ANTIINFLAMMATORY ACTIVITY OF THE FRUIT OF *Kigelia pinnata* DC

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Summary

Extracts obtained from fruit of *Kigelia pinnata* (family: Bignoniaceae) are used as folk remedy worldwide for the treatment of various inflammatory ailments and rheumatism. Effects of the methanolic extract of *Kigelia pinnata* fruit was studied using various in vivo models of inflammation in mice and rats and observed potent inhibiting activity in formaldehyde-induced paw edema, acetic acid–induced vascular permeability, cotton pellet-induced granuloma, estimation of plasma MDA levels and carrageenan induced peritonitis models. MEKP (100, 200 and 400 mg/kg, p.o) exhibited a dose-dependent and significant inhibition ($p<0.01$) in all the experimental models. The anti-inflammatory activity observed was comparable to the respective standard drugs. Preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates, glycosides, proteins and alkaloids. Acute toxicity studies were performed and produced no mortality in dose up to 2000 mg/kg, p.o. The results gave a scientific basis to the traditional uses of *K.pinnata* particularly involved in inflammation.

Key words: *Kigelia pinnata*, vascular permeability, granuloma, peritonitis, edema.

Introduction

Kigelia pinnata DC (Bignoniaceae) cultivated in many parts of India as an ornamental and roadside tree (syn: *Kigelia africana* Benth) colloquially called as "Sausage tree" with maroon red flowers, long pendulous panicles and gourd like fruits. In folklore medicine, the fruits are used as dressing ulcers, to treat syphilis, rheumatism and as galactagogue in lactating women [1, 2]. The bark is traditionally used to treat syphilis and gonorrhea [3]. *K. pinnata* also has been reported for its anti-implantation [4], molluscicidal [5] and antimicrobial [6] activities. The extracts of stem bark and fruit are reported for their cytotoxic activities and showed promising results in treating melanoma and renal carcinoma [7]. Inhabitants of Kurukshetra district in Haryana use fruit paste for antileprotic activity [8]. Steroids, iridoiods and coumarins have been isolated from root bark [9] and flavonoids and iridoids from the fruits and leaves [10]. The phytochemical studies revealed the presence of quercetin, kaempferol, β-setosterol, napthoquinones, iridoids and flavonoids [11]. The present study was designed to investigate and evaluate the pharmacological basis for the use of *K. pinnata* DC fruit in folklore medicine for the treatment of pain and inflammation. The anti inflammatory activity of methanolic extract of the fruit of the plant (MEKP) was evaluated on formaldehyde-induced paw edema, acetic acid–induced vascular permeability, cotton pellet-induced granuloma, estimation of plasma MDA levels and carrageenan induced peritonitis models.

Materials and methods

Plant Material

The fruits of *K.pinnata* were collected from Kurnool, Andhra Pradesh, India. It was authenticated by a botanist Smt G.B.Rajya Lakshmi of the Dept of Botany, Govt.Degree College for Women, Kurnool. A voucher specimen of this plant material with No.KU/UCPSc/12/2006 has been retained in the Dept of Pharmacognosy and Ethnopharmacology, University College of Pharmaceutical Sciences, Warangal.

Preparation of Extract

The fruits were dried under shade, made into coarse powder and subjected to maceration process at room temperature in methanol for 7 days with occasional shaking. The methanolic extract collected was concentrated under reduced pressure at $50^0 – 55^0$ C and stored in a vacuum desiccator. The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Animals

Wistar rats (150-250 g) and albino mice (20-27 g) of either sex were maintained under standard husbandry conditions and had free access to food and water *ad libitum* and were acclimatized to the laboratory environment for a period of one week prior to the experimental session. All the animals were divided into different groups each consists of six animals were fasted overnight prior to the experiments. All the experiments were performed after obtaining prior permission from Institutional Animal Ethics Committee.

Drugs and Chemicals

Formaldehyde (S.D.Fine chemicals Ltd. Mumbai), acetic acid (Ranbaxy laboratories Ltd., Punjab), diclofenac sodium (Dr. Reddy Labs, Hyderabad), indomethacin (Sun Pharma, Mumbai), ibuprofen (Natco Pharma, Hyderabad), evans blue (Sigma, St.Louis, Missouri, USA), gum acacia (Hi-media, Mumbai) and methanol (BDH, Mumbai). All other chemicals were of analytical grade and procured locally.

Phytochemical screening

The methanolic extract was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids and glycosides by employing standard phytochemical tests [12].

Acute toxicity study

Acute oral toxicity was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No 425 [13].

Formalin - induced acute inflammatory model [14]

Formalin 0.1 ml (2% in distilled water) was injected into sub planter area of left hind paw. The extracts at doses of 100, 200 and 400 mg/kg or diclofenec sodium 10 mg/kg were given 1 h prior to formalin injection. The paw volume was determined by plethysmographic method in order to measure degree of inflammation as shown in Table.1.

