GASTROPROTECTIVE EFFECT OF RADISH “RAPHANUS SATIVUS” L. ON EXPERIMENTAL GASTRIC ULCER MODELS IN RATS

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Abstract
Raphanus sativus L. ‘Radish’ locally known as Fijl, is a Brassicaceous plant of cabbage family which has been reputed for its benefic medicinal properties. The present study was undertaken to evaluate the freshly squeezed Radish juice (FRJ) for its anti-gastric ulcer activity in experimental models, besides preliminary qualitative phytochemical screening. Oral administration of FRJ in doses of 2 and 4 ml/200 g b.w. significantly inhibited gastric ulcer formation induced by necrotizing agents (ethanol, sodium hydroxide and sodium chloride) hypothermic restraint-stress and indomethacin. The FRJ also replenished the ethanol induced depleted gastric wall mucus secretion and nonprotein-sulfhydryl (NP-SH) concentrations in rats. The phytochemical screening showed the presence of flavonoids, anthocyanins and sulfated constituents. In conclusion, the results indicate that the fresh juice of Radish possesses gastroprotective potential related to the mucus secretion stimulation (potentiation of defensive factors); and increase in NP-SH concentration is probably due to prostaglandin-inducing abilities, mediated through its antioxidant activity.

INTRODUCTION
Radish, Raphanus sativus L. (Brassicaceae Family) commonly known as Fijl or muli is a common pungent ingredient used in various

Rezumat
Raphanus sativus L. (Fam. Brassicaeae), hrean, este o plantă utilizată în medicina tradițională pentru proprietățile sale terapeutice deosebite. Lucrarea prezintă rezultatele unor studii experimentale, efectuate pe animale de laborator (Șobolani albi), de testare a acțiunii antiucleroase a sucului proaspăt extras din această plantă. Efectul farmacologic a fost urmărit în diferite condiții experimentale: ulcer indus prin factori necrozați (etanol, hidroxid de sodiu, clorură de sodiu), ulcer de stres și ulcer indus prin administrare de indometacin.

- Gastroprotection
- Radish
- Raphanus sativus
abdominal disorders. Almost all parts of the plant including leaves, seeds and roots are utilized in medicine [14, 18]. The fresh juice obtained from leaves are diuretic, laxative, roots are used for urinary complaints, haemorrhoids, gastrodynic pains and various gastric ailments. The seeds are expectorant, digestive, diuretic, laxative and carminative and the root extract has been reported to have antiurolithiatic properties [19, 31]. There were no available scientific reports in literature on the traditional claims of this plant material in regard to its antiulcer potential. The present study deals with the protective effect of Radish juice on acute experimental models of gastric ulceration in rats, to substantiate the use of radish in Unani and Arab traditional medicine in gastric disorders.

MATERIALS AND METHODS

The fresh radish was purchased from the local vegetable market and authenticated by an expert taxonomist. A voucher specimen (# 77207) has been deposited at the Herbarium of the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

1. Animals:
Wistar albino rats of either sex, approximately of same age, weighing 150-200 g were obtained from Animal Care Center, College of Pharmacy, King Saud University. They were maintained under standard conditions of temperature, humidity and light (12 h dark, 12 h light) and provided with Purina chow and having free access to water. Before testing, the animals were fasted for 36 h with access to water ad libitum. The experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2. Dose selection and route of administration:
The freshly squeezed juice of radish (FRJ) was administered at oral doses of 2 ml and 4 ml per 200 g b.w. These doses were selected based on preliminary experiments that showed pharmacological effects in animals. Moreover, the herbal medicine practitioners commonly used the radish in the form of juice, therefore, the present dosage form was adopted in this study.

3. Gastric lesions induced by necrotizing agents (Cytoprotection):
The necrotizing agents were administered in doses of 1 ml per 200 g b.w. (80% ethanol, 0.2 M NaOH and 25% NaCl). Radish juice was given 30 min before the administration of necrotizing agents. One hour after the administration of ethanol and alkalis the rats were sacrificed and examined for lesions in the stomach. The scoring of lesions in the stomach were observed as follows: the patchy lesions of stomach induced by ethanol were
scored according to the method described by Robert et al. (1983) using the following scale: 0 = normal mucosa; 1 = hyperemic mucosa or up to 3 small patches; 2 = from 4 to 10 small patches; 3 = more than 10 small or up to 3 medium-sized patches; 4 = from 4 to 6 medium-sized patches; 5 = more than 6 medium-sized or up to 3 large patches; 6 = from 4 to 6 large patches; 7 = from 7 to 10 large patches; 8 = more than 10 large patches or extensive necrotic zones. “Small” was defined as up to 2 mm across (max. diameter), “medium-sized” between 2 and 4 mm across and “large” more than 4 mm across (Valcavi et al., 1982).

