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Original Research Article

Investigation of Analgesic, Anti-inflammatory and antipyretic potential of ethanolic extract of arial parts of *Flemingia chappar* Graham

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ABSTRACT:
The principal aim of the investigation was to explore the analgesic and antiinflammatory potential of ethanolic extract of aerial parts of Flemingia chappar Graham. Results of acute toxicity studies as per OECD guidelines 420 and 425 and subacute toxicity studies as per OECD guidelines 407 suggest that extract can be considered under category 5. Analgesic potential was investigated on male swiss albino mice by hot plate, tail flick, tail immersion, tail clip and acetic acid induced writhing methods. Anti-inflammatory effect was tested on wistar rats using carragenan, dextran, histamine and cotton pellets as inducing agents. Antipyretic activity was studied on normal body temperature and yeast induced pyrexia on rats. With the help of Infra-red, NMR and mass spectrums the isolated compounds FAC_1, FAC_2, FAC_3 and FAC_4 was found to be β-Sitosterol, Quercetin, Acacetin and Rutin respectively in appreciable quantities, which may plausibly responsible for the activities studied.

Key words: Flemingia chappar, antiinflammatory, analgesic, flavanoids, glycosides.

Introduction

Inflammation and pain is the condition results as responsive reaction of vascularised living tissue to local injury [(1)]. The inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischemia, antigen–antibody interactions and thermal or other physical injury. Each type of stimulus provokes a characteristic pattern of response that represents a relatively minor variation. The response usually is accompanied by some familiar clinical signs such as erythema, edema, hyperalgesia and pain. A large number of NSAID's as potential analgesics and anti-inflammatory agents are used in the market. However, on chronic usage majority of NSAIDs produces acute adverse reactions on GIT, liver and kidneys, hence necessitated Scientists across the world to search for safer herbal alternatives with analgesic and anti-inflammatory effects. Many herbal formulations were introduced into market with greater patient compliance.

Flemingia chappar are erect Shrubs, ca. 1 m tall. Branchlets slender, densely brown villous. Leaves simple; stipules narrowly ovate, ca. 2 mm, deciduous; petiole ca. 1.5 cm, densely deciduous brown villous; leaf blade orbicular-cordate, 4-4.5 cm, papery or thinly leathery, glabrous or sparsely pubescent except veins, abaxial surface

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with dense, orange, sessile glands, basal veins 3, lateral veins 3 pairs, base slightly cordate, apex rounded or obtuse. Inflorescence an axillary or terminal thyrse; inflorescence axis 3-7 cm, densely brown hairy; cymules each enclosed by concave bract; bracts 1.5-2 × 1.8-3.8 cm, membranous, glabrous, with obvious reticulate veins, persistent, apex emarginate. Flowers are 6-9 mm; pedicel 0.4-1.6 cm and densely hairy. Calyx is 5-lobed, 4-5 mm, densely pubescent, with orange glands; lobes lanceolate, subequal to tube. Standard with lobe as long as broad, contracted above auricles, and obovate or obcordate; wings oblong; keel slightly curved. Legume elliptic, 10-15 × 6-10 mm, densely brown villous. Fl. Dec-Mar, fr. Mar-May.

Traditional healers of Jashpur region of chhattisgarh uses roots of *Flemingia chappar* in the treatment of epilepsy,diabetes, insomnia, acidity and stomach disorders. In Bihar the people of santhal tribes use 1 to 2 drops of juice extracted from pressed seeds put in the eyes as a remedy in eye troubles and to remove cataract. In Madhya Pradesh the *Flemingia chappar* Ham is known as Galphule, in Gamharia (Raigarh) of Madhya Pradesh, the leaf juice mixed with seven drops of mustard oil and a little amount of jaggery is used in eye pain by the tribal people. Leaves collected in the month of April were used in this study.

In the current research, an effort was made to study the analgesic and anti-inflammatory potential of ethanolic extract of *Flemingia chappar* Graham by following standard pharmacological screening methods. Preliminary phytochemical investigation was carried out on the extracts in order to derive plausible scientific evidence to substantiate its pharmacological potential.

Materials and methods

Plant material

Fresh arial parts of *Flemingia chappar* Graham was obtained during April 2009 from Madhya Pradesh India. The plant was authenticated by Dr. B.Prathibha Devi, Professor & Head, Department of Pharmacy, Osmania University, Hyderabad. A.P. and the specimens have been preserved in our research lab (GPWKDP/2009/YP/01). The collected plant was cleaned immediately and shade-dried for a week, powdered mechanically, sieved (10/44) and stored in airtight containers.

