

## Antiinflammatory and Analgesic Activity of *Caesalpinia pulcherrima* Linn. Leaf Extracts

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The dried leaves of *Caesalpinia pulcherrima* are known to possess antiinflammatory and analgesic properties. The present work was carried out to confirm and validate the same, using ethyl acetate and ethanol as solvents in succession and the two different extracts obtained were subjected for screening on albino rats for analgesic and antiinflammatory activity. The results were also compared with pentazocine and diclofenac sodium tablets respectively. The ethyl acetate extract showed better analgesic and antiinflammatory activities compared with ethanolic extract.

**Key Words:** *Caesalpinia pulcherrima*, Analgesic, Anti-inflammatory activity.

### INTRODUCTION

*Caesalpinia pulcherrima* Linn. (Family Caesalpiniaceae) is an exotic drought resistant shrub or a small tree mostly distributed throughout India<sup>1,2</sup>. The various parts of the plant are known to possess different pharmacological properties, i.e., seeds in powder form used as a remedy for stomachache<sup>3</sup>; infusion of flowers prescribed in bronchitis, tonic, emmenagogue and it has anticancer properties<sup>4</sup>; stem bark contains caesalpin, pulcheralpin, 6-methoxybenzoquinone and 4'-methylisoliquiritigenin<sup>5</sup>. In the present work, the antiinflammatory and analgesic activity of the extracts were investigated.

### EXPERIMENTAL

**Preparation of Extracts:** Fresh leaves were collected from Kolli hills and authenticity was confirmed by Siddha research unit, Kolli hills, Nammakal District, Tamilnadu. The leaves were dried under shade, pulverized and passed through sieve No. 40 to get the desired coarseness. The powdered leaves (500 g) were extracted with ethyl acetate and ethanol in a soxhlet extractor. The extracts

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were then distilled separately under reduced pressure to yield solid masses (EAE 3.6%, EE 2.8%) which were completely free from solvents. The solid masses were used for analgesic and antiinflammatory activity.

**Acute Toxicity Studies:** The LD<sub>50</sub> values for the prepared extracts were determined in albino mice by arithmetical method<sup>6</sup>. The LD<sub>50</sub> values for both the extracts were found to be 30 mg/kg.

#### (a) Antiinflammatory activity

**Carrageenin induced paw oedema method:** Albino rats of either sex were arranged in four groups of ten each, weighing between 150–200 g; one group served as control (received 0.75% CMC, 5 mL/kg), second group served as standard (received 5 mg/kg of diclofenac sodium), while the other groups received the ethyl acetate and the ethanolic extract of *Caesalpinia pulcherrima* (20 mg/kg)<sup>7</sup> respectively by oral route; oedema was produced by the method described by Winter *et al.*<sup>8</sup> The paw volume was measured at 0 to 4 h after the injection of carrageenin. The apparatus used for the measurement of rat paw volume was plethysmograph. Drug pretreatment was given 1 h before the injection of carrageenin. The relative potency of extracts under investigation was calculated based upon the percentage inhibition of inflammation<sup>9</sup>.

**Cotton pellet granuloma method:** Albino rats were divided into four groups, each consisting of ten animals. One of the groups of albino rats served as a control (received 0.75% CMC, 5 mL/kg), other group served as standard (received 5 mg/kg of diclofenac sodium) and the remaining groups were treated with the ethyl acetate and ethanolic extract of the *Caesalpinia pulcherrima* (20 mg/kg) orally respectively. After shaving off the fur on the back, the rats were anaesthetized with pentobarbitone (30 mg/kg). Through a single incision on the dorsal surface, sterilized preweighed cotton pellets were implanted in both axillae and groin region according to the method of D'Arcy *et al.*<sup>10</sup>, with slight modification. The drugs were administered daily for 10 days (0 to 9 days). On the tenth day the pellets were dissected out and dried at 60°C and the pellet weight was determined. The yield of ethyl acetate and ethanolic extracts of *Caesalpinia pulcherrima* were 0.778 and 0.784%, respectively.

#### (b) Analgesic activity

The analgesic activity was screened and evaluated by two different methods.

**Hot Plate Method:** In Jassen method<sup>11</sup>, the test compounds were given orally to the groups of ten mice each. One group received pentazocine (10 mg/kg, i.p.) and other group received vehicle. The time of reaction to pain stimulus of the mice based on the plate heated at 55 ± 5°C was recorded at 15, 30, 45 and 60 min after the administration.

**Tail Flick Method:** Tail flick response<sup>12</sup> was evoked by placing the rat tail over a wire heated electrically. The intensity of heat was adjusted (current 3.0 A) so that the base line tail flick latency averaged 3 to 4 in all the animals. Cut off

time 20 s in order to avoid injury to the tail. The percentage of analgesia was calculated.

### Statistical Analysis

All values are expressed as mean  $\pm$  standard error. Test of significance was analyzed by Student's 't' test<sup>13</sup>.

## RESULTS AND DISCUSSION

Table-1 shows the effect of drug treatment and carrageenin induced oedema suppressant effect of 20 mg/kg of ethyl acetate and ethanolic extract were  $0.683 \pm 0.003$  and  $0.726 \pm 0.008$ , respectively. In the cotton pellet granuloma model, inflammation and granuloma develop during a period of several days. This model is an indication for the proliferating phase of inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are the basic sources of granuloma formation. Hence, decrease in granulome weight indicates the suppressant of proliferative phase, which was effectively inhibited by ethylacetate extract of *Caesalpinia pulcherrima*.

Tables 2 and 3 show the antiinflammatory activity of *Caesalpinia pulcherrima* by plethysmograph and by cotton pellet granuloma method, respectively.

Tables 4 and 5 reveal that the ethylacetate and the ethanolic extract possess remarkable analgesic activity. The percentages of analgesia of ethyl acetate and ethanolic extract were 40.5 and 24.4% in tail flick method and but 69.5 and 64.4% in the hot plate method.

TABLE-1  
DETERMINATION OF LD<sub>50</sub> VALUE

Group	Dose mg/kg	No. of animals	Dose difference (a)	Dead	Mean mortality (b)	Product (a $\times$ b)
1	100	4	—	0	—	—
2	200	4	100	3	1.5	150
3	300	4	100	3	3.0	300
4	400	4	100	4	3.5	350
						800

LD<sub>50</sub> = (maximum dose) - sum of product (a  $\times$  b)/No. of animals used

LD<sub>50</sub> = 400 - 800/4    LD<sub>50</sub> = 400 - 200    LD<sub>50</sub> = 200 mg/kg

1/10 of LD<sub>50</sub> = Therapeutic dose = 20 mg/kg.

TABLE-2  
ANTI-INFLAMMATORY ACTIVITY OF *CAESALPINIA PULCHERRIMA*  
BY USING PLETHYSMOGRAPH

S. No.	Animal group	Paw volume (mL $\pm$ SEM and % of inhibition)				
		0 min	60 min	120 min	180 min	240 min
1.	Control	0.356 $\pm$ 0.005	0.546 $\pm$ 0.013	0.760 $\pm$ 0.014	0.836 $\pm$ 0.008	0.933 $\pm$ 0.005
2.	Standard	0.366 $\pm$ 0.006	0.368 $\pm$ 0.003 32.96%	0.373 $\pm$ 0.005† 50.92%	0.374 $\pm$ 0.015‡ 58.13%	0.380 $\pm$ 0.003‡ 59.72%
3.	Ethyl acetate extract (20 mg/mL)	0.383 $\pm$ 0.005	0.430 $\pm$ 0.008 21.2%	0.580 $\pm$ 0.005* 23.68%	0.630 $\pm$ 0.003† 27.51%	0.683 $\pm$ 0.003‡ 26.79%
4.	Ethanollic extract (20 mg/kg)	0.366 $\pm$ 0.015	0.523 $\pm$ 0.020 4.21%	0.670 $\pm$ 0.016 11.84%	0.696* $\pm$ 0.006 16.22%	0.726† $\pm$ 0.008 22.18%

‡P < 0.001, †P < 0.01, \*P < 0.02.

TABLE-3  
ANTI-INFLAMMATORY ACTIVITY OF *CAESALPINIA PULCHERRIMA*  
BY COTTON PELLET GRANULOMA METHOD

S.No.	Drugs	Dose	Granulation wt. in mg (Mean $\pm$ SEM)	% granulation wt. in mg (Mean $\pm$ SEM)
1.	Control (0.75% CMC)	5 mL/kg	308.12 $\pm$ 0.64	—
2.	Diclofenac sodium	5 mg/kg	162.28 $\pm$ 2.42	44.6 $\pm$ 1.8*
3.	Ethyl acetate extract	20 mg/kg	182.25 $\pm$ 2.96	38.4 $\pm$ 2.6*
4.	Ethanollic extract	20 mg/kg	262.48 $\pm$ 4.26	Non significant

\*P < 0.01.

TABLE-4  
ANALGESIC ACTIVITY OF *CAESALPINIA PULCHERRIMA*  
BY TAIL FLICK METHOD

S. No.	Group	Response time (Mean $\pm$ SEM)				
		0 min	15 min	30 min	45 min	60 min
1.	Control	2.00 $\pm$ 0.00	2.25 $\pm$ 0.25	1.50 $\pm$ 0.57	1.50 $\pm$ 0.52	2.00 $\pm$ 0.57
2.	Standard pentazocine (10 mg/kg)	2.20 $\pm$ 2.00	6.40 $\pm$ 0.48	7.60 $\pm$ 0.18	10.90 $\pm$ 0.18†	13.80 $\pm$ 0.12†
3.	Ethyl acetate extract (20 mg/kg)	2.80 $\pm$ 0.25	6.80 $\pm$ 0.25	7.50 $\pm$ 0.16	8.00 $\pm$ 0.25*	9.30 $\pm$ 0.12†
4.	Ethanollic extract (20 mg/kg)	2.60 $\pm$ 0.25	6.75 $\pm$ 0.25	7.25 $\pm$ 0.23	7.85 $\pm$ 0.32	6.40 $\pm$ 0.29†

\*P < 0.01, †P < 0.001.

TABLE-5  
ANALGESIC ACTIVITY OF *CAESALPINIA PULCHERRIMA*  
BY HOT PLATE METHOD

S. No.	Group	Basal reaction time (Mean $\pm$ SEM)				
		0 min	15 min	30 min	45 min	60 min
1.	Control	2.03 $\pm$ 0.12	2.00 $\pm$ 0.03	2.15 $\pm$ 0.01	2.00 $\pm$ 0.03	2.14 $\pm$ 0.13
2.	Standard pentazocine (10 mg/kg)	2.14 $\pm$ 0.11	6.69 $\pm$ 0.04	7.92 $\pm$ 0.16*	9.26 $\pm$ 0.05*	11.49 $\pm$ 0.01†
3.	Ethyl acetate extract (20 mg/kg)	2.06 $\pm$ 0.01	4.78 $\pm$ 0.01	6.14 $\pm$ 0.20	6.89 $\pm$ 0.07	7.02 $\pm$ 0.03†
4.	Ethanollic extract (20 mg/kg)	2.21 $\pm$ 0.02	3.26 $\pm$ 0.01	4.16 $\pm$ 0.02	5.16 $\pm$ 0.14	6.02 $\pm$ 0.02†

\*P < 0.01, †P < 0.001.

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