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Anti-inflammatory and antinociceptive activity of pods of *Caesalpinia pulcherrima*

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ABSTRACT

Present study evaluates the Anti-inflammatory and antinociceptive activities of various extracts of pods of *Caesalpinia pulcherrima* using various experimental models. The analgesic activity pods of *Caesalpinia pulcherrima* carried out using acetic acid-induced writhing in mice and tail flick test in rats. The anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema and cotton pellet-granuloma formation in rats. The effects of the administration of reference standard (diclofenac) were also evaluated. Two different extracts (Petroleum ether and Methanolic) of *Caesalpinia pulcherrima* at the dose level of 100, 200 and 400 mg/kg, p.o. were tested. Treatment with Methanol extract (100, 200, and 400 mg/kg, p.o.) showed significant ($p < 0.01$) inhibition of carrageenan induced rat paw edema. Maximum inhibition was observed at 400 mg/kg dose as compared to the control, cotton pellet granuloma formation and acetic acid-induced writhing; however, pet ether and methanolic extracts (400 mg/kg, p.o.) were found to be more effective in increasing latency period in tail flick method. The results obtained indicate that *C. pulcherrima* has analgesic and antiinflammatory activities that supports the folk medicinal use of the plant.

Key words: Acetic acid, carrageenan, granuloma formation, *Caesalpinia pulcherrima*, tail flick.

INTRODUCTION

The use of herbal extracts and nutritional supplements either as alternative or complimentary medicine to the conventional chemotherapy for treatment of inflammatory diseases is well documented in Ayurveda, which is an alternative medicinal system that has been practiced primarily in the Indian subcontinent for 5000 years (Dahanukar et al., 2000). *Caesalpinia pulcherrima* L. Swartz (Leguminosae) is an ornamental plant due to its variety of flowers, which appear yellow, pink, offwhite, and red with yellow margins (Roach et al., 2003). It is a common medicinal plant in India, Taiwan and South- East Asian countries. In alternative medicine, the different parts of this plant have been used as an anti-inflammatory, abortifacient, emmenagogue, bronchitis and malarial infection while fruits are employed to cure diarrhea and dysentery. Phytochemical investigations on *Caesalpinia pulcherrima* have revealed the presence of various phytoactive constituents such as glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols. (Srinivas et al., 2003; Chiang et al., 2003). *Caesalpinia pulcherrima* L. Swartz (Caesalpinaceae) is an ornamental plant due to its variety of flowers, which appear yellow, pink, off-white, and red with yellow margins (Roach et al., 2003). Its seeds have shown Antiviral activity (Chiang et al., 2003) stem shown cytotoxic activity (Pherson et al., 1983), Leaves shown Antitumor activity (Chiang et al., 2003), Antimicrobial activity (Ragasa et al., 2002), Antiviral activity (Chiang et al., 2003), Flowers shown Antimicrobial, Antifungal

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activity (Sudhakar *et al.*, 2006) fruits shown Antiviral activity (Chiang *et al.* 2003), Bark shown Antimicrobial, Cytotoxic (Nasimul *et al.*, 2003).

An inflammatory response implicates macrophages and neutrophils, which secrete a number of mediators (eicosinoids, oxidants, cytokine and lytic enzymes) responsible for initiation, progression and persistence of acute or chronic state of inflammation (Lefkowitz *et al.*, 1999). Prostaglandin E2 (PGE2) and nitric oxide (NO) are most important amongst these mediators and are produced in macrophages by cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), respectively (Harris *et al.*, 2002; MacMicking *et al.*, 1997). PGE2 is implicated in inducing the production of various chemoattractants and proinflammatory cytokines (Harris *et al.*, 2002), while NO is responsible for vasodilatation, increase in vascular permeability and oedema formation at the site of inflammation (Moncada *et al.*, 1991). NO along with superoxide (O₂^{•-}) and the products of their interaction, also initiates a wide range of toxic oxidative reactions causing tissue injury (Hogg, 1998). Likewise, the neutrophils too produce oxidants and release granular constituents comprising of lytic enzymes performing important role in inflammatory injury (Yoshikawa and Naito, 2000). Inhibition in the release of these mediators is a potential strategy to control inflammation and is implicated in mechanism of action of a number of antiinflammatory drugs including the representative ones like dexamethasone (Bourke and Moynagh, 1999).

