ISSN:2348-5388

Journal of Fundamental Pharmaceutical Research
contents available at www.jfpronline.info

Evaluation of Analgesic activity of Ethanolic extracts of *Fumaria Officinalis Linn.* in experimental animals

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Submission Info:

Available online
01/07/2014

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Abstract:
Pain is a common and distressing feature of many diseases such as tumour, surgical procedures, physical trauma, noxious chemical stimulation etc. It is mostly a warning signal and primarily protective but excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation. Preliminary phytochemical investigations of different extracts of leaves of *Fumaria officinalis Linn.* were studied. The petroleum ether extract contains phytosterols, saponins and fixed oils. The chloroform extract contains proteins. The ethanolic extract contains carbohydrates, saponins, flavonoids, phytosterols, tannins and phenolic compounds. Acute toxicity studies are in hot plate method the extract at 200mg significantly ($p < 0.001$) increased the PRT while the reference drug Standard: Diclofenac sodium- 10 mg/ kg b. wt. and the extract at the dose of 500mg/ kg significantly. The tail withdrawal response or tail flick time was significantly ($p < 0.0001$) increased from 3.583±0.2386 seconds in the control group (10ml/ kg normal saline) 13.75±0.2141in the Standard: Diclofenac sodium- 10 mg/ kg b. wt. treated group and 12.42±0.2386 seconds in the highest dose of the extract (500 mg/kg). The percentage inhibition of writhing was also dose dependently increased from zero in the control group (normal saline) to 33% in the group that received 500mg/ kg of the extract. In Acetic Acid Induced Writhing Method there was significant difference between the group that were given 200mg/ kg and those treated with the reference drug Diclofenac sodium and the extract at the dose of 500 mg/ kg had a better analgesic activities than other extract treated group.

Key Words: *Fumaria officinalis*; Acute toxicity; Tail flick time; Diclofenac Sodium; Analgesic activity.

Introduction:

Pain is a common and distressing feature of many diseases such as tumor, surgical procedures, physical trauma, noxious chemical stimulation etc [1].

It is mostly a warning signal and primarily protective but excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation [2]. Analgesics are drugs used to relieve pain and the existing ones have serious adverse effect such as additive potential, drowsiness, nausea, respiratory depression as seen in opiates [3] and gastrointestinal bleeding and ulceration as seen with Non steroidal anti-inflammatory drugs (NSAIDS) [4]. These make the search for new analgesic drugs a necessity and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity [5]. *Fumaria officinalis Linn.* is one of such plants commonly known as "Puttapatra" belongs to the family *Fumaraceae.*

There are numerous plants and polyherbal formulations claimed to have analgesics activities. Nearly 150 phytoconstituents from 181 plants have been claimed to possess Analgesic activity. At the same time surprisingly, we do not have readily available statisfactory plant drugs/formulations to treat pain. However, number of medicinal preparations has been advocated in traditional system of medicine, especially in ayurveda, for treating pain. Their usage is in vogue since centuries and are quite often claimed to have offer significant relief. In addition usage of many folklore remedies, mainly plant products, is also quite common throughout India. Only a small portion of the analgesics plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy [6].

One such plant, possessing analgesics activity is *Fumaria officinalis Linn.* aerial part of this plant has been used in India as analgesics. Hence in the present study we have selected this plant to scientifically evaluate analgesics activity. It has been reported to possess laxative, diuretic, choleretic, antispasmodic, chronic eczema and antileprotic, blood purification [7,8].

Materials & Methods

Plant Material: Arial parts of the plant of *Fumaria officinalis Linn.* were collected from the Nilgiri Hills,Tamil nadu. The plants were authenticated by Dr. Rajan, (Field Botanist, Central council for research in Homeopathy, Govt. of India, Ooty.The voucher specimen (Ref No: 05/P.colog/2007- 2008) of the plant material has been deposited in the Department of Pharmacology. The plants were dried in shade at 4 to 5 days at 25 ºC, reduced to fine powder to particle size no 40. Around 1kg of herb of *Fumaria officinalis* was subjected to continuous soxhlet extraction with Petroleum ether (60-80 °C) for 32 h. The same marc was successively extracted with Chloroform (60 - 70 °C) and Ethanol (72 - 86 °C) for 24 h. The extracts were concentrated on water bath (50 ºC). After concentrated preparation, the dried powder extract was stored at 4 °C. The yield of the petroleum extract, chloroform extract and ethanolic extract were found to be 4% (w/w), 2.6% (w/w) and 9.8% (w/w) respectively. Ethanolic extract were used for the experimental study.