4. Hypothermic restraint stress-induced ulcers:
The method described by Levine (1971) was adopted with slight modifications. The animals were fasted for 36 h but access to water ad libitum. Thirty minutes after the oral administration of radish juice (2 and 4 ml/200 g b.w.), the rats were immobilized in restraint cages and placed inside a ventilated refrigerator maintained at 3±1°C for 3 h. The animals were then sacrificed and the stomachs were excised. They were examined for ulceration and the severity of intraluminal bleeding according to the following arbitrary scale described by Chiu et al. (1984). 0 = no blood detectable; 1 = thin blood follows the rugae; 2 = thick blood follows the rugae; 3 = thick blood follows the rugae with blood clots in certain areas and 4 = extensive covering of the whole gastric mucosal surface with thick blood.

5. Determination of gastric wall mucus:
Gastric wall mucus was determined according to the modified procedure of Corne et al. (1974). The glandular segment of the stomach was separated from the rumen of the stomach, weighed, and transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 mmol/L sucrose solution buffered with 0.05 ml sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue, and excess dye was removed by two successive rinses with 10 ml of 0.25 mmol/L sucrose, first at 15 min and then after 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 mmol/L magnesium chloride which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 4000 rpm for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated.

6. Gastric lesions induced by indomethacin:
Indomethacin was suspended in 1.0% carboxy-methycellulose (CMC) in water (6 mg/ml) and administered orally to the 36 h fasted rats in a dose of 30 mg/kg, body weight. Control rats were treated similarly with an
equivalent amount of the vehicle [3]. The radish juice was given 30 min prior to indomethacin administration at a dose of 2 and 4 ml/rat. The animals were sacrificed 6 h after the treatment. The stomachs of the animals were excised off the body, rinsed with normal saline and studied according to the method described by Szabo et al., 1985.

7. Estimation of non-protein sulfhydryls:
Gastric mucosal non-protein sulfhydryls (NP-SH) was measured according to the method of Sedlak and Lindsay, 1968. The glandular part of the stomach was homogenized in ice-cold 0.02 mmol/L ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 ml of the homogenates were mixed in 15 ml test tubes with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 rpm. Two milliliters of supernatant mixed with 4 ml of 0.4 mol/L Tris buffer at pH 8.9. 0.1 ml of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured within 5 min after addition of DTNB at 412 nm against a blank reagent.

Statistical Analysis: The readings shown are means ± standard error of means. The mean determination of treatment groups was compared statistically with that of control by using ANOVA followed by Tukey-Kramer method.

RESULTS AND DISCUSSION

1. Effect of fresh radish juice on gastric lesions induced by necrotizing agents
The treatments of rats with 80% ethanol, 0.2 M NaOH and 25% NaCl produced extensive gastric lesions mainly confined to glandular part of the stomach in all the control (only necrotizing agents treated) animals. The ulcer index in ethanol, sodium hydroxide and sodium chloride treated groups was 6.66±1.03, 6.40±0.89 and 6.83±1.16, respectively. Pretreatment of rats with FRJ at the dose of 2 ml/200 g b.w. significantly prevented the gastric mucosal lesions (p < 0.001). The higher dose of FRJ (4 ml/ 200 g b.w.) also significantly reduced the ulcer index as 1.66±0.81 (p<0.001), 0.6±0.54 (p<0.001) and 1.00±1.09 (p<0.001) in ethanol, sodium hydroxide and sodium chloride groups, respectively, as compared to control group (Table I).
Table I
Effect of freshly Radish juice (FRJ) on gastric lesions induced by various necrotizing agents

<table>
<thead>
<tr>
<th>Treatment (n = 6)</th>
<th>FRJ ml/ 200g b.w. orally</th>
<th>Ulcer Index (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80% EtOH</td>
</tr>
<tr>
<td>Control 1</td>
<td>–</td>
<td>6.66±1.03</td>
</tr>
<tr>
<td>FRJ group 1</td>
<td>2</td>
<td>2.66±1.50***</td>
</tr>
<tr>
<td>FRJ group 2</td>
<td>4</td>
<td>1.66±0.81***</td>
</tr>
</tbody>
</table>