MATERIALS AND METHODS

Plant Material

Pods of *Caesalpinia pulcherrima* Fam. Caesalpinaceae were collected from local region of Nashik, India in October 2008. The plant material was identified and authenticated by Dr. P. G. Diwakar Botanical survey of India, Pune (Ref no. BSI/WC/Tech/2009 /370).

Preparation of Extract

The plant material were cleaned, dried under shade and pulverized by using grinder. 500g of the powder of plant was successively extracted with Petroleum ether, chloroform, and methanol in order of their increasing polarity using Soxhlet apparatus. The yield of extracts as follows. The extracts were obtained for *Caesalpinia pulcherrima* Petroleum ether as 1.21 %, Chloroform as 2.46 %, Methanol as 13.32 %. From the Preliminary Phytochemical study revealed that presence of sterols, glycosides, Alkaloids, Triterpenoids, Flavonoids and tannins in the extracts.

Experimental animals

Albino rats of Wistar strain (150--200 g) and Swiss albino mice (25--30 g) of either sex were used in the entire study and were procured from Haffkine Institute, Mumbai. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C; relative humidity 60%--70%) in a 12 h light-dark cycle. The animals were fed with standard laboratory

diet of Pranav agro pvt .Ltd. and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethical Committee.

(A) Studies on anti-inflammatory activity

Evaluation of Carrageenan induced rat paw edema of extract of Caesalpinia pulcherrima

The anti-inflammatory activity using carrageenan induced hind paw edema was carried out as described by Winter *et al.*, (1962). Anti-inflammatory activity was evaluated using the Carrageenan induced rat paw oedema according to the technique of Winter *et al.* After 16h of fasting, the rats of 150-200 gm were divided into eight groups of six each. Group I served as control group and received distilled water (DW), orally. Group II received Diclofenac as standard at a dose of 5 mg/Kg. Group III, IV and V animals received Pet Ether extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg ; Group VI, VII, VIII received methanol extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg; After 1 h, 0.1 ml of 1% w/v Carrageenan suspension was injected subcutaneously in to the plantar surface of the right hind paw. The paw volume was measured using a Digital plethysmometer PLM-01 (Orchid Scientifics, India) immediately and 3 h after carrageenan injection. (Pandurangan *et al.*, 2008)

Granuloma formation induced by cotton pellet in rats

After 16h of fasting, the rats of 150-200 gm were divided into eight groups of six each. Group I served as control group and received distilled water (DW), orally. Group II received Diclofenac as standard at a dose of 5 mg/Kg. Group III, IV and V animals received Pet Ether extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg ; Group VI, VII, VIII received methanol extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg; orally for consecutive six days (Winder *et al.*, 1962; Swingle *et al.*, 1972). The cotton pellet weighing 50±1 mg was sterilized in an autoclave (Lab hosp, Mumbai, India) handled with sterile instrument. The pellet was inserted in each animal on the back. Control group received vehicle.

The animals were sacrificed on seventh day and cotton pellet along with granuloma mass were collected, it was weighted and dried at 60^oc. Results of the assay were calculated as % inhibition of dry weight of granuloma formation by using the formula: 100 (A-B)/A, where, A= gain in dry weight of control pellet (mg), B= gain in dry weight of drug treated (mg).

(B) Studies on analgesic activity

(a) Tail flick latency period in rats

Male rats of 150-200 g. rats were divided into eight groups containing six animals in each group. Group I served as control group and received distilled water (DW), orally. Group II received Diclofenac as standard at a dose of 5 mg/Kg. Group III, IV and V animals received Pet Ether extract of *Caesalpinia*

pulcherrima at a dose of 100, 200 and 400 mg/kg ; Group VI, VII, VIII received methanol extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg orally; A tail flick response was evoked by placing each rat tail over the wire heated electrically, using Analgesimeter (Space Scientific, Nashik, India). The intensity of heat was adjusted so that baseline tail flick latency averaged 3-4 sec in all animals. Cut off time was 15 sec in order to avoid injury to tail. The extracts and reference standard Diclofenac were administered orally in their respective doses 1 hr prior to the test (Davies *et al.*, 1946).

