

## Anti-inflammatory and anti-nociceptive activities of *Fumaria indica* whole plant extract in experimental animals

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The 50% ethanolic extract of *Fumaria indica* was investigated for its anti-inflammatory and antinociceptive potential in animal models. Oral administration of *F. indica* dry extract (100, 200 and 400 mg kg<sup>-1</sup>) exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan and histamine induced hind paw oedema,  $p < 0.05$ ) and chronic cotton pellet granuloma models of inflammation,  $p < 0.01$ ). The extract (400 mg kg<sup>-1</sup>) exhibited maximum anti-inflammatory effects of 42.2 and 42.1% after 3 h with carrageenan and histamine, respectively. The same dose of extract showed 38.9% reduction in granuloma mass in a chronic condition. A significant anti-nociceptive activity was evidenced in mice; 6.6–67.7% ( $p < 0.01$ ) protection in mechanical, 33.9–125.1% ( $p < 0.05$ ) protection in thermal induced pain and 22.2–73.9% ( $p < 0.05$ ) protection in acetic acid-induced writhing.

**Keywords:** *Fumaria indica* (Fumariaceae), anti-inflammatory activity, anti-nociceptive activity

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*Fumaria indica* (Hausskn.) Pugsley, syn: *F. parviflora* Lam. (Fumariaceae) is a small, scandent, branched annual herb growing wild in plains and lower hills. The plant is considered to be diuretic, diaphoretic, anthelmintic, laxative and is used to purify blood and in liver obstruction in ethnopharmacology (1, 2). Pharmacological studies show that *F. indica* possesses antipyretic, antidiarrhoeal and hypoglycemic properties (3–5). It is a smooth muscles relaxant and has hydrocholeretic, by stimulating bile excretion, and hepatoprotective effects (6–10). Phytochemical investigation revealed the presence of alkaloids, viz. protopine (6), parfumine, cryptopine, copticine, fumariline (11), fumaramine, fumaritine, paprafumicin, paprarine, papracinine, papraline, reddeanine (12), fumarophycine (13), narlumicine, narceimine, narlumidine (14); steroids, viz.  $\beta$ -sitosterol, stigmasterol, campesterol; organic acids viz. caffeic acid and fumaric acid (9, 13).

As there is no reference in literature to the anti-inflammatory aspects, it was considered worthwhile to study the anti-inflammatory and anti-nociceptive activity of the *Fumaria indica* whole plant.

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## EXPERIMENTAL

### *Plant material and extraction*

Plants of *F. indica* were collected from the rural area around Lucknow, India. The plant material was identified, authenticated taxonomically and a voucher specimen was preserved in Pharmacognosy and Ethnopharmacology Division (NBR-21) for future reference. Air-dried powdered material of *F. indica* (1000 g) was exhaustively extracted with 10 volumes of 50% ethanol. This process of extraction was repeated four times, the extract was filtered, concentrated on rotavapour (Büchi, USA) and then freeze-dried (Freezone<sup>®</sup> 4.5, Labconco, USA) under high vacuum (1.33 Pa) and at temperature of  $-40 \pm 2$  °C (yield 9.85%, *m/m*).

### *High performance thin layer chromatography (HPTLC)*

The 50% ethanolic extract of *F. indica* was analyzed by TLC, which showed the presence of phenolics. Caffeic acid and 50% ethanolic extract of *F. indica* were spotted using a Camag Linomat IV spotter on the precoated silica gel 60/UV<sub>254</sub> HPTLC plates (Merck, India) as stationary phase. The plate was eluted with toluene/ethyl acetate/formic acid (70:30:10) as mobile phase. After development, the plates were dried and densitometrically scanned on a TLC scanner III at 284 nm using WinCat software (CAMAG, Switzerland); the peak area was recorded and the calibration curve was prepared by plotting the peak area against the concentration of caffeic acid applied.

