IN VIVO AND IN VITRO INVESTIGATIONS OF PHARMACOLOGICAL POTENTIALS OF CASSIA OBTUSIFOLIA PLANT

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ABSTRACT

Objectives: Cassia obtusifolia L. belonging to the Family Caesalpiniaceae, proposed to have abundant pharmacological potential and widely consumed as laxative, diuretic, and stomachic. The aim of the present research was to study the anti-inflammatory, analgesic, antipyretic, and antioxidant potentials of C. obtusifolia plant.

Methods: Various acute and chronic animal models such as Carrageenan-induced paw edema, tail immersion method, acetic acid-induced writhing, and in vitro methods were used to study the profound pharmacological and antioxidant potentials.

Results: The results for pharmacological study were statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparisons using INTA software.

Conclusion: The present study reveals that C. obtusifolia possesses comparable anti-inflammatory, analgesic, antipyretic, and antioxidant potential.

Keywords: Cassia obtusifolia, Anti-inflammatory, Antioxidant, Carrageenan, Antipyretic.

INTRODUCTION

Drugs presently used for the management of pain and inflammatory conditions are either narcotics, for example, opioids or non-narcotics, for example, salicylates and corticosteroids, and for example, hydrocortisone. Being synthetically designed these drugs possess well-recognized side and toxic effects. On the contrary, many medicines from plant origin had been used for long time. Exploring the healing power of plants, people from centuries have been trying to alleviate and treat diseases with different plant extracts and formulations [1]. Plants represent a large intact source of structurally novel compounds that might serve as the basis for the development of novel drugs. Screening of the plants for their biological activity merely depends on their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a specific compound against a specific disease is a challenging extensive process [2]. Literature research for several medicinal plants possesses active constituents with significant anti-inflammatory and analgesic activities.

Cassia obtusifolia L. belonging to Caesalpiniaceae family, also known as sicklepod is extensively used as a traditional medicine [3]. The foremost active components of C. obtusifolia include anthraquinones, naphthopyrones, and lactones which are assumed to possess various medicinal potentials. The plant is also widely consumed as laxative, diuretic, stomachic, and digestive aid [4].

Hence, an attempt has been made to conduct studies for ascertaining anti-inflammatory, antipyretic, and analgesic potential of the medicinal plant C. obtusifolia using different animal models. Different in vitro methods were also used to study the antioxidant potential of C. obtusifolia plant.

METHODS

Chemicals
All AR grade solvents such as ethanol, ethyl acetate, sodium carbonate, sodium phosphate, hydrogen peroxide, and trichloroacetic acid were procured from Merck Life Science Private Ltd, Vikhroli (East), Mumbai, India. Gallic acid, Folin–Ciocalteu reagent;1, and 1-diphenyl-2-picrylhydrazyl (DPPH) were procured from SD-Fine Chem Ltd., Fischer Scientific Pvt. Ltd, Pune, India, and HiMedia Lab. Ltd., Mumbai, respectively. Marketed tablet formulations of indomethacin, pentazocine, and paracetamol were used as standard drugs that were procured from the local market.

Procurement and authentication of plant
The leaves of the plant were collected from fields of Sant Tukaram Nagar, Pimpri, Pune, and authenticated by the Botanical Survey of India, Pune, and were given the voucher specimen number SMK-1.

Extraction procedure
Leaves of C. obtusifolia Linn. (2 kg) were dried under shade and grinded to get the coarse powdered material. The extraction of the powdered leaves was carried out by the maceration (water) process. Then, the solution was filtered and to this filtrate, alcohol was added to get precipitate of the polysaccharides. Then, the resulting solution was filtered and the filtrate was evaporated to $1/4^{th}$ of the total volume. After evaporating $1/4^{th}$ of the total volume of the solution, it was successively extracted with ethyl acetate. The ethyl acetate extract was evaporated to get the brownish-yellow colored flavonoid-rich fraction of C. obtusifolia Linn. (FRCO) (1% w/w) which gave a positive response to the Shinoda test for the flavonoids [5-7].

Pharmacological screening

The pharmacological screening was performed on Swiss albino mice of either sex weighing 18–25 g and Wistar rats of either sex weighing 150–200 g at Padm. Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune. The animals were kept in the departmental animal house in well cross-ventilated room at 27±2°C, light and dark cycles of 12 h, respectively, for 1 week before and during the experiments. All the animals of either sex were housed in groups of five under standard laboratory conditions with free access to a standard pellet diet and water *ad libitum*. All experiments were conducted in accordance with the guidelines of the local animal ethical committee (Registration no. 198/CPCSEA).

Preparation of test solutions

FRCO was accurately weighed and suspended in distilled water using 1% gum acacia. Other standard solutions such as indomethacin, pentazocine, and paracetamol were suspended in normal saline solution.