Acetic acid-induced Vascular Permeability test.

Whittle's method was used with some modifications[15]. In brief, male mice weighing 20-27 g were fasted for 10 h prior to the experiments and were given the test drugs and vehicle orally. Each animal was given an intravenous injection of a 1% solution of Evans blue as 0.1 ml/10 g at 30 min after the oral treatment. The vascular permeability inducer, 0.1 ml/10 g of 0.6% acetic acid in saline, was injected intraperitoneally at 30 min after Evans blue injection. After 20 min, the mice were killed by dislocation of the neck and 10 ml of normal saline was injected intraperitoneally, after which the washing solution was collected in tubes and then centrifuged at 2000 rpm for 10 min. The absorbance of the supernatant was read at 610 nm with a spectrophotometer. The control group was treated similarly except that they received an oral dose of vehicle alone. The vascular permeability was expressed in terms of the amount of total dye (µg/ mouse) which was leaked into the intraperitoneal cavity.

Cotton pellet-induced granuloma[16]**:**

The cotton pellets-induced granuloma in rats was studied according to the method D'Arcy *et al*. (1960). The animals were divided into five groups of six animals in each group. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle (2% gum acaia). Group 2 received the standard drug, indomethacin (10 mg/kg body weight) and the extract MEKP at the concentration of 100, 200 and 400 mg/kg body weight was administered orally to groups 3, 4 and 5, respectively for seven consecutive days from the day of cotton pellet implantation. On

8th day the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60^0 C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The anti-proliferative effect of MEBV was compared with control.

Plasma MDA (Malondialdehyde) estimation

After seven days drug treatment in cotton pellet granulation method, 3-5 ml of blood was collected from inner canthus of eye from each animal using capillary tube, in a vial containing EDTA as an anticoagulant. Plasma was separated by centrifugation at 3000 rpm for 10 min. It was stored at -20^0C and used to estimate MDA levels. The reduced levels of MDA were taken as indicator of anti-lipoperoxidative activity, which can be taken as index of reduced oxidative stress.

Carrageenan Induced Peritonitis

Inflammation was induced by the modified method of Griswold et al., 1987 [17]. Male swiss albino mice weighing 20-25 g were divided into five groups (n=6). Group I served as control, Group II served as standard and was dosed with indomethacin (10 mg/kg, p.o) and group III to V were dosed with MEKP at the doses of 100, 200, 400 mg/kg p.o. The control (2% gum acacia), standard drug and extract doses were administered orally one hour prior to the induction of peritonitis. After one hour, carrageenan (0.25 ml, 0.75% w/v in saline) was injected intraperitonially. Four hours later, the animals were sacrificed by cervical dislocation and 2 ml of Ca^{2+} and Mg^{2+}free phosphate buffered saline (PBS) was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined [18, 19]. The percentage of leukocyte inhibition was calculated using the following formula:

% of Leukocyte Inhibition (% L. I) = (1- T/C) X 100

Where 'T' represents the treated groups' leukocyte count and

'C' represents the treated control group leukocyte count.

Inhibition of Neutrophil migration was calculated by the following equation:

Inhibition of Neutrophil Migration = 100-{(N T/NC) X 100}

Where NT = Neutrophil counts of treated groups

NC = Neutrophil counts of control groups.

Statistical analysis

The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by Dunnet's t-test. P value of < 0.05 was considered as statistically significant.

Results

Phytochemical screening

Preliminary phytochemical screening of the plant extract revealed the presence of flavonoids, carbohydrates, glycosides, proteins and alkaloids.

Test for Acute Toxicity

In the acute toxicity study no mortality was observed during the 24 hr period at the doses tested and the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, atoxia, diarrhoea or increased diuresis.

Formaldehyde-induced paw edema

In formaldehyde induced paw edema method, the oral administration of MEKP in graded doses (100, 200 and 400mg/kg) produced significant reduction in paw volume in dose dependent manner in comparison to control. The maximum effect was seen in the oral dose of 400mg/kg which showed significant ($p<0.01$) reduction as 43.05% in paw volume in comparison to control. The anti-inflammatory activity in this dose of the test drug was comparable to standard diclofenac (10 mg/kg p.o.). The maximum anti-inflammatory effect was observed at 3 h in all the doses of test drug.