### *Animals*

Male Sprague-Dawley rats (150–175 g) and albino mice (25–30 g) were obtained from the animal colony of the National Laboratory Animal Centre, Lucknow, India. They were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions at  $26 \pm 2$  °C and relative humidity 44–56% and 10 h light 14 h dark cycles each day for one week before and during the experiments. All animals were fed the standard rodent pellet diet (Amrut, India) and drank water *ad libitum*. All studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India.

### *Anti-inflammatory activity of 50% ethanolic extract of F. indica*

The rats were divided into five groups of six animals each. The test groups received the dry extract of *F. indica* (suspended in 1% caboxymethylcellulose, CMC) at doses of 100, 200 and 400 mg kg<sup>-1</sup>, *p.o.*; the reference group received phenylbutazone (100 mg kg<sup>-1</sup>, in 1% CMC, *p.o.*) as positive control and the negative control animals received the vehicle only (1% CMC, 10 mL kg<sup>-1</sup>, *p.o.*).

*Carrageenan induced paw oedema.* – The test was used to determine the anti-inflammatory activity of the extract by the method of Winter *et al.* (17). The animals pretreated with extract or phenylbutazone one hour before were injected with 0.1 mL of 1%  $\lambda$  carra-

geenean (in 1% CMC) solution into the sub-plantar side of right hind paw. Paw volume was measured by dislocation of the water column in a plethysmometer (Ugo Basile, Italy) immediately after  $\lambda$  carrageenan application (time zero) and 3 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

*Histamine-induced hind paw oedema.* – This experiment was conducted as per the methodology used by Parmar and Ghosh (16). Right hind paw oedema was induced by the sub plantar injection of 0.1 mL of histamine (1 mg mL<sup>-1</sup> in 1% CMC). Extract and phenylbutazone were administered 1 h prior to the inflammatory insult. The paw volume compared to that of the negative control animals was recorded after 3 h and considered as anti-inflammatory response.

*Cotton pellet-induced granuloma.* – The test was performed on the rats using the cotton pellet induced granuloma method (17). The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. Groups of 6 animals received either extract or phenylbutazone or vehicle (1% CMC) orally for seven consecutive days from the day of cotton pellet insertion. On the eighth day animals were anesthetized again and cotton pellets were removed and dried to constant mass (18).

#### *Anti-nociceptive activity of 50% ethanolic extract of F. indica*

Anti-nociceptive activity was assessed by analgesy meter induced pain (19), hot plate reaction time (20) and abdominal writhing test using acetic acid (21) applied to male albino mice. Animals of the negative control received the vehicle only (1% CMC, 10 mL kg<sup>-1</sup>, *p.o.*).

*Analgesy meter induced pain.* – Analgesy meter induced pain was tested in mice using an analgesy-meter (Ugo Basile). This method involved the application of force to the paw using the analgesy meter, which exerts a force at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and constant motor rate was used to drive the plunger onto the paw. When the mice struggled, the instrument was switched off and the force at which the animal felt pain was read on a scale. The pre and post treatment weight causing pain was determined for each mouse. *F. indica* extract as test drug (100, 200, and 400 mg kg<sup>-1</sup>, *p.o.*) and acetylsalicylic acid (ASA, 25 mg kg<sup>-1</sup>, *p.o.*) as reference drug in 1% CMC were administered 30 min before the test.

*Hot plate reaction time.* – Mice were screened by being placed on a hot plate maintained at 55 ± 1 °C and recording the reaction time in seconds for fore paw licking or jumping. Only mice that reacted within fifteen seconds and did not show large variation on three separate occasions, each fifteen minutes apart, were taken for the test. Reaction time (paw licking, jumping) was measured initially and 30 min after intraperitoneal injection of 10 mg kg<sup>-1</sup> pentazocine (PZ) as a reference drug and *F. indica* extract in 1% CMC (100, 200, and 400 mg kg<sup>-1</sup>) (20).

