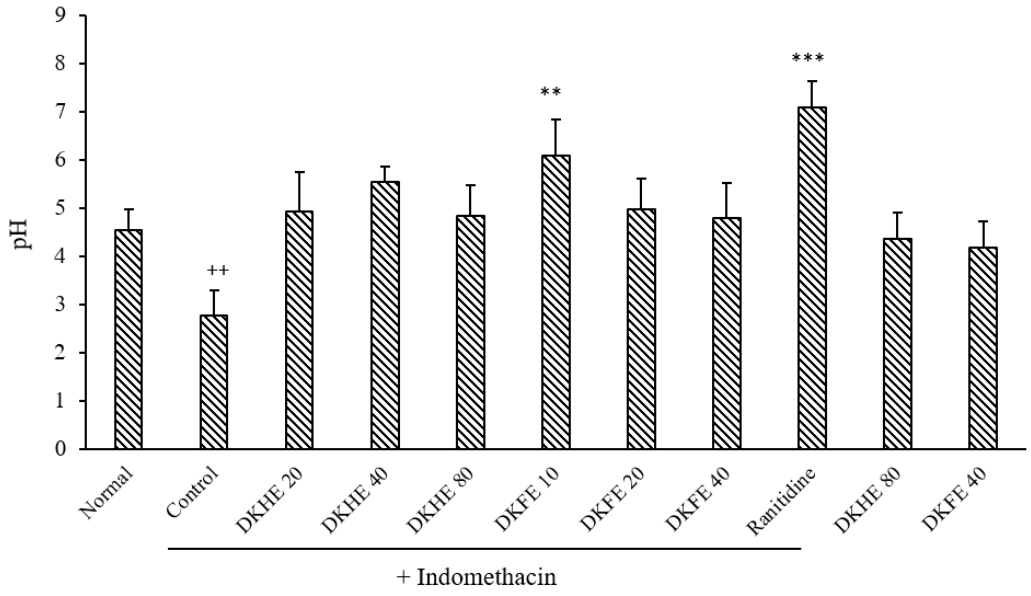

Fig. 1. pH of gastric secretions in rats. Normal rats received normal saline/tween (5 mL/kg/day), control rats with gastric ulcer induced by indomethacin (30 mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. $^{**}P < 0.01$ and $^{***}P < 0.001$ show significant difference with the control group; $^{++}P < 0.01$ *vs* the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract.