Acetic acid-induced vascular permeability

In vascular permeability test the oral administration of MEKP at doses of 100, 200 and 400 mg/kg inhibited the increase of dye leakage into the peritoneal cavity in mice (Table.2). Its potency was comparable to that of 10 mg/kg of indomethacin. MEKP showed a significant inhibition of vascular permeability. Its inhibitory effect at doses of 100, 200 and 400 mg/kg was 20.5, 23 and 31.2% respectively, in comparison with the control group. The standard drug showed 39.5% of inhibition. The anti-inflammatory activity of MEKP was less effective than the standard drug.

Cotton pellet-induced granuloma

In cotton pellet granuloma test MEKP extract in graded doses (100, 200 and 400 mg/kg.b.w.) showed significant reduction of both wet as well as dry weights of granuloma in dose dependent manner, when compared to control with maximum effect in 400 mg/kg dose of the test drug showing 42.7 and 46.71% reduction of wet and dry weights of granuloma respectively. The anti-inflammatory activity in this dose was also comparable with indomethacin (10 mg/kg) as shown in Table.3.

Plasma MDA estimation

In oxidative stress model, MEKP (400 mg/kg x 7 days, orally) produced significant ($p<0.01$) reduction in plasma MDA levels which was 33.5% in comparison to diseased control. However, standard drug reduced greater reduction of MDA levels (52.5%) as shown in fig.1.

Table.1. Effect of the methanolic extract of ***Kigelia pinnata*** fruit on formaldehyde-induced rat paw edema

S.No	Group	Dose	Increase in paw volume (ml)					
		(mg/kg)	1h	2h	3h	4h	5h	24h
1.	Control	---	0.305±0.011	0.345±0.008	0.374±0.010	0.304±0.004	0.230±0.005	0.200±0.003
2.	Diclofenec sodium	10	0.208±0.003**	0.221±0.005**	0.203±0.006**	0.209±0.003**	0.142±0.002**	0.110±0.003**
3.	*K.pinnata*	100	0.250±0.003**	0.243±0.003**	0.238±0.005**	0.232±0.003**	0.208±0.005*	0.171±0.002**
4.	*K.pinnata*	200	0.236±0.011**	0.227±0.008**	0.226±0.007**	0.208±0.010**	0.189±0.003**	0.120±0.002**
5.	*K.pinnata*	400	0.221±0.012**	0.213±0.009**	0.213±0.009**	0.187±0.007**	0.162±0.006**	0.111±0.003**

Values are mean ± S.E.M. ($n = 6$).

** Experimental groups were compared with control ($p< 0.01$).

Table.2. Effect of methanolic extract of ***Kigelia pinnata*** fruit on acetic acid-induced vascular permeability in mice

S.No	Group	Dose (mg/kg)	Amount of dye leakage (OD)	Inhibition (%)
1.	Control	---	1.38±0.035	
2.	Indomethacin	10	0.835±0.018**	39.5
3.	*B.vulgaris*	100	1.097±0.034**	20.5
4.	*B.vulgaris*	200	1.063±0.036**	23.0
5.	*B.vulgaris*	400	0.95±0.063**	31.2

Values are mean ± S.E.M. *(n = 6).*
** Experimental groups were compared with control *(p< 0.01).*

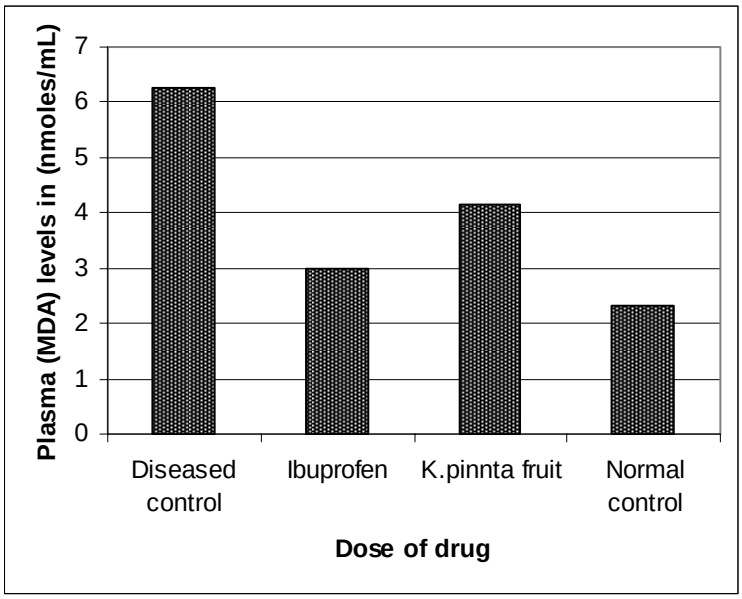

Fig.1. Effect of methanolic extract of *Kigelia pinnata* fruit on oxidative stress by plasma estimation of malondialdehyde (MDA)

Table.3. Effect of the methanolic extract of ***Kigelia pinnata*** fruit on cotton pellet-induced granuloma in rats