(b) Acetic acid-induced writhing in mice

Male mice of 20-40 g. were divided into eight groups containing six animals in each group. Group I served as control group and received distilled water (DW), orally. Group II received Diclofenac as standard at a dose of 5 mg/Kg. Group III, IV and V animals received Pet Ether extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg ; Group VI, VII, VIII received methanol extract of *Caesalpinia pulcherrima* at a dose of 100, 200 and 400 mg/kg orally.

The writhing syndrome was elicited by intraperitoneal injection of acetic acid (0.1ml of 0.6% solution) and numbers of writhes displayed from 5 to 20min were recorded (Koster *et al.*, 1959). The extracts and reference standard Diclofenac were administered orally in their respective doses 30 min prior to the test.

STATISTICAL ANALYSIS

Results of all the above estimations have been indicated in terms of mean \pm SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons test using GraphPad InStat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $P < 0.05$.

RESULTS

Effect *Caesalpinia pulcherrima* extracts on rat paw edema induced by carrageenan

In the present study two different extracts were evaluated for anti-inflammatory activity using carrageenan-induced rat paw edema and the data was compared with that of control. (Table-1) Vehicle treated rats and Diclofenac (5 mg/kg p.o.) treated rats showed increase of paw volume as 2.4 ± 0.01 ml and 1.45 ± 0.002 ml respectively after 3h. Treatment with Petroleum Ether extract of *Caesalpinia pulcherrima* (100, 200, and 400 mg/kg, p.o.) showed a significant inhibition of paw volume after 1h, 2 h and 3 h. ($p < 0.01$). Treatment with Methanol extract (100, 200, and 400 mg/kg, p.o.) showed significant ($p < 0.01$) inhibition of carrageenan induced rat paw edema. Maximum inhibition was observed at 400 mg/kg dose as compared to the control. It was observed that the methanolic extracts of *Caesalpinia pulcherrima* (400 mg/kg, p.o.) exhibits maximum anti inflammatory activity against carrageenan

induced hind paw edema. The inhibition obtained with *Caesalpinia pulcherrima* and was 52.66% .

Table-1: Effect *Caesalpinia pulcherrima* extracts on rat paw edema induced by carrageenan.

Group	Treatment (mg/kg)	Mean increase in paw volume (ml)				% Decrease in paw volume at 3 h
		0 h	1 h	2 h	3 h	
I	Control	0.90 \pm 0.024*	1.49 \pm 0.014*	1.90 \pm 0.013*	2.40 \pm 0.014*	-
II	Diclofenac (05)	0.89 \pm 0.008*	1.01 \pm 0.01*	1.29 \pm 0.01*	1.45 \pm 0.028**	62.66
III	P.E(100)	0.87 \pm 0.037**	1.52 \pm 0.027*	1.67 \pm 0.032**	1.90 \pm 0.017**	31.33
IV	PE (200)	0.88 \pm 0.016*	1.26 \pm 0.028*	1.52 \pm 0.042**	1.82 \pm 0.029*	37.33
V	PE (400)	0.90 \pm 0.023*	1.33 \pm 0.021*	1.48 \pm 0.015*	1.71 \pm 0.019*	46.00
VI	ME(100)	0.87 \pm 0.028**	1.54 \pm 0.037**	1.72 \pm 0.030**	1.85 \pm 0.016*	39.66
X	ME(200)	0.88 \pm 0.030**	1.24 \pm 0.031**	1.46 \pm 0.024**	1.72 \pm 0.034**	44.00
XI	ME(400)	0.90 \pm 0.027**	1.19 \pm 0.017*	1.39 \pm 0.022**	1.61 \pm 0.018*	52.66

Data were analyzed using ANOVA and expressed as Mean \pm SEM ($N = 5$) followed by Dunnett's test and differences between means were regarded significant at * ($P < 0.05$), ** ($P < 0.01$)

granuloma formation in rats

The Effect of *Caesalpinia pulcherrima* extracts on cotton pellet granuloma formation is shown in Table 2.