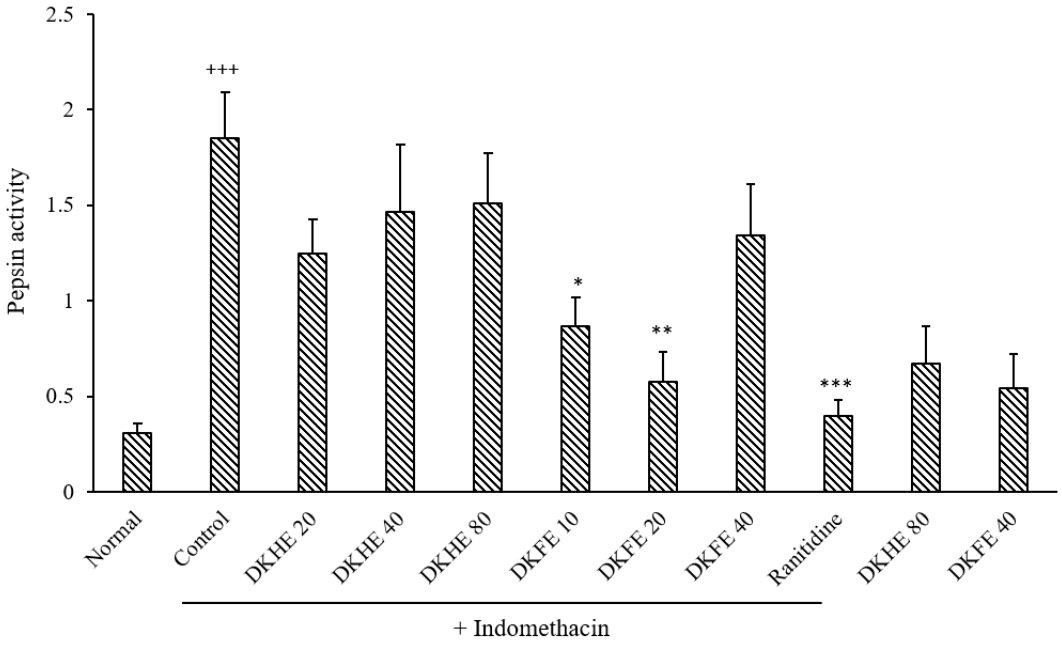

Fig. 2. Pepsin activity assessments in rat stomach. Normal rats received normal saline/tween (5mL/kg/day), control rats with gastric ulcer induced by indomethacin (30 mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicate significant differences in comparison with the control group; $^{+++}P < 0.001$ against the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract.

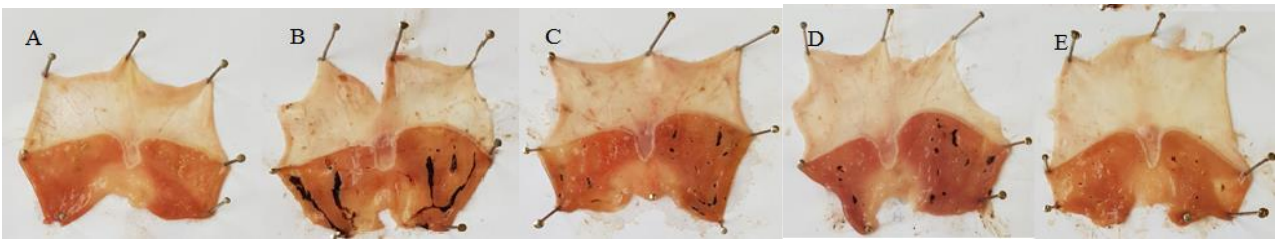

Fig. 3. Macroscopic photos of gastric tissue in rats. (A) Normal stomach treated with normal saline/tween, (B) ontrol stomach with representative ulcers (as dark spots or linear forms) induced by indomethacin (30 mg/kg), (C) gastric ulcer treated with *Dracocephalum kotschyi* hydroalcoholic extract (20 mg/kg), (D) gastric ulcer treated with *Dracocephalum kotschyi* flavonoid extract (10 mg/kg), and (E) gastric ulcer treated with ranitidine (30 mg/kg).

Effect of DKHE and DKFE on pepsin activity

Another factor was assessed in this research was pepsin activity. Pepsin activity was sharply increased (about 6 folds) in the control group that received indomethacin in comparison with the normal group (Fig. 2). Oral administration of DKHE produced no significant effect on pepsin activity however, DKFE at 10 and 20 mg/kg significantly reduced indomethacin-induced pepsin activity (Fig. 2). Oral administration of examined extracts alone caused no significant changes in pepsin activity when compared to the normal group (Fig. 2). It could be found that ranitidine fully inhibited pepsin activity induced by indomethacin and reversed it even up to the normal group value (Fig. 2).

Effect of DKHE and DKFE on macroscopic parameters

In the normal group, no sign of peptic ulcer or erosion was seen in the macroscopic parameters. In the group that received indomethacin, there was clear induction of peptic ulcer in spot or linear forms all over the stomach lining (Fig. 3). Both DKHE and DKFE reduced the severity and extent of indomethacin-induced ulcer (Fig. 3). DKHE and DKFE significantly reduced indomethacin-induced ulcer numbers when lower doses of extracts were applied (Fig. 4).

