Evaluation of Analgesic and Anti-inflammatory Activity of *Ficus racemosa* Linn. Stem Bark Extract in Rats and Mice

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Abstract

Present work was undergone to investigate analgesic and anti-inflammatory effect of *Ficus racemosa* Linn. Stem bark extract in rats and mice. *Ficus racemosa* Linn. was studied for its analgesic activity on acetic acid induced writhing test in mice, tail flick test in rats and hot plate method in mice. The anti-inflammatory effects were investigated by employing acute inflammatory model i.e carrageenan-induced hind paw oedema, egg albumin induced paw oedema in rats, also studied for its preliminary phytochemical screening and acute toxicity studies, revealed presence of flavonoids, tannins and polyphenolic compounds, triterpenoids, coumarins, phytosterols, carbohydrates.

The extract did not produce mortality up to 5000 mg/kg p.o. Ethanol extract at the maximum dose (500 mg/kg) showed comparatively significant ($p< 0.05$) activity in tail flick method, significant inhibition of the writhes in writhing test, showed more significant ($p< 0.05$) response at 90, 120 and 180 min in hot plate method, comparatively significant ($p< 0.01$) inhibition of paw volume in carrageenan and egg albumin induced paw oedema method to that of standard diclofenac sodium (100 mg/kg). Petroleum ether extract is non-significant in all the cases of analgesic and anti-inflammatory methods. While, hydro-alcoholic extract (100, 300, 500 mg/kg) showed quiet more significant ($p< 0.01$) response in analgesic and anti-inflammatory method to that of respective standard. The results obtained suggest marked analgesic and anti-inflammatory activity of ethanolic extract (500 mg/kg) and hydro-alcoholic extract (100, 300, 500 mg/kg). The results obtained support the stem bark is useful in inflammatory and painful conditions like leaves and unripe fruits of the same plant *Ficus racemosa* Linn.

Keywords: *Ficus racemosa*, Moraceae, Analgesic, Anti-inflammatory activity, Acute and Chronic models.

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INTRODUCTION

Ficus racemosa Linn. Syn. *Ficus glomerata* Roxb. is a moderate to large sized tree belonging to the family Moraceae. It is a very common plant distributed throughout the India. The different parts of the plant are employed in native medicine for treating diarrhea, dysentery and diabetes, hypocholesterolemic[1,2], spermatozoic. Unripe fruits and leaves of the same plant in treating inflammation, root bark as anti-oxidant[3,4]. *Ficus racemosa* also been reported to produce anti filarial, antibacterial activities[5,6], in treating wounds and ulcers[7,8]. Hence in the present investigation evaluation of anti-inflammatory activity of stem bark extract of *Ficus racemosa* Linn. (FRSE) was taken up.

MATERIAL AND METHODS

Plant Material and preparation of the extract

The stem bark of *Ficus Racemosa* Linn. was procured from botanical garden of Bhimashankar, Maharashtra, Pune, India. Plant was authenticated by Botanical Survey of India (BSI), Maharashtra, Pune, India where a voucher specimen is deposited for future reference. The grinded bark powder was exhaustively extracted for 24 hrs with respective solvent in soxhlet apparatus with frequent shaking. The extract was filtered and concentrated and made free from solvent using distillation assembly. It was further concentrated over low flame for about 15 min. to a thick consistency and dried in hot air oven for 48 hr at 50° C. The dried extract was kept in desiccator for further use.

Preliminary Phytochemical Screening9

Ficus Racemosa stem bark was studied for its preliminary Phytochemical screening for detection of the various plant constituents.

Preparation of the test drugs

All the test drugs were prepared by suspending the test drugs in 5 % gum acacia having water as vehicle. The doses of the test drugs were selected according to the assessment of the toxicity. All the test drugs were administered by oral route using catheter.

Experimental Animals

Healthy strain of Wister albino rats (150–250 g) and Swiss albino mice (18–20 g) of either sex were used as experimental models for testing of activity. The animals were housed in standard conditions of temperature (± 25°C), 12 hr light per day cycle and having relative humidity 45–55 % in the animal house. The animals were fed with the standard pellet diet (Hindustan Liver rats pellets), and water ad *libitum*. The all experimental protocol was approved by the institutional animal ethical committee (IAEC), and then experimental studies were undergone according to their rules and regulations. (1197/c/08/CPCSEA)

Acute oral toxicity[10]

For the testing of acute toxicity, the animals were kept on fasting for a night prior to the experimental procedure. The "Up and Down" or "Staircase" method was adopted and LD_{50}dose was found to be 5000 mg/kg and accordingly doses of hydro-alcoholic, alcoholic and petroleum ether extracts were fixed to 100, 300, 500 mg/kg for further study.

