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Anti-Inflammatory Activity of Ethanolic Leaf Extracts from *Adenanthera pavonina* (L) in Rats

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ABSTRACT

Ethanolic extracts from the leaves of *Adenanthera pavonina* were assessed at doses of 250 and 500 mg/kg for anti-inflammatory effects using both acute and chronic inflammatory models. The anti-diarrheal activity was also evaluated to support any possible evidence of prostaglandin synthesis inhibition. It was found that the doses possessed inhibitory effects on the acute phase of inflammation as seen in carrageenan-induced hind paw edema as well as in a subacute study of cotton pellet-induced granuloma formation. The anti-inflammatory activity elicited by the leaf extracts may be due to the influence of the active constituents such as beta-sitosterol and stigmasterol. A possible mechanism may also be due to the inhibition of prostaglandin synthesis which is also evidenced by the delay in the formation of wet faeces. Further study is required to postulate the exact molecular mechanism involved in this process of inhibition of inflammation by these leaf extracts.

Key words: Adenanthera, anti-inflammatory, cotton pellet, paw edema

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INTRODUCTION

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world due to their potent pharmacological activities and low toxicity. *Adenanthera pavonina* (L) is used in Indian folk medicine in the treatment of inflammation, cholera, general paralysis, and blood pressure.[1-3] A previous study had demonstrated potent anti-inflammatory activity in its seeds.[4] The leaves are reported to contain glucosides of beta-sitosterol and stigmasterol, which are reported to possess pronounced anti-inflammatory and analgesic activities.[5,6] It is therefore of interest to investigate ethanolic extracts of *Adenanthera pavonina* for any anti-inflammatory activity.

MATERIALS AND METHODS

Plant material

The plant material was collected from Tambaram; Chennai in the month of May. The specimen was identified by a resident botanist and the voucher specimen was deposited in the Department of Pharmacology, CLBMCP; Chennai.

Preparation of ethanolic extract of *Adenanthera pavonina*

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction.

A weighed quantity (100 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with petroleum ether (40% v/v) and the residual marc was dried and extracted with ethanol (95% v/v).The extract was evaporated under reused pressure using a rotovac evaporator until all the solvent had been removed to give an extract sample with a yield of 18.46% w/w in relation to the dried starting material.

Experimental animals

Inbred adult Wistar rats (150-200 g) of either sex were obtained from the animal house of CLBMCP, Chennai. The animals were maintained in a well-ventilated room with 12:12 h light/dark cycles in polypropylene cages. Standard pelleted feed and drinking water were provided *ad libitum* throughout the experimental period. The animals were acclimated to laboratory conditions one week prior to the initiation of experimental work. The protocol was approved by the Ethics Committee and the CPCSEA.

Acute oral toxicity study

The animals were fasted overnight and were administered a 5000 mg/kg body weight dose of the extract. The animals were further subjected to observation for four hours; any signs and symptoms of mortality were recorded.[7]

Carrageenan induced hind paw edema

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control, group 2 received indomethacin 20 mg/kg suspended in 1% sodium carboxymethyl cellulose. Groups 3 and 4 were treated with 250 and 500 mg/kg of ethanolic extracts of *Adenanthera pavonina* (EEAP) suspended in Tween 80/ethanol/saline (1:1:10).[8] Edema was induced by injecting 0.1 mL of a 1% solution of carrageenan in saline into the subplantar aponeurosis of the right hind paw of the rats. The vehicle, extracts, and the standard drugs were administered 60 min prior to the injection of the phlogestic agent. The volumes of edema of the injected and the contralateral paws were measured at 1, 2, 3, 4, 5 h after the induction of inflammation using a plethysmograph to calculate the percentage of anti-inflammatory activity.[9,10]

Cotton pellet granuloma

The animals were grouped as described above to study the anti-inflammatory activity. The groups were fasted and treated with drugs/doses similar to that of carrageenan-induced hind paw edema. Sterile cotton pellets each weighing 30 ± 5 mg were prepared and sterilized in a hot air oven at 123°C for 3 h. Each animal was placed under light ether anesthesia and subcutaneously implanted with four cotton pellets, one each into both the axillae and the groin region under aseptic conditions. The drugs were administered orally for seven days starting from the day of implantation of the pellets. All the animals had free access to drinking water and food. On the 8th day, all the animals were sacrificed and the implanted cotton pellets were recovered, cleaned of surrounding tissues, and blotted with filter paper. These cleaned pellets were weighed and dried in a hot air oven overnight at 70°C and the dry weights were noted.[11]

Castor oil-induced diarrhea

The animals were grouped as described above to study anti-inflammatory activity. The groups were fasted and treated with drugs/doses similar to that of carrageenan-induced hind paw edema. The drugs were administered one hour before the administration of the cathartic agent, castor oil 0.5 mL/g, *p.o.* after which the animals were placed individually in a propylene cage lined with filter paper. The appearance of the first wet faeces and the total weights of the wet and dry faeces were noted up to the 4th hour after administration of the drug.[12]

Statistical analysis

The values are expressed as mean ± S.E.M. Statistical analysis was performed using ANOVA (One-way) followed by Dunnett's *t*-test. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Acute toxicity studies revealed that the extract is nontoxic up to 5000 mg/kg, demonstrating the safety profile of the extract.