Acute toxicity study

Acute toxicity study was carried out on Swiss albino mice which were divided into three groups, each containing six animals. Group I animals were treated with vehicle (1% gum acacia) and were served as control group, while the Groups II and III animals were administered with aqueous extract of *C. obtusifolia* and flavonoid-rich FRCO orally in varying doses of 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, and 2.50g/kg, respectively. The animals were continuously observed for 72 h to detect changes in the autonomic or behavioral responses such as alertness, spontaneous activity, irritability, and urination. Any mortality during experimentation and in the following 7 days was also recorded [8].

Anti-inflammatory activity

Anti-inflammatory activity was studied using acute and chronic anti-inflammatory models such as carrageenan-induced paw edema, acetic acid-induced vascular permeability, and cotton pellet-induced granuloma.

Carrageenan-induced paw edema

The acute hind paw edema was produced by injecting 0.1 ml of carrageenan (1% suspension in saline) locally into the subplantar aponeurosis of the right hind paw of rats. Animals were divided into five groups, six rats per group. FRCO was administered orally at different doses (50, 100, and 200 mg/kg) along with the standard drug indomethacin (10 mg/kg, p.o.) and 1% acacia solution (p.o.). FRCO and indomethacin were administered 1 h before injection of carrageenan in the right hind paw subplantar of each rat. The rat pedal volume up to the ankle joint was measured using plethysmometer (Ugo Basile, 7140, Italy) at 0, 1, 2, 3, 4, 5, 6, and 24 h after the injection of carrageenan and the percent inhibition was calculated using the formula;

$$\text{Percent inhibition} = Vc - Vt/Vc \times 100$$

Where Vc and Vt represent the mean increase in paw volume in control and treated groups, respectively [9].

Acetic acid-induced vascular permeability

The mice were intravenously injected with 10 ml/kg of 1% Evans Blue dye solution in saline, followed by intraperitoneal injection of 10 ml/kg of 0.7% acetic acid. The FRCO (50 mg/kg, 100 mg/kg, and 200 mg/kg), indomethacin (10 mg/kg), and 1% acacia solution (p.o.) were administered orally 1 h before the injection of Evans blue. Twenty minutes after the injection of acetic acid, the mice were sacrificed by cervical dislocation. Peritoneal fluids were collected by washing with 5 ml of normal saline and then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 590 nm using a spectrophotometer (Shimadzu 7140) [10-12].

Cotton pellet-induced granuloma

The test samples, indomethacin, and 1% acacia solution in distilled water were administered orally once daily for 7 days. Sterilized cotton pellet weighing 20 mg were put subcutaneously into the groin region of rats on the 1st day. The animals were sacrificed on the 8th day. The pellets, which by then were surrounded by granuloma tissue, and the thymus were dissected out carefully and dried at 600°C to a constant weight. The wet weight and dry weight were determined. The percentage of inhibition was expressed by comparing the mean weight in the test groups with that in the control group.

Analgesic activity

Analgesic activity was studied using four different models, such as acetic acid-induced writhing, hot plate method, formalin test, and tail immersion test [11,13].

Acetic acid-induced writhing test

In the writhing test, male mice were divided into five groups. The mice were treated with 0.6% acetic acid solution in normal saline injected intraperitoneally at a dose of 10 ml/kg. The numbers of writhes were counted starting from 5 min after injection that lasted for 15 min. The response consisted of abdominal wall contractions, pelvic rotation, followed by hind limb stretches. The test samples, indomethacin, and 1% acacia solution (p.o.) were administered orally 1 h before acetic acid injection. The percentage of analgesic activity was calculated as follows:

$$\text{Percentage analgesic activity} = Nc - Nt/Nc \times 100$$

Where, Nc is the average number of stretches of the control group, Nt is the average number of stretches of the test drug group [11,14].

Hot plate method

The hot plate test was carried out at a fixed temperature of 55±5°C. The reaction consisted of paw licking and jumping. The time in seconds between the platform and reaction was recorded as the response latency. The mice exhibiting latency time >30 s or <5 s were excluded from the study. The latency time was determined at 0 min, 30 min, 60 min, 90 min, and 120 min after administration of the test samples, pentazocine (10 mg/kg i.p.), and 1% acacia solution (p.o.) [10,14,15].

Formalin test

Albino mice of either sex were divided into five groups. Group I considered as normal control received orally with the vehicle 1% gum acacia, Group II with standard indomethacin (5 mg/kg, i.p), Groups III, IV, and V were given orally with different doses, i.e., 50 mg/kg, 100 mg/kg, and 200 mg/kg of FRCO. One hour after the above treatment, 20 µl of 2.5% formalin was injected into the subplantar region of each animal. Duration of paw lickings was monitored for 0–5 min (Early phase) and at 20–25 min (Late phase) after formalin challenge [16].