*Acetic acid-induced writhing test.* – In the writhing test, 0.6% acetic acid (10 mg kg<sup>-1</sup>, *i.p.*) was injected and the number of writhes and stretching with a jerk of the hind limb were counted for a period of 15 min. Acetylsalicylic acid (ASA, 25 mg kg<sup>-1</sup>, *p.o.*) or the *F. indica* extract in 1% CMC (100, 200, and 400 mg kg<sup>-1</sup>) were administered orally 30 min before acetic acid injection (21).

### Statistical analysis

All the data are presented as mean ± SEM and one-way analysis of variance (ANOVA) and Newman-Keuls Multiple Comparison Test were applied for determining the statistical significance between different groups.

## RESULTS AND DISCUSSION

### Phytochemical screening

Preliminary qualitative phytochemical screening of *F. indica* showed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and triterpenoids.

Earlier studies on this plant by Sousek *et al.* (12) reported the presence of organic acids. Hence, we made an attempt to quantify the caffeic acid in *F. indica*.

Concentration of caffeic acid (396 µg g<sup>-1</sup> extract) present in 50% ethanolic extract of *F. indica* was estimated.

### Anti-inflammatory activity of 50% ethanolic extract of *F. indica*

Treatment with different doses of *F. indica* showed a dose-dependent inhibition of swelling caused by λ carrageenan after 3 h equivalent to 13.6 to 42.2% protection in comparison with the negative control, whereas phenylbutazone at the dose of 100 mg

Table I. Effect of 50% ethanolic extract of *F. indica* on carrageenan and histamine induced paw oedema in rats

| Treatment  | Carrageenan induced      |                | Histamine induced        |                |
|--|--------------------------|----------------|--------------------------|----------------|
|  | Paw volume (mL)          | Inhibition (%) | Paw volume (mL)          | Inhibition (%) |
| Negative control (1% CMC, 10 mL kg <sup>-1</sup> ) | 1.18 ± 0.05              | –              | 1.26 ± 0.03              | –              |
| <i>F. indica</i> (100 mg kg <sup>-1</sup> )        | 1.02 ± 0.04 <sup>a</sup> | 13.6           | 1.11 ± 0.05 <sup>a</sup> | 11.2           |
| <i>F. indica</i> (200 mg kg <sup>-1</sup> )        | 0.89 ± 0.03 <sup>b</sup> | 24.7           | 1.00 ± 0.02 <sup>b</sup> | 20.6           |
| <i>F. indica</i> (400 mg kg <sup>-1</sup> )        | 0.67 ± 0.03 <sup>b</sup> | 43.2           | 0.73 ± 0.03 <sup>b</sup> | 42.1           |
| Phenylbutazone (100 mg kg <sup>-1</sup> )          | 0.66 ± 0.05 <sup>b</sup> | 44.1           | 0.71 ± 0.06 <sup>b</sup> | 43.2           |

Mean ± SEM for six rats per group.

<sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.001$  compared to negative control.

kg<sup>-1</sup> showed 44.1% anti-inflammatory activity (Table I). The *F. indica* extract at doses of 100, 200 and 400 mg kg<sup>-1</sup> reduced the oedema induced by histamine by 11.2 to 42.1% respectively, whereas phenylbutazone decreased by oedema 43.2% (Table I). The study of *F. indica* extract on cotton pellet granuloma in rats indicated that *F. indica* (100, 200 and 400 mg kg<sup>-1</sup>, *p.o.*) significantly ( $p < 0.01$ ) reduced the granuloma formation by percent inhibition of 16.7 to 38.9% compared to the control. Phenylbutazone showed significant ( $p < 0.001$ ) inhibition of granuloma formation by percent inhibition of 40.1% (Table II).

