Evaluation of antisecretory, gastroprotective and in-vitro antacid capacity of Fumaria indica in rats

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Abstract

Fumaria indica is used for its anthelmintic, antidyspeptic, cholagogue, diaphoretic, diuretic, laxative, stomachic, tonic properties and claimed to possess various properties for the ailments of blood, skin, gastrointestinal systems and central nervous system. The present study was undertaken to evaluate antisecretory, gastroprotective and in-vitro antacid capacity of ethanol extract from F. indica in rats. Evaluation of F. indica extract as antisecretory was carried out by pyloric ligation induced ulcer model. The gastroprotective effect was carried out by absolute ethanol induced ulcer model. Integrity of gastric mucosa was evaluated by estimation of GSH and gastric mucus level. The in-vitro antacid capacity was evaluated by titration method. Ethanol extract of F. indica at 200 mg kg-1 orally showed inhibition of secretion in pyloric ligation model. GSH level (1.67 µg mg-1 protein), gastric wall mucus (240.76 µg g-1 wet glandular tissue) and percentage protection (77.59%) of ulcer were significantly (P<0.05) increased in absolute ethanol induced ulcer model. The in-vitro antacid capacity of ethanol extract of F. indica was compared with the standard. Conclusively, it appears that F. indica possess antisecretory (inhibition of acid secretion), gastroprotective (potentiation of defensive factors) and in-vitro antacid activity.

Key words

Antacid, Antisecretory, Fumaria indica, Gastroprotective

Introduction

Fumaria is a genus of herbs distributed in Asia, Europe and Africa. Fumaria indica (Hausskn.) Pugsley (F. indica) is locally known as "Pitpapra" or "Shahtrah" in India (Chopra et al., 2002). In traditional system of medicine, plant is used for its anthelmintic, antidyspeptic, cholagogue, diaphoretic, diuretic, laxative, stomachic, tonic properties and claimed to possess various properties for the ailments of blood, skin, gastrointestinal systems and central nervous system (Gupta et al., 2012). Review of literature for phytoconstituents revealed the presence of fumarophyicine; steroids, viz. β-sitosterol, stigmasteryl, campesterol; organic acids viz. caffeic acid and fumaric acid (Sousek et al., 1999) and Sanguinarine, d-bicuculline, funaridine, parfumidin, d-tetrahydrocpltisine, cplistine, nonacasonal, d-8-OMe-dihydrosguainarine and oxysanguinarine (Asolkar et al., 2005). The aqueous extract of seeds of F. indica has show antibacterial activity against six stains of Enterobacteriaceae, of which Klebsiella pneumonia was the most susceptible bacterium while Salmonella typhimurium and Escherichia coli were the most resistant bacteria (Parekh and Chand, 2007). Fuyuziphine, an alkaloid isolated from F. indica at 100-750 ppm possess antifungal properties (Pandey et al., 2007).

Extract of whole plant of F. indica possessed significant anti-inflammatory activity in acute and chronic models of inflammation along with central and peripheral anti-nociceptive activity (Rao et al., 2007). Ethanol extract of F. indica posses antioxidant and free radical scavenging activity (Fazal et al., 2011). F. indica showed hepatoprotective activity against antitubercular drug induced hepatotoxicity in albino rats (Nimbkar et al., 2000). Further, Rathi et al. (2008) found that protopine was responsible for hepatoprotective activity. Fifty percent ethanol extract of F. indica was investigated for its neuropharmacological, antidepressant activity and general effects on central nervous system and showed that F. indica had the potential of increasing...
pentobarbital induced sleeping time, decrease onset of sleeping time in rats with decrease in locomotor and anticonvulsant activity (Singh et al., 2010). *F. indica* also possess chemopreventive (Hussain et al., 2012a, 2012b) and spasmodic and spasmodolytic effects (Gilani et al., 2005).

On the basis of literature survey, the present study was undertaken to evaluate antisecretory, gastroprotective and in-vitro antacid capacity of ethanol extract of *F. indica* in rats.