Tail immersion test

The basal reaction time of each mouse was determined using tail-withdrawal response when one-third of the tail was immersed in a water bath at 51±1°C. Mice with basal reaction time between 5 and 10 s were selected 24 h before the test. The animals were treated with gum acacia 1%, pentazocine (10 mg/kg, i.p.), and FRCO (50, 100 and 200 mg/kg p.o.) and 30, 60, 90, and 120 min later the reaction time was evaluated. The cutoff time for immersion was 180 s [17].

Antipyretic activity

The antipyretic property of FRCO was tested in rats in which hyperthermia was introduced. Initial rectal temperature was recorded using Digital Tele Thermometer (Model No. 461E, INCO, Ambala, India). Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at the dose of 1 ml/100 g of body weight. When rectal temperature was at peak (16 h after injection of yeast suspension), the rectal temperature was again recorded. Test and control drug was given orally. After 1 h, temperature was recorded followed by 1 h interval for consecutive 5 h.

In vitro antioxidant activity

In vitro antioxidant activity was studied by total phenolic content, DPPH free radical scavenging activity, hydrogen peroxide assay, and reducing power assay.

Total phenolic content

2 ml sample solution, 10 ml water, and 2 ml Folin–Ciocalteu reagent were mixed up and volume was made up to 25 ml by adding sodium carbonate solution. After incubation, the absorbance of the solution was measured at 750 nm. Instead of sample solution, gallic acid was added and same procedure was repeated for the standard gallic acid solution. From the stock solution of gallic acid, different concentrations were prepared 250, 500, 750, 1000, and 1250 μl and calibration curve was plotted. Line equation obtains from the calibration curve was used for determining % of total polyphenols with that of gallic acid [18,19].

DPPH free radical scavenging activity

1 ml of different concentrations of extract solution were taken in different vials. To this, 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37°C for 20 min. Absorbance was measured against methanol as a blank at 517 nm and absorbance of DPPH was taken as control. Percent antiradical activity was calculated using the following formula [20]:

$$\text{\% Scavenging activity} = OD_{\text{Control}} - OD_{\text{Test}} \text{ sample} / OD_{\text{Control}} \times 100$$

Hydrogen peroxide assay

1 ml different concentrations of extract solution were taken in different vials. Different concentrations (25–200 μg/ml) of FRCO were added to 0.6 ml of H_2O_2 (40 mM). The absorbance of the solution was read at 230 nm after 10 min against a blank containing FRCO in phosphate-buffered without H_2O_2. The concentration of H_2O_2 was determined spectrophotometrically at 230 nm. The percent scavenging of H_2O_2 by FRCO was calculated using the formula [21]:

$$\text{\% Scavenging} = \text{Absorbance of control} - \text{Absorbance of sample} / \text{Absorbance of control} \times 100$$

Reducing power assay

1 ml of FRCO (dilution) was mixed with 1 ml of 0.2 M sodium phosphate-buffered (pH 6.6) and 1 ml of 1% potassium ferricyanide. The reaction mixtures were incubated 50 OC for 20 min. Then, 1 ml of 10% trichloroacetic acid was added into the reaction mixture. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of distilled water and 200 μl of 0.1% $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed using the following formula [22].

$$\text{\% Scavenging} = A_0 - A_1 / A_0 \times 100$$

Where A_0 is the absorbance of the blank and A_1 is the absorbance of test.

Statistical analysis

Values were presented as mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparison using INTA software. The level of significance was set as $p < 0.05$ and $p < 0.01$.
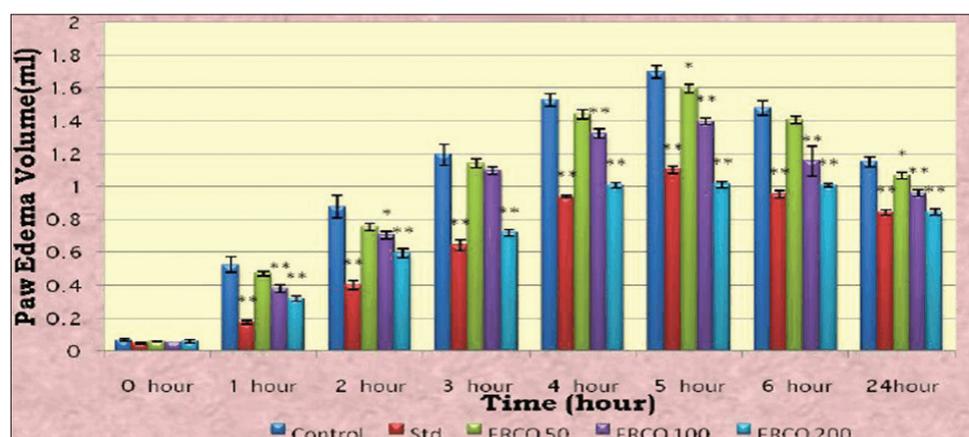