Table II. Effect of 50% ethanolic extract of *F. indica* on cotton pellet induced granuloma in rats

| Treatment  | Dry mass (mg)           | Inhibition (%) |
|--|-------------------------|----------------|
| Negative control (1% CMC, 10 mL kg <sup>-1</sup> ) | 36.8 ± 1.9              | –              |
| <i>F. indica</i> (100 mg kg <sup>-1</sup> )        | 30.6 ± 1.2 <sup>a</sup> | 16.9           |
| <i>F. indica</i> (200 mg kg <sup>-1</sup> )        | 27.3 ± 1.6 <sup>b</sup> | 26.0           |
| <i>F. indica</i> (400 mg kg <sup>-1</sup> )        | 22.5 ± 1.5 <sup>b</sup> | 38.9           |
| Phenylbutazone (100 mg kg <sup>-1</sup> )          | 22.1 ± 1.1 <sup>b</sup> | 40.1           |

Mean ± SEM for six rats per group.

<sup>a</sup>  $p < 0.01$ , <sup>b</sup>  $p < 0.001$  compared to negative control.

### Anti-nociceptive activity of 50% ethanolic extract of *F. indica*

The experimental data of the force-induced pain indicates that the mice treated with *F. indica* extract exhibited resistance against pain after 30 min equivalent to 17.4 to 57.3% at different doses compared to the control (Fig. 1). *F. indica* extract at the dose of 100 mg kg<sup>-1</sup> increased the hot plate reaction time by 33.9%. However, dose levels of 200 and 400 mg kg<sup>-1</sup> increased the reaction time significantly ( $p < 0.01$ ) and percent protection by 73.2% and 125.1%, respectively (Fig. 2). *F. indica* extract showed inhibition of the writhing response induced by acetic acid dose dependently (100, 200 and 400 mg kg<sup>-1</sup>), which resulted in greater inhibition of stretching episodes; the protection ranged from 27.0 to 53.5%, whereas and acetylsalicylic acid blocked the writhing response by 63.1% (Table III).

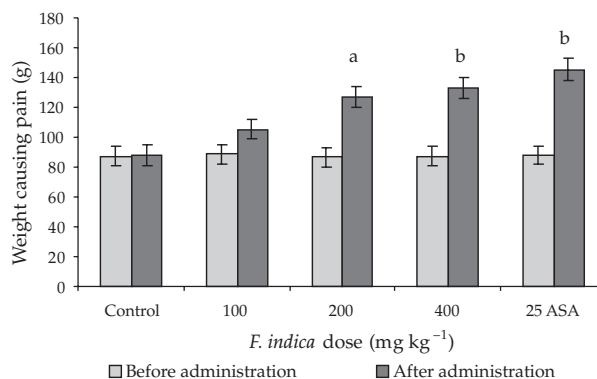


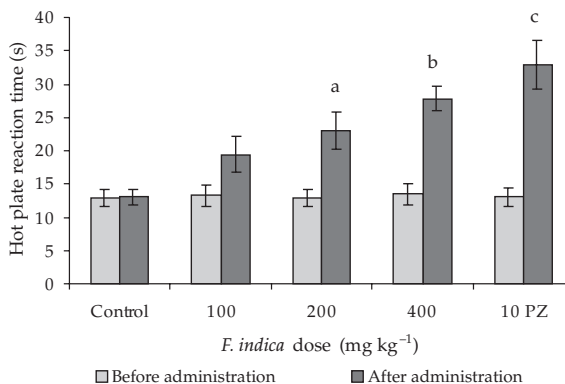
Fig. 1. Effect of 50% ethanolic extract of *F. indica* on force induced pain in mice.

Mean ± SEM for six mice per group.

<sup>a</sup>  $p < 0.01$ , <sup>b</sup>  $p < 0.001$  compared to negative control.

Fig. 2. Effect of 50% ethanolic extract of *F. indica* on hot plate reaction time in mice.

Mean  $\pm$  SEM for six mice per group. <sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.01$ , <sup>c</sup>  $p < 0.001$  compared to negative control.