***p<0.001, ANOVA followed by Tukey-Kramer

2. Effect of fresh radish juice on hypothermic restraint stress-induced gastric lesions

Animals subjected to restraint plus cold for 3 hr showed the presence of considerable ulcerogenicity in the form of hemorrhagic mucosal lesions in the stomach, which were confined to glandular segment only. There was also evidence of intraluminal bleeding. Pretreatment of rats with FRJ significantly reduced the intensity of ulceration and intraluminal bleeding at high dose (Table II). However, the lower dose of FRJ caused an insignificant decrease in ulcer index and bleeding.

Table II
Effect of FRJ on hypothermic restraint stress induced intraluminal bleeding and gastric lesion in rats

<table>
<thead>
<tr>
<th>Treatment (n = 6)</th>
<th>FRJ ml/ 200g b.w orally</th>
<th>Intraluminal Bleeding</th>
<th>Gastric lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Score (Mean±S.E.)</td>
<td>Ulcer Index (Mean±S.E.)</td>
</tr>
<tr>
<td>Control 2</td>
<td>–</td>
<td>3.00±0.89</td>
<td>18.33±3.66</td>
</tr>
<tr>
<td>FRJ group 3</td>
<td>2</td>
<td>0.8 ±0.83</td>
<td>6.6±6.14</td>
</tr>
<tr>
<td>FRJ group 4</td>
<td>4</td>
<td>0.33±0.51*</td>
<td>2.66±1.50*</td>
</tr>
</tbody>
</table>

*p<0.05. ANOVA followed by Tukey-Kramer

3. Effect of fresh radish juice on ethanol-induced changes in gastric wall mucus

The treatment of rats with ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (200.54±22.54 µg/g) as compared to control group of rats (330.24±32.61 µg/g). Pretreatment of rats with FRJ at 2 ml/rat (267.31±39.98 µg/g) insignificantly increased the phenomenon and at higher dose (4 ml/ 200g b.w) (320.42±24.87 µg/g)
significantly enhanced Alcian blue binding capacity of gastric mucosa (p<0.01) respectively (Table III).

**Table III**

Effect of FRJ on 80% ethanol-induced gastric wall mucus concentration changes

<table>
<thead>
<tr>
<th>Treatment (n = 6)</th>
<th>Dose ml/200g b.w orally</th>
<th>Non-protein sulfhydryls (NP-SH) concentration (µmol/100 mg wet tissue)</th>
<th>Gastric wall mucus (µg/g of Alcian blue wet glandular tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – 3.96±0.41</td>
<td>3.96±0.41</td>
<td>330.24±32.61</td>
<td></td>
</tr>
<tr>
<td>80% Ethanol only – group 5</td>
<td>–</td>
<td>1.82±0.14***</td>
<td>200.54±22.54***</td>
</tr>
<tr>
<td>FRJ + 80% ethanol – group 6</td>
<td>2</td>
<td>2.34±0.20*</td>
<td>267.31±39.98b</td>
</tr>
<tr>
<td>FRJ + 80% Ethanol – group 7</td>
<td>4</td>
<td>3.62±0.28**</td>
<td>320.42±24.87**</td>
</tr>
</tbody>
</table>

a: As compared to control group. b: As compared to 80% ethanol group. **p < 0.01. ANOVA followed by Tukey-Kramer.

4. Effect of fresh radish juice on ethanol-induced mucosal NP-SH depletion:

The level of NP-SH in the gastric mucosa of control rats was 3.96±0.41 µmol/100 mg of tissue, significantly decreased to 1.82±0.1 µmol/100 mg following the administration of ethanol. Pretreatment of rats with RFJ at higher dose (4 ml/rat) significantly replenished the ethanol-induced depletion of NP-SH concentration (p<0.01, Table III).