Extraction

About 5000 grams of crude drugs were extracted using AR grade solvents Petroleum ether (60 – 80°C), Benzene, Chloroform, Acetone, Ethyl acetate and Ethanol (95%) by successive soxhlation method until the phytoconstituents were completely exhausted. All the extracts were concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50°C, comminuted in a ball mill and preserved in air tight containers kept in desiccators prior to its studies and labeled as FCE.

Preliminary phytochemical investigation:

A preliminary phytochemical investigation was carried out for all the extracts obtained from the *Flemingia chappar* Graham[(2)] using analytical grade chemicals, solvents and reagents. The respective yields and the preliminary phytochemical investigation results were given in Table 1.

PHARMACOLOGICAL STUDIES

Acute toxicity studies: OECD Guidelines No. 420[3]

Female wistar rats (nulliparous and non-pregnant) of 8 to 10 weeks old weighing 200 – 250gms supplied by National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water *ad libitum.* The studies were performed according to OECD Guidelines 420 and the protocol was approved by the Institutional Animal Ethics Committee (Reg. No. VNCP/1472/PO/a/CPCSEA).

Sighting study

Animals were fasted over-night prior to dosing and weighed. The test substance was administered to single animals in a sequential manner following the flow charts in Annex 2 of OECD 420. The starting dose for the sighting study was selected from the fixed dose levels of 300 mg/kg (as there is no evidence from *in vivo* and *in vitro* data). The next dose used for this study was 2000 mg/kg. The Test substances were administered in a constant volume of 2 mL/100g body weight in the form of suspension. After the substance has been administered, food was withheld for a further 3-4 h. A period of at least 24 hours was allowed between the dosing of each animal. All animals were observed for at least 14 days.

Main study

A total of five female wistar rats were used for each dose level investigated and the animals were made up of one animal from the sighting study dosed at the selected dose level together with an additional four animals. The time interval between dosing at each level was 3 or 4 days.

Acute toxicity studies: OECD Guidelines No. 425[4]

Animals were divided into two groups of 3 animals each. Group I was treated with vehicle (distilled water) and was kept as a control. Group II was treated with 5000 mg/kg dose according to their body weight. Blood and tissue were collected on 14th day. Hematological and biochemical parameters were measured in treated group as well as in control group. The organs were quickly blotted and weighed in a digital balance. Gross necropsy of heart, liver and kidney were observed.

Sub acute toxicity studies: OECD Guidelines No. 407[5]

The plant extract at the dose of 250, 500 and 1000 mg/kg body weight were administered orally to 4 groups of six rats respectively to every 24 h for 28 days and control received vehicle at the same volume. The toxic manifestation such as body weight, mortality, and food and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with ether. The heparinised blood samples were collected for determining haematological parameters and the serum from non-heparinised blood was carefully collected for determining clinical blood chemistry. Animals were sacrificed after blood collection and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examination.

Analgesic activity:

Analgesic activity of ethanolic extract of *Flemingia chappar* Graham (FCE) at doses 100,200 and 400 mg/kg,

p.o was studied by five different methods.

a. Hot plate method

The study was carried out according to the method of Eddy [6]. Mice that showed nociceptive responses within 10 sec, when placed on a Eddy's hot plate (Techno, Lucknow, India) maintained at 55 ± 0.5 °C were selected for study. The mice so selected were then grouped into five (6 in each group) namely I, II, III, IV and V. The group I was treated with 2% v/v, aq. Tween 80, 10 ml / kg p.o which served as control and the II, III and IV groups were treated with the FCE 100, 200 and 400 mg/kg, p.o respectively and group V was treated with morphine 2 mg/kg s.c. After 30 minutes of the above treatment each mouse was placed gently on the hot plate maintained at 55 ± 0.5 °C and the reaction time was noted. The reaction time was taken as the time interval between the animals placed on the plate till the moment it began to lick its forepaws or jump. Four consecutive trials after a gap of 5 minutes were done and the mean value was calculated.

b. Tail Immersion Method:

The study was performed according to the method of Luiz et al [7]. The animals were treated and grouped similarly as described in hot plate method. Each mouse was held in position in suitable restrainer with the tail extending out. After 30 minutes of the above treatment each mouse 3-4 cm length of the tail was marked and immersed in the water bath thermostatically maintained at 51°C. The withdrawal time (in seconds) of the tail from hot water was noted as the reaction time/tail flick latency. Four consecutive trials after a gap of 5 minutes were done and the mean values were calculated.