EEAP caused significant ($P < 0.001$) reduction in paw edema from the third hour at the 250 mg/kg dose level, whereas significant ($P < 0.001$) reduction in paw edema was observed from the second hour at the 500 mg/kg dose level [Table 1]. There is good evidence that the early or first phase of transient permeability is due to the release of histamine and can thus be suppressed by antihistamines. The mediation of the delayed or second phase of exudation is more controversial and complex, and has been attributed in part to kinins, prostaglandins, neutrophils, and lipoxygenase products of arachidonic acid metabolism.[13] The probable mechanism of anti-inflammatory action of EEAP may be due to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than

Table 1: Effect of EEAP on Carrageenan-induced hind paw edema

Treatment	Dose (mg/kg)	Mean difference in paw volume (mL)				
		1 h	2 h	3 h	4 h	5 h
Vehicle	-	0.16 ± 0.06	0.23 ± 0.09	0.28 ± 0.07	0.32 ± 0.07	0.38 ± 0.06
Standard [Indomethacin]	20	0.14 ± 0.03 a† (12.5)	0.16 ± 0.05 a* (30.43)	0.11 ± 0.04 a* (60.71)	0.10 ± 0.04 a* (68.75)	0.10 ± 0.03 a* (73.68)
EEAP	250	0.15 ± 0.04 a† (6.25)	0.21 ± 0.04 a† (8.69)	0.18 ± 0.06 a* (35.71)	0.18 ± 0.06 a* (43.75)	0.17 ± 0.04 a* (55.26)
EEAP	500	0.14 ± 0.06 a† (12.5)	0.20 ± 0.05 a* (13.04)	0.16 ± 0.04 a* (42.85)	0.16 ± 0.03 a* (50.00)	0.15 ± 0.06 a* (60.52)

EEAP: Ethanolic extract of *Adenanthera pavonina;* Values are mean ± SEM, $n = 6$ animals in each group; a represents comparisons between Group 2, 3, 4 *vs* Group 1; Values within parentheses represent the percentage protection; Statistical evaluation by one-way ANOVA followed by Dunnett's *t*-test; Symbols represent statistical significance: *-$P < 0.001$; #-$P < 0.01$; †- nonsignificant

the lipoxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan. The inhibition of prostaglandins was further confirmed by the delay in the time of appearance of wet faeces. Prostaglandins contribute to pathophysiological functions in the gastrointestinal tract. Castor oil increases the peristaltic activity and produces permeability changes in the intestinal mucous membranes to electrolytes and water, effects that are associated with prostaglandin release.[12] The delay of castor oil-induced diarrhea may be related to the inhibition of prostaglandin synthesis which may also influence the possible mechanism of anti-inflammatory activity mediated through possible prostaglandin inhibition.[14]

The cotton pellet method has been widely employed to assess the transudative, exudative, and proliferative components of chronic inflammation. EEAP caused significant ($P < 0.001$) reduction in the wet and dry weights at the 250 and 500 mg/kg dose levels [Table 2]. Subcutaneous implantation of cotton pellets into rats results in the formation of granuloma at the site of the implant. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed.[15] The granuloma formed by day seven is characterized by the formation of a vascularized fibrous capsule containing fibroblasts and infiltrating mononuclear cells.[16] The cellular infiltration is divided into two phases: An acute phase lasting for about two days in which polymorphonuclear leukocytes are the predominant feature, followed by another period of increasing numbers of mononuclear cells, mainly macrophages, and cell proliferation.[11]

Drugs possessing anti-inflammatory activity have been shown to delay castor oil-induced diarrhea, suggesting the involvement of prostaglandins in this mechanism. Around 44 NSAIDs were tested and their selective potencies in castor oil-induced diarrhea and the carrageenan-induced test were found to be well correlated.[17] EEAP significantly delayed the time of appearance of wet faeces [Table 3] which could be due to inhibition of prostaglandin synthesis.

Table 2: Effect of EEAP on Cotton pellet granuloma

Treatment	Dose (mg/kg)	Wet weight (mg)	Dry weight (mg)
Vehicle	-	399.16 ± 6.50	95.66 ± 3.52
Standard [Indomethacin]	20	281.83 ± 4.54 a*	36.00 ± 1.80 a*
EEAP	250	354.00 ± 3.25 a*	48.83 ± 2.72 a*
EEAP	500	342.16 ± 3.46 a*	40.33 ± 2.10 a*

EEAP: Ethanolic extract of *Adenanthera pavonina;* Values are mean ± SEM, $n = 6$ animals in each group; a represents comparisons between Group 2, 3, 4 *vs* Group 1; Statistical evaluation by one-way ANOVA followed by Dunnett's *t*-test; Symbols represent statistical significance: *- $P < 0.001$; #-$P < 0.01$; †- nonsignificant

Table 3: Effect of EEAP on Castor oil-induced diarrhea

Treatment	Dose (mg/kg)	Time of first appearance of wet faeces (min)	Total wet faeces at 4th hour (mg)
Vehicle	-	36.33 ± 1.68	30.16 ± 2.24
Standard	20	73.83 ± 1.66 a*	12.5 ± 1.25 a*
EEAP	250	50.66 ± 2.52 a*	22.5 ± 1.82 a*
EEAP	500	63.5 ± 2.23 a*	20.66 ± 1.62 a*

EEAP: Ethanolic extract of *Adenanthera pavonina;* Values are mean ± SEM, $n = 6$ animals in each group; a represents comparisons between Group 2, 3, 4 *vs* Group 1; Statistical evaluation by one-way ANOVA followed by Dunnett's *t*-test. Symbols represent statistical significance: *-$P < 0.001$; #-$P < 0.01$; †- nonsignificant

CONCLUSION

It is clearly evident that the ethanolic leaf extract of *Adenanthera pavonina* possesses significant anti-inflammatory activity in rats. This may be due to the presence of the reported active constituents and their influence on the prostaglandin pathway. Further studies are needed to determine the exact molecular mechanism involved in the process of protection against inflammation.

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