Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20160101

Research Article

Evaluation of analgesic activity of *Ficus racemosa* leaf extract using acetic acid induced writhing method in mice

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Received: 11 December 2015
Accepted: 07 January 2016

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ABSTRACT

Background: To evaluate the analgesic activity of *Ficus racemosa* leaf extract and its comparison with standard drug using acetic acid induced writhing method in mice.
Methods: Chloroform extract of leaves was obtained using soxhlet apparatus. Isolated fraction of extract (FRE) was used for the experiments. Swiss albino mice of either sex, average weight 20-25 gms were used for experiments. Animals were divided into 6 groups consisting of 6 animals each.1% acetic acid was administered intra peritoneally to the experimental animals to create pain sensation. Each group received particular treatment 30 minutes prior to administration of acetic acid. Each mouse of all groups were observed individually for counting number of writhes they made in 15 minutes commencing just 5 minutes after the administration of acetic acid. Diclofenac was taken as standard drug.
Results: FRE at the dose of 50 mg/kg did not shows any significant analgesic activity while at the dose of 100 and 200 mg/kg it showed significant analgesic activity as compare to control group. FRE at dose of 200 mg/kg showed significant analgesic activity as compare to diclofenac in writhing model. Diclofenac in combination with 50 mg/kg dose of FRE produced significant analgesic activity when compared to control value or either treatment alone.
Conclusions: FRE endowed with peripheral analgesic properties in dose dependent manner. And also enhances the analgesic effect of diclofenac. However further study is needed in order to understand the precise mechanism.

Keywords: *Ficus racemosa*, Analgesics, Diclofenac sodium, Albino mice

INTRODUCTION

Pain is ill defined, disabling accompaniment of many medical conditions. NSAIDs are most popular and most commonly used analgesics for mild to moderate pain. But chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration.[1] Opioid analgesics are also very effective in relieving pain. But the adverse effect produced by opioids are very severe and life threatening. So pain, which is one of the most common problem occurring amongst human population, still requires some better drugs with high efficacy and less side effects. Parallel to this, the holistic approach of herbs have accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects.

Ficus racemosa Linn (*Moraceae*) is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree, without much prominent aerial roots found throughout greater part of India in moist localities and is often cultivated in villages for its edible fruit.[2] All parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in the traditional system of medicine in India. Apart from the usage in traditional medicine, scientific studies indicate *F. racemosa* to possess various biological effects such as hepatoprotective[3], chemopreventive[4], antidiabetic[5], anti-inflammatory,[6] antipyretic,[7] antitussive[8] and antidiuretic.[9]

Therefore, the present study was designed to investigate the analgesic effect of the chloroform extract of leaves in mice using acetic acid induced writhing.

METHODS

Collection and extraction of leaves

Leaves of *F. racemosa* were collected from local area near MGM Medical College, Indore [M.P.], India. The identification and authentication was carried out by department of Botany, Holkar Science College, Indore [MP]. After authentication, in the month of August fresh leaves of almost same size were collected in bulk, washed under running tap water to remove dust and adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 40 and taken for further studies.

Preparation of FRE

For the preparation of extract 100 gm of dried coarse powdered leaves were charged in to the soxhlet's apparatus (hot extraction) and extracted successively with chloroform. The successive chloroform extract (deep brown colour) was filtered & dried under reduced pressure to get a solid mass free from the solvent. The crude extract thus obtained was further fractionated. The solvent fractionation was done with alcohol and acetone. The insoluble fraction of alcohol and acetone fractionation was dried and passed through column chromatography. The mobile phase was consisting of chloroform and the stationary phase was consisting of silica gel (200-400 mesh). The eluent was collected and dried to obtain whitish powder (FRE).[10] The yield was 0.21% with respect to dry starting material.

Phytochemical screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferricchloride solution, gum with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic colour changes using standard procedures.[11]

Experimental animals

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the central animal house, M G M Medical College, Indore [MP]. They were kept under standard hygienic conditions, at 20 ± 2^0C temperature, relative humidity (60 ± 10%) with 12 hours day and night cycle, with food and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 5 days before the start of the experiment.