c. Tail flick method

The method described by Kulkarni [8] was followed in this experiment. The animals were treated and grouped similarly as described in hot plate method except group V was treated with aspirin 20 mg/kg, p.o. After 30 minutes of the above treatment the basal reaction time for each mouse was noted by placing the tip (last-1 - 3cm) of the tail on the radiant heat source of the analgesiometer (Techno, Lucknow, India) and the time of withdrawal of tail from the heat source (Flicking response) shown within 5 -6 sec were selected for study. A cutoff period of 10 - 12 sec was observed to prevent the damage to the tail. Four consecutive trials after a gap of 5 minutes were done and the mean value was taken.

$$\text{Inhibition (\%)} = \frac{\text{Reaction time (Control)} - \text{Reaction time (Treated)}}{\text{Reaction time (Control)}} \times 100$$

d. Caudal compression (Tail clip method)

The method followed in this study was as described by Bianchi and Franceschini [9]**.** The animals were treated and grouped similarly as described in Tail flick method. The pressure exerted by the clip was so adjusted that it was just sufficient to respond in all mice. All the mice were screened by applying a metal artery clip to the base of the tail with its jaw sheathed with thin rubber tubing. The animals that did not attempt to dislodge the clip within 4-5 seconds were discarded for the experiment. The grouping and treatment of animals was as followed in hot plate method. The time to dislodge the clip of each mouse was noted. Four consecutive trials after a

gap of 5 minutes were done and the mean value was taken.

% Inhibition = [Post-treatment Latency] − [Pre-treatment Latency] X 100 / [Cut-off Time − Pre-treatment Latency]

e. Acetic acid induced writhing test

The method described by Koster *et al* [10] was followed in this study. The animals were treated and grouped similarly as described in Tail flick method. Thirty minutes after the above treatment each mouse was injected 10 ml / kg of 0.7 % aqueous acetic acid intraperitoneally. Each mouse was placed in a plastic transparent observation cage and number of abdominal constriction was cumulatively counted from 5 to 15 minutes. Results were expressed as percent inhibition of analgesia.

Inhibition (%) = Average number of writhes (Control) – Average number of writhes(Test) ×100 / Average number of writhes (Control)

Anti inflammatory activity

Anti-inflammatory activity of ethanolic extract of *Flemingia chappar* Graham (FCE) at doses 100, 200 and 400 mg/kg, p.o was studied by four different methods.

a. Carrageenan – induced rat paw edema

The study was conducted according to the method of Winter *et al* [11]. Male albino Wistar rats weighing 100 – 250 g were housed in wire netted cages in a controlled room temperature 22 ± 1ºC, relative humidity 60 – 70 % and with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum.* The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in the experiments were of analytical grade. Five groups of six animals each were used for the experiment. Group I of animals were administered with 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extact of *Flemingia chappar* (FCE) 100, 200 and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was treated with Indomethacin 20 mg/kg, p.o. One hour after oral administration, edema was induced by subplantar injection (left hind paw) of 0.1ml of 1% freshly prepared suspension of carragenan (Sigma Chemical Co., USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured at 3 hour after induction of inflammation using Plethysmometer. The percent inhibition of inflammation were calculated by using formula

Percentage of inhibition inflammation= (A-B/A) X100

Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

b. Histamine induced rat paw edema

In this model edema was induced by subplantar injection (hind paw) of 0.05ml of 1% w/v, freshly prepared solution of histamine to all animals, which were grouped and treated similarly as followed in carragenan induced rat paw edema method. The volume of the injected and the contra lateral paws were measured 3 h after induction of inflammation using Plethysmometer according to the method described by Winter *et al* [11].

c. Dextran induced rat paw edema

In this model edema was induced by subplantar injection of 0.05 ml of

freshly prepared 1% w/v solution of dextran into the right hind paw of the rats, which were grouped and treated similarly as followed in carragenan induced rat paw edema method [12].

Inhibition (%) = Increase in paw oedema (control) – Increase in paw edema (treated)×100

Increase in paw oedema (control)

d. Chronic test

Four groups of six animals each were used for the experiment. The rats were anaesthetized under ether anesthesia and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. Group I animals was given 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extract of *Flemingia chappar* Graham(FCE) at 100,200 and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was given with the standard drug Indomethacin (20 mg/kg, p.o). The treatment was continued for seven consecutive days from the day of cotton pellets implantation. The animals were anaesthetized again on 8th day and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37˚C for 24 h and dried at 60˚C to constant weight. The increment in the dry weight of the cotton pellets was taken as a measure of granuloma formation [13].

Antipyretic activity:

Male albino Wistar rats weighing 200–250g were housed in wire netted cages in a controlled room temperature 22±1˚C, relative humidity 60–70 % and with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum.* The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in this experiment were of analytical grade.