Mouse Writhing Assay[11,12]

The method of Ghule et al. was used FRSE petroleum ether, ethanolic, hydro-alcoholic extracts (100, 300, 500 mg/kg) p.o administered to respective groups and Aspirin at the dose of 25 mg/kg i.p administered to the standard group, Control group receives 5 % gum acacia and 30 min later the animals of these groups were administered with 0.5 ml of 1% acetic acid dissolved in 0.9 % saline by i.p. The number writhes were counted per 15 min.

Tail Flick Method [11,12]

To evaluate anti-nociceptive activity of herbal extract, rat tail flick apparatus (INCO, India) was used. In tail flick method Wister rats (150–250 g) randomly distributed in to eleven groups. The first group served as control and animal were administered with 5 % gum acacia, group II to group X animals received with FRSE petroleum ether, ethanolic, hydro-alcoholic extracts (100, 300, 500 mg/kg) p.o respectively. Group XI was standard and animals were received with ibuprofen (100 mg/kg), the reaction time was noted at 15, 30, 60, 90, 120 and 180 min time interval after drug administration.

Hot Plate Method [11,12]

In hot plate method Swiss Albino mice (18–25 g) randomly distributed in to eleven groups. The first group served as control and animal were administered with 5 % gum acacia, group II to group X animals received with FRSE petroleum ether, ethanolic, hydro-alcoholic extracts (100, 300, 500 mg/kg) p.o respectively. Group XI was standard and animals were received with Pentazocin (10 mg/kg), the basal reaction time was noted before and 30, 60, 90, 120 and 180 min after the administration of the test drug.

Carrageenan-induced Paw Oedema in rats [12,13,14]

For evaluation of acute inflammation, this model employed and method of *Winter et al*. was followed for the measurement of oedema volume by using and instrument plethysmograph. In this experiment healthy Wister rats of either sex were used having weight in the range of 150–250 g. The animals were starved overnight before previous to dosing. To ensure uniform hydration of the animals, the rats were provided with water *Ilibitum*. The first group served as control and animal were administered with 5 % gum acacia, group II to group X animals received with FRSE petroleum ether, ethanolic, hydro-alcoholic extracts (100, 300, 500 mg/kg) p.o respectively. Group XI was standard and animals were received with diclofenac sodium (100 mg/kg). The phlogestic agent carrageenan was prepared by suspending the drug (1%) into the normal saline vehicle and acute inflammation was produced by administration of 0.1 ml of the above suspension to all the animals of each five groups, through injection into the right hind paw at the sub plantar region.

A mark was made at the region of the malleolus of the paw and the paw was immediately immersed in the Plethysmometer, up to the mark, and the paw volume was measured, serves as reading for 0 hr. The readings were taken similarly and paw volumes were measured for 1, 2, 3 and 4 hr respectively. The average paw swelling in the groups of the test animals of different solvent

extracts treated were compared with control group which is treated with vehicle and standard group animals those have received diclofenac sodium. Mean increase in paw volume was determined.

Egg albumin-induced Paw oedema in rats[12,13,14]

This test was performed by *Winter et al*, The first group served as control and animal were administered with 5 % gum acacia, group II to group X animals received with FRSE petroleum ether, ethanolic, hydro-alcoholic extracts (100, 300, 500 mg/kg) p.o respectively. Group XI was standard and animals were received with diclofenac sodium (100 mg/kg). After 30 min, each group was injected with 0.5 ml raw egg albumin sub-plantar to the left hind paw. A plethysmometer was used to measure the volume of the paw oedema for the period of 120 min with readings take at 30 min interval, i.e. 30, 60 and 90 min after albumin administration.

Statistical Analysis

All data were expressed as Mean ± SEM and analyzed statistically by using Dunnett's 't-test'. Difference was considered significant at P value less than 0.05.

RESULTS

Acute Toxicity Studies

In the acute toxicity test, sign of toxicity included lethargy, jerk, convulsions and death not observed in acute dose of 5000 mg/kg p.o in mice. For further study of FRSE for the analgesic and anti-inflammatory activity the dose selected were 100, 300, 500 mg/kg.