Acute Toxicity study

The acute toxicity was determined for the isolated fraction of chloroform extract of *Ficus racemosa* (FRE) on albino mice using fixed dose method of OECD Guideline no. 420 given by CPCSEA (Committee for the purpose of control and supervision of experiments on animals). The animals were divided into two groups of six in each. The animals were fasted overnight prior to the acute experimental procedure. Gum acacia (2% w/v) was used as vehicle to suspend FRE. Control group received 2% gum acacia (2 ml/kg) and the other group received FRE (300 mg/kg). All animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.[12] No obvious sign of morbidity was observed. So the same procedure was repeated with 2000 mg/kg dose of FRE. And again no sign of any morbidity was observed.[12] So limit dose for FRE was considered as 2000 mg/kg.

Preparation of drugs for animal experimentation

FRE is almost insoluble in water. So the suspension of FRE and solutions of all the other drugs, to be given orally to the experimental animals as standard or in combination, were prepared in 2% gum acacia. Gum acacia here acted as a vehicle. Control groups were given a 2% gum acacia suspension (in the standard dose of 10 ml/kg).

Analgesic activity

Principle

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal using radiant heat as a source of pain; chemical using irritants such as acetic acid and bradykinin and physical pressure using tail compression. In the laboratory, commonly used procedures are tail flick method (tail withdrawal from the radiant heat); acetic acid induced writhing method and the hot plate method.

Writhing method was used for the evaluation of peripheral analgesic activity. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction which is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limb are taken as reaction parameter to chemically induced pain.

Drugs/Groups

Group I 2% gum acacia (10 ml/kg, P.O.), group II diclofenac (5 mg/kg), group III FRE (50 mg/kg) and group IV diclofenac sodium and FRE (5 mg/kg + 50 mg/kg). Acetic acid 1 % v/v (0.1 ml/ 10 gm).

Procedure

Abdominal constrictions were induced by 1 % v/v glacial acetic acid solution (10 ml/kg, I.P.) in mice pre-treated with vehicle or one of the test substances. The number of abdominal writhing were measured over 20 min after the injection of acetic acid. Results are expressed as percentage inhibition of abdominal constrictions with respect to control. The same procedure was repeated with FRE at a dose of 100 and 200 mg/kg.

Statistical analysis

Results were expressed as mean ± SEM and analysed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied followed by post hoc multiple Tukey's comparison test. P value less than 0.05 (P<0.05) was considered as statistically significant.

RESULTS

Table 1: Result of phytochemistry of *Ficus racemosa* leaves.

Chemical constituents	Present/absent
Steroid	+
Alkaloid	+
Tannin	++
Carbohydrate	-
Gum	+
Glycoside	-
Flavonoid	+++
Saponin	-

+ present, - absent

Table 2: Effect of FRE in acetic acid induced writhing in mice.

Drug treatment	Dose (P.O.)	Number of writhes in 20 minutes	% inhibition with respect to control
Control (2% Gum acacia)	10 ml/kg	36.50±1.60	-
Diclofenac	5 mg/kg	18.17±1.47*	50.02
FRE_{50}	50 mg/kg	31.67 ±1.28	13.23
FRE_{100}	100 mg/kg	13.00± 1.15*	64.38
FRE_{200}	200 mg/kg	12.33± 0.80*†	66.21
One way ANOVA	F P	73.55 < 0.001	

One way ANOVA followed by multiple Tukey's comparison test; Values are mean ± SEM, n= 6 in each group; df = 4, 25; *P< 0.05 as compared to control; †P< 0.05 as compared to diclofenac group.

FRE at a dose of 50mg/kg did not show a significant analgesic activity as compared to control group (P>0.05). At the dose of 100 mg/kg it showed a highly significant analgesic activity as compare to control group (P<0.01). At the dose of 200 mg/kg it showed a highly significant decrease in number of writhes as compared to control as well as diclofenac treated group (P<0.01). FRE at a dose of 200 mg/kg showed 66.21 % inhibition in number of writhes as compared to 50.02% inhibition by diclofenac. Diclofenac sodium showed significant decrease in number of writhes (45.48%) as compared to control group (P<0.05) (Table 2).

FRE at a dose of 50 mg/kg did not show any significant decrease in the number of writhes (P>0.05). Diclofenac sodium in combination with FRE 50 produced highly significant decrease in number of writhes (66.48%) when compared to control value or either of the treatment alone (P<0.01) (Table 3).

Table 3: Effect of FRE, diclofenac and their combination in acetic acid induced writhing.