Effect on Normal body temperature in rats

Four groups of six animals in each group were used in the study. The initial rectal temperatures were recorded by inserting thermocouple to a depth of 2 cm into the rectum. Rectal temperatures were recorded at 1, 2, 3, 4, and 5 hrs[14].

Effect on yeast induced pyrexia in rats

The room temperature was maintained at 22 – 24ºC throughout the experiment. Initial rectal temperatures for all the rats were recorded as described above. A 20% suspension of 20 ml/kg Brewer's yeast in normal saline was given as subcutaneously to induce fever[44]. The site of injection was massaged gently in order to spread the suspension beneath the skin. Animals were fasted after the administration of yeast. Eighteen hours post challenge; the rise in rectal temperature was recorded. Animals that showed an increase of 0.3–0.5^{0}C in rectal temperature were selected for further studies. Five groups of six animals in each group were taken. Rectal temperatures were recorded at 1, 2, 3, 4 and 5 hrs post dosing[14].

Statistical analysis

All results were expressed as the mean ± SEM. The results were analyzed for statistical significance by one way ANOVA test using computerized GraphPad InStat version 3.05, Graph pad software Inc., San Diego, U.S.A.

Isolation of phytochemical constituents from FCE

Preparation of sample

Sample preparation is the most important step in the development of

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analytical methods for the analysis of botanicals and herbal preparations. The ethanolic extract of the test extract (FCE) were fractioned by extraction with petroleum ether (40°- 60°C) (fraction-I), ethyl ether (fraction-II) and ethyl acetate (fraction-III) in succession. Each of the steps was repeated thrice to ensure complete extraction. Fraction III of each of the test samples was hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 5 hours. The mixture was filtered and the filtrate was extracted thrice with ethyl acetate in a separating funnel. All the ethyl acetate layers were mixed, washed with distilled water several times until neutrality is attained, and then concentrated in vacuon and then subjected to chromatographic examination.

Preparation of column

A glass column (60cmX6cm), stationary phase (Silica gel G (0.2-0.3 mm thick) and mobile phase (Ethanol: Ammonium hydroxide, 200:1) was used for the isolation procedures. The glass column was cleaned thoroughly in water and then rinsed with acetone and fixed vertically in a stand. A wad of glass wool is placed at the bottom of the column to prevent the passage of solid support. Slurry of silica gel G was prepared with the mobile phase in a beaker and poured into the glass column slowly to ensure even packing. 2 -3 cm of solvent system is always maintained at the top of the column to avoid the drying. Fresh solvent system Ethanol: ammonium hydroxide, 200:1 is poured on the top of the column and eluted once; 2 cm of solvent system is maintained at the top of the column.

Sample loading

20 gms of the sample was weighed and mixed with silica gel G and kept air dried, the dried material was loaded on the top of the column and a wad of glass wool was placed above it to prevent the disturbance of solvent addition. Now the mobile phase was poured slowly at the top of the column and observed for the separation. The flow rate was adjusted to 30 drops per minute. 50 ml each of the eluents were collected in small beakers. 121 such fractions were collected and the volumes of the eluents were reduced by evaporation.

TLC Profiles of the eluents

Thin layer chromatographic characterization has been performed for all the fractions individually. Sample from each fraction was separately applied 1 cm above the edge of the TLC plates (Merck) along with standard reference samples. These plates were developed in an airtight chromatography chamber containing about 200 ml of solvent mixture of benzene, acetic acid and water (125:72:3). The developed plates were air dried and visualized under UV light after exposure to ammonia fumes by placing at the mouth of bottle containing concentrated ammonium hydroxide held in contact with each spot for about 5-10 seconds. Fluorescent spots corresponding to that of standard markers were marked. The developed plates were sprayed with 5% ferric chloride solution, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber to observe the colour of the spots. R_f values were calculated for isolated samples and compared with coinciding standard. The fractions showed similar hR_f values were mixed together. The combined fractions having similar hR_f value were subjected to the separation process.

Preparative thin layer chromatography (PTLC)

Glass plates (20 x 20 cm) thickly coated (0.4-0.5 nm) with silica gel 'G'

(BDH, 500 µ thick) and activated at 100°C for 30 minutes and cooled at room temperature were used for preparative thin layer chromatography (PTLC). The combined fractions having hRf values were applied on separate plates and the plates were developed in an airtight chromatography chamber containing about 200 ml of solvent mixture of benzene, acetic acid and water (125: 72:3), air dried and visualized under UV light. Each of the spots coinciding with those of standard reference compounds was marked. The marked spots were scrapped and collected separately along with the silica gel 'G' and eluted with ethanol.