Animals: Wister Albino rats (150 - 200 g) and Albino mice (20 – 25g) of either sex procured from Bioneeds animal house, Dhavas pet, Tumkur, were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26 ± 2 °C. They were fed with standard diet supplied by Pranav agro industries Ltd. Sangli. The study has got the approval (Ref: IAEC/PP/05/2007-2008) from the Institutional Animal Ethical Committee (IAEC). All the animal experiments are conducted in accordance with the guidelines of the CPCSEA (Reg No. 997/c/06/ CPCSEA) guide for care and use of laboratory animals. After procuring the animals were acclimatized for 10 days under standard husbandry conditions as: Relative humidity 45 - 55%, and 12 h light and dark cycle.

Preliminary Phytochemical Screening: The preliminary phytochemical Screening was carried out on the petroleum ether, chloroform, and ethanolic extracts of leaves of *Fumaria officinalis* for qualitative

identification. Tests for common phytochemicals were carried out by standard methods described in practical Pharmacognosy[9, 10].

Acute Toxicity Study: The albino mice of 20– 25 g body weight of either sex were selected to find out the acute toxicity study of ethanolic extract of *Fumaria officinalis* leaves. The dose of 5, 50, 300 and 2000 mg/kg were selected based on the fixed dose (OCED Guideline No. 420) method of CPCSEA. The extract was administered by intraperitonially. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

Evaluation of Analgesics Activity:

Hot Plate Method :

The study was done using the effect of hot plate induced pain in mice [11] mature mice mere randomly divided into 5 groups (1-5) of 6 mice per group, fasted for 12 hours with clean drinking water provided *ad libitum.* The pre drug PRT (Pre Reaction Time) was assessed by placing each mouse upon a heated metal plate (Hot plate) maintained at the temperature of about 55-60^{0}C within a restraining Square box. The PRT for each mouse was determined using a stop watch to measure the time it took the mouse to flick or lick the hind paw or jump about. The cut off time was put at 20 seconds. This served as the control reaction time. The mice were then treated with 10ml/kg normal saline for the control group (group I); Standard: Diclofenac sodium- 10 mg/ kg b. wt. for the Standard group (group II) and 100, 200 and 500mg/ kg *Fumaria officinalis* extract for groups III, IV and V respectively (treatment groups) respectively. 30 minutes after drug and extract administration the PRT for each mouse was again determined using the same method as above. [12]

Tail Flick Method:

The experiment was carried out by measuring tail withdrawal time from hot water [13]. Thirty mice were randomly divided into five groups (1-5) of 6 mice per group and fasted for 12 hours. The mice were pretreated I hour before the experiment with 10ml/ kg normal saline ; group II, (Standard) was given Standard: Diclofenac sodium- 10 mg/kg b. wt. and 100, 200 and 500mg/ kg of *Fumaria officinalis* extract for groups III, IV and V respectively (extract treated groups) respectively. About 3-5cm of the tail of each mouse was dipped into a water bath containing warm water maintained at the temperature of 50±10C and the time taken for the mouse to flick the tail known as the pain reaction time (PRT) was recorded for all the mice [14].