Table 2: Effect of *Caesalpinia pulcherrima* extracts in granuloma formation induced by cotton pellet in rats.

Treatment (mg/kg)	Average weight of cotton pellet	Average weight of cotton pellet with granuloma	% Inhibition
Control	50 \pm 0.01	125.10 \pm 5.91**	
Diclofenac (05)	50 \pm 0.01	70.52 \pm 3.21*	44.13
Pet. Ether (100)	50 \pm 0.01	110.48 \pm 2.98*	12.07
Pet. Ether (200)	50 \pm 0.01	97.15 \pm 3.11*	22.09
Pet. Ether (400)	50 \pm 0.01	80.10 \pm 4.19**	36.06
Chloroform (100)	50 \pm 0.01	107.31 \pm 3.16*	14.10
Chloroform (200)	50 \pm 0.01	99.08 \pm 4.61*	20.17
Chloroform (400)	50 \pm 0.01	95.01 \pm 3.91**	24.32

Data were analyzed using ANOVA and expressed as Mean \pm SEM ($N = 5$) followed by Dunnett's test and differences between means were regarded significant at * ($P < 0.05$), ** ($P < 0.01$).

The extracts significantly inhibited cotton pellet granuloma. The percent inhibition for diclofenac Standard was found to be 44%. The percent inhibition for Petroleum ether extract of *Caesalpinia pulcherrima* was 12% ,22% , 36 % at doses of 100,200, and 400 mg/kg, respectively. The percent inhibition for Methanol extract of *Caesalpinia pulcherrima* was 14% ,20% , 24 % at doses of 100,200 and 400 mg/kg, respectively.

Effect of *Caesalpinia pulcherrima* extracts on tail flick latency period

Treatment of methanolic extract of *Caesalpinia pulcherrima* 100,200 and 400 mg/kg, p.o. significantly inhibited nociception in rats by 16 % ,21% and 23% respectively. [table- 3]

Effect of *Caesalpinia pulcherrima* extracts in acetic acid induced writhing in mice

The effect of different extracts of *Caesalpinia pulcherrima* against acetic acid induced writhing in mice. It was

Table 3: Effect of *Caesalpinia pulcherrima* extracts in tail flick latency period.

Treatment (mg/kg)	Tail flick latency (in s)	% Analgesia
Control	4.21 ± 0.34	--
Diclofenac (05)	8.36 ± 0.88**	39.38
Pet. Ether (100)	6.50±0.22 *	21.22
Pet. Ether (200)	6.10±0.31*	17.51
Pet.Ether(400)	5.09±0.41**	08.15
Chloroform (100)	5.98±0.28*	16.40
Chloroform (200)	5.16±0.32*	08.08
Chloroform (400)	4.70±0.21*	04.54

Data were analyzed using ANOVA and expressed as Mean ± SEM ($N = 5$) followed by Dunnett's test and differences between means were regarded significant at * ($P < 0.05$), ** ($P < 0.01$)

observed that mice treated with Petroleum ether extract of *Caesalpinia pulcherrima* was shown protection against 5% ,18% , 38 % at doses of 100,200 and 400 mg/kg, respectively, shows significant ($P < 0.01$) protection compared to control group, however methanol extract of *Caesalpinia pulcherrima* was shown protection against 23% ,36% , 47 % at doses of 100,200 and 400 mg/kg, respectively, was found to be more significant ($P < 0.01$) in protecting acetic acid induced writhing compared to control group. Diclofenac shown 58.18% protection against acetic acid induced writhing in mice.