Comparison of ulcer area showed that in the animal group treated with DKHE (20, 40, and 80 mg/kg) the ulcer area was reduced 62, 55, and 29%, respectively. In a similar way, DKFE (10, 20, and 40 mg/kg) reduced indomethacin-induced ulcer area by 75, 48, and 35%, respectively (Fig. 5). Ranitidine reduced both ulcer number (61%) and ulcer area (91%) induced by indomethacin in a meaningful manner (Figs. 4 and 5).

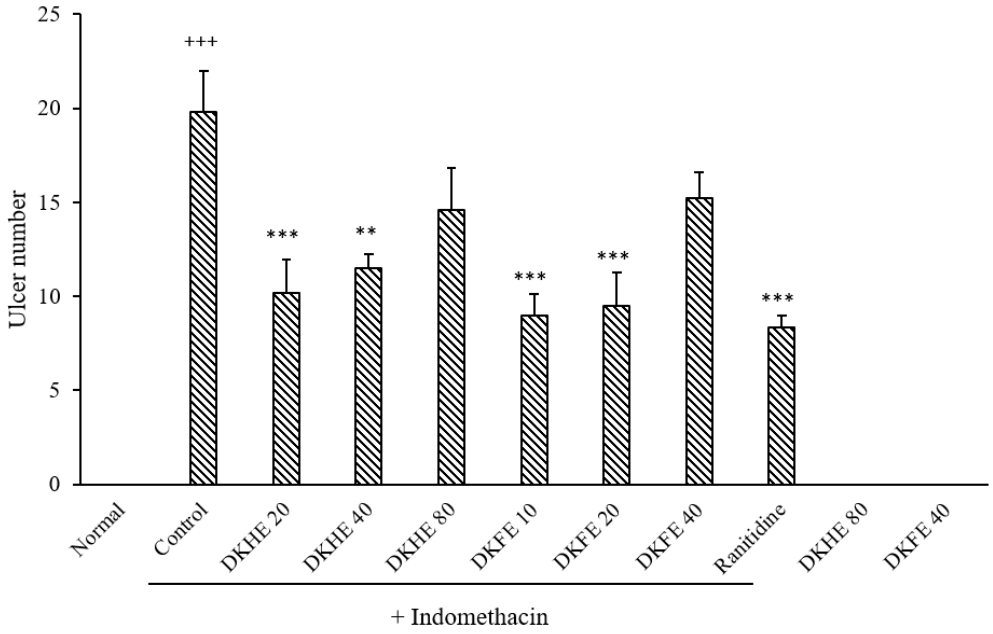

Fig. 4. Numerical assessment of ulcers in rat's stomach. Normal rats received normal saline/tween (5 mL/kg/day), control rats with gastric ulcer induced by indomethacin (30mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. $^{**}P < 0.01$ and $^{***}P < 0.001$ show significant differences in comparison with the control group; $^{+++}P < 0.001$ in contrast with the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract.