Fig. 1: Effects of FRCO on carrageenan-induced hind paw edema. *p<0.05, **p<0.01, compared with the control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean
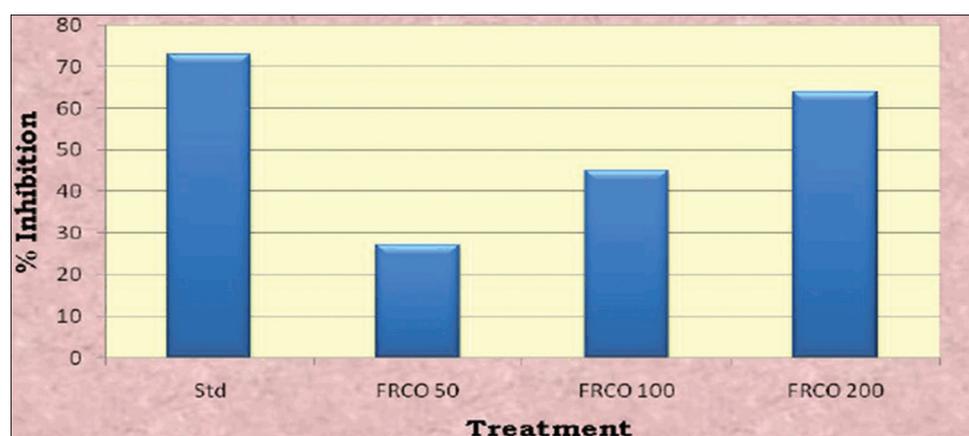

Fig. 2: Effect of FRCO on acetic acid-induced vascular permeability in mice. Where, N = 5 Mean ± SEM = Mean ± Standard error of mean

RESULTS AND DISCUSSION

Acute toxicity study

There was no toxic reaction or mortality observed. Flavonoid-rich fraction was found to be safe up to 2000 mg/kg p.o. Thus, based on the results of preliminary toxicity testing of the doses of 50, 100, and 200 mg/kg p.o. were chosen for further experiments.

Anti-inflammatory activity

Effect of FRCO on carrageenan-induced hind paw edema in rats

In carrageenan-induced hind paw edema, FRCO (50, 100, and 200 mg/kg, p.o.) reduced carrageenan-induced edema formation significantly at 1st, 2nd, 5th, and 6th h. A significant inhibition in edema formation was observed by both FRCO and indomethacin at 24th h (Fig. 1).

Effects of FRCO on acetic acid-induced vascular permeability

The FRCO treatment inhibited acetic acid-induced vascular permeability, and the same was reflected in increased leakage of dye. The amount of dye leakage was significantly ($p<0.01$) reduced by all the doses (50, 100, and 200 mg/kg, p.o.) of extract. The dose of indomethacin (10 mg/kg p.o.) prevented significantly dye leakage and the effect was greater than the FRCO (Table 1 and Fig. 2).

Effects of FRCO on cotton pellet-induced granuloma

In cotton pellet-induced granuloma in rats, the FRCO treatment orally inhibited the inflammatory effect on both the phases of inflammation and effect was found to be dose-related. Indomethacin (10 mg/kg p.o.) treatment also elicited inhibitory effects on both exudate and granulation phases of inflammation. The indomethacin effect was greater than the FRCO (Fig. 3).

Analgesic activity

Effects of FRCO on acetic acid-induced writhing test

The FRCO at the dose 50, 100, and 200 mg/kg, p.o. inhibited acetic acid-induced writhing in mice. Indomethacin showed greater inhibition in acetic acid-induced writhing as compared to FRCO (Table 2 and Fig. 4).

Effects of FRCO on hot plate method

The FRCO at the dose 50, 100, and 200 mg/kg, p.o. significantly inhibited the latency period. Pentazocine showed greater inhibition in the hot plate method model of analgesic as compared to FRCO (Table 3).

Effects of FRCO on the formalin test method

In the formalin test method, the FRCO treatment orally inhibited both phases, i.e., early and late phase and effect was found to be dose related. Indomethacin 5 mg/kg i.p shows an inhibitory reaction greater than the FRCO (Fig. 5).

Effects of FRCO on tail–flick test

The ability of FRCO to induce the central analgesia was evaluated by temperature-based tests. As per the results, in tail–flick test the FRCO treatment orally increase the time duration of flicking of tail when
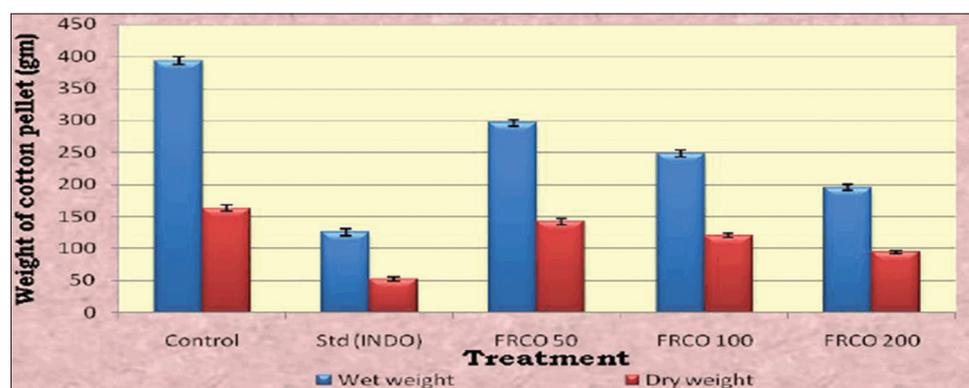