Preliminary Phytochemical Screening

Preliminary Phytochemical screening of the FRSE showed the presence of tannins, triterpenoids, flavonoids, coumarins, phenolic compounds phytosterols and carbohydrates, proteins.

Analgesic Studies

The effect of FRSE on acetic acid induced writhing is demonstrated in Table 1. the ethanolic FRSE at higher dose 500 mg/kg showed significant (16.12 %) inhibition of writhes and 100, 300 mg/kg ethanolic extracts were insignificant. Hydro alcoholic FRLE showed dose dependent inhibition of writhes and maximum inhibition (41.66 %) was observed for 500 mg/kg. Standard aspirin (25 mg/kg) showed maximum inhibition of writhes (68.0 %). Petroleum ether FRSE was insignificant at all doses for inhibition of writhes induced by acetic acid in mice.

The analgesic activity of FRSE was evaluated using hot plate test. The results are presented in Table 2 shows the time course of analgesic effect of FRSE. Oral administration of hydro-alcoholic extracts at (100, 300, 500 mg/kg) and ethanolic FRSE (300, 500 mg/kg) resulted significant prolongation of latency time in hot plate test, except left over pet ether extracts. These effects reached their peak at 120 min after dose administration and then decreased. At 120 min mean latency time of ehanolic FRSE 500 mg/kg is 5.61, in case of hydro alcoholic FRSE 100, 300, 500 mg/kg is 8.91, 5.43, 10.25 second compared with control. Pet ether FRSE and ethanolic (100, 300 mg/kg) was non

Table 1. Effect of *Ficus racemosa* Linn. extracts in acetic acid induced writhing test

GROUP	TREATMENT	Number of writhes (per 15 min)	Inhibition (%)
I	Control	37.2±0.71	---
II	Aspirin (25 mg/kg)	11.9±0.57	68.01
III	Pet ether (100 mg/kg)	36.8±0.61	1.07
IV	Pet ether (300 mg/kg)	37.3±1.28	---
V	Pet ether (500 mg/kg)	36.7±0.40	1.34
VI	Ethanol (100 mg/kg)	35.8±0.83	3.76
VII	Ethanol (300 mg/kg)	33.6±0.87	9.677
VIII	Ethanol (500 mg/kg)	31.2±0.48	16.12
IX	Hydro-alcoholic(100 mg/kg)	29.4±0.56	20.96
X	Hydro-alcoholic(300 mg/kg)	24.6±0.55	33.87
XI	Hydro-alcoholic(500 mg/kg)	21.7±0.38	41.66

Values are mean ± SEM (n = 6), experimental group here compared with Control,

*, **, represent $p < 0.05$, $p < 0.01$ respectively, by Dunnett's test

Table 2. Effect of *Ficus racemosa* Linn. extracts in tail flick method

GROUP	TREATMENT	0 min	30 min	60 min	90 min	120 min	180 min.
I	Control	1.988±0.103	2.025±0.129	2.04±0.113	2.07±0.150	2.06±0.11	2.055±0.109
II	Ibuprofen100 mg/kg	2.056±0.118	6.511±0.222**	7.95±0.131**	8.78±0.162**	7.19±0.16**	5.87±0.10**
III	Pet ether100 mg/kg	2.14±0.021	2.16±0.025	2.02±0.045	2.16±0.048	2.24±0.068	2.12±0.071
IV	Pet ether300 mg/kg	2.32±0.45	2.23±0.13	2.17±0.81	2.21±0.26	2.08±0.29	2.56±0.31
V	Pet ether500 mg/kg	2.45±0.81	2.54±0.85	2.32±0.11	2.38±0.31	2.40±0.36	2.35±0.63
VI	Ethanol100 mg/kg	2.37±0.31	2.78±0.396	2.82±0.93	2.91±0.86	2.58±0.64	2.51±0.84
VII	Ethanol300 mg/kg	2.21±0.17	2.69±0.37	2.93±0.42	3.10±0.45	3.25±0.36	2.54±0.12
VIII	Ethanol500 mg/kg	2.18±0.71	3.76±0.12*	3.85±0.28*	3.99±0.31*	4.15±0.24*	3.14±0.28*
IX	Hydro-alcoholic100 mg/kg	2.008±0.132	4.438±0.248**	5.75±0.138**	6.77±0.137**	7.203±0.124**	5.96±0.24**
X	Hydro-alcoholic300 mg/kg	2.198±0.181	5.328±0.217**	6.71±0.21**	7.70±0.21**	8.48±0.17**	6.62±0.21**
XI	Hydro-alcoholic500 mg/kg	2.163±0.183	6.048±0.232**	7.58±0.26**	8.60±0.18**	9.21±0.15**	8.06±0.15**