The present study demonstrates the anti-inflammatory activity of the 50% ethanolic extract of *F. indica* in different models of inflammation—acute exudative and proliferative phases of inflammation. Dose of 400 mg kg<sup>-1</sup> shows anti-inflammatory and anti-nociceptive effect to standard drugs. Caffeic acid is one of the phenolics present in *F. indica*, which was reported to possess anti-inflammatory activity (22). On the other hand,  $\beta$ -sitosterol has also been reported as an anti-inflammatory, analgesic and antipyretic agent (23, 24). Therefore, caffeic acid and  $\beta$ -sitosterol may be responsible for the anti-inflammatory and anti-nociceptive effects exhibited by *F. indica*. The ability of the *F. indica* extract in analgesic activity may be due to the involvement of prostaglandins and other mediators in a different order of magnitudes. The capacity of prostaglandin to sensitize pain receptors to mechanical and chemical stimulation appears to result from a lowering of the threshold after *F. indica* treatment.

Table III. Effect of 50% ethanolic extract of *F. indica* on acetic acid induced writhing in mice

| Treatment  | Number of writhings         | Inhibition (%) |
|--|-----------------------------|----------------|
| Negative control (1% CMC, 10 mL kg <sup>-1</sup> ) | 26.7 $\pm$ 2.7              | —              |
| <i>F. indica</i> (100 mg kg <sup>-1</sup> )        | 19.5 $\pm$ 2.2 <sup>a</sup> | 27.0           |
| <i>F. indica</i> (200 mg kg <sup>-1</sup> )        | 15.3 $\pm$ 1.2 <sup>b</sup> | 42.6           |
| <i>F. indica</i> (400 mg kg <sup>-1</sup> )        | 12.4 $\pm$ 1.7 <sup>c</sup> | 53.5           |
| Acetylsalicylic acid (25 mg kg <sup>-1</sup> )     | 9.9 $\pm$ 1.6 <sup>c</sup>  | 63.1           |

Mean  $\pm$  SEM for six mice per group.

<sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.01$ , <sup>c</sup>  $p < 0.001$  compared to negative control.

## CONCLUSIONS

Based on the present study, it can be concluded that *F. indica* has potential anti-inflammatory activity against both exudative (carrageenan and histamine induced in-

flammation) and proliferative (cotton pellet induced granuloma) phases of inflammation; the extract also showed anti-nociceptive activity, mediated both centrally and peripherally. *F. indica* extract significantly raised the pain threshold. This offers a new perspective in the treatment of pain. Further studies are in progress and are aimed to identify all active constituents responsible for the anti-inflammatory and anti-nociceptive properties.

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## S A Ž E T A K

### Protuupalno i analgetsko djelovanje ekstrakta cijele biljke *Fumaria indica* na eksperimentalnim životinjama

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Na animalnom modelu ispitivano je protuupalno i analgetsko djelovanje ekstrakta biljke *Fumaria indica* sa 50%-tnim etanolnom. Peroralna primjena suhog ekstrakta *F. indica* (100, 200 i 400 mg kg<sup>-1</sup>) pokazuje značajno i o dozi ovisno protuupalno djelovanje na akutni (edem šape uzrokovan karagenom i histaminom,  $p < 0.05$ ) i kronični upalni proces (granulomi uzrokovani pamučnim peletama,  $p < 0.01$ ). Najveći protuupalni učinak u karagenskom, odnosno histaminskom testu od 42,2 i 42,1% dobiven je s dozom 400 mg kg<sup>-1</sup> nakon 3 h. Ista doza ekstrakta pokazala je 38,9% smanjenje mase granuloma. Značajno analgetsko djelovanje dokazano je pokusima na miševima: 6,6–67,7% ( $p < 0,01$ ) zaštita od mehanički izazvane boli, 33,9–125,1% ( $p < 0,05$ ) zaštita od termički izazvane boli i 22,2–73,9% ( $p < 0,05$ ) zaštita od kemijski izazvane boli octenom kiselinom.

*Ključne riječi:* *Fumaria indica* (Fumariaceae), protuupalno djelovanje, analgetsko djelovanje

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