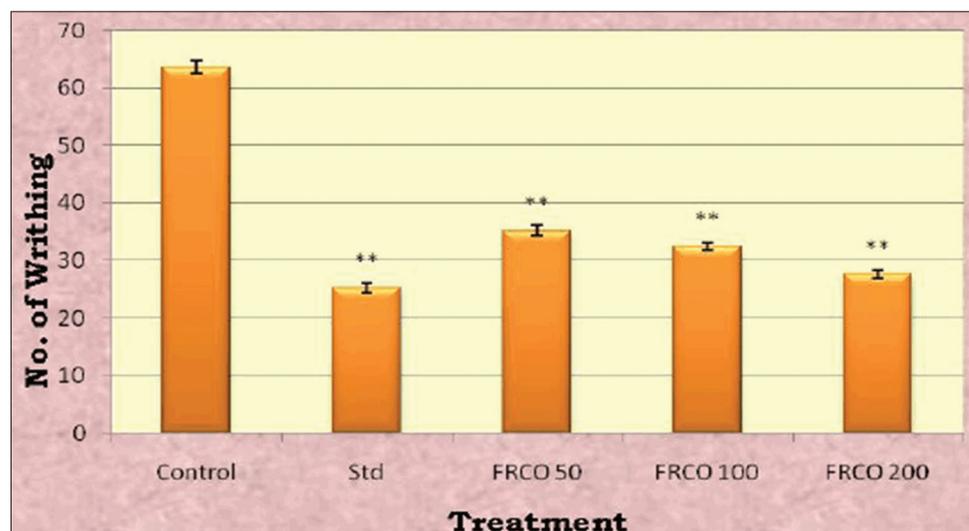**Fig. 3: Effect of FRCO on cotton pellet-induced granuloma in rats. Where, N = 5 Mean ± SEM = Mean ± Standard error of mean**

Fig. 4: Effect of FRCO on acetic acid-induced writhing test. *p<0.05, **p<0.01, compared with the control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean

Table 1: Effect of FRCO on acetic acid-induced vascular permeability in mice

Name of groups	OD	Amount of dye leakage	% Inhibition
Control	0.1101±0.001	26.25 µg/ml	-
Std. (INDO)	0.0321±0.007	07.75 µg/ml	73
FRCO 50	0.0835±0.001	20.12 µg/ml	27
FRCO 100	0.0605±0.002	14.62 µg/ml	45
FRCO 200	0.0450±0.003	10.87 µg/ml	64

OD: Optical density, FRCO: Flavonoid-rich fraction of *C. obtusifolia* Linn. Where, N=5 Mean±SEM=Mean±Standard error of mean

Table 2: % Inhibition of FRCO on acetic acid-induced writhing test

S. No.	Group	Dose (mg/kg)	Number of writhing	% inhibition
1.	Control	--	63.6±1.030	--
2.	Std. (INDO)	10	25.2±0.860 **	60
3.	FRCO 50	50	35.2±0.969 **	44
4.	FRCO 100	100	32.4±0.678 **	49
5.	FRCO 200	200	27.6±0.748 **	56

*$p < 0.05$, **$p < 0.01$, compared with control group (ANOVA followed by Dunnett's test). Where, N=5, Mean±SEM=Mean±Standard error of mean

deep into the water bath. The pretreated animals increased the basal reaction time. At high doses (200 mg/kg) of FRCO and pentazocine (10 mg/kg i.p) increase the percentage reaction time effectively. Pentazocine (10 mg/kg i.p) shows an inhibitory reaction greater than the FRCO (Fig. 6).

Antipyretic activity
Effect of FRCO on pyrexia-induced by brewer's yeast in rats
In the antipyretic activity, FRCO at the dose level of 50, 100, and 200 mg/kg significantly reduced the fever induced by the brewer's yeast in rats. FRCO reduced the fever particularly at 5th and 6th h in dose-dependent manner, whereas paracetamol reduced the fever right from the 2nd to the 6th h (Tables 4 and 5).

In vitro antioxidant activity
Total phenolic content
Total polyphenolic content was observed to be 31.50 mg/g of gallic acid. It was observed that gallic acid being the major component of ethanolic extract and was mainly responsible for antioxidant activity.