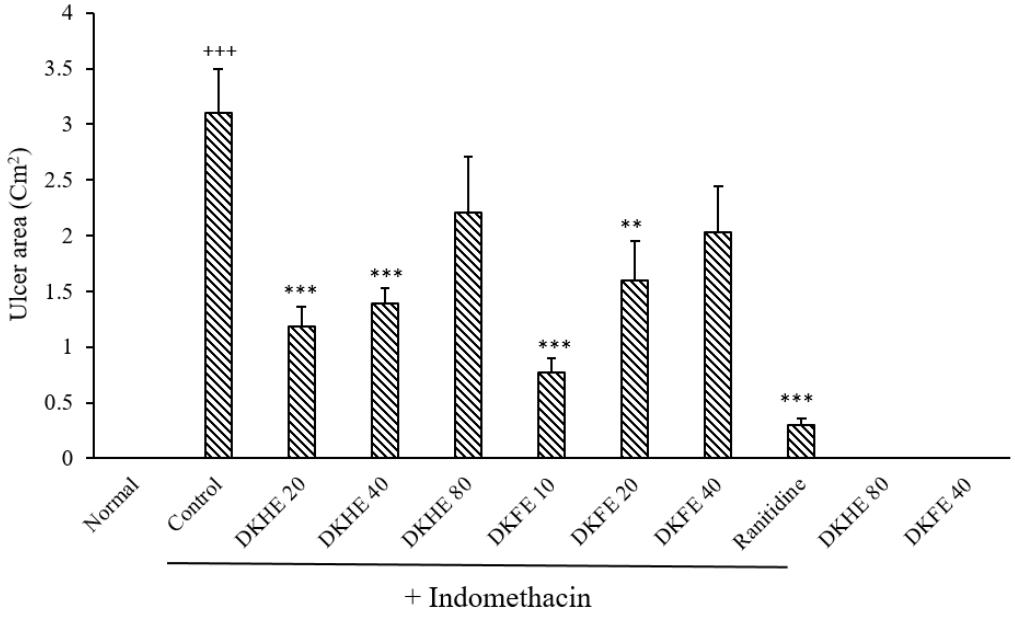

Fig. 5. Assessment of ulcer area in rat's stomach. Normal rats received normal saline/tween (5 mL/kg/day), control rats with gastric ulcer induced by indomethacin (30 mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. $^{**}P < 0.01$ and $^{***}P < 0.001$ show significant differences with the control group; $^{+++}P < 0.001$ *vs* the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract.

Table 2. Microscopic features of rat stomach tissue in different groups. Normal rats received normal saline/tween (5 mL/kg); control, the gastric ulcer was induced by indomethacin (30 mg/kg). DKHE, DKFE, and ranitidine (30 mg/kg) were given orally prior to indomethacin.

Groups/ dose (mg/kg)	Ulcer severity Median (range) (0-12)
Normal rats	0 (0-0)
Control	11 (8-12) +++
DKHE 20 + indomethacin 30	4 (4-8) ***
DKHE 40 + indomethacin 30	8 (2-8) **
DKHE 80 + indomethacin 30	10 (4-12)
DKFE 10 + indomethacin 30	4 (3-9) ***
DKFE 20 + indomethacin 30	8 (3-10) **
DKFE 40 + indomethacin 30	10 (4-12)
Ranitidine 30	3 (2-6) ***
DKHE 40	0 (0-0)
DKFE 80	0 (0-0)

DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract. $^{**}P < 0.01$ and $^{***}P < 0.001$ show statistically significant difference compared with the control group; $^{+++}P < 0.001$ significant difference *vs* normal group, (n = 6). Two later groups (DKHE and DKFE) were compared with the normal group.

Effect of DKHE and DKFE on microscopic parameters

As it is shown in Table 2, tissue samples were unaffected in the normal group. In the control group that received indomethacin orally and treated with vehicle, inflammation severity, hemorrhagic spot, sub-mucosal edema, superficial mucosal ulcers were found as the most severe changes in gastric tissue (Table 2).

All doses of DKHE and DKFE fractions reduced microscopic features significantly (at least $P < 0.01$) compared to the control group, although in groups that received DKHE 80 mg/kg and DKFE 40 mg/kg improving pathological parameters were not significant (Table 2, Fig. 6). In the reference group that received ranitidine, these pathological features were improved significantly compared to the control group (Table 2, Fig. 6). On the other side, DKHE (80 mg/kg) and DKFE (40 mg/kg) alone couldn't produce any sign of ulcer or erosion in gastric mucosa indicating its safety as complementary herbal medicine (Table 2, Fig.6).