**Materials and Methods**

**Preparation of plant extract** : Fresh plants of *Fumaria indica* (Hausskn.) Pugsley (*F. indica*). (Family: Fumariaceae) were collected from the garden of department in January 2011 and were cleaned, dried under shade at room temperature and powdered. Powder was passed through 20 mesh size. Plant material was first defatted with petroleum ether in soxhlet and then extracted with 50% ethanol. The obtained semisolid material was filtered and the filtrate was dried in rota evaporator to yield 10.09% (w/w). Ethanol extract of *Fumaria indica* was stored in desiccator for further preliminary phytochemical screening and pharmacological evaluation.

**Preliminary phytochemical screening** : Ethanol extract of *F. indica* was subjected to preliminary qualitative tests for carbohydrates, proteins, amino acids, steroids, glycosides, phenolics and tannins, alkaloids and flavonoids (Trease and Evans, 1987; Khandelwal, 2011).

**Animals** : Wistar albino rats of either sex were obtained from animal house of the department. They were housed in an environmentally regulated room for a 12 hrs light: 12 hrs dark cycle at 25 ºC and had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee of Institute and experiments were conducted according to CPCSEA, India (CPCSEA-837/ac/2004) guidelines on use and care of experimental animals.

**Acute toxicity study** : Different doses (5, 50, 300 and 2000 mg/kg, p.o.) of ethanol extract of *F. indica* were selected for acute toxicity test in animals (OECD, 2001). Three female rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urination incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes. Mortality, if any, was determined over a period of 2 weeks.

**Pharmacological evaluation** : For the assessment of activity, two dose level were chosen in such a way that high dose was approximately one-tenth of the maximum dose during acute toxicity studies, and a low dose which was 50% of the one-tenth dose (100, 200 mg kg⁻¹, p.o.).

**Pyloric ligation induced ulcers** : Ethanol extract of *F. indica* at 100 and 200 mg kg⁻¹ and ranitidine at 50 mg kg⁻¹ were administered orally for 7 days in their respective groups. Control group of animals received suspension of 1% (w/v) carboxy methyl cellulose (CMC) in distilled water and rats were kept for 18 hrs fasting. Pyloric ligation was carried out as described by Shay et al. (1945). Stomach was dissected out and gastric juice was drained in a small beaker for estimation of volume of gastric juice, pH, free acidity, total acidity and cut opened along the greater curvature and ulcer index was determined using the following scoring system: 0=normal mucosa, 0.5=blushing, 1=spot ulcers, 1.5=haemorrhage streaks, 2=3 mm<ulcers<5 mm and 2.5=ulcers>5 mm (Kulkarni, 1999; Card et al., 1960).

**Absolute ethanol induced ulcers** : The experiment was performed according to the method of Morimoto et al. (1991) and De-Andrade et al. (2007) with some modification. Rats were randomly divided into 4 equal groups (n=6/group) and treated orally in the following manner: each rat in group 1 received 1ml of 1 % CMC solution. Animals in groups 2, 3 and 4 were administered with 100 and 200 mg kg⁻¹ of ethanol extract of *F. indica* and ranitidine 50 mg kg⁻¹ p.o., respectively. One hour after treatment, all the rats received 1ml of absolute ethanol to induce gastric ulcer. One hour later, the animals were sacrificed by cervical dislocation, and each stomach were removed and opened along the greater curvature. Each stomach was gently rinsed with water to remove the gastric contents and ulcers were graded as mentioned earlier.

**Estimation of non-protein sulphydryl content in stomach tissues** : All groups of rats treated were utilised to estimate reduced glutathione (GSH) content in stomach tissues as non-protein sulphydryls according to the method described by Sedlak and Lindsay (1968).

**Estimation of gastric wall mucus** : Mucus of gastric wall was determined according to the method of Corne et al. (1974). The glandular segment from stomach was removed, weighed and incubated in tubes containing 1% Alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 hrs. Alcian blue binding extract was centrifuged and absorbance of supernatant was measured at 498 nm. The quantity of Alcian blue extracted (μg g⁻¹ of glandular tissue) was then calculated.