DPPH free radical scavenging activity
In DPPH radical scavenging activity, FRCO and ascorbic acid showed significant DPPH free radical scavenging activity at different concentrations (50, 100, 150, 200, and 250 µg/ml) in dose-dependent manner. IC_{50} value was found to be 137 and 200 g/ml for ascorbic acid and FRCO, respectively (Table 6 and Fig. 7).
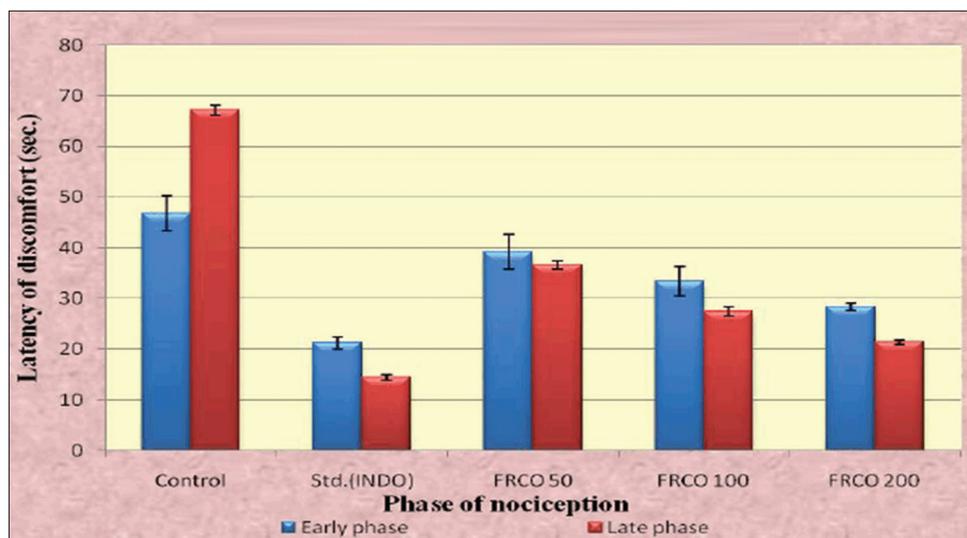
Table 3: Effect of FRCO in hot plate method

Group	Dose mg/kg	Time (h)				
		0 h	0.5 h	1 h	1.5 h	2 h
Control	-	6.56±0.060	6.42±0.037	6.7±0.044	6.62±0.048	6.7±0.044
Std(PENT)	10	6.62±0.048	14.08±0.080**	15.98±0.058**	16.74±0.067**	15.96±0.050**
FRCO 50	50	6.7±0.044	8.68±0.058**	9.4±0.063**	11.8±0.054**	11.12±0.066**
FRCO 100	100	6.18±0.048	9.2±0.044**	11.2±0.054**	12.7±0.083**	12.2±0.044**
FRCO 200	200	6.44±0.040	9.74±0.074**	12.04±0.081**	13.16±0.074**	12.38±0.086**

*$p < 0.05$, **$p < 0.01$, compared with control group (ANOVA followed by Dunnett's test). Where, N=5 Mean±SEM=Mean±Standard error of mean

Table 4: Effect of FRCO on pyrexia-induced by brewer's yeast in rats

Group	Dose mg/kg	Temperature (°C) (Mean ± SEM)					
		1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	-	37.34±0.097	37.42±0.058	37.48±0.073	37.40±0.054	37.52±0.086	37.5±0.031
Control	-	39.42±0.073	39.6±0.044	39.44±0.067	39.28±0.066	38.06±0.050	39.08±0.050
Std. (PARA)	150	39.2±0.077	38.9±0.070	38.68±0.037	38.34±0.050	38.10±0.044	37.52±0.086
FRCO 50	50	39.34±0.060	39.3±0.083	39.24±0.067	38.92±0.037	38.92±0.048	38.60±0.070
FRCO 100	100	39.4±0.034	39.36±0.067	39.36±0.040	38.98±0.058	38.62±0.020	38.46±0.040
FRCO 200	200	39.28±0.058	39.22±0.131	38.78±0.058	38.52±0.177	38.50±0.063	38.38±0.058

*$p < 0.05$, **$p < 0.01$, compared with control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean

Table 5: Effect of FRCO on pyrexia-induced by brewer's yeast in rats

Group	Dose mg/kg	Difference in temperature (°C) (Mean ± SEM)					
		1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	-	0.46±0.04	0.54±0.024	0.6±0.04	0.6±0.031	0.54±0.050	0.62±0.037
Control	-	2.32±0.106	2.5±0.083	2.36±0.050	2.18±0.02	1.96±0.074	2.00±0.070
Std. (PARA)	150	2.22±0.142	1.62±0.058**	1.52±0.080**	0.98±0.080**	0.94±0.060**	40.52±0.050**
FRCO 50	50	2.24±0.040	2.36±0.02	1.76±0.024	1.82±0.037	1.74±0.037 *	1.5±0.031**
FRCO 100	100	2.18±0.037	2.3±0.031	2.16±0.024	2.14±0.024*	1.4±0.031**	1.22±0.037**
FRCO 200	200	2.26±0.040	2.3±0.031	1.5±0.158*	1.76±0.024**	1.46±0.024**	0.76±0.024**

*$p < 0.05$, **$p < 0.01$, compared with control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean. FRCO: Flavonoid-rich fraction of *C. obtusifolia* Linn.

