



Anti-inflammatory activity of *Adenanthera pavonina* Linn. leaves

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Abstract

Antiinflammatory activity of *Adenanthera pavonina* Linn. leaves were evaluated using formalin induced rat paw oedema model for acute inflammation and cotton pellet granuloma model for chronic inflammation. The leaves of the plant was extracted with methanol by cold maceration method. The methanolic extract was fractionated with water. The extract and its aqueous fraction were screened for its anti inflammatory activity at the dose levels of 200mg/kg and 400mg/kg. Compared with control both showed significant activity against acute and chronic inflammation. Aqueous fraction of methanolic extract significantly inhibit the paw oedema in acute model and granuloma formation in chronic model with respect to the methanolic extract. The results indicates that the antiinflammatory activity of *Adenanthera pavonina* leaf extract could be through a inhibition of elevated prostaglandin biosynthesis and reduction of proliferative mass inflamed cells. The present results of anti inflammatory activity supports the folklore claim of *Adenanthera pavonina* leaves.

Keywords: *Adenanthera pavonina*, formalin, cotton pellet granuloma, anti inflammatory

1. Introduction

The plant *Adenanthera pavonina* Linn. commonly known as “anaikundumani” in Tamil, belongs to the family Mimosaceae [1]. It is a large sized deciduous tree, ranges in height from 6-15 meters found naturally in India. Earlier claims reported that bark and leaves are astringent, vulnerary and aphrodisiac and are used in ulcers, pharyngopathy, vitiated condition of vata and gout and rheumatism. It also reported that the heart wood is astringent, aphrodisiac, haemostatic and is useful in dysentery, hemorrhages and vitiated conditions

of vata and gout [2]. The root is emetic in nature [3]. Earlier phytochemical investigation showed that the leaves contain octacosanol, dulcitol, glucosides of betasitosterol, flavones and stigmasterol [4] and alcoholic extract of leaves contains an alkaloid [5]. The bark contains beside stigmasterol glycosides, butein, chalcone, dihydromyricetin, 2, 4-dihydrobenzoic acid, robinetin and saponins which on hydrolysis and methylation afforded methyl echinocystate and methyloleanolate. The wood contains robenetin, chalcone, butein and the flavones. The seeds

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contain non protein amino acid, methelene glutamine and traces of ethledine glutamic acid [6]. Based on ethnopharmacological and phytochemical reports , in the present work an attempt was made to evaluate anti inflammatory activity of *Adenanthera pavonina* leaf by acute and chronic model

2. Materials and Methods

2.1 Plant material and extraction

The plant specimen for the proposed study was collected from Chennai, Tamilnadu in the month of July. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, (PARC) Tambaram, Chennai. The leaves were shade dried and coarsely powdered. About 500gms of powder was extracted with methanol by cold maceration method. The solvent was filtered and distilled off. Final traces of solvent was removed under vacuum. The percentage yield of methanolic extract was 25.05 %w/w. The total methanolic extract thus obtained was fractionated with water and its percentage was 8.1% w/w.

2.2 Chemicals and reagents

Plant extracts used in the present study were methanolic extract and its aqueous fraction of *Adenanthera pavonina* Leaves. Formalin (Sigma chemical), Indomethacin (Novartis). and solvents from Qualigen Fine chemicals

2.3 Experimental animals

Adult male *wistar* rats weighing 150-175 gms were used in the present pharmacological studies. The inbred animals were taken from the animal house in department of Pharmacology, Vels university, Pallavaram. Chennai. The animals were maintained in well-ventilated room temperature with natural 12 hours day light and 12 hours night cycle in the propylene cages. They were fed standard rodent pellet diet from Poultry Research Station, Nandanam , Chennai.

and water *ad libitum* through out the experimental period. The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature .The study was carried out with the approval of Animal Ethics Committee as per CPCSEA guidelines.

Acute method

Formalin Induced Paw Oedema [7]

Rats were divided into six groups each group consistinig of six.

Group 1 vehicle control : 2% of tween 80 (1ml/kg body weight p.o.)