Purification of the eluent

Eluted fractions were filtered, dried and again cochromatographed with standard markers. The marked spots were scrapped and collected separately along with the silica gel 'G' and eluted with ethanol. The eluent was subjected to repeated filtration through whatman filter paper saturated with ethanol to ensure complete separation from silica gel G. The fraction was left air dried, the residue was collected and subjected to single crystal growth.

Procedure for obtaining single crystal

The residue obtained was completely dissolved in HPLC grade Ethanol, and single crystal development was assisted by slow diffusion of benzene to this solution, stoppered tightly and kept undisturbed. After 7 days, the crystals observed were gently removed from the mother liquor and washed with benzene, weighed and packed individually in separate vials.

Analysis of isolated compounds

Physico chemical properties of the isolated material:

Physical properties such as Colour, odour, melting point, solubility in different solvents were recorded. Chemical nature of the compounds was also recorded by subjecting to different qualitative chemical tests. The IR spectra of the isolated compounds were recorded in JASCO – FTIR Spectrophotometer using potassium bromide disc in the region $2000cm^{-1}$ to $200cm^{-1}$. The ^{1}H NMR, ^{13C} NMR spectrums of the isolated compounds was recorded in Bruker DPX-300 NMT Spectrometer using $CDCl_3$ as solvent and respective internal standards. Mass spectrums of isolated compounds were recorded in JEOL JMS 600 in FAB mode.

RESULTS AND DISCUSSION

Results in Table 1 suggest that the plant contain several chemical constituents such as steroids and flavanoids in appreciable quantities.

Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 days. All observations were systematically recorded individually for each animal. Observations include changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Individual weights of animals should be determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and recorded. At the end of the test surviving animals were weighed and then humanely killed. All animals were subjected to gross necropsy and pathological changes were recorded. Microscopic examination of organs was

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also done for evidence of gross pathology in animals surviving 24 or more hours after the initial dosing.

Table 1. Results of preliminary phytochemical studies.

Constituent	FCP	FCC	FCA	FCEA	FCE
% Yield (w/w)	1.6	2.5	4.9	6.4	16.9
Alkaloids					
Mayer`s test	-	-	-	-	-
Dragendorff`s test	-	-	-	-	-
Wagner`s test	-	-	-	-	-
Hager`s test	-	-	-	-	-
Carbohydrates					
Molish`s test		+	++	++	+++
Fehling`s test		+	++	++	++
Benedict`stest		+	++	++	++
Glycosides					
Borntrager`s test	-	-	-	-	-
Saponins					
Foam test	+	+	+	+	+++
Flavonoids					
Ferric chloride test	+	+	+	++	++
Shinoda test	+	+	+	+	+
Lead acetate test	+	+	+	+	+
Alkaline reagent test	+	+	+	+	++
Steroids					
Libermann-Burchard test	+	+	+	+	+++
Salkowski test	+	+	+	+	+++
Proteins					
Millon`s test	++	++	++	++	++
Biuret test	+	+	+	+	++
Ninhydrin test	++	++	++	++	++

Results of Acute toxicity studies of FCE were conducted as per OECD guidelines 425. The FCE did not show any sign and symptoms of toxicity or mortality up to 5000 mg /kg body weight on oral administration, thus these extracts could be considered as category 5. Body weight before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, behavioral pattern, sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were observed. The onset of toxicity and signs of toxicity were not seen in the rats upto 72 hr of observation period. This indicates the safety of extract. Hence, the 100, 200 and 400 mg/kg dose were selected for the further study.

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Table 2. Analgesic activity studies of FCE on male swiss albino mice

	Group I	Group II		Group III		Group IV		Group IV	
Material administered	2%Tween 80	FCE						Morphine	
Dose	10 ml/kg	100 mg/kg		200 mg/kg		400 mg/kg		2 mg/kg	
Route of administration	Oral							Subcutaneous	
Method	Reaction time in sec. (mean ± SEM)	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition
Hot plate	6.4 ± 0.8	7.8 ± 0.3*	21.88	9.2 ± 0.2*	43.75	11.1 ± 0.4*	73.44	11.80 ± 0.32*	84.38
Tail Immersion	2.2±0.1	4.2±0.17**	90.91	4.9±0.2**	122.73	5.0±0.2**	127.27	5.6±0.311**	154.55
Tail flick	3.3 ± 0.3	4.9 ± 0.19*	48.48	5.6 ± 0.2*	69.70	5.8 ± 0.3*	75.76	6.16 ± 0.42*	86.67
Tail clip	5.1 ± 0.4	6.6 ± 0.24*	29.41	7.3 ± 0.6*	43.14	8.7± 0.6*	70.59	9.16 ± 0.41*	79.61
	Material administered							Aspirin 20 mg/kg p.o.	
Acetic acid induced writhing	53.3 ± 3.5	25.9 ± 2.44*	51.41	21.5 ± 2.1*	59.66	16.9 ± 3.2*	68.29	12.76 ± 3.46*	76.06

Figures in parentheses indicate the percentage inhibition of pain compared to control. N= 6 *p< 0.001,**p<0.05>0.02: Student's t-test.