**Antacid capacity** : In the in-vitro model, acid neutralizing capacity of an antacid was performed and number of mEq of acid consumed was calculated by the formula:

\[ \text{Total mEq} = (30 \times N_{\text{HCl}}) - (V_{\text{NaOH}} \times N_{\text{NaOH}}) \]

Where, \(N_{\text{HCl}}\) and \(N_{\text{NaOH}}\) are normalities of hydrochloric acid and sodium hydroxide, and \(V_{\text{NaOH}}\) is the volume of sodium hydroxide used for titration and expressed in terms of mEq of acid consumed per mg of the substance tested (USP, 2007).
**Effects on liver**: The functioning of liver was assayed by evaluating alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin direct and bilirubin total.

**Histopathology**: Samples of stomach and liver from different groups were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections of about 5µm were cut and stained with haematoxylin and eosin. These were examined under microscope for histopathological changes such as degeneration, haemorrhage, oedematous appearance, erosion and necrosis.

**Statistical analysis**: The results were expressed as mean±SEM and were analyzed using one-way analysis of variance followed by Dunnett’s test using GraphPad Prism 5.0 (Graph-Pad Software Inc., San Diego, California, USA). The value of P<0.05 was considered statistically significant.

**Results and Discussion**

Preliminary qualitative phytochemical screening show the presence of carbohydrates, steroids, glycosides, phenolics and tannins, alkaloids and flavonoids. The ethanol extract of *F. indica* was found to be safe up to 2000 mg kg⁻¹ with no signs of mortality or change in behaviour pattern and suggested that the plant extract was not toxic but safe.

Percentage inhibition of ulcer was 59.77% and 77.59% at 100 and 200 mg kg⁻¹ of ethanol extract of *F. indica* (Table 1). Pyloric ligation in rats produced an increased volume of gastric secretion. The volume of gastric secretion reduced in 100 mg kg⁻¹ ethanol extract of *F. indica* (P<0.05) and also in 200 mg kg⁻¹ ethanol extract of *F. indica* which was comparable to ranitidine (P<0.001). The pH of ethanol extract of *F. indica* (200 mg kg⁻¹) and ranitidine increased to 4.35 and 4.40 from 3.67 (control) (Fig. 1).

Free acidity and total acidity decreased due to ethanol extract of *F. indica* (P<0.001). The pH of ethanol extract of *F. indica* was found to be safe up to 2000 mg kg⁻¹ with no signs of mortality or change in behaviour pattern and suggested that the plant extract was not toxic but safe.

Table 1: Effect of ethanol extract from *F. indica* on pylorus ligation induced ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Ulcer index</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml</td>
<td>14.50 ± 0.76</td>
<td>-</td>
</tr>
<tr>
<td><em>F. indica</em></td>
<td>100</td>
<td>5.83 ± 0.40</td>
<td>59.77</td>
</tr>
<tr>
<td><em>F. indica</em></td>
<td>200</td>
<td>3.25 ± 0.44</td>
<td>77.59</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>50</td>
<td>3.08 ± 0.24</td>
<td>78.74</td>
</tr>
</tbody>
</table>

Values are mean of six replicate ±SEM. ANOVA followed by Dunnet test with control group. Significance represented as *** (P<0.001)

Ethanol extract of *F. indica* (200 mg kg⁻¹) exhibited 68.63% gastro protection as compared to ranitidine (71.81%) in absolute ranitidine increased to 4.35 and 4.40 from 3.67 (control) (Fig. 1). and 1.72 µg mg⁻¹ protein) and gastric wall mucus (240.76 µg g⁻¹ wet glandular tissue and 242.24 µg g⁻¹ wet glandular tissue) significantly increased in both ethanol extract of *F. indica* (200 mg kg⁻¹) and ranitidine; which decreased due administration of ethanol (P<0.05) as shown in Fig. 3 and 4.