Fig. 5: Effects of FRCO on formalin test method. *p<0.05, **p<0.01, compared with the control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean

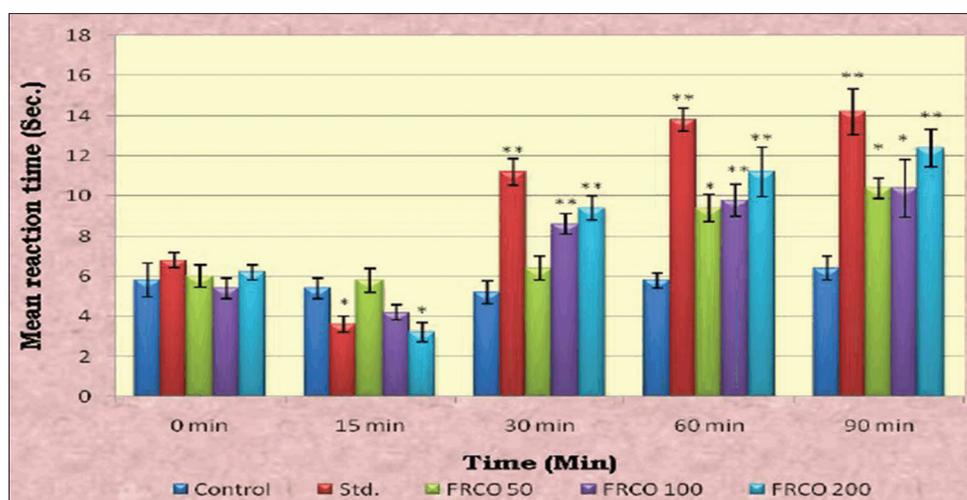**Fig. 6: Effect of FRCO on tail-flick test. *p<0.05, **p<0.01, compared with the control group (ANOVA followed by Dunnett's test). Where, N = 5 Mean ± SEM = Mean ± Standard error of mean**
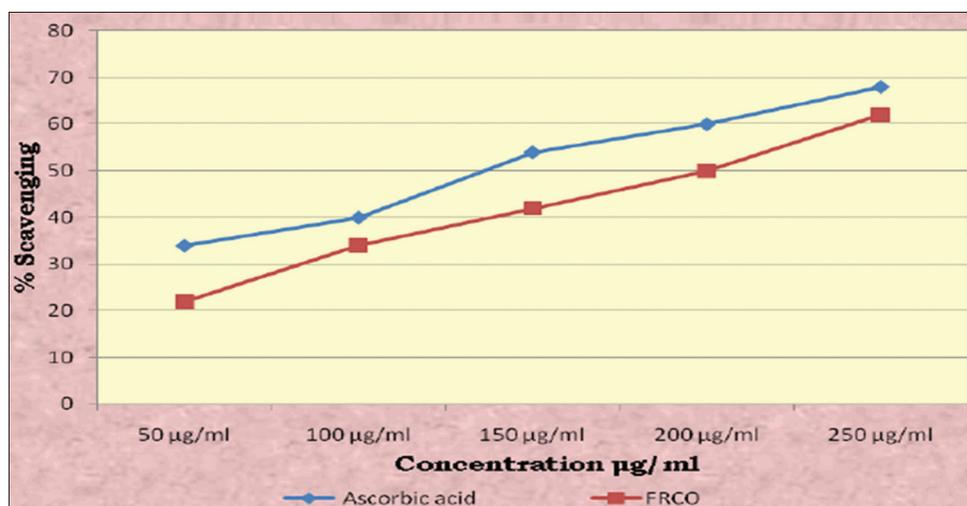