S.No	Treatment	Dose	Weight of cotton	% Inhibition
	Weight of cotton pellet (mg) ***(Dry)***	% Inhibition (mg/kg)	pellet (mg) ***(Wet)***	
1.	Control	---	142.0 ± 1.91	
	41.5 ± 2.11			
2.	Indomethacin	10	75.67 ± 1.75**	46.71
	23.83 ± 0.95**	42.58		
3.	*K.pinnata*	100	102.2 ± 2.43**	28.0
	30.0 ± 0.96**	27.7		
4.	*K.pinnata*	200	92.67 ± 1.36**	34.74
	27.83 ± 1.17**	32.9		
5.	*K.pinnata*	400	81.33 ± 3.55**	42.7
	24.5 ± 0.76**	41.0		

Values are mean ± S.E.M. *(n=6).*

** Experimental groups were compared with control *($p<0.01$).*

Carregeenan induced peritonitis

The MEKP also inhibited peritoneal leukocyte migration at the rate of 40.5, 63.1 and 78.9% at the doses of 100, 200 and 400 mg/kg, respectively, whereas the inhibition produced by indomethacin (10 mg/kg) 60.7% was found to be in carrageenan-induced peritonitis model as shown in Table.4. The inhibition of neutrophils infiltration of MEKP was 33.5, 45.7 and 64.9% respectively, where as indomethacin shows 65.3%.

Table.4. Effect of methanolic extract of ***Kigelia pinnata*** fruit on leukocytes migration and neutrophils migration in peritoneal exudation in carrageenan-induced mice

S.No	Group	Dose (mg/kg)	Leukocytes (10^5 mL $^{-1}$)	Leukocyte Inhibition	Neutrophils (10^5 mL $^{-1}$)	% Inhibition of Neutrophil migration
1.	Control		4.07±0.08	----	2.45±0.1	---
2.	Indomethacin	10	1.6±0.14**	60.7	0.85±0.03**	65.3
3.	*K.pinnata*	100	2.42±0.10**	40.5	1.63±0.07**	33.5
4.	*K.pinnata*	200	1.50±0.11**	63.1	1.33±0.05**	45.7
5.	*K.pinnata*	400	0.86±0.05**	78.9	0.86±0.05**	64.9

Values are mean ± S.E.M. (*n=6*).
** Experimental groups were compared with control (*p<0.01*).

Discussion

Inflammatory events involve micro-vascular changes with increased vascular permeability, flow of exudation, including plasmatic protein and amplification of endogenous chemical mediators [20]. The extract of *K.pinnata* fruit showed significant inhibition of formalin-induced rat paw edema. The formalin injection into rat paw produces localized inflammation and pain. This nociceptive effect is biphasic in nature: an early neurogenic component followed by a later tissue-mediated response [21]. Inhibition of formalin-induced paw edema in rats is one of the most suitable tests to evaluate anti-proliferative activity and to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis [22, 23]. The vascular permeability was induced by acetic acid, which could cause an increase in peritoneal fluids of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), serotonin, and histamine [24]. This leads to a dilation of the arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated, and edema forms. Indomethacin and MEKP showed significant inhibition of acetic acid-induced vascular permeability in mice. This result suggested that MEKP probably has an anti-inflammatory property like indomethacin (nonselective COX inhibitor), acting through inhibition of the inflammatory mediators of the acute phase of inflammation.

Cotton pellet granuloma method is used to evaluate the transudative and proliferative components of chronic inflammation. The wet weight of the cotton pellet correlates with the transuda and the dry weight of the cotton pellet correlates with the amount of the granulamatous tissue [25]. The present data support the hypothesis of the greater effect of the MEKP on the inflammation mediators in the immediate response of inflammation in rats. The present study showed significant reduction in MDA levels by MEKP. The oxidative stress is the condition where Reactive Oxygen Species (ROS) generation exceeds endogenous antioxidant defense [26] and it is well-known that in chronic and sub-acute inflammation ROS play an important role in modulating the extent of inflammatory response and consequent tissue and cell injury [27]. MDA is a metabolic product of lipidperoxidation, the level of which is increased in oxidative stress. Therefore, reduction of oxidative stress by anti-lipo peroxidative activity might possibly be the mechanism of anti-inflammatory action of MEKP in model of sub-acute inflammation.

Intraperitonial injection of carrageenan leads to inflammation of the peritoneum resulting from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorphonuclear cells by a variety of processes including adhesion and cell mobility [28]. Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility.

In conclusion, the methanol extract of *Kigelia pinnata* fruit significantly attenuated the inflammation produced by various inflammatory methods. The present study indicates that the fruit of *K.pinnata* possesses anti-inflammatory property thereby validating its local use by alternative medical practitioners.

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