In-vitro antacid capacity of ethanol extract of *F. indica* and gelusil were found as 23 mEq and 26 mEq of the acid consumed per gram of each, respectively. Blood serum level of alanine transaminase (ALT) (p<0.01) were increased in pylorus ligated rats to 31.42 (U L⁻¹) and were decreased to 24.24 (U L⁻¹) and 24.73 (U L⁻¹) respectively, at both dose level (100 and 200 mg kg⁻¹) of ethanol extract of *F. indica*. Similarly, in alcohol induced ulcer model the level of ALT was again raised 32.23 ± 2.32 (U L⁻¹) and decreased to 24.12 ± 4.26 and 23.62 ± 3.52 in rats pre-treated with rats with 100 and 200 mg kg⁻¹, ethanol extract of *F. indica*, respectively. Other parameters, AST, ALP, total protein, albumin, bilirubin direct and bilirubin total were found in normal range (data not shown). Also, Pylorus ligation caused histopathological lesions including degeneration, haemorrhage and oedematous appearance of the gastric tissue. Pre-treatment with ethanol extract of *F. indica* (100 and 200 mg kg⁻¹) and ranitidine (50 mg kg⁻¹) to rats presented significant protection against all such damage to mucosa.

Pylorus ligation induced ulcer is widely used method for studying the effect of drugs on gastric secretion. Obstruction made by ligating the pyloric end of the stomach causes accumulation of gastric acid, leading to the development of gastric ulcers (Khare et al., 2008, Okpo et al., 2011 and Savegnago et al., 2006). Reduced gastric ulceration was indicated by reduction in ulcer index in the pylorus ligation model. The results suggested that *F. indica* possessed antisecretory potency as well as acid neutralizing effect. These properties suggested one of the mechanism through which ethanol extract of *F. indica* was capable to guard stomach mucosa from pylorus ligation induced damage.

Ethanol is known to a cause gastric damage by altering defensive factors, including decreasing mucus production and blood circulation within mucosa. In addition, the gastric damage caused by ethanol may be due to generation of reactive species, decreased cell proliferation and an exacerbated inflammatory

Table 2: Effect of ethanol extract from *F. indica* on ethanol induced ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Ulcer index</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml</td>
<td>18.33 ± 1.15</td>
<td>-</td>
</tr>
<tr>
<td><em>F. indica</em></td>
<td>100</td>
<td>6.83 ± 1.09</td>
<td>62.72</td>
</tr>
<tr>
<td><em>F. indica</em></td>
<td>200</td>
<td>5.75 ± 0.91</td>
<td>68.63</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>50</td>
<td>5.17 ± 0.48</td>
<td>71.81</td>
</tr>
</tbody>
</table>

Values are mean of six replicate ±SEM. ANOVA followed by Dunnet test with control group. Significance represented as *** (P<0.001)
response (Choi et al., 2009; Ineu et al., 2008). Generation of reactive species and a concomitant reduction of antioxidant ability are accountable for cell damage and death due to their extreme reactivity. Reactive oxygen species attack essential cell constituents such as proteins, lipids, nucleic acids and formation of toxic compounds (Kaharaman et al., 2003). In the experiment, ulcers were protected by administration of ethanol extract of F. indica to rats through increased level of mucus and GSH, which play a role in scavenging free radicals.

Increased mucus secretion by gastric mucosal cells in pylorus ligation method can prevent gastric ulceration by at least improving the buffering of acid in gastric juice and by acting as an effective barrier to back diffusion of H+ ion (Frondoza et al., 2004 and Khushtar et al., 2009). Reduced glutathione is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radicals by oxidative stress (Bafna and Balaraman, 2005). Administration of ethanol extract of F. indica at 200 mg kg−1 dose showed significant increase in reduced glutathione level, as compared to control animals, which suggested the efficacy in preventing free radical-induced

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damage. In-vitro antacid capacity of ethanol extract of F. indica was comparable with gelusil and correlate with acid neutralization in pylorus ligation. Histopathologically, pre-treatment with ethanol extract of F. indica at dose (200 mg kg\(^{-1}\)) presented significant protection against all such damage to mucosa in both the models.

In conclusion, it appears that F. indica possess antisecretory (inhibition of acid secretion) and gastroprotective (potentiation of defensive factors) activity. Probably, these effects are due, partly at least, to presence of flavonoids, glycosides phenolics and tannins in the ethanolic extract of plant.

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References