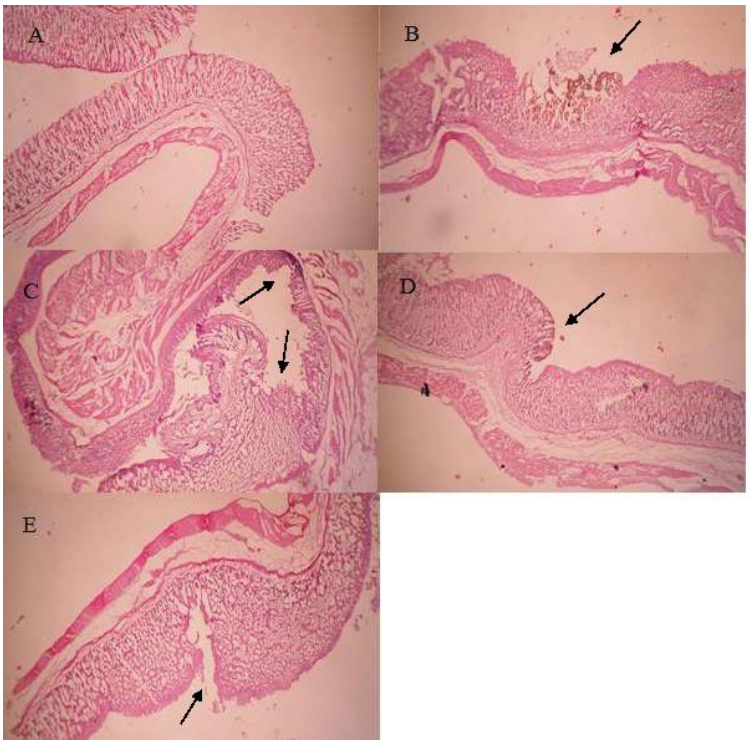

Fig. 6. Microscopic evaluation of gastric tissue injuries induced by indomethacin in rats. (A) Normal tissue treated with normal saline/tween, (B) control group that received indomethacin (30 mg/kg), (C) gastric ulcer treated with *Dracocephalum kotschyi* hydroalcoholic extract (20 mg/kg), (D) gastric ulcer treated with *Dracocephalum kotschyi* flavonoid extract (10 mg/kg), and (E) gastric ulcer treated with ranitidine (30 mg/kg). Black arrows show improvement and healing of ulcers in the extract-treated groups versus the control.

Effects of DKHE and DKFE on MPO activity

Measurement of MPO activity has been used as an indication of tissue inflammation. In the normal group, treated with vehicle, only a residual MPO activity was observed, while in the indomethacin-treated control group MPO activity has substantially been elevated (Fig. 7). Administration of DKHE and DKFE produced variable changes in MPO activity in comparison with the control group albeit two lower doses of DKHE (20 and 40 mg/kg; $P < 0.05$) and the middle dose of DKFE (20 mg/kg) were practically effective ($P < 0.01$) in this regard. Ranitidine also inhibited MPO activity significantly ($P < 0.01$) (Fig.7). DKHE and DKFE did not present a different picture of MPO activity versus the normal group when applied alone ($P > 0.05$) (Fig. 7).

Effect of DKHE and DKFE on MDA value

MDA indicates lipid oxidation and acts as another marker for tissue inflammation and oxidative stress. As it is shown in Fig. 8, only a basal level of lipid oxidation was seen in the normal tissues. Animals treated with oral indomethacin represented a great increase in MDA level over the treatment course. Pretreatment of animals with DKHE (20 mg/kg) and DKFE (10 mg/kg) significantly inhibited lipid oxidation induced by indomethacin (Fig. 8). By increasing oral doses of the extracts, the antioxidant activity of the extracts was unexpectedly diminished. Ranitidine reduced lipid oxidation activity induced by indomethacin significantly ($P < 0.001$) (Fig. 8). DKHE and DKFE alone didn't alter MDA value in comparison to normal tissue ($P > 0.05$) (Fig. 8).

