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Anti-inflammatory activity of heartwood extracts of *Caesalpinia pulcherrima* (Caesalpiniceae)

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Abstract

Objective: To investigate the Anti-inflammatory Activity of methanolic and petroleum ether extracts of heartwood of *Caesalpinia pulcherrima*. Method: Anti-inflammatory Activity of methanolic and petroleum ether extracts of heartwood of *Caesalpinia pulcherrima* (30, 100, 300 mg/kg, p.o.) was studied in rats using Carrageenan induced hind paw edema method. Result: Both methanolic and petroleum ether extracts showed maximum anti-inflammatory effect when compared with control group. From these methanol extract (30, 100, 300 mg/kg, p.o.) significantly ($p<0.05$) inhibited Carrageenan induced hind paw edema. Conclusion: The methanolic extract of heartwood of *Caesalpinia pulcherrima* contain bioactive principles, which possess anti-inflammatory activity.

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

1.Introduction

Caesalpinia pulcherrima (Caesalpiniceae) popularly known as Guleture and Peacock flower in India, is a shrub or small tree up to 5m in height abundantly cultivated throughout India [1]. Literature survey reveals the presence of diterpenoids [2], isovouacapenol C, pulcherrimin-A in roots [3]. Stems contain peltogynoids, bonducellin, and 6-methoxypulcherrimin, homoisoflavonoids [4]. Flowers showed the presence of lupeol, β-sitosterol, flavonoids, and myricetin [5]. The leaves contain hydrocyanic acid, tannins [6], and benzoic acid [7]. The different parts of this plant are employed in traditional medicine as emmenagogue, abortifacient, purgative and stimulant. It also shows antipyretic, antimicrobial and antituberculur activities and used in bronchitis, asthma and malarial fever [8]. Flowers of *Caesalpinia pulcherrima* have been reported to posses antiviral [9] and antioxidant effects [10]. Hence in the present investigation evaluation of anti-inflammatory activity of heartwood extracts of *Caesalpinia pulcherrima* was taken up.

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2. Materials and Method

The heartwood of *Caesalpinia pulcherrima* was collected from Nashik, Maharashtra, India. The plant was authenticated by Mr.S.G. Pradhan, botanical survey of India, Pune (Voucher no. BSI/WC/Tech/2003/597). And preserved in the herbarium of the department.

2.1 Preparation of Extracts

The heartwood of *Caesalpinia pulcherrima* was shade dried, coarsely powdered (1kg) and successively extracted with petroleum ether (60-80°), chloroform and methanol in the increasing order of polarity in a soxhlet extractor. The yield of the crude extracts was 2.02%, 3.58%, 13.13% for petroleum ether, chloroform and methanolic extracts respectively. Methanolic extract was treated with acetone; acetone soluble part of methanolic extract was used. The crude extracts were subjected to preliminary Phytochemical screening; which showed the presence of tannins, flavonoids, sterols, triterpenes and glycosides.

2.2 Test Animals

Wistar rats (100-150gm) were obtained from Serum Institute, Pune. Animals were housed in groups of five at an ambient temperature of 25 ± 1°C. Animals had free access to food and water. Animals were deprived of food but not water 4 h before the experiment. The Institutional Animal Ethical Committee approved the protocol of this study.

2.3 Chemicals and Drugs

Aspirin (Research Lab, Mumbai), Carrageenan (Sigma, Mumbai), Pet ether, methanol and acetone were obtained from Modern Scientific, Nashik.

2.4 Test Samples and Standards

Pet ether extract (30, 100, 300, and 500 mg/kg), methanolic extract (30, 100, 300, and 500 mg/kg), Carrageenan and Aspirin were prepared in 2% gum acacia suspension before oral administration.

2.5 Anti-inflammatory Activity

Carrageenan Induced Rat Paw Edema

The method of Winter *et al*. (1962) was used to study acute inflammation. Rats in groups of five each were treated with vehicle, Pet ether extract (30, 100, and 300 mg/kg, p.o.), methanolic extract (30, 100, 300, and 500 mg/kg) one hour prior to Carrageenan injection. 0.1ml of 1% Carrageenan was injected into the subplantar tissue of left hind paw of each rat. Swelling of Carrageenan injected foot was measured at 0, 1, 2, 3, 4 hr using Plethysmometer (UGO Basile, Italy) [11]. The right hind paw was injected with 0.1 ml of vehicle. Aspirin (20 mg/kg p.o.) was used as reference agent.