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Table 2 and Fig 1 represent the results of analgesic activity studies by five different methods. Several tests (acute and sub -acute) which differ with respect to stimulus quality, intensity and duration, were employed in evaluating the analgesic effect of the FCE to ascertain the analgesic properties of a substance using behavioural nociceptive tests [12]. In the hot plate method, the test drug FCE showed 21.88 & 43.75% and 73.44% of inhibition at the doses of 100,200 and 400 mg/kg respectively, whereas the percent inhibition for morphine was 84.38. The effect of test drug FCE on tail flick test was observed as 48.48%, 67.7% and 75.76% for 100,200 and 400mg/kg respectively whereas morphine showed 86.67% of inhibition under similar conditions. In tail clip method, the test drug FCE showed 29.41, 43.14% and 70.59% inhibition at the doses of 100,200 and 400 mg/kg respectively. In Tail immersion method, the withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The tail flick latency of FCE at 100 mg/kg at 60 minutes was comparable with that of standard drug morphine 2 mg/kg s.c. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The test drug FCE showed significant effect in various acute (phasic) pain models, namely, hot plate, tail flick and tail clip tests suggest that the effect on these pain models may act via centrally mediated pain control.

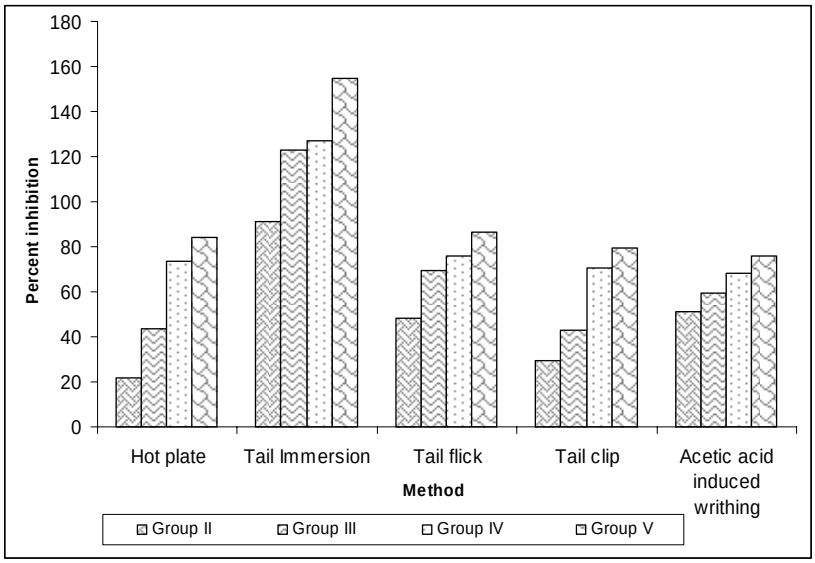

Fig.1. Analgesic activity of FCE on male swiss albino mice

The abdominal writhing response induced by acetic acid is sensitive process to establish peripherally acting analgesics. Local peritoneal receptors are responsible for abdominal writhing action. Intraperitoneal administration of acetic acid causes an increase in of PGE_2 and $PGF_2\alpha$ and produce analgesia by inducing capillary permeability and liberating endogenous substances like serotonin, histamine, prostaglandins, bradykinin, and substance P that sensitize pain nerve endings. It has been suggested that acetic acid stimulates the valinoid receptors and bradykinin B_2 receptors in the pathway comprising sensory afferent C-fibers [13]. Therefore, the observed

activity may be due to interfering the synthesis or release of endogenous substances or desensitization of nerve fiber which carry pain sensation. In Acetic acid induced writhing assay the test drug FCE at the doses of 100, 200 and 400 mg/kg p.o. exhibited 51.41, 59.66 & 68.29% of inhibition respectively. The commercial drug Aspirin at the dose of 100 mg/kg p.o. exhibited 76.06% inhibition under similar experimental conditions. The results suggest that FCE also possess significant peripherally mediated analgesic effect. Hence it can be concluded that the FCE possesses analgesic properties, which are mediated via peripheral and central inhibitory mechanisms.

