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Original Article

Screening of *Caesalpinia pulcherrima* Linn Flowers for Analgesic and Anti-inflammatory Activities

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Summary: The flowers of *Caesalpinia pulcherrima* were extracted with methanol to determine their analgesic and anti-inflammatory activities. Intraperitoneal administration of methanolic extract (75, 150 and 225 mg/kg) produced significant analgesic activity in acetic acid-induced writhing, tail immersion test and hot plate tests and anti-inflammatory effect against carrageenan-induced paw edema in experimental animals.

Industrial relevance: The herbal medicines are getting more importance in the treatment of inflammation because of the side effect of the current therapy used to treat those inflammation using synthetic drugs. Herbal medicines have less side effects and less costly when compared to the synthetic drugs. The present study will help the industry to produce herbal drug with less side effect, less costly affordable and more effective in the treatment of pain and inflammation. Finally the phytochemical screening or elucidation of the bioactive compounds from the plant would be effective drug against pain and inflammation.

Keywords: Acute toxicity; Analgesic; *Caesalpinia pulcherrima;* Inflammation; Prostaglandins

Introduction

Nature has provided a complete store-house of remedies to cure all ailments of mankind (Kokate et al., 2002; Ravi et al., 2009). This is where; nature provides us drugs in the form of herbs, plants, and algae to cure the incurable diseases without any side effects (Trease and Evans, 1983). *Caesalpinia pulcherrima* belonging to the family Leguminosae is an ornamental plant widely used for treatment of various ailments across India. It is known as Gulmohor, Krishnachura, and Mayirkonnai by the Hindi, Bengali, and Tamil people respectively. Medicine men in the Amazon Rainforest have long known some of the medicinal uses for *Caesalpinia pulcherrima*, which is known as *ayoowiri*. The juice from the leaves is said to cure fever, the juice from the flower is said to cure sores, and the seeds are said to cure bad cough, breathing difficulty, and chest pain. Four grams from the root is also said to induce abortion in the first trimester of pregnancy (Schiebinger, 2004). In Eastern Himalaya the flowers, leaves-sap, and other parts are used to treat swelling, earache, muscular and rheumatic pain and various cardiovascular diseases. Various constituents like cassane diterpenoids, and flavonoids has been isolated from the plant (Pranithanchai et al., 2009; Rao et al., 2005). Hence, this study has been undertaken to evaluate the analgesic and anti-inflammatory activities of the methanolic extract of *Caesalpinia pulcherrima* L. flowers (MCP).

Materials and Methods

Plant material: The flowers of *Caesalpinia pulcherrima* were collected in the month of April from the Eastern part of India (Sikkim Himalayas). The Herbarium Specimen of plant was deposited in the department of Pharmacognosy and it has been identified from Himalayan Pharmacy Institute, Majhitar, Sikkim, India.

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Extraction: The flowers of *Caesalpinia pulcherrima* were dried in shade and powdered (no. 60 mesh) and 100 g of the dried powder was Soxhlet extracted successively with petroleum ether, chloroform, and methanol. The weight of methanolic extract after drying was calculated as 12.63 g.

Animals: Swiss albino mice of both sexes weighing between 25-30 g were used for acute toxicity study and analgesic experiments. Male Sprague-Dawley rats weighing 200-250 g were used for anti-inflammatory activity. They were housed in standard environmental condition like, ambient temperature (25 ^{o}C ± 1 ^{o}C), relative humidity (55±5%), and 12/12h light dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The institute animal ethical committee has given the approval for conducting animal experiments (HPI/08/60/IAEC/0061).

Acute toxicity study: The acute toxicity study of MCP was performed in mice by graphical method (Turner, 1965; Veerappan et al., 2007). The dead animals obtained from primary screening studies, LD_{50} value determination experiments, and the acute studies were subjected to post mortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen, and brain was carefully noted and any apparent and significant features or differences from the normal was recorded.

Writhing test: Writhing was induced in mice *(N=6)* by intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period as previously reported (Bose et al., 2007; Koster et al., 1959; Hendershot and Forsaith, 1959). Animals were treated through i.p. route 30 min before injection of acetic acid with MCP (75, 150, and 225 mg/kg), or acetylsalicylic acid (ASA) (200 mg/kg).

Tail immersion test: Six mice in each group were administered i.p. with vehicle (0.9%, sodium chloride), pentazocine (30 mg/kg), and MCP (75, 150, and 225 mg/kg). The distal part of the tail was immersed in hot water maintained at 55 ± 1 °C. The time taken to withdraw the tail was noted as reaction time (Bose et al., 2007). A cut off time of 10 sec was maintained at 55 C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45, and 60 min after treatment.