5. Effect of fresh radish juice on gastric lesions induced by indomethacin:

To study the anti-ulcerogenic effects of FRJ on indomethacin-induced gastric lesions in rats, two doses of FRJ were used (2 and 4 ml/200g b.w.). Data on ulcer index in pretreated rats with both doses are reported in Table IV. Indomethacin treatment significantly increased ulcer index in the gastric mucosa when compared with the corresponding values with FRJ treated rats (38.16±6.08 vs 14.00±4.56, P<0.01) at higher dose. However, the lower dose has shown an insignificant reduction of the intensity of the gastric ulceration.
Table IV
Effect of FRJ on gastric lesions induced by indomethacin (indo) in rats (mean ± S.D.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals (n)</th>
<th>Dose ml/rat orally</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 4 (indo only)</td>
<td>6</td>
<td>–</td>
<td>38.16±6.08</td>
</tr>
<tr>
<td>FRJ + Indo - group 8</td>
<td>6</td>
<td>2</td>
<td>20.66±5.35</td>
</tr>
<tr>
<td>FRJ + Indo - group 9</td>
<td>6</td>
<td>4</td>
<td>14.00±4.56**</td>
</tr>
</tbody>
</table>

**p<0.01. ANOVA followed by Tukey-Kramer

The preventive effect of radish juice was investigated in rats using different experimental models of peptic ulcer disease, which operate by distinct mechanisms of ulcerogenesis. Ethanol and strong alkalies induces the formation of ulcers [15, 26]. Necrotizing agents induced gastric lesion formation that may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury [10, 27]. It is known that necrotizing agents that induce ulcers are inhibited by agents which enhance mucosal defensive factors such as PGE₂ [23]. The ulcerative index was significantly lower in the FRJ-treated groups compared to control group (only treated with necrotizing agents), confirming the cytoprotective potential of the juice, that maybe due to prostaglandin generation or by its antioxidative properties.

Hypothermic-restraint stress ulcers have been widely used experimentally for the evaluation of anti-ulcer activity in animals because of data reproducibility [25, 16]. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion [11] and reduction in mucus production [12]. Disturbances in gastric mucosal microcirculation [9] and alterations leading to abnormal motility [32] have also been considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions. However, an important factor in the genesis of stress ulcer is vagal over-activity that increases gastric acid secretion and is often termed the aggressive factor [8]. The present data showed that pre-treatment with radish juice significantly protects the gastric mucosa against hypothermic restraint-stress induced ulcers in rats, by possible decreased acid secretion and/or by its antioxidant properties [20].

The gastric wall mucus is thought to play an important role as a defensive factor against gastrointestinal damage [6]. The determined gastric wall mucus was used as an indicator for gastric mucus secretion. Pretreatment with the FRJ in the present study, significantly replenished the depleted ethanol induced gastric wall mucus suggests that the
The gastroprotective effect of the radish juice is mediated at least partly by the preservation of gastric mucus secretion. Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin is known to induce gastric ulceration, which are attributed mainly to inhibition of biosynthesis of cytoprotective PG, resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway [21]. These agents break the mucosal permeability to H\textsuperscript{+} and Na\textsuperscript{+} ions, and drop in the transmucosal potential difference and induce the formation of erosions and ulcers [7, 33]. In the present assay, FRJ was also able to produce a significant inhibition of the gastric mucosal injury induced by indomethacin, indicating a probable local increase in PG synthesis. It has also been reported that leukotriene antagonist and lipoxygenase inhibitors are capable of inhibiting alcohol as well as NSAID-induced gastric ulceration in rats [17]. These results further indicate that FRJ may enhance gastric mucosal defensive factors, such as mucus and/or PG. Therefore, the protection offered by FRJ against necrotizing and NSAID-induced gastric ulceration could also be due to inhibition of the 5-lipoxygenase pathway or to the antagonistic activity of leukotriene [2].

Non-protein sulfhydryls (NP-SH) are thought to be involved in protecting gastric mucosa against various chemicals [27]. Decreased levels of endogenous sulfhydryls have been associated with tissue damage by ethanol, indomethacin and various chemical agents [28, 29]. In the present study, it was observed a significant reduction in gastric NP-SH content after 80\% ethanol administration. Pretreatment of rats with FRJ significantly prevented NP-SH depletion.

**CONCLUSION**

This study provides evidence that the radish juice possesses an antigastric ulcer effect, being presumably attributed to its phenolic, terpenoidal and sulphurated constituents through preventing the accumulation of excessive free radicals and protecting the gastric tissue against noxious chemical challenges. This may be related to its PG generating, antioxidant and/or preserving mucus secreting properties and by strengthening the mucosal barrier integrity, which is the first line of defense against endogenous and exogenous ulcerogenic agents. Thus, the results of the present study support the folkloric claims that use of radish is beneficial in gastropathy.
Acknowledgement
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