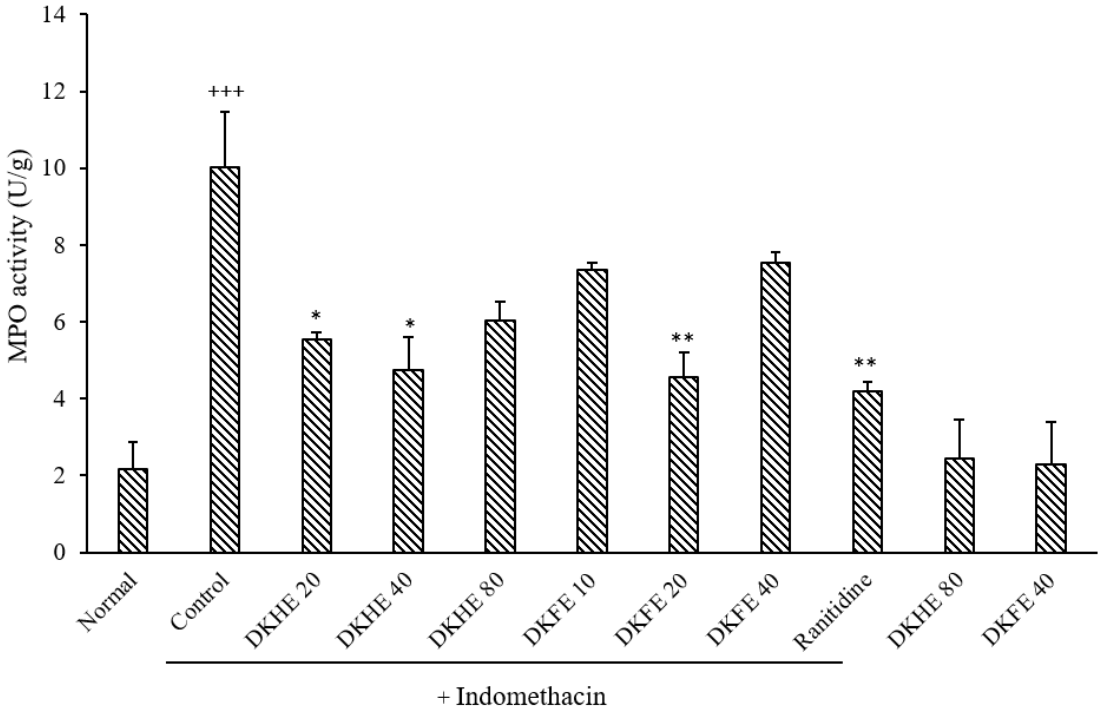

Fig. 7. MPO activity assessments in rat's stomach. Normal rats received normal saline/tween (5 mL/kg/day), control rats with gastric ulcer induced by indomethacin (30 mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. *$P < 0.05$ and **$P < 0.01$ show significant differences compared with the control group and $^{+++}$ $P < 0.001$ against the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract; MPO, myeloperoxidase.