Drug treatment	Dose (P.O.)	Number of writhes in 20 minutes	% inhibition with respect to control
Control (2% Gum acacia)	10 ml/kg	33.33 ±1.229	-
Diclofenac	5 mg/kg	18.17±1.47*	45.48
FRE_{50}	50 mg/kg	28.83±1.04	13.50
Diclofenac + FRE_{50}	5 + 50 mg/kg	11.17±0.79*†	66.48
One way ANOVA	F P	75.17 < 0.001	

One way ANOVA followed by multiple tukey's comparison test; Values are mean ± SEM, n= 6 in each group, df = 3, 20; *P< 0.05 as compared to control; †P< 0.05 as compared to diclofenac group.

DISCUSSION

Ficus racemosa is a moderate sized avenue tree found throughout India. It is popular in indigenous system of medicine like ayurveda, siddha, unani and homoeopathy. In the traditional system of medicine various plant parts such as bark, root, leaves, fruits and latex are used in dysentry, diarrhea, diabetes, stomachache, piles and as carminative and astringent and also as antioxidant and anticancer agent.[13]

After an extensive literature search, it has been observed that, a lot of work has been done on the crude extract of bark of *F.racemosa* while research work on its leaves is scarcely available. We therefore, planned to explore the presence of any CNS activity in the leaf extract. We obtained crude chloroform extract of leaves of *F.racemosa* using soxhlet apparatus and subjected to

fractionation. The isolated fraction of chloroform extract of *F.racemosa* leaves (FRE) was used for our studies.

Pain, being the most unpleasant sensory and emotional experience worldwide, needs utmost attention for treatment and research purpose. Amongst all modalities available for the pain management, Non- steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs; although effective but associated with dreadful adverse effects of severe gastritis, peptic ulcer, nausea, vomiting, idiosyncrasy, etc. The anti-inflammatory and antinociceptive activities of nonsteroidal anti-inflammatory drugs (NSAIDs) are attributed to inhibition of the cyclooxygenase (COX) enzymes, thus blocking the synthesis of prostaglandins that promote inflammatory responses and enhanced sensitivity to pain at the peripheral site of tissue injury.

In order to evaluate any acute effect of FRE for presence of analgesic activity, we selected acetic acid induced writhing model for peripheral activity.

The study for analgesic effect using acetic acid induced writhing method reveals that FRE showed significant analgesic action ($P< 0.01$) at two dose levels i.e. 100 and 200 mg/kg b. wt as compare to control group (Table 2). Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs.[14] Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid[15] via cyclooxygenase (COX), and prostaglandin biosynthesis.[16] In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.[17] The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability.[18] The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.[16,19] The significant pain reduction by FRE might be due to the presence of analgesic principles acting through the prostaglandin pathways.

The abdominal writhing induced by acetic acid was also reported to be less selective[20] and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents.[21] Preliminary qualitative phytochemical screening reveals the presence of tannins, gums, flavonoids and alkaloids in FRE (Table 1). Therefore, it is assumed that these compounds or any of these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins.[22,23] There is also reports on the role of tannins in anti-nociceptive activity.[24]. Besides alkaloids are well known for their ability to inhibit pain perception.[25]

The experimental data suggests no analgesic activity of FRE at 50 mg/kg b.wt. dose, so we used this dose for studying any influence of sub therapeutic dose of FRE on analgesic activity of diclofenac using the same models selected for evaluating its per se effects. Interestingly, FRE produced highly significant ($P<0.01$) improvement in analgesic activity of the diclofenac. If we look for the reasons for the enhanced activity with a sub therapeutic dose of FRE, it appears that it might be due to increase in bioavailability of diclofenac. This enhancement may be either due to increase in absorption or reduction in metabolism of drug.

CONCLUSION

Our study concludes that FRE endowed with peripheral analgesic properties in dose dependent manner. It also enhances the analgesic effect of the standard drugs.

However, further study is needed in order to understand the precise mechanism. In future experiments, studies can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the peripheral analgesic effect.

ACKNOWLEDGEMENTS

We are grateful to Dr. Sanjay Vyas, Professor of Botany, Holkar Science College, Indore [M.P] for his help in Identification of leaves of *Ficus Racemosa* tree.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Rai N, Vyas S, Phadnis P. Evaluation of analgesic activity of *Ficus racemosa* leaf extract using acetic acid induced writhing method in mice. Int J Basic Clin Pharmacol 2016;5:60-4.