

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF *KIGELIA PINNATA* LEAF EXTRACT IN WISTAR RATS

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ABSTRACT

Aim of the study: The main aim of the present study is to evaluate the anti-inflammatory activity of leaf extract of *Kigelia pinnata* on wistar rats. **Materials & Methods:** Anti-inflammatory activity of the leaf extract of *Kigelia pinnata* at a dose of 200mg/kg & 400mg/kg was evaluated against the standard drug indomethacin at a dose of 10 mg/kg i.p. Wistar rats of either sex of five numbers in each group was undertaken for study and evaluated by carrageenan-induced paw edema and cotton pellet-induced granuloma methods. **Results:** Both doses of leaf extract of *Kigelia pinnata* has significant reduction in the carrageenan induced paw edema ($P < 0.001$) in a dose dependent manner (Table 1) when compared to control and shows the reduction of the weight of cotton pellet granuloma in a dose dependent manner (Table 2) in the cotton pellet induced granuloma model of inflammation in rats. **Conclusion:** The leaf extract of *Kigelia pinnata* have potential anti-inflammatory activity so, it can be recommended for further studies.

Keywords: *Kigelia pinnata* leaf, Anti-inflammatory activity, Carrageenan-induced paw edema, Cotton pellet-induced granuloma, Indomethacin

INTRODUCTION

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammatory diseases are very common throughout the world. Rheumatoid arthritis is one of the oldest known diseases of mankind affecting the majority of population no substantial progress has been made in achieving a permanent cure and different types of rheumatic diseases are a major cause of morbidity of the working force. (Sanja *et al.*, 2009). Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. These mediators even in small quantities can elicit pain response. Antiinflammatory drugs make up about half of analgesics, relieving pain by reducing inflammation as opposed to opioids which affect the central nervous system.

The greatest disadvantage in presently available potent synthetic drugs for the treatment of inflammation lies in their toxicity and reappearance of symptoms after discontinuation. Therefore the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

Kigelia pinnata (family: Bignoniaceae) known as "sausage tree" cultivated in many parts of India as an ornamental and roadside tree. *Kigelia pinnata* is a multipurpose medicinal plant with many attributes and considerable potentials. The plant has traditional uses which include anticancer, antiulcer, anti-aging, antioxidant and antimalarial activities. It is also widely applied in the treatment of genital infections, gynaecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle-cell anaemia, psoriasis, eczema, central nervous system depression, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and tumours etc (Olatunji *et al.*, 2009). The stem bark and fruit extract showed activity against melanoma and carcinoma cell lines (Houghton *et al.*, 1994). Extracts of rootbark and stembark exhibited antitrypanosomal activity (Moideen *et al.*, 1999). The fruits and barks are ground and boiled in water and taken orally for the treatment of stomach ailments.

Kigelia pinnata is extensively used in Indian traditional medicine for several diseases including inflammatory disorders but there is no scientific evidence available for such activity. Based on the traditional uses the aim of present study was to investigate the anti-inflammatory activity of leaf extract of *Kigelia pinnata* on wistar rats (Carey *et al.*, 2010).

MATERIAL AND METHODS

Plant material

Fresh leaves of *Kigelia pinnata* have been collected from Ramnagar region. After collection, the plant material was authenticated by Dr. K. S. Negi (Principal Scientist), National Bureau of Plant Genetic Resources, Bhowali, Nainital, Uttarakhand (Ref. No. Ph./ M. Pharm. Project/ 526). The leaves were dried in shade, powdered and subjected to maceration with methanol at room temperature for 72 hr with occasional shaking and concentrated by removing the solvents by drying.

Phytochemical screening

Freshly prepared *K. pinnata* leaf extract was subjected to standard phytochemical screening tests for various constituents by standard methods (Rajgopal *et al.*, 2001).

Animals

Wistar rats weighing between 150-200 gm of either sex were used in the experimental study. The animals were kept properly in polypropylene cages and provided with food and water. Healthy and fresh animals were used in the experiment and they were kept on fasting overnight prior to the experimentation. The experimental protocol was approved by the Institutional Animal Ethical Committee.

Carrageenan-induced paw edema

This method is the most commonly used method for the evaluation of anti-inflammatory drugs. Wistar rats were divided into four groups having five animals in each group. Animals of group-1 (carrageenan control group) received normal saline solution, animals of group-2 (standard drug treated group) received indomethacin (10 mg/kg, i.p.), animals of group-3 received *K. pinnata* extract (200 mg/kg, p.o.) and animals of group-4 received *K. pinnata* extract (400 mg/kg p.o.). Vehicle, standard drug and test compound were administered 30 min prior to carrageenan injection. After 30 min, 0.1 ml of 1% (w/v) solution of carrageenan in 0.9% normal saline solution was injected s.c. into the plantar region of right hind paw and the paw volume of each rat from all groups was measured at 0, 30, 60, 120, 180 and 240 min after carrageenan challenge.