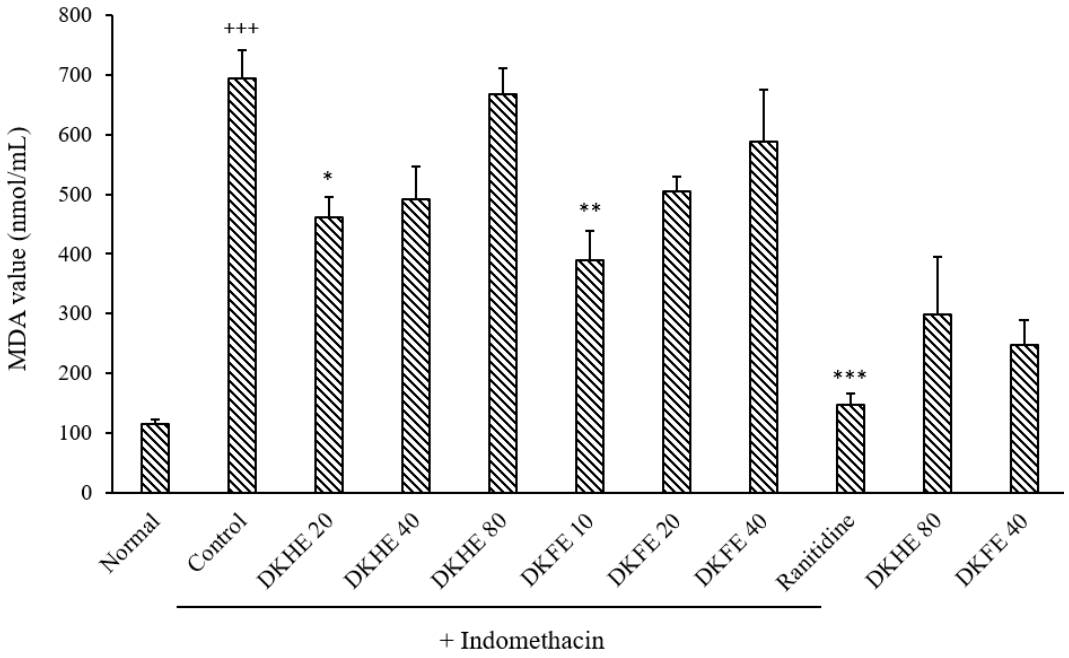

Fig. 8. MDA level assessment in rat's stomach. Normal rats received normal saline/tween (5 mL/kg/day), control rats with gastric ulcer induced by indomethacin (30mg/kg). DKHE at 20, 40, and 80 mg/kg, DKFE at 10, 20, and 40 mg/kg, and ranitidine at 30 mg/kg were used. Collected data are presented as mean ± SEM, n = 6. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ show significant difference with the control group; $^{+++}P < 0.001$ against the normal group. DKHE, *Dracocephalum kotschyi* hydroalcoholic extract; DKFE, *Dracocephalum kotschyi* flavonoid extract; MDA, malondialdehyde.

DISCUSSION

Peptic ulcer is a common problem of anti-inflammatory drugs such as NSAIDs and corticosteroids (1). The underlying cause of peptic ulcer induction by these drugs is based on their ability to inhibit prostaglandin production (32). Indomethacin is a potent inhibitor of cyclooxygenase enzymes which is responsible for the synthesis of prostaglandins and thereby reduces prostaglandin necessary for the maintenance of stomach protective layers (33,34). *D. kotschyi* possessed anti-inflammatory activities and its anti-inflammatory action has been likely attributed to its flavonoid's contents (4,17). It has been suggested that some flavonoids inhibit prostaglandin production (35). *D. kotschyi* is enriched in flavonoids but so far there is no report about the net effect of *D. kotschyi* extract on the stomach ulcer. Therefore, the objective of this research was to find out if, similar to chemical anti-inflammatory agents, *D. kotschyi* extract has the potential to cause peptic ulcer or inversely its consumption might be beneficial for patients with a history of peptic ulcer alike many flavonoids-enriched herbals (4). Therefore, a validated animal model of induced peptic ulcer by indomethacin was currently used in order to address these questions (28). As expected, indomethacin produced evident damages to the lining of the stomach wall. However, *D. kotschyi* extracts alone at doses with anti-inflammatory and anti-spasmodic activity, did not cause any kind of stomach ulcer. On the other hand, DKHE and DKFE extracts prevented stomach ulcer induced by indomethacin indicating that they might exert a sort of protective function against peptic ulcer. By considering pepsin activity and pH of gastric secretions, it was found out that pepsin was likely more affected by herbal extracts however, pH was not changed significantly. This might present a piece of evidence that the protective effect of herbal extracts was mediated through a direct reaction of extract ingredients with pepsin molecules rather than hydrochloric acid suppression. This protection was inversely dependent on the dose and with increasing the dose, it tends to be reversed as previously shown by other experiments (17). This may indicate that there are some constituents within the extracts that either antagonize the ulcer

healing effect or potentiating nonspecific anti-inflammatory action of extract at larger doses on the GI tract (36).