Acetic Acid Induced Writhing Method:

This study was carried out using acetic acid induced abdominal writhing reflex pain model [15, 16]. Thirty mature mice were randomly divided into 5 groups (1-5) of 6 mice per group, fasted for 12 hours and treated as follows, Group I (negative control group) received 10ml/kg normal saline, group II (Standard) received Standard: Diclofenac sodium- 10 mg/kg b. wt.; groups III, IV and V received 100, 200 and 500mg/ kg of *Fumaria officinalis* extract respectively. One hour after drug and extract administration, 0.6% glacial acetic acid (10ml/kg) was administered intraperitoneally (I.P) to all the mice to induce abdominal contortions or writhings. The analgesic effect was assessed in each mouse for 30 minutes and recorded. The degree of analgesia was calculated using the following formula [17].

Mean of control group= mean of treated group x 100 Mean of control group 1. This represents the percentage of inhibition of writhing.

Data Analysis: The results were presented as mean ±SEM. The analysis was done using one way analysis of variance (ANOVA) and the difference between the means tested using Post Hoc LSD and T- test. The value of $p<0.05$) were considered statistically significant.

Results:

Preliminary Phytochemical Screening:

Preliminary phytochemical investigations of different extracts of leaves of *Fumaria officinalis Linn.* were studied. The petroleum ether extract contains phytosterols, saponins and fixed oils. The chloroform extract contains proteins. The ethanolic extract contains carbohydrates, saponins, flavonoids, phytosterols, tannins and phenolic compounds.

Acute Toxicity Study:

In the acute toxicity study ethanolic extract of leaves *Fumaria officinalis* were found to be toxic (2/3 mice died) at a dose of 2000 mg/ kg, intraperitonially. Hence, LD50 cut off value of ethanolic extract was fixed as 2000 mg/kg body weight. So, that 1/20th, 1/10th and 1/4th of the LD50 cut off value that is, 100, 200 and 500 mg/ kg body weight were selected as screening dose for analgesic activity.

Hot Plate Method:

The result of the effect of *Fumaria Officinalis* on hot plate induced pain in mice is presented in Table 1. The result showed that there was no significant difference in mean among the groups but after administration, the extract generally significantly ($p < 0.005$) increased the post drug PRT. In the individual group there was no significant difference between the pre and post drug PRT in group I (normal saline treated group) and group 3 (200mg/ kg of the extract). The extract at 200mg significantly ($p <0.001$) increased the PRT while the reference drug Standard: Diclofenac sodium- 10 mg/ kg b. wt. and the extract at the dose of 500mg/ kg significantly ($p <0.0001$) increased the PRT when the pre and post drug PRT are compared. Also the extract at 500mg/ kg had a near to same analgesic effect like Diclofenac sodium in this study.

TABLE 1: Effect of *Fumaria Officinalis* on Hot Plate induced Pain in Mice

Group	Treatment & Dose	Time in Minutes					
		0	30	60	90	120	180
I	Control 10ml/kg normal saline (i.p)	3.4 ± 0.1483	3.567± 0.05578	4.250± 0.07638	4.117± 0.04773	3.617± 0.1352	3.750± 0.06708
II	Standard: Diclofenac sodium- 10 mg/kg b. wt.	4.1± 0.1549***	6.540± 0.09274***	9.233± 0.07601***	12.23± 0.08433***	15.15± 0.07188***	17.58± 0.1249***
III	Extract Treated 100mg/kg of *Fumaria officinalis*	3.4± 0.1483	4.300± 0.08563***	5.917± 0.06009***	7.333± 0.1085***	8.533± 0.1229***	10.57± 0.1054***
IV	Extract Treated 200mg/kg of *Fumaria officinalis*	3.66± 0.1382	4.567± 0.1647***	6.133± 0.04944***	8.233± 0.07601***	11.25± 0.8003***	12.55± 0.2291***
V	Extract Treated 500mg/kg of *Fumaria officinalis*	3.95± 0.0562	5.567± 0.1282***	7.117± 0.04773***	10.30± 0.06831***	13.30± 0.2683***	15.30± 0.23092***

Values are Mean ± SEM, (n = 6 in each group). Figures in parenthesis are percent protection as compared to Control group and all values were significantly different ($P< 0.01$). Experimental groups were compared with Carrageenan control: *$P<0.05$ and **$P<0.01$,***$P<0.01$.