Values are mean ± SEM (n = 6), experimental group here compared with Control,

* represent $p < 0.05$, by Dunnett's test

** represent $p < 0.01$, by Dunnett's test

Table 3. Effect of *Ficus racemosa* Linn. extracts in hot plate method

GROUP	TREATMENT	0 min	30 min	60 min	90 min	120 min	180 min
I	Control	1.72±0.088	1.73±0.092	1.731±0.095	1.753±0.085	1.906±0.096	1.966±0.13
II	Pentazocin	1.77±0.084	4.361±1.61**	8.341±0.33**	6.86±0.035**	5.23±0.12**	4.43±0.058**
III	Pet ether100 mg/kg	1.70±0.13	1.83±0.61	1.78±0.53	1.86±0.19	1.81±0.93	1.92±0.95
IV	Pet ether300 mg/kg	1.93±0.42	1.87±0.23	1.94±0.26	1.98±0.85	1.85±0.68	1.71±0.58
V	Pet ether500 mg/kg	1.87±0.32	1.82±0.97	1.91±1.21	1.84±1.01	1.93±0.56	1.88±0.25
VI	Ethanol100 mg/kg	1.56±0.123	1.72±0.67	1.96±1.68	1.96±1.34	1.84±0.87	1.76±0.167
VII	Ethanol300 mg/kg	1.63±0.11	2.34±0.76	2.89±0.45	2.11±0.23	3.08±0.81	2.1±0.15
VIII	Ethanol500 mg/kg	1.72±0.45	2.65±0.41	3.23±0.71*	5.61±0.78**	4.89±0.19**	4.78±0.68**
IX	Hydro-alcoholic 100 mg/kg	1.46±0.117	2.486±0.176*	4.32±0.098**	5.911±0.223**	4.33±0.12**	3.433±0.153 **
X	Hydro-alcoholic 300 mg/kg	1.66±0.107	1.748±0.248	2.33±0.123	8.43±0.17**	6.71±0.071**	4.195±0.061**
XI	Hydro-alcoholic 500 mg/kg	1.77±0.128	4.303±0.259**	5.088±0.176**	10.25±0.182**	7.65±0.074**	6.97±0.22**

Values are mean ± SEM (n = 6), experimental group here compared with Control,

* represent $p < 0.05$, by Dunnett's test

** represent $p < 0.01$, by Dunnett's test

significant. Pentazocin significantly increased response latency time of animal with maximum effect was observed at1h after treatment.

The results of analgesic activity evaluated by tail flick method are presented in Table 3. Hydro-alcoholic FRSE at all doses showed dose dependent increase in tail flick latency period and hydro-alcoholic FRSE (500 mg/kg) and ibuprofen (100 mg/kg) showed maximum increase in tail flick latency period. Ethanol FRSE 500 mg/kg showed significant increase in tail flick latency period, petroleum ether FRSE at all doses and ethanolic FRSE (100, 300 mg/kg) was found insignificant.

Anti—inflammatory studies

The activity of FRSE on against carrageenan induced paw edema is shown in Table 4. Hydro alcoholic FRSE 100,300, 500 mg/kg is highly significant, ehanolic FRSE 300, 500 mg/kg is quiet significant in reduction of paw oedema induced by carrageenan. While pet ether FRSE was not showed any significant effect. Maximum reductions in rat paw oedema at 2h, 4h after administration of hydro alcoholic FRSE and standard diclofenac sodium.

The effect of FRSE on egg albumin induced hind paw oedema in rats is shown in Table 5. The results showed

Table 4. Effect of *Ficus racemosa* Linn. extracts in carrageenan induced rat paw oedema