DISCUSSION

Caesalpinia pulcherrima L. Swartz (Caesalpinaceae) is an ornamental plant due to its variety of flowers, which appear yellow, pink, off-white, and red with yellow margins (Roach et al., 2003). Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported (Bhujbal et al., 2008) hence the analgesic and anti-inflammatory effects produced by these extracts may be attributed due to the flavonoids and steroids. However, its pharmacological actions and mechanisms have not been precisely documented in spite of its increasing usage recently. Present work reported the potential effects of the pods of *Caesalpinia pulcherrima*, as an anti-inflammatory and analgesic agent using both *in vivo* and *in vitro* models.

Carrageenan-induced paw edema and cotton pellet granuloma formation in rats reflect the edematous stages during acute and chronic inflammation. (Matsuda et al., 1992, Vogel et al., 2002). In the present study, two different extracts of pods of *Caesalpinia pulcherrima* were tested. Carrageenan induced rat paw edema has been a popular inflammatory model to investigate nonsteroidal anti-inflammatory effect of compounds (El-Shenawy et al., 2002) Serotonin, histamine, bradykinin, and prostaglandin have been identified as a mediators for carrageenan induced rat paw edema. (Mohan et al., 2002)

Petroleum ether and methanolic extracts were found to possess a prominent anti-inflammatory activity, showing inhibition to the paw edema induced by carrageenan during the three time points from 1 to 3 h. In cotton pellet granuloma model Petroleum ether and methanolic extracts showed significant inhibition. The effectiveness of these extracts at 1 and 3 h in carrageenan induced paw edema indicates their antagonist effect at Serotonin, histamine, bradykinin and prostaglandin. Because the release of serotonin and

histamine occurs 1 h after carrageenan whereas bradykinin and prostaglandin are released 2 and 3 h, respectively, after carrageenan injection. (Di Rosa et al., 1971). The cotton pellet granuloma is a model of chronic inflammation, and dry weight has been shown to correlate with the amount of granulomatous tissue formed. (Thangam et al., 2003). In the present study animals treated with Petroleum ether and methanolic extracts showed significant inhibition of granuloma formation. Diclofenac was found to be more effective in preventing granuloma formation compared to extracts respectively. Since inflammation is also associated with pain, majority of anti-inflammatory drug possess analgesic activity. The peripheral analgesic effect of drugs may be mediated through inhibition of cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action may be mediated through inhibition of central pain receptors.

This hypothesis is in line with previous reports (Eddy et al., 1953 Williamson et al., 1996) who have postulated that acetic acid-induced writhing and tail flick methods are useful techniques for evaluation of peripherally and centrally acting analgesic drugs, respectively. Present study also showed the effects of *C.pulcherrima* extracts on acetic acid-induced writhing and tail flick latency test. Treatment of *C.pulcherrima* extracts (400 mg/kg, p.o.) significantly inhibited nociception. Whereas petroleum ether and methanolic extract (400 mg/kg, p.o.) inhibited pain perception respectively in tail flick latency test and acetic acid-induced writhing. These results indicated extracts might produce the analgesic effect peripherally as well as centrally.

Flavonoids isolated from some medicinal plants have been proven to possess antinociceptive and/ or anti-inflammatory effects. (Duke et al., 1992). It has been shown by Meli *et al.*,1990, Dicarlo *et al.*,1994 that flavonoids also inhibit gastric motility in a dose - dependent, manner. It is therefore possible that the inhibitory effects on anti-nociceptive and anti-inflammatory effects observed in these extracts may be attributed in part to its flavonoid content. Flavonoids also inhibit the phosphodiesterases involved in cell activation. (Meli *et al.*,1990). Much of this effect is upon the biosynthesis of protein cytokines that mediates adhesion of circulating leukocytes to sites of injury. Flavonoids inhibit biosynthesis of prostaglandins, which are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways. (Moroney *et al.*, 1988). Protein Kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes. (Manthey *et al.* 2001) (Rajnarayana *et al.*,2001)

CONCLUSION

From the present study, it is concluded that Extracts of pods of *C.pulcherrima* are capable of inhibiting inflammatory reactions as well as pain. The results provided experimental evidence for its traditional use in treating various diseases associated with inflammation and pain.

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