Hot plate test: The method originally described by Woolfe and Mac Donald (Woolfe and MacDonald, 1944). In this method the groups of 6 mice of either sex were used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. This can be a copper plate or a heated glass surface. The temperature of the surface was controlled for 55 ±1°C. The animals were placed on the hot plate and the time until either licking or jumping occurs, were recorded by a stop-watch. The latency was recorded before and after 30, 60, 120, and 180 min following i.p. administration of the standard or the MCP (75, 150, and 225 mg/kg). Pethidine (5 mg/kg, i.p.) was used as standard drug for comparing analgesic effect.

Carrageenan induced paw edema : Anti-inflammatory activity was assessed by the method described by Winter (Winter et al., 1962). Male Sprague-Dawley rats of either sex weighing 200 – 250 g were divided into 5 groups (N=6). Group I received normal saline (control), Group II, III and IV received MCP (75,150, and 225 mg/kg, i.p. respectively). Group V received diclofenac (reference standard 10 mg/kg, i.p.) (Brooks et al., 1991). Animals were treated with MCP and diclofenac and subsequently 1 h after treatment; 0.1ml of 1% suspension of carrageenan in normal saline was injected into the subplantar region of left hind paw to induce edema. The paw volume was measured at 0, 1, 2, 3, and 4hr after carrageenan injection using digital paw edema meter (520-R, IITC Life Science -USA).

Statistical analysis: The results were statistically analysed using one way *ANOVA* followed by Dunnet's *t*-test. p values < 0.05 were considered significant.

Results and discussion

The dead animals obtained from the acute toxicity experiments were found with effects of respiratory arrest and convulsion. Apart from this characteristic observation, no other significant observation deviant from the normal was seen in these dead animals. The LD_{50} value of MCP calculated as 1496.23 mg/kg. Intraperitoneal administration of the MCP (75, 150, and 225 mg/kg) significantly reduced the number of writhings induced by acetic acid in mice, the MCP 150 mg/kg and MCP 225 mg/kg were most active (figure 1). The activity was comfrontable to that of ASA (200 mg/kg) used as reference drug. Moreover the MCP (75, 150, and 225 mg/kg) induced protection in tail immersion test being the MCP 150 mg/kg and MCP 225 mg/kg were found more active upto 1 h, that is comparable to standard pentazocine (30 mg/kg, i.p.) (Table 1). The tested samples protected mice against both chemical and thermal induced noxious stimuli, which were evidenced from both the tail immersion and acetic acid induced writhing tests. Variation in order of activity for MCP (75, 150, and 225 mg/kg) in acetic acid induced writhing and tail immersion tests indicated that the different constituents present in MCP may be responsible for central and peripheral analgesia. Acetic acid which is used as an inducer for writhing syndrome (Koster et al., 1959; Hendershot and Forsaith, 1959) causes algesia by releasing of endogenous substances, which then excite the pain nerve ending; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is possible that

MCP exerts an analgesic effect probably by inhibiting the synthesis of prostaglandins. Based on these results, we concluded that intraperitoneal administration of the MCP results in analgesic activity. Presence of phytoconstituents like terpenoids and flavonoids has been previously found to be responsible for analgesic activity in plants (Trease and Evans, 1983). The presence of the said constituents in MCP may be responsible for observed activities. However, the tested extract has also been effective in the hot plate test (Table 2). As the hot plate test is a specific central antinociceptive test (Santos et al., 2007), it is possible that MCP exerts its effect through central mechanism.

The most widely used primary test to screen new anti-inflammatory agent's measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent (Winter et al., 1962). Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages (Brito and Antonio, 1998; Gupta et al., 2006). The MCP reduced the carrageenan induced paw edema in rats, and MCP 150 and 225 mg/kg was found most active (Table 3). It may be due to inhibition of cyclooxygenase enzyme followed by prevention of inflammatory mediators release. The presence of the said constituents in MCP may be responsible for observed activities. These findings seem to justify the use of the plant in traditional Indian medicine in the treatment of pain and inflammation. Further investigations needed to isolate and characterize the active component of the plant extract.