The results of anti-inflammatory studies for four different models were summarized in Table 3 and Fig 2. Most of the investigators reported that inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents [16]. The sub planter injection of carrageenan (1% w/v) developed edema of high intensity and persisted for 3 h after injection in the control groups. The oral administration of FCE at the doses of 100, 200 and 400 mg/kg p.o. showed significant and dose dependent inhibition (31.86, 43.76 and 50.09% respectively). The commercial anti-inflammatory drug, Indomethacin showed 55.68% of inhibition at the dose of 20mg/kg p.o. The development of carrageenan induced oedema is bi-phasic. The first phase is attributed to the release of histamine, serotonin and kinins, whereas, the second phase is related to the release of prostaglandins [11, 17]. The inhibitory action of the drug (FCE) on carrageenan induced paw edema in rats may be mediated through either any of the mediators alone or in combination. Hence FCE was further investigated against paw edema induced by individual agents like Histamine and Dextran and showed a maximum inhibition of and 47.36, 36.45% respectively at the dose of 400 mg/kg. The drug FCE also exhibited significant anti–inflammatory effect in the cotton pellet induced granuloma test (58.51% for 400mg/kg, p.o.). This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation [18, 19]. It was observed that the gain in weight of the pellets was linear with the time. This linearity was continued for eight days and then leveled off. Therefore, seven days was chosen for the experiments[11]. Results suggest that the FCE at doses of 100, 200 and 400 mg/kg p.o. significantly reduced the edema produced by several inducers and are comparable with many standard drugs suggested in each model. It has been reported by many researchers that flavanoids inhibit eicosanoids synthesis by inhibiting both cyclooxygenase and lipoygenase activities [20,21], as well as hamper the non enzymatic peroxidation of polyunsaturated fatty acids required for the activation of these oxygenases [22]. Quercetin and other flavonoids inhibit leukotrienes synthesis and histamine, prostaglandins release, as well as acts as superoxide scavengers [23].

Fever may be a result of enhanced formation of cytokines such as IL - 1β, IL – 6, interferons α and β and TNFα that increases the synthesis of PGE2 in hypothalamic area. Increase in cyclic AMP which in turn triggers the hypothalamus to elevate body temperature by promoting increased heat generation and decreased in heat loss. Regulation of body temperature requires a delicate balance between the production and loss of heat. Hypothalamus regulates the set point at which body temperature is maintained. In the present study, the antipyretic effect of FCE was studied on normal body temperature and yeast induced pyrexia.

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Table 3. Anti-inflammatory activity studies of FCE on male albino Wistar rats

	Group I	Group II		Group III		Group IV		Group V	
Material administered	2%Tween 80	FCE						Indomethacin	
Dose	10 ml/kg	100 mg/kg		200 mg/kg		400 mg/kg		20 mg/kg	
Route of administration	Oral								
Inducing agent	Paw volume after 3 hours (mean ± SEM)	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition
Carrageenan	53.7 ± 3.91	36.59 ± 2.34 **	31.86	30.2 ± 2.57 *	43.76	26.8 ± 1.84 *	50.09	23.8 ± 2.02 *	55.68
Histamine	47.3 ± 2.56	32.9 ± 1.34*	30.44	28.2 ± 1.24*	40.38	24.9 ± 1.3*	47.36	24.7 ± 0.9*	47.78
Dextran	41.70 ± 2.34	31.28 ± 1.23**	24.99	29.55 ± 1.16**	29.14	26.5 ± 1.6**	36.45	26.60 ± 1.54*	36.21
	Weight of granuloma formation (mg)	Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)	
Cotton wool	80.16 ± 5.78	51.6 ± 4.48*	35.63	39.57 ± 5.61*	50.64	33.26 ± 3.21*	58.51	31.16 ± 4.49*	61.13

p - value was calculated by comparing with the control by students t-test, **p*< **0.001,*****p*<0.05>0.02:N=6