More interestingly, a similar pattern of anti-inflammatory and anti-ulcerative action has been seen on the colitis-induced ulcer with *D. kotschyi* extract (17). Measurement of MPO activity is widely used for the assessment of gastrointestinal inflammation (31,37). Oral administration of *D. kotschyi* extracts reduced enzymatic activity of MPO, however, the effect was not fully consistent with the hypothesis that flavonoids are the main ingredients for this purpose. Therefore, it can be concluded that the reduction in peptic ulcer extent and severity observed by *D. kotschyi* extracts administration partially might be due to its anti-inflammatory property and some other constituents like alkaloids, essential oils, tannins, etc. (7,12,20). MDA is a highly reactive natural compound that is associated with lipid peroxidation which is an indicator of tissue injury (38). Reduction in MDA levels by *D. kotschyi* extracts indicates a reduction in tissue oxidative stress. Increased levels of MDA have been reported in patients with rheumatism (39). Therefore, it seems that the anti-ulcer properties of *D. kotschyi* extracts could be at least, in some part, due to antioxidant ingredients within the extract. Antioxidant activity is generally attributed to phenolic compounds which flavonoids and flavonols are among the most characterized in extracts. Since the lowest doses of extracts exhibited the greatest decline in MDA activity, therefore it seems that extracts also contain peroxidant constituents tend to limit this capacity for healing effects of extracts. In this research, flavonoids fraction was also studied because they have been reported as potential anti-inflammatory reagents (21). Flavonoids such as apigenin, luteolin, acatein, and their glucopyranoside derivatives, calycopterin, xanthomicrol, isokaempferide, and limonene are among the most abundant and important flavonoids are present in extracts (7). The anti-inflammatory effect of flavonoids is suggested to be due to inhibition of pro-inflammatory cytokine-induced chemokine expression (40). Apigenin is one of the flavonoids constituents identified in the hydroalcoholic extract of *D. kotschyi* (36). The anti-ulcerative effect of apigenin has already been reported in an animal model of colitis (17). The anti-inflammatory effect of apigenin is reported to be due to inhibition of nitric oxide synthase and cyclooxygenase-2 enzymes induction (41). Inhibition of interleukin-4 production and suppression of tumor necrosis factor-α elevations might also be involved (42). Luteolin is another flavonoid constituent found in *D. kotschyi* extract with similar structures and anti-inflammatory properties to apigenin (22). Similarities between hydroalcoholic and flavonoids-enriched extracts given in the current study suggesting that flavonoids are not the sole active ingredients involved in the anti-inflammatory and anti-ulcer action of *D. kotschyi* extracts. Moreover, phytochemical analysis of a hydroalcoholic extract of *D. kotschyi* has shown that flavonoids account for a small percentage of this fraction. Therefore, further investigation for the existence of active components within the total and aqueous extract is recommended.

CONCLUSION

Unlike corticosteroid and NSAIDs, oral administration of *D. kotschyi* extracts neither caused peptic ulcer nor aggravated indomethacin-induced peptic ulcer. In fact, *D. kotschyi* extracts had a protective effect against indomethacin-induced ulcer. So, it can be suggested that consumption of *D. kotschyi* extracts preparation would be likely safe in patients with a history of peptic ulcer. However, there is a long way to introduce this medicinal plant for clinical application, and more detailed mechanistic, clinical trial, and toxicological studies are warranted for this purpose.

Acknowledgements

This study was financially supported by the Vice-Chancellor for Research and Technology in Isfahan University of Medical Sciences under Grant No. 398383.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

H. Sadraei drafted the primary manuscript and contributed to data analysis and depicting the graphs and tables. I. Yousefi drafted the proposal and carried out all the experimental procedures as well as analysis of the data and representing the results. S.E. Sajjadi introduced the candidate plant while supervised the preparation of extracts and their standardization. A. Talebi prepared suitable tissue samples for pathological studies and their interpretation. M. Minaiyan represented the idea, drafted the final modified manuscript and supervised all the steps of procedures, data analysis and their interpretation.

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