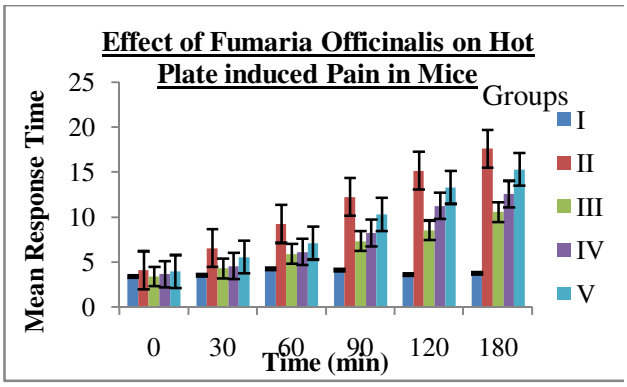
Tail Flick Response in mice:

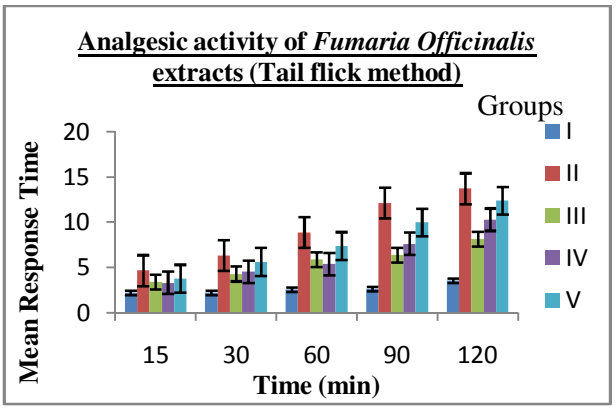
The tail withdrawal response or tail flick time was significantly ($p < 0.0001$) increased from 3.583±0.2386 seconds in the control group (10ml/ kg normal saline) 13.75±0.2141in the Standard: Diclofenac sodium- 10 mg/ kg b. wt. treated group and 12.42±0.2386 seconds in the highest dose of the extract (500 mg/kg). Table 2.

TABLE 2: Analgesic activity of *Fumaria Officinalis* extracts (Tail flick method)

Group	Treatment & Dose	Basal Reaction Time (Sec)	Reaction time in minutes (Sec)				
			15 Min	30 Min	60 Min	90 Min	120 Min
I	Control 10ml/kg normal saline (i.p)	2.5	2.250± 0.2045	2.250± 0.2141	2.583± 0.2386	2.667± 0.1667	3.583±0.2386
II	Standard: Diclofenac sodium- 10 mg/kg b.wt.	2.45	4.700± 0.08563***	6.367± 0.1563***	8.917± 0.2386***	12.17± 0.2108***	13.75±0.2141***
III	Extract Treated 100mg/kg of *Fumaria officinalis*	2.63	3.453± 0.1387***	4.333± 0.1667***	5.917± 0.1537***	6.417± 0.3005***	8.167±0.3575***
IV	Extract Treated 200mg/kg of *Fumaria officinalis*	2.25	3.350± 0.1607***	4.583± 0.2386***	5.417± 0.2007***	7.667± 0.3073***	10.33±0.2108***
V	Extract Treated 500mg/kg of *Fumaria officinalis*	2.68	3.817± 0.07923***	5.667± 0.1667***	7.417± 0.1537***	10.00± 0.2582***	12.42±0.2386***

Values, expressed as ml, are mean ± SEM from 6 animals in each group, % inhibition shown in parenthesiscomparison groups II, III, IV & V as group I,# – significantly different at $P < 0.05$;
@ – significantly different at $P < 0.01$;*– significantly different at $p<0.001$; ns – Not significant