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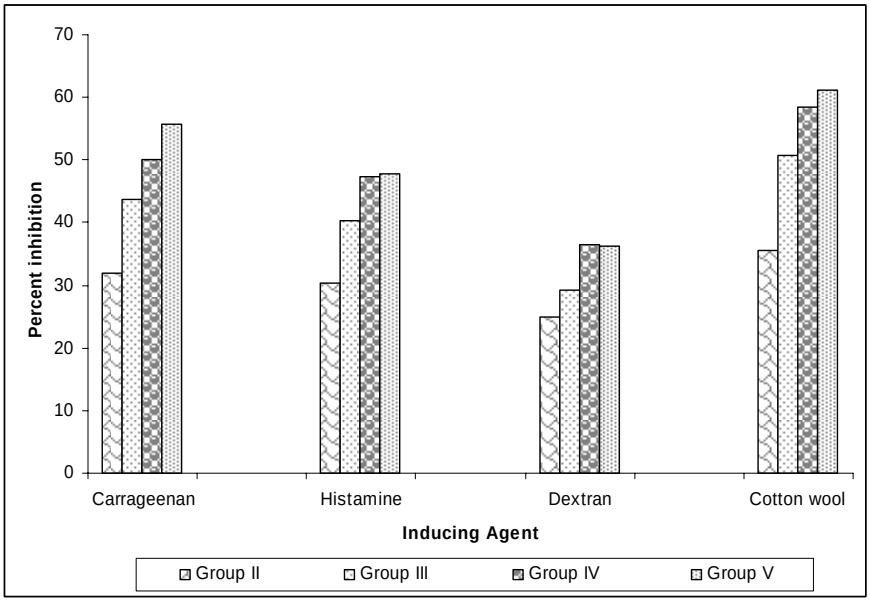

Fig 2: Anti-inflammatory activity of FCE on male albino Wistar rats

Table 4: Effect of FCE & GAE on normal body temperature in rats

Group	0h	1h	2h	3h	4h	5h
I	37.93±0.01	37.63±0.01	37.47±0.03	37.34±0.05	37.67±0.03	37.67±0.01
II	37.66±0.04	37.56±0.03	37.74±0.04	37.98±0.08	37.58±0.03	37.28±0.03
III	37.52±0.09	37.32±0.04	37.37±0.04	37.65±0.06	37.52±0.06	37.57±0.07
IV	37.26±0.03	37.35±0.05	37.35±0.02	37.57±0.04	37.47±0.08	37.59±0.09

Table 5: Effect of FCE & GAE on Yeast induced Pyrexia in rats

Group	0h	19h	20h	21h	22h	23h	24h
I	37.93±0.01	39.22±0.07	39.02±0.02	39.12±0.10	39.6±0.06	39.7±0.12	38.58±0.1
II	36.82±0.02	39.12±0.02	37.3±0.06(↓79.13)	37.1±0.22(↓87.83)	36.9±0.09(↓96.52)	36.8±0.05(↓100.87)	36.8±0.01(↓100.87)
III	37.52±0.01	39.22±0.04*	38.99±0.3*(↓13.53)	38.45±0.36*(↓45.29)	38.34±0.2*(↓51.76)	38.23±0.10*(↓58.24)	38.14±0.02*(↓63.53)
IV	37.32±0.02	39.29±0.01*	38.80±0.07*(↓24.87)	38.31±0.21*(↓49.75)	38.15±0.12*(↓57.87)	37.92±0.01*(↓69.54)	37.84±0.02*(↓73.60)
V	37.22±0.04	39.60±0.1**	38.60±0.09*(↓42.02)	38.2±0.23*(↓58.82)	38.05±0.45*(↓65.13)	37.84±0.02*(↓73.95)	37.64±0.12**(↓82.35)

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Table No: 6. Isolation of Chemical constituents from FCE

Fraction no.	Weight of Residue (g)	Compound Isolated
1-10	1.880	waxy residue
11-13	1.235	waxy residue
14-20	0.960	greenish coloring matter
21-26	0.113	White powder FCE-1 (passed the test for steroids)
27-35	0.254	waxy residue
36-39	0.418	waxy residue
40-45	0.526	waxy residue
46-55	0.715	greenish coloring matter
56-62	0.570	Yellow powder FCE-2 (passed the test for flavonoids)
63-66	0.202	Yellow powder FCE-3 (passed the test for flavonoids)
67-71	0.070	Yellow powder FCE-4 (passed the test for flavonoids)
72-78	0.220	Intangible mass
79-84	0.110	Intangible mass
85-89	0.080	greenish coloring matter
90-98	0.524	greenish coloring matter
99-108	0.154	greenish coloring matter
109-116	1.095	greenish coloring matter
117-121	0.498	greenish coloring matter

CONCLUSION

Ethanolic extract of *Flemingia chappar* Graham was systematically evaluated for its analgesic and anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the FCE found to possess comparable efficacy with that of standard analgesics and anti-inflammatory drugs. With the help of Infra-red, NMR and mass spectrums the isolated compounds FAC_1, FAC_2, FAC_3 and FAC_4 was found to be β-Sitosterol, Quercetin, Acacetin and Rutin respectively in abundant quantities. *Flemingia chappar,* an abundantly available shrub in Madhya Pradesh is certainly a nature's treasure for mankind for prevention and treatment of inflammation associated with pain and fever.

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