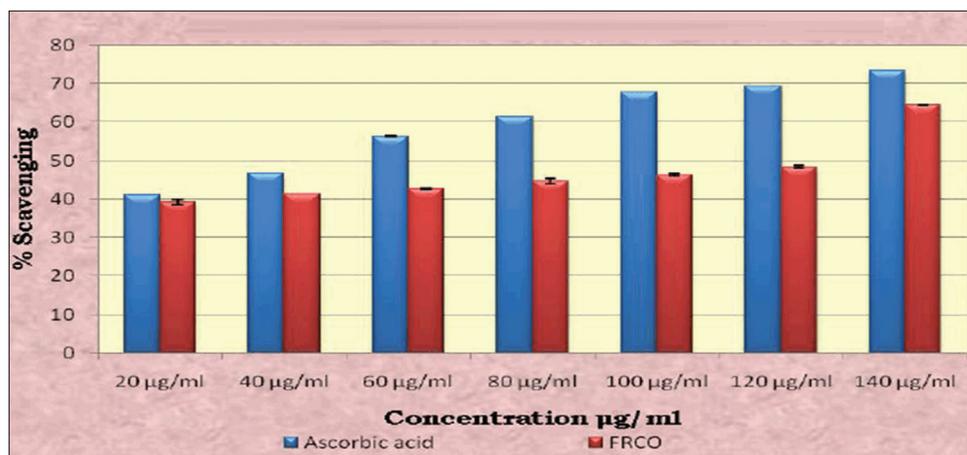**Fig. 7: Effect of FRCO in DPPH free radical scavenging assay**

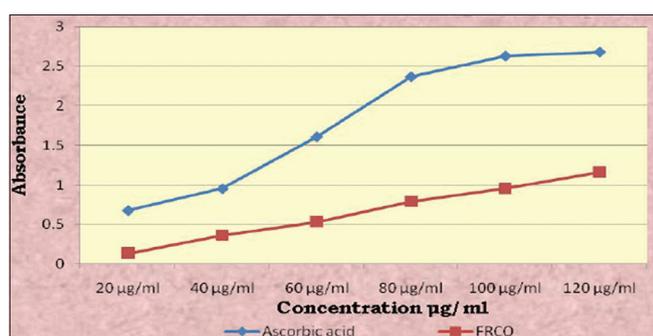Fig. 8: Effect of FRCO in hydrogen peroxide assay

Fig. 9: Effect of FRCO in reducing power assay

Table 6: Effect of FRCO in DPPH free radical scavenging assay

Concentration μg/ml	% Scavenging ascorbic acid	IC_{50} value	% Scavenging FRCO	IC_{50} value
50 μg/ml	34±2.000	137	22±2.000	200
100 μg/ml	40±2.000		34±2.000	
150 μg/ml	54±3.464		42±3.464	
200 μg/ml	60±3.464		50±4.000	
250 μg/ml	68±2.000		62±3.464	

Table 7: Effect of FRCO in hydrogen peroxide assay

Concentration μg/ml	Ascorbic acid		FRCO	
	% Scavenging	IC_{50} value	% Scavenging	IC_{50} value
20	41.04±0.006	48	39.33±0.667	127
40	46.66±0.004		41.33±0.002	
60	56.33±0.002		42.66±0.333	
80	61.33±0.002		44.66±0.666	
100	67.66 ±0.0007		46.33±0.333	
120	69.33±0.006		48.33±0.333	
140	73.32±0.002		54.49±0.130	

FRCO: Flavonoid-rich fraction of *C. obtusifolia* Linn.

Table 8: Effect of FRCO in reducing power assay

Concentration (μg/ml)	Absorbance of Ascorbic acid	Absorbance of FRCO
20	0.674±0.0117	0.1341±0.0020
40	0.9495±0.0038	0.3632±0.0170
60	1.6078±0.0083	0.5342±0.0021
80	2.3690±0.0248	0.7930±0.0010
100	2.6314±0.0040	0.9544±0.0010
120	2.6817±0.0014	1.1616±0.0500

Effect of FRCO in hydrogen peroxide assay

In hydrogen peroxide assay, FRCO and ascorbic acid, showed significant radical scavenging activity at different concentrations (20, 40, 60, 80, 100, 120, and 140 μg/ml) in dose-dependent manner. IC_{50} value was found to be 48 and 127 g/ml for ascorbic acid and FRCO, respectively (Table 7 and Fig. 8).

Reducing power assay

In reducing power assay, FRCO and ascorbic acid showed significant radical power ability at different concentrations (20, 40, 60, 80, 100, and 120 μg/ml) in dose-dependent manner (Table 8 and Fig. 9).

CONCLUSION

The *C. obtusifolia* plant belonging to Caesalpiniaceae family consisted of flavonoids as the major chemical constituents. These flavonoid-rich fractions were profound to have certain pharmacological potential. Thus, the present study reveals the anti-inflammatory, analgesic, antipyretic, and antioxidant potential of flavonoid-rich fractions of *C. obtusifolia* plant. The results of the *in vitro* assays and animal models conclude that *C. obtusifolia* has comparable pharmacological potentials.

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AUTHORS' CONTRIBUTIONS

Dr. S.M Kewatkar and Dr. Vivek Paithankar contribution included designing and performing laboratory work, analyzing the results, and preparing the paper. Dr. Santosh Bhujbal, Dr. S.P. Jain, and Dr. D.H. Nagore contribution included data interpretation and identification of the compounds. All the authors have read the final manuscript and approved the submission.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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