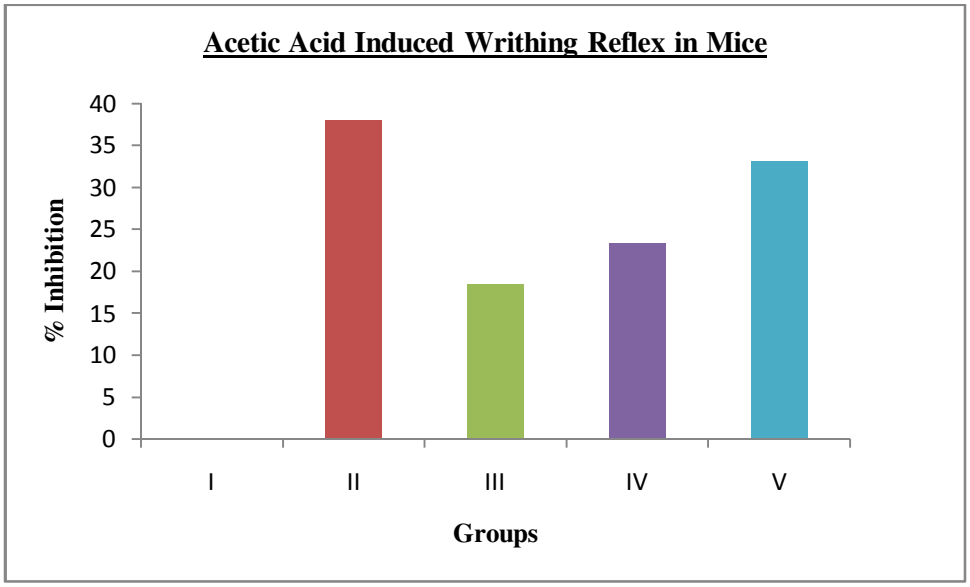


Acetic acid- induced writhing reflex:

The results of the analgesic effect of *Fumaria officinalis* extract an acetic acid induced writing reflex method is presented in Table 2. The results showed that the extract at the doses used just like the reference drug Standard: Diclofenac sodium- 10 mg/ kg b. wt. significantly ($p< 0.0001$) reduced the mean number of abdominal constrictions or writhing in a dose dependent manner when compared to the control group. The percentage inhibition of writhing was also dose dependently increased from zero in the control group (normal saline) to 33% in the group that received 500mg/ kg of the extract. There was significant difference between the group that were given 200mg/ kg and those treated with the reference drug Diclofenac sodium and the extract at the dose of 500 mg/ kg had a better analgesic activities than other extract treated group.

Table3: Effect of *Fumaria Officinalis* on Acetic Acid Induced Writhing Reflex in Mice

Group	Treatment & Dose	Mean writhing ± SEM recorded in 15 minutes	% Inhibition
I	Control 10ml/ kg normal saline (i.p)	50.66667±1.061969	0
II	Standard: Diclofenac sodium- 10 mg/ kg b. wt.	31.41667±0.800174	37.9934210
III	Extract Treated 100mg/ kg of *Fumaria officinalis*	41.33333±0.918937	18.421052
IV	Extract Treated 200mg/ kg of *Fumaria officinalis*	38.83333±0.945751	23.355263
V	Extract Treated 500mg/ kg of *Fumaria officinalis*	33.91667±1.392939	33.05921



Discussion:

Certain noxious stimuli are painful and reflex movements or behaviors resulting from such stimuli are indicative of a pain threshold. The stimulus may be thermal, electrical, mechanical or chemical [18] and this informed the adoption of the three analgesic models viz. Acetic acid-induced writhing, tail flick and hot plate methods.

The acetic acid-induced abdominal contortions or writhing reflex model is a sensitive method for screening analgesic effects of compounds [19]. Some chemicals such as acetic acid could induce abdominal contortions in laboratory animals [20]. The writhing reflex seen in this experiment was produced by injection of 0.6% glacial acetic acid. Intraperitoneal injection of acetic acid produces writhing reflex in the animals by activation of the chemo-sensitive nociceptors. The percent reduction in the number of abdominal contortions indicates the level of analgesia in the acetic acid writhing reflex model [21].

ACKNOWLEDGEMENTS:

The authors would like to express their gratitude to Mr. Premnath Reddy, Chairman and Mrs. Shalini Reddy, Director, Acharya Institutes, Bangalore for providing necessary facilities and support.

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