2.6 Statistical Analysis

All values shown as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test $P<0.05$ was considered statistically significant.

3. Result

The results of anti-inflammatory activity of heartwood extracts of *Caesalpinia pulcherrima* against Carrageenan induced inflammation as shown in Table1 and Table 2.

The heartwood extracts of *Caesalpinia pulcherrima* (30, 100, and 300mg/kg p.o.) significantly ($p<0.05$) inhibited Carrageenan induced paw edema. But the acetone soluble part of methanol extract showed maximum inhibition of paw edema when compared to the control group. The anti-inflammatory activity produced by all the extracts of *Caesalpinia pulcherrima* was found to be more effective than the reference standard aspirin. The percentage inhibition of edema at 4hr after *Carrageenan challenge* produced by pet ether and methanolic extracts

Table 1. Effect of Pet ether (30, 100, 300, and 500 mg/kg) on Carrageenan Induced Rat Paw Edema

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Decrease in paw volume at 4 h
	0 h	1 h	2 h	3 h	4 h	
Control	0.65 ± 0.02	1.12 ± 0.01	1.28 ± 0.01	1.59 ± 0.004	1.84 ± 0.04	-
Aspirin (20)	0.74 ± 0.06	1 ± 0.03*	1.08 ± 0.03*	1.18 ± 0.03*	1.1 ± 0.03*	20.98
Pet ether extract (30)	0.62 ± 0.05	0.69 ± 0.03*	0.86 ± 0.02*	1.21 ± 0.009*	1.03 ± 0.01*	33.33
Pet ether extract (100)	0.52 ± 0.01	0.6 ± 0.004*	0.77 ± 0.008*	0.98 ± 0.004*	0.87 ± 0.02*	42.28
Pet ether extract (300)	0.55 ± 0.05	0.61 ± 0.03*	0.70 ± 0.008*	0.90 ± 0.006*	0.70 ± 0.004*	46.60

n = 5. The observations are mean ± SEM. *p<0.05, as compared to control (ANOVA followed by Dunnett's test).

Table 2. Effect of methanolic extracts (30, 100, 300, and 500 mg/kg) on Carrageenan Induced Rat Paw Edema

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Decrease in paw volume at 4 h
	0 h	1 h	2 h	3 h	4 h	
Control	0.65 ± 0.02	1.16 ± 0.01	1.91 ± 0.01	2.12 ± 0.03	2.18 ± 0.01	-
Aspirin (20)	0.74 ± 0.06	1 ± 0.03*	1.08 ± 0.03*	1.1 ± 0.03*	1.1 ± 0.03*	37.10
Methanol extract (30)	0.62 ± 0.01	0.68 ± 0.01*	0.74 ± 0.02*	0.92 ± 0.01*	0.80 ± 0.02*	53.80
Methanol extract (100)	0.52 ± 0.01	0.63 ± 0.01*	0.70 ± 0.004*	0.85 ± 0.02*	0.77 ± 0.03*	57.37
Methanol extract (300)	0.44 ± 0.02	0.51 ± 0.02*	0.66 ± 0.02*	0.79 ± 0.03*	0.68 ± 0.02*	62.16

n=5. The observations are mean ± SEM. *p<0.05, as compared to control (ANOVA followed by Dunnett's test).

of *Caesalpinia pulcherrima* and standard aspirin was 20.98 and 37.10 respectively.

4. Discussion

Edema represents the early phase of inflammation in Carrageenan induced paw edema and is the simplest and most widely used acute inflammatory model for studying anti-inflammatory agents [12]. The development of Carrageenan induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, serotonin and kinins in the first hr after injection of Carrageenan in 3 hrs and second phase is related to release of prostaglandin like substance in >4 hrs [13].

All the extracts of *Caesalpinia pulcherrima* showed significant anti-inflammatory activity at 4 hrs against Carrageenan induced rat paw edema. The involvement of endogenous substances such as PGs may be minimized in this model. In the present study, maximum anti-inflammatory effect of heartwood of *Caesalpinia pulcherrima* may be attributed to the presence of flavonoids as evident by preliminary phytochemical investigation [14, 15].

Thus, it can be concluded that methanolic extracts (30, 100, 300, and 500 mg/kg) of the heartwood of *Caesalpinia pulcherrima* posses anti-inflammatory properties which are probably mediated via inhibition of prostaglandin synthesis and may have a potential benefit for the management of pain and inflammation.

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