Cotton pellet-induced granuloma

In this study wistar rats were divided into four groups having five animals in each group and treatments were given as per carrageenan-induced paw edema method. The animals were administered with vehicle, standard drug and test drug thirty min prior to the cotton pellets implantation. After thirty min of first dosing 10 ± 0.5 mg of sterile cotton pellet was inserted one near each axilla region by making small subcutaneous incision in anaesthetized animals. Vehicle, standard drug and test drug were administered for seven consecutive days. On the eighth day, the animals were sacrificed by excessive anaesthesia and the cotton pellets were removed surgically. Pellets were separated from extraneous tissue and weighed immediately for wet weight and then

dried at 60° C until the weight become constant. The percent inhibition increase in the weight of the cotton pellets was calculated by:

$$\% \text{ Inhibition} = \frac{[Wc - Wd]}{Wc} \times 100$$

Wc = Difference in pellet weight of the control group

Wd = Difference in pellet weight of the drug treated group

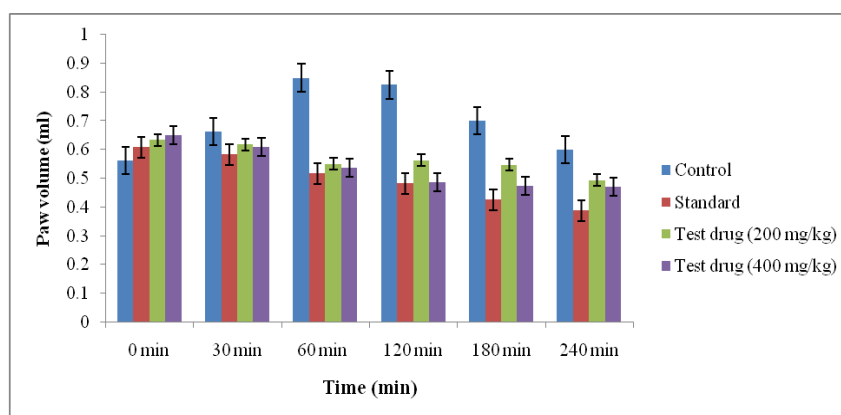
Statistical analysis

All data were expressed as mean \pm SEM and analyzed by One-way analysis of variance (ANOVA) followed by Tukey's test. $P < 0.001$ was considered statistically significant.

Table 1: Anti-inflammatory activity of *Kigelia pinnata* by Carrageenan-induced paw edema

Groups	Dose	Paw volume after drug/ extract administration (ml)					
		0 min	30 min	60min	120 min	180 min	240 min
		mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM
Control	0.01 ml	0.56 \pm 0.08	0.66 \pm 0.05	0.85 \pm 0.02	0.82 \pm 0.03	0.70 \pm 0.50	0.60 \pm 0.06
Standard	10 mg/kg	0.61 \pm 0.03	0.58 \pm 0.03	0.51 \pm 0.02*	0.48 \pm 0.01*	0.42 \pm 0.03	0.39 \pm 0.03
Test drug	200mg/kg	0.63 \pm 0.05	0.62 \pm 0.06	0.55 \pm 0.05*	0.56 \pm 0.05*	0.55 \pm 0.05	0.49 \pm 0.004
Test drug	400 mg/kg	0.65 \pm 0.06	0.61 \pm 0.045	0.53 \pm 0.04*	0.48 \pm 0.03*	0.47 \pm 0.02	0.47 \pm 0.02

Values are expressed as mean \pm SEM (n=5), * $p < 0.001$ denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.



Graph - 1

Table 2: Anti-inflammatory activity of *Kigelia pinnata* by cotton pellet induced granuloma.

Groups	Dose	Wet weight of cotton pellet (mg)	% Inhibition	Dry weight of cotton pellet (mg)	% Inhibition
		mean \pm SEM		mean \pm SEM	
Control	0.01 ml	112.88 \pm 2.6	-	40.34 \pm 1.5	-
Standard	10 mg/kg	57.18 \pm 1.1*	49.34	16.290 \pm 0.42*	59.62
Test drug	200 mg/kg	83.42 \pm 2.0*	26.098	27.35 \pm 0.99*	32.20
Test drug	400 mg/kg	61.91 \pm 1.5*	45.15	18.73 \pm 0.84*	53.60

Values are expressed as mean \pm SEM (n=5), * $p < 0.001$ denotes significance with respect to the control group using one way ANOVA followed by Tukey's test

RESULTS

The phytochemical analysis of *Kigelia pinnata* leaf extract revealed the presence alkaloids, carbohydrates, saponins, flavonoