



## Anti-inflammatory and neuropharmacological activities of *Caesalpinia pulcherrima* leaves

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### Abstract

The crude methanolic extracts of leaves of *Caesalpinia pulcherrima* were evaluated for its anti-inflammatory and neuropharmacological activities. When given orally to rats at dose of 200 and 400 mg/kg, the extract showed a significant ( $P < 0.001$ ) anti-inflammatory activity against carrageenin induced paw edema in rats comparable to the standard drug phenyl butazone. The extract of *Caesalpinia pulcherrima* leaves also potentiated the pentobarbital induced sleeping time in mice, and decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test.

**Keywords:** Anti-inflammatory, Antidiarrhoeal, Neuropharmacological, *Caesalpinia pulcherrima*

### Introduction

*Caesalpinia pulcherrima* (L.) is a perennial large shrub or small tree found throughout Bangladesh. It has several medicinal properties, used in treatment of ulcer, fever, tumors, asthma and skin diseases (Kirtikar and Basu, 1987). *C. pulcherrima* also has uses in the folk medicine: the stem is used as an abortifacient and emmenagogue, while decoctions of the leaves, roots, and bark are used as a febrifuge and to treat liver disorders as well as ulcers from mouth and throat (Quisumbing, 1978). Previous studies on this plant have resulted in the isolation of several diterpenoids (Pranithanchai *et al.*, 2009, Roach *et al.*, 2003), flavonoids (Maheswara *et al.*, 2006, Rao *et al.*, 2005),

peltogynoids (Srinivas *et al.*, 2003), homoisoflavonoids (Das *et al.*, 2009). Some of the constituents were found to possess antitumour (Che *et al.*, 1986), antimicrobial properties (Ragasa *et al.*, 2002). Few of them biological activity has yet been reported on the leaf part of this plant. Due to the presence of different chemical compounds, the leaf part of this plant may possess some pharmacological activities. That is why anti-inflammatory and neuropharmacological activities of the crude extract of leaf of *Caesalpinia pulcherrima* were investigated in the present study.

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## Experimental

**Plant material.** Leaves of *Caesalpinia pulcherrima* were collected in 2007 from the district of Jessore, Bangladesh and were authenticated by the experts at National Herbarium (accession number: 35762). The leaves were dried and powdered with the help of a suitable grinder and then extracted with 90% methanol (200 ml each time) in a soxhlet apparatus. The extract was evaporated to dryness by rotary evaporator to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get a dried extract (yield: about 15%). This extract was used for pharmacological screening.

**Animals.** Animal studies were performed in accordance with the declaration of Helsinki and the European Community guidelines for the ethical handling and the use of laboratory animals and through the clearance of Institutional Animal Ethics Committee (IAEC). For neuropharmacological activity study, Swiss-albino mice of either sex, weighing 20-25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used. On the other hand, Wistar rats of either sex, weighing 180-200 g, purchased from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for anti-inflammatory activity study. The animals were provided with standard laboratory food and tap water *ad libitum* and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

**Drugs.** Carrageenin (Sigma Chemicals, USA), Phenylbutazone (Square Pharmaceuticals Ltd, Bangladesh).

**Chemical group test.** The crude methanolic extract of leaves of *Caesalpinia pulcherrima* was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans, 1989). In each test 10% (w/v) solution of the extract

in methanol was taken unless otherwise mentioned in individual test.

**Anti-inflammatory activity.** Anti-inflammatory activity of *Caesalpinia pulcherrima* was tested by using carrageenin induced rat paw edema model (Winter *et al.*, 1962; Ahmed *et al.*, 2004). Rats were randomly divided into four groups, each consisting of six animals. Group I was kept as 'control' giving 1%(v/v) tween-80 solution in water; group II was kept as 'positive control' and was given the standard drug phenylbutazone at a dose of 100 mg/kg of body weight; group III and IV were test groups, treated with extracts at the doses of 200 and 400 mg/kg of body weight respectively. Control vehicle, standard drug and the extracts were given orally 1h prior to the injection of 0.1 ml of 1% freshly prepared suspension of carrageenin. The paw volume was measured by using a plethysmometer just before and 1, 2, 3, 4, 5 h after the carrageenin injection.

**Neuropharmacological activity.**

*i) Pentobarbital induced hypnosis.* Pentobarbital itil-induced hypnosis test was carried out by the method of Williamson *et al.*, 1996. The test animals were divided into three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *Caesalpinia pulcherrima* at dose of 250 and 500 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with distilled water containing 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The total sleeping time were recorded for both control, as well as for treated groups.

*ii) Exploratory behavior.* This experiment was performed by (i) Open field test (Gupta *et al.*, 1971) (ii) Hole cross test (Takagi *et al.*, 1971) and (iii) Hole board test (Nakama *et al.*, 1972). The test animals were divided into

three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *Caesalpinia pulcherrima* (prepared by distilled water and tween-80) at dose of 250 and 500 mg/kg of body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The observations were made on 0 min before injection and 30, 60, 120 and 240 min after injections of the test samples and control.

**Statistical analysis:** Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

## Results

**Chemical group test.** Results of different chemical tests on the methanolic extract of *Caesalpinia pulcherrima* showed the presence

of alkaloids, steroids, flavonoids, saponins, gums and tannins (Table 1).

**Anti-inflammatory activity.** The experimental findings from the carrageenin-induced rat paw edema model showed that the methanolic extract of *Caesalpinia pulcherrima* reduced the paw volume significantly ( $P < 0.001$ ) from 1h to 5h. The extract showed highest effects at the third hour where the inhibition was about 29% and 41% at dose of 200 and 400 mg/kg respectively, which were comparable to the standard drug phenylbutazone, where the inhibition was about 51% at the dose of 100 mg/kg of body weight (Table 2).

**Neuropharmacological activity.**

*i) Pentobarbital induced hypnosis Test*

Table 3 showed the effect of *Caesalpinia pulcherrima* on pentobarbital induced hypnosis in mice. The total sleeping time was about 64 and 98 min at dose of 250 and 500 mg/kg of body weight respectively where as in control group it was about 29 min.

**Table 1.** Phytochemical properties of *Caesalpinia pulcherrima* crude extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve

Key: +ve = Presence -ve = Absence

**Table 2.** Effect of methanolic extract of *Caesalpinia pulcherrima* on carrageenin induced rat paw edema

Animal group / Treatment	Time after carrageenin injection				
	1 hr	2 hr	3 hr	4 hr	5 hr
	Increase in Paw Edema volume (ml) × 1000 ± S.E.M.				
Control (1% Tween 80) 10 ml/kg; p.o.	135.00 ± 0.70	163.20 ± 1.03	245.80 ± 0.74	276.40 ± 0.65	235.00 ± 0.86
Positive control Phenylbutazone 100 mg/kg; p.o.	95.20 ± 0.76**	101.50 ± 0.99** *	118.2 ± 1.24***	179.00 ± 1.04**	145.74 ± 0.78**
Test group-1 Methanol extract 200 mg/kg; p.o.	115.10 ± 1.15*	127.60 ± 1.67*	176.50 ± 0.87**	221.00 ± 0.69*	182.8 ± 0.87*
Test group-2 Methanol extract 400 mg/kg; p.o.	100.80 ± 1.05*	114.3 ± 0.87**	145.3 ± 0.94***	201.70 ± 1.55**	162.60 ± 1.04**

Values are expressed as mean ± S.E.M (n=6); \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  vs. control; p. o., per oral

**Table 3.** Effect of methanolic extract of *Caesalpinia pulcherrima* on pentobarbital induced hypnosis in mice

Animal group	Treatment	Time of onset of sleep (min)	Total sleeping time (min)
I (Control)	0.1% Tween 80 solution	7.425±0.143	29.43±2.01
II (Test group-I)	MeOH. Extract 250 mg/kg.	7.13±0.0598*	64.57±2.39**
III (Test group-II)	MeOH. Extract 500 mg/kg	7.29±0.122*	98.86±7.93**

Values are Mean ± SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.001$  vs. control, Student's t-test; Me.= Methanol

**Table 4:** Effect of *Caesalpinia pulcherrima* on exploratory behavior in mice

Group	Response at				
	0 min	30 min	60 min	120 min	240 min
Effect on Open Field Test					
I (Control)	85.27±2.09	92.74±1.84	96.56±1.94	105.15±1.32	106.84±1.47
II (Me. Ext.) 250 mg/kg	84.83±1.67*	78.56±1.14*	71.24±1.45*	63.58±1.87*	61.92±3.27*
III (Me. Ext.) 500 mg/kg	85.67±2.01*	69±2.49*	56±2.89*	45.32±3.04*	41.66±3.38*
Effect on Hole Cross Test					
I (Control)	8.51±0.66	9.87±0.45	9±0.49	9.34±0.78	8.32±0.55
II (Me. Ext.) 250 mg/kg	6.27±0.67*	6.15±0.50*	4.0±0.49*	3.66±0.36*	2.0±0.29*
III (Me. Ext.) 500 mg/kg	8.42±0.65*	6.0±0.38*	3.85±0.27*	2.19±0.25*	1.18±0.19*
Effect on Hole Board Test (Head dipping)					
I (Control)	14.52±0.64	21.15±1.15	22.57±1.05	18.84±0.69	14.0±0.84
II (Me. Ext.) 250 mg/kg	13.0±0.54*	11.18±0.37*	10.41±0.51*	8.309±0.41*	5.12±0.31*
III (Me. Ext.) 500 mg/kg	14.26±0.82*	10.84±0.69*	8.14±0.64*	5.18±0.58*	2.45±0.54*

Values are expressed as mean ± S.E.M.(n=7); Me., methanolic. \*,  $P < 0.001$  vs. control.

ii) *Exploratory behavior Test.* Test for exploratory behavior in mice was performed by (i) Open field test (ii) Hole cross test and (iii) Hole board test. It was observed that the extract decreased the number of open field score (Table 4), caused decrease in the number of hole crossed from one chamber to another chamber (Table 4), and also decreased head dip responses (Table 4) in mice at dose of 250 and 500 mg/kg of body weight from 30 min to 240 min.

## Discussion

The most widely used primary test for the screening of new anti-inflammatory agents is the carrageenin-induced rat paw edema model (Winter *et al.*, 1962). The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar *et al.*, 1969) and the delayed edema is due to the release of

bradykinin and prostaglandins (Di Rosa *et al.*, 1971; Flower *et al.*, 1985). It has been reported that the second phase of edema is sensitive to steroidal and non-steroidal anti-inflammatory agents (Di Rosa *et al.*, 1971). The extract reduced the paw volume significantly from 1h to 5h in which the highest effects were found at the third hour. These results tend to suggest the probable anti-inflammatory activity of the extract.

Central depressants elicit their effect by interfering with the functions of the cerebral cortex.

An important method of investigating the probable cortical manifestation of a drug is to check its effect on the pentobarbital narcosis as pentobarbital has multifarious effects on the cerebral cortex (Bowman and Rand, 1980). The pentobarbital sleeping time test was performed to find out whether the water extract of the plants have any effect on the cerebral cortex. Pentobarbital shorten the onset of sleep and increases sleep duration. The methanolic extract of *Caesalpinia pulcherrima* reduced the onset of sleep and potentiated the pentobarbital induced sleeping time in mice, which suggests its central depressant activity (Perez *et al.*, 1998), thus, suggesting the probable tranquilizing action (Capasso *et al.*, 1996).

It has been experimentally proven that, in the absence of a special task to perform, the behaviour of a given animal tend to maintain that inner activation level that is, at times, inconsistent with the actual level of activation of the animals. In order to get as accurate a picture as possible, on the effect of the drug on exploration, the open field test was performed. The extract also made mice to reduce their behavioural exploration, which further support the central sedative properties of the extract. The overall results tend to predict the CNS depressant action of the extract.

In conclusion, it could be suggested that the crude methanolic extract of

*Caesalpinia pulcherrima* might possess anti-inflammatory and central nervous system depressant activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential confirm its pharmacological properties.

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