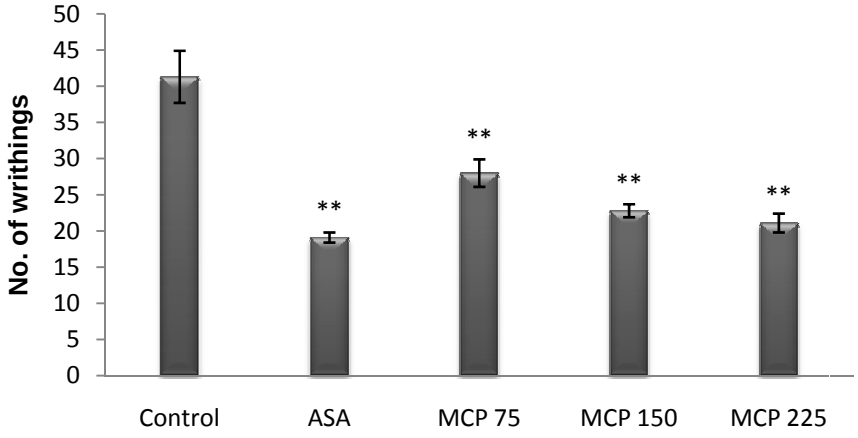

Figure 1. Effect of MCP on acetic acid-induced writhing in mice

Table 1. Effect of MCP on tail immersion test in mice

Groups	Dose	Average tail withdrawing time (s)				
	(mg/kg)	0 min	15 min	30 min	45 min	60 min
Control	_	2.6±0.33	2.5±0.22	2.4±0.24	2.8±0.58	2.6±0.21
Pentazocine	30	2.8±0.30	5.8±0.30**	8.2±0.58**	8.2±0.48**	8.3±0.33**
MCP	75	2.6±0.33	3.6±0.21*	5.0±0.44**	5.4±0.40**	3.5±0.22
MCP	150	2.8±0.16	4.1±0.30**	6.4±0.40**	6.8±0.37**	6.8±0.30**
MCP	225	2.7±0.19	3.8±0.40*	7.4±0.50**	7.6±0.50**	7.8±0.40**

Values are expressed as mean ± SEM, *N=6*
*P<0.05; **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's *t*-test)

Table 2. Effect of MCP on thermic stimulus induced pain (hot plate test) in mice

Groups	Dose	Reaction time in seconds at time (h)				
	(mg/kg)	0	0.5	1	2	3
Control	_	7.1±0.47	7.8±0.54	8.3±0.33	8.8±0.16	9.5±0.22
Pethidine	5	7.8±0.47	9.1±0.47	10.6±0.34**	13.3±0.49**	15.1±0.60**
MCP	75	7.6±0.49	8.3±0.49	9.1±0.30	10.3±0.33*	10.6±0.61
MCP	150	8.1±0.40	8.5±0.34	10.1±0.30*	11.8±0.47**	12.6±0.42**
MCP	225	7.5±0.42	9.1±0.74	10.1±0.60*	12.3±0.33**	13.1±0.54**

Values are expressed as mean ± SEM, *N=6*
$^{*}P<0.05$; $^{**}P<0.01$ compared with vehicle control (ANOVA followed by Dunnet's *t*-test)

Table 3. Effect of MCP on carrageenan-induced paw edema in rats

Groups (n=6)	Dose	Paw volume (ml)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Normal Saline	10 ml/kg	0.33±0.007	0.49±0.005	0.46±0.004	0.48±0.005	0.47±0.004
MCP	75 mg/kg	0.31±0.01	0.46±0.006*	0.45±0.007	0.46±0.004	0.45±0.005
MCP	150 mg/kg	0.31±0.01	0.41±0.008**	0.41±0.008**	0.39±0.008**	0.39±0.008**
MCP	225 mg/kg	0.33±0.007	0.38±0.008**	0.37±0.006**	0.35±0.004**	0.34±0.004**
Diclofenac	10 mg/kg	0.32±0.009	0.34±0.008**	0.33±0.007**	0.31±0.008**	0.31±0.004**

Values are expressed as mean ± SEM, *N=6*.
$^{*}P<0.05$; $^{**}P<0.01$ compared with vehicle control (ANOVA followed by Dunnet's *t*-test)

Conclusion

The therapeutic efficacy of *Caesalpinia pulcherrima* extensively used in Indian System of Medicine has been established through modern testing and evaluation. The medicinal applications of this plant, countless possibilities for investigation still remain in relatively newer areas of its function. Hence, phytochemical substances of these plants will enable to exploit its therapeutic use. Therefore, further studies may carry out to prove the potential of this plant. The plant is becoming the endangered species now so more work can be done on agricultural and climatic conditions to grow this plant.

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