Group 2 (standard) : Indomethacin (10 mg/kg p.o) suspended in tween 80 (2%w/v)

Group 3 (test) : Methanolic extract of leaves of *Adenanthera pavonina* (200 mg/kg p.o) (MEAP) suspended in tween 80 (2%w/v)

Group 4 (test) : Methanolic extract of leaves of *Adenanthera pavonin* (400 mg/kg p.o) MEAP) suspended in tween 80 (2%w/v)

Group 5 (test) : Aqueous fraction of methanolic extract of *Adenanthera pavonina* (AFAP) (200 mg/kg p.o) suspended in tween 80 (2%w/v)

Group 6 (test) : Aqueous fraction of methanolic extract of *Adenanthera pavonina* (400 mg/kg p.o) (AFAP) suspended in tween 80 (2%w/v)

After 30 minutes of administration of test drugs and standard drug orally, oedema was induced by injecting 0.1 ml of 2 % of formalin in the subplantar region of left hind paw. The hind paw volumes of the left legs were measured at the time intervals 1st , 2nd ,4th and 24th hours by plethysmometer after induction. Percentage inhibition was calculated by using formula

$$\% \text{ inhibition } = \frac{C-T}{C} \times 100$$

C - Mean difference between right and left paw volume in control group.

T- Mean difference between right and left paw volume in test and standard.

The result was tabulated in terms of paw volume in hours and percentage of inhibition in Table 1 and also is shown in Fig-1.

Chronic Method

Cotton Pellet Granuloma method [8]

Rats were divided into four groups each consisting of six.

Group 1 Vehicle control : 2 % w/v tween 80 (1ml/kg body wt. p.o.)

Group 2 (standard) : Indomethacin(10 mg/kg p.o) suspended in tween 80 (2%w/v)

Group 3 (test) : Methanolic extract of leaves of *Adenanthera pavonina* (400 mg/kg p.o) (MEAP) suspended in tween 80 (2%w/v)

Group 4 (test) : Aqueous fraction of methanolic extract of *Adenanthera pavonina* (400 mg/kg p.o) (AFAP) suspended in tween 80 (2%w/v)

Inflammation was produced by cotton pellet induced granuloma in rats. Sterile cotton (50±1mg) soaked in 0.2 ml of distilled water was implanted subcutaneously under ether anesthesia. The treatment was given up to seven days after cotton implantation. The animals were sacrificed on the 8th day and the granuloma tissue with cotton pellet was dissected and dried at 60°C overnight and the dry weight was noted. The weight of the cotton pellet before implantation is subtracted from the weight of the dried dissected pellets.

% inhibition

C- dryweight of cotton of control group animals.

T- dryweight of cotton of test group animals

The result was given as percentage of inhibition of granuloma in Table 2 and also in Fig-2

2.4 Statistical analysis

All results were reported as mean ± S.E.M. These results were further analysed by using one way ANOVA followed by Dunnet's 't' test to calculate significance of the results. "p" – value less than 0.05 and 0.01 were considered significant.

3. Results

3.1 Formalin induced paw edema model

The methanolic extract and its aqueous fraction of *Adenanthera pavonina* leaves significantly ($P<0.01$ and $P<0.05$) inhibit the paw volume. The percentage inhibition of oedema produced by methanolic extract and its aqueous fraction at higher dose (400 mg/kg) was found to be 52.3% and 62.8% respectively. Compared to Indomethacine (10 mg/kg) which showed 62.5% after 24 hours of formalin injection. The results are shown in Table-1

3.2 Cotton pellet granuloma

The anti-inflammatory activity of the methanolic extract and its aqueous fraction of *Adenanthera pavonina* leaves by chronic inflammation were found by this method. Indomethacine was used as standard. The percentage decrease in the dry weight of granulomatous tissue by the various samples in rats was found out and all the values are compared by using t-test. MEAP, AFAP (400 mg/kg) significantly inhibit the granuloma formation up to 40.07% and 43.55% respectively as compared to control where as the Indomethacine (10 mg/kg) produces 54.77% inhibition. Results were shown in Table-2 and Fig-2

