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Research Article

TO EVALUATE THE ANALGESIC ACTIVITY OF FICUS RACEMOSA LEAF EXTRACT IN ALBINO MICE USING EDDY'S HOT PLATE METHOD.

NIKET RAI[1*], SAVITA VYAS[1], PRADEEP PHADNIS[1]

[1]Department of pharmacology, M.G.M. Medical College, Indore, M.P. -452001, India. Email: drniketrai@gmail.com

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ABSTRACT

Objective-To evaluate the analgesic activity of ficus racemosa leaf extract in albino mice and its comparison with standard drug using Eddy's hot plate method.

Method-Chloroform leaf extract was obtained using soxhlet apparatus. Isolated fraction of extract (FRE) was used for the experiments. Swiss albino mice of either sex, average weight 20-25gms were used for experiments. Animals were divided into 6 groups consisting of 6 animals each. In hot plate method, Basal reaction time was recorded. Each group received particular treatment and reaction time was recorded after half, one, two and four hours. Pentazocine was taken as standard drug.

Result-FRE at the dose of 50mg/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 200mg/kg showed significant analgesic activity as compare to pentazocin. Pentazocin in combination with 50mg/kg dose of FRE produced significant analgesic activity when compared to control value or either treatment alone.

Conclusion-FRE endowed with central analgesic properties in dose dependent manner. And also enhances the analgesic effect of pentazocin. However further study is needed in order to understand the precise mechanism.

Keywords: ficus racemosa, analgesics, pentazocin, albino mice, Eddy's hot plate.

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].Opioids 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 posses 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 of Ficus racemosa in mice using Eddy's hot plate method.

MATERIAL AND METHODS-

Drugs: Pentazocine- Fortwin® injection (Ranbaxy pharmaceuticals Ltd.), Potassium hydroxide pellets (Ranbaxy pharmaceuticals Ltd), Chloroform (Sara Fine Chemicals, Baroda), Ethanol (Bengal chemicals).

Equipments / instruments: Eddy's hot plate (Biotechnics India), Analytical balance (AND Japan), Electronic weighing machine (Eagle India).

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 [M.P.]. 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 100gm 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 color 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 [M.P.]. They were kept under standard hygienic conditions, at 20 ± 2^0C temperature, relative humidity (60 ± 10%) with 12 hour 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.

Ethical approval-The study was approved by the Institutional Animal Ethics committee (IAEC), M.G.M. Medical College, Indore. *(IAEC approval no. 03 dated 31/07/10.*

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. 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 Eddy's hot plate method.

In the hot plate model, the animals are placed on the Eddy's hot plate which consists of an electrically heated surface. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics do not generally affect these responses [13].

Drugs/Groups: Group I 2% gum acacia (10ml/kg, p.o.), Group II pentazocine (5mg/kg, p.o.) , Group III FRE (50 mg/kg, p.o.), Group IV FRE (100 mg/kg, p.o.), Group V FRE (200 mg/kg, p.o.) and Group VI pentazocine and FRE (5mg/kg + 50mg/kg, p.o.).

Procedure: Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1oC. Responses such as jumping, withdrawal and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time [14].

The time obtained was considered the basal/normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals.

After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0.5, 1, 2 and 4 hrs interval after drug administration.

Statistical analysis

Results were expressed as mean ± SEM and analyzed 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.

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

Steroid	+
Alkaloid	+
Tannin	++
Carbohydrate	-
Gum	+
Glycoside	-
Flavonoid	+++
Saponin	-

+ present, - absent.

Table 2: **Effect of FRE, pentazocine and their combination in mice using the "Eddy's hot plate method"**

Groups	Dose	Pre Treatment	Reaction time in seconds 0.5hr	1 hr	2 hr	4 hr
Control (2%gum acacia)	10 ml/kg	3.70±0.30	3.37±0.44	4.16±0.22	4.34±0.36	4.06±0.52
Pentazocine	5 mg/kg	3.89±0.24	6.44±0.72*	7.06±0.61*	7.47±0.67*	7.10±0.38*
FRE_{50}	50mg/kg	3.34±0.30	3.57±0.45	4.10±0.06	4.97±0.31	5.31±0.97
FRE_{100}	100mg/kg	3.53±0.27	6.19±0.71*	6.92±0.76*	7.51±0.64*	7.33±0.52*
FRE_{200}	200mg/kg	3.57±0.32	6.62±0.57*	7.13±0.64*	7.84±0.40*	7.62±0.25*
Pentazocine +FRE_{50}	5+50 mg/kg	3.65±0.44	8.80±0.35*†	9.39±0.23*†	9.87±0.06*†	9.52±0.24*†
One way ANOVA	F	0.32	13.33	16.57	19.47	12.36
	P	>0.05	<0.05	<0.05	<0.05	<0.05

One way ANOVA followed by multiple tukey's comparison test.

Values are mean ± SEM, n= 8 in each group, df = 5, 24

* P< 0.05 when compared to control group

† P< 0.05 when compared to pentazocine group

RESULTS

[Table 2] FRE at a dose of 50mg/kg did not show any significant increase in reaction time when compared to control or pentazocine group (P>0.05). FRE at a dose of 100 and 200mg/kg showed significant increase in reaction time as compared to control group (P<0.05). The peak effect was seen at 2 hrs. But analgesic effect of FRE at a dose of 100 and 200mg/kg was not significantly better than pentazocine (P>0.05). Pentazocine showed a significant increase in reaction time as compared to control group which lasted for whole study period (P<0.05). The peak effect of pentazocine was seen at 2

hrs (7.47 sec) and after 4 hrs the effect started to decline (7.10 sec). Pentazocine in combination with FRE_{50} produced highly significant increase in reaction time as compared to control or either of the treatment alone (P<0.01).

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 [15].

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 Eddy's hot plate model for central analgesic activity.

The FRE (100 and 200 mg/kg) and pentazocine (5 mg/kg) presented a longer latency time than the control group in the hot plate test in a dose related manner [Table no. 2]. The hot plate method is considered to be selective for the drugs acting centrally. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity [16]. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [17]. Therefore, FRE must have a central activity. 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 pentazocin 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 pentazocin. 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 pentazocin. This enhancement may be either due to increase in absorption or reduction in metabolism of drug.

CONCLUSION

Our study concludes that FRE endowed with central 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 central analgesic effect.

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