	Average volume of mercury displaced (ml)			
Treatment	**0 h**	**1 h**	**2 h**	**4 h**
Control	0.25±0.02	0.51±0.02	0.62±0.03	0.71±0.03
Petroleum ether (100mg/kg)	0.25±0.02	0.45±0.03	0.57±0.03	0.56±0.03
Petroleum ether (300mg/kg)	0.24±0.02	0.47±0.03	0.55±0.03	0.55±0.03
Petroleum ether (500mg/kg)	0.25±0.02	0.45±0.03	0.52±0.02	0.51±0.02
Ethanol (100mg/kg)	0.25±0.02	0.42±0.02	0.50±0.03	0.51±0.03
Ethanol (300mg/kg)	0.25±0.02	0.32±0.02	0.43±0.03	0.45±0.03
Ethanol (500mg/kg)	0.24±0.02	0.30±0.02*	0.41±0.02*	0.40±0.02*
Hydro-alcoholic (100mg/kg)	0.24±0.03	0.34±0.02*	0.32±0.02*	0.30±0.02**
Hydro-alcoholic (300mg/kg)	0.26±0.02	0.31±0.02**	0.29±0.04**	0.28±0.04**
Hydro-alcoholic (500mg/kg)	0.25±0.02	0.28±0.02**	0.26±0.03**	0.26±0.02**
Diclofenac Sodium (100mg/kg)	0.24±0.02	0.25±0.02**	0.27±0.02**	0.26±0.02**

Values are mean ± SEM (n = 6), experimental group here compared with Control,

* represent $p < 0.05$, by Dunnett's test

** represent $p < 0.01$, by Dunnett's test

Table 5. Effect of *Ficus racemosa* Linn. extracts in egg-albumin induced rat paw oedema

	Average volume of mercury displaced (ml)		
Treatment	**30 min**	**60 min**	**90 min**
Control	0.48±0.02	0.54±0.03	0.61±0.03
Petroleum ether (100mg/kg)	0.45±0.03	0.51±0.03	0.58±0.03
Petroleum ether (300mg/kg)	0.47±0.03	0.56±0.03	0.59±0.03
Petroleum ether (500mg/kg)	0.41±0.03	0.48±0.02	0.52±0.02
Ethanol (100mg/kg)	0.46±0.02	0.53±0.03	0.56±0.03
Ethanol (300mg/kg)	0.43±0.02	0.42±0.03	0.45±0.03
Ethanol (500mg/kg)	0.36±0.02*	0.39±0.02*	0.41±0.02*
Hydro-alcoholic (100mg/kg)	0.36±0.02*	0.33±0.02*	0.38±0.02*
Hydro-alcoholic (300mg/kg)	0.38±0.02*	0.37±0.04**	0.39±0.04*
Hydro-alcoholic (500mg/kg)	0.27±0.02**	0.26±0.03**	0.28±0.02**
Diclofenac Sodium (100mg/kg)	0.26±0.02**	0.28±0.02**	0.29±0.02**

Values are mean ± SEM (n = 6), experimental group here compared with Control,

* represent $p < 0.05$, by Dunnett's test

** represent $p < 0.01$ respectively, by Dunnett's test

that the hydro-alcoholic FRSE and diclofenac sodium caused dose dependent and significant inhibition of egg-albumin induced rat paw oedema over a period of 90 min and ethanolic FRSE 500 mg/kg also showed significant results while petroleum ether FRSE at all doses and ethanolic FRSE (100, 300 mg/kg) showed non significant inhibition of paw oedema.

DISCUSSION

The results presented here may help to establish the scientific basis for utilization of stem bark of *Ficus racemosa Linn*. For the treatment of pain and inflammation in folk medicine as that of leaves and fruit extracts of the same plant reported earlier research. In the present investigation here is study of stem bark for the claimed application of the same plant.

In this work, we have demonstrated the effect of petroleum ether, ethanolic and hydro-alcoholic stem bark extracts of *Ficus racemosa Linn*. (FRSE) at 100, 300, 500 mg/kg p. o doses on acetic acid induced writhing test, hot plate method and tail flick test for analgesic effect and carrageenan-induced paw oedema as well as egg albumin-induced paw oedema for anti-inflammatory effect of test drug. The hydro-alcoholic FRSE showed analgesic and anti-inflammatory effects in laboratory animals at the

dose dependent manner and ethanolic FRSE produced significant results at higher dose 500 mg/kg and 100, 300 mg/kg showed non significant results in all models. Petroleum ether FRSE found insignificant in analgesic and anti-inflammatory models.

The results supported the traditional use of this plant in some painful and inflammatory conditions. Because of presence of biologically active principles i.e flavonoids, tannins, phenolic compounds and phytosterols in the same plant from Phytochemical investigation suggests that one of above constituent or in combination together is responsible for producing the analgesic and anti-inflammatory effects. Further studies are in progress to isolate and characterize the active principle from the stem bark of the *Ficus racemosa Linn*.

The oral LD_{50} obtained with this plant extract also suggested that it may have a reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lack of any reported side effect with the traditional use of this plant.

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