Table 1 Effect of MEAP and AFAP on formalin induced paw edema in rats

Treatment % Inhibition	Dosemg/kg	Mean Oedema volume (ml)								
		1hr	2hr	4hr	24hr	1hr	2hr	4hr	24hr	
Vehicle control	1ml 2% Tween80	0.12±0.0022	0.14±0.0032	0.22±0.0052	0.16±0.0034	-	-	-	-	
Standard	10	0.07±0.003	0.08±0.0025	0.12±0.004	0.06±0.002	41.66	42.85	45.45	62.5	
(Indomethacine)	MEAP	200	0.09±0.0012 ^{a**}	0.11±0.003 ^{a**}	0.15±0.005 ^{a**}	0.08±0.004 ^{a**}	24.14	27.42	31.81	42.7
	MEAP	400	0.08±0.002 ^{a*}	0.07±0.0021 ^{a*}	0.16±0.0012 ^{a**}	0.045±0.001 ^{a**}	33.33	35.34	37.29	52.3
	AFAP	200	0.08±0.0013 ^{a*}	0.12±0.0024 ^{a*}	0.15±0.006 ^{a**}	0.08±0.006 ^{a*}	26.14	32.42	37.5	48.5
	AFAP	400	0.09±0.0026 ^{a**}	0.11±0.0026 ^{a*}	0.14±0.004 ^{a**}	0.05±0.0065 ^{a**}	35.33	37.34	42.29	62.8

Comparison made between Control and test, ^a P<0.01, ^b P<0.05. Comparison made between Standard and test, ^{**} P<0.01, ^{*} P<0.05

MEAP- Methanolic extract of *Adenanthera pavonina* Linn. AFAP- Aqueous fraction of *Adenanthera pavonina* Linn.

Data was analysed by one way ANOVA followed by Dunnet's 't' test, n=6

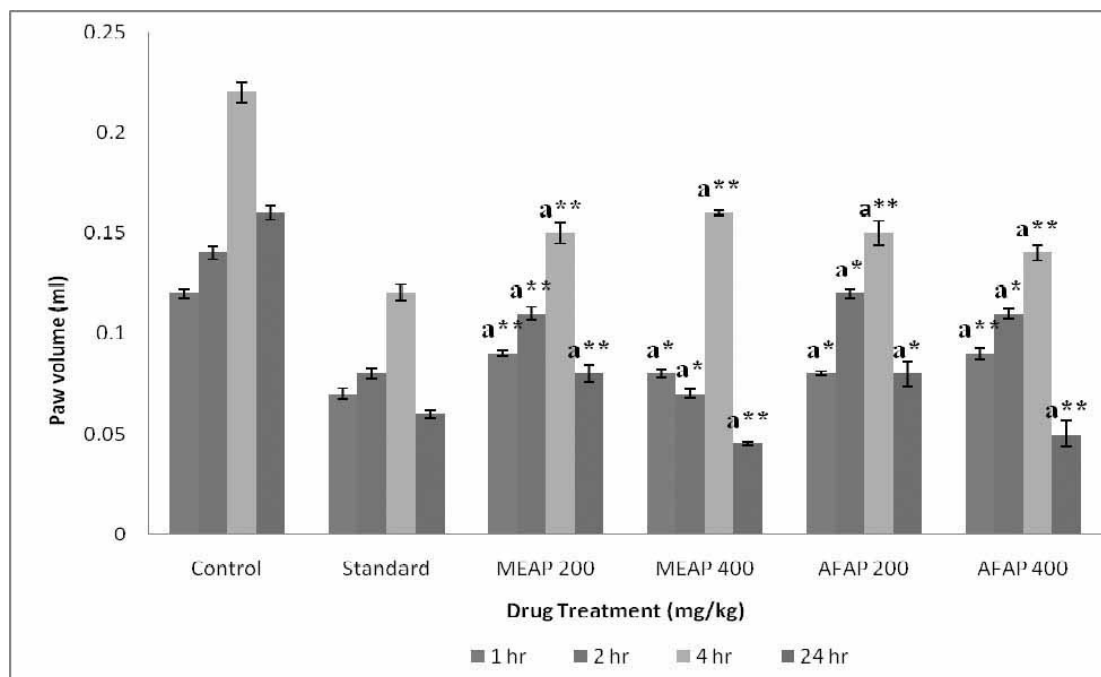


Fig-1 Effect of MEAP and AFAP by formalin induced paw edema method

MEAP- Methanolic extract of *Adenanthera pavonina* AFAP- Aqueous fraction of *Adenanthera pavonina*

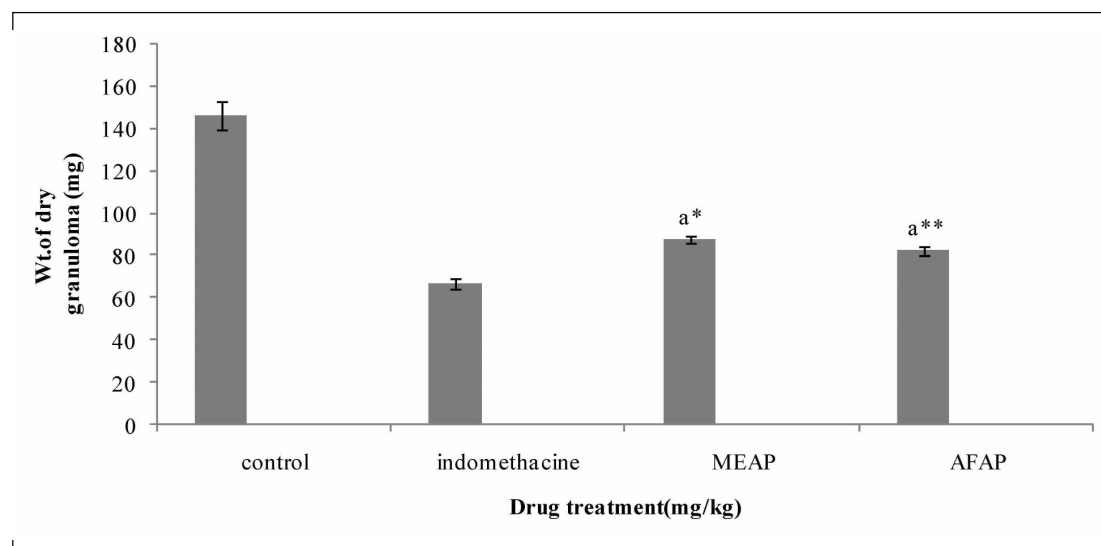


Fig-2 Effect of MEAP and AFAP[on cotton pellet granuloma method

MEAP- Methanolic extract of *Adenanthera pavonina*

AFAP- Aqueous fraction of *Adenanthera pavonina*

Table-2 Effect of MEAP and AFAP on cotton pellet granuloma in rats

Treatment	Dose(mg/kg)	Weight of dry granuloma(mg)	% Inhibition
Vehicle control	1 ml tween 80 (2%)	148.16±6.73	-
MEAP	400	87.58±1.9364 ^{a*}	40.07
AFAP	400	82.45±1.94 ^{a**}	43.55
Standard(Indomethacine) 10		67.00±2.470	54.77

Comparision made between Control test, ^a P<0.01, ^b P<0.05

Comparision made between Standard test, ^{**} P<0.01, ^{*} P<0.05

MEAP- Methanolic extract of *Adenanthera pavonina*

AFAP- Aqueous fraction of *Adenanthera pavonina*

Data was analysed by one way ANOVA followed by Dunnet's 't' test n=6

4. Discussion

The methanolic extract and its aqueous fraction of *Adenanthera pavonina* leaves significantly inhibit the paw edema in formalin induced inflammation in rats. As compared to methanolic extract, its aqueous fraction inhibited the oedma formation significantly.

Formalin induced inflammation is one of the most commonly employed techniques for screening of the anti inflammatory effect by inducing oedema in the hind paw of the rat by injecting an irritant (formalin) and testing the ability of the drug to inhibit the induced oedema. Formalin induces long lasting inflammation which continues over more than 24 hours. In this study the tested extract showed significant anti inflammatory effect, when compared to the control. Various physiological substances such as autocooids, which include histamine, serotonin, bradykinin, substance P, eicosanoids (prostaglandin, Thromboxane and leucotrienes), the platelet activation factor as well as cytokinins and lymphokines are involved in the process of inflammation. The mechanism of anti inflammatory effect of the test extract can be attributed to the inhibition of one of the above mentioned mediators of inflammation.

Cotton pellet induced granuloma method has been widely employed to assess the transudative,

exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. 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. [9]. Methanolic extract and its aqueous fraction of *Adenanthera pavonina* leaves inhibits monocyte infiltration and fibroblast proliferation. The results indicates the efficacy of methanolic extract and its aqueous fraction in granuloma formation. But effect was found more in case of aqueous fraction. This is reflected by the inhibition of the increased number of fibroblast and by synthesis of collagen and mucopolysaccharids during granuloma.

The result may be concluded that the reduction of edema in acute and chronic inflammatory condition may be due to its free radical scavenging property. The free radical scavenging property of the plant may be due to the presence of alkaloids. So the present study supports folklore claim of the decoction of leaves of the plant in treatment of inflammatory associated diseases such as gout and rheumatism. *Adenanthera pavonina* Linn may be considered as natural source in Ayurveda for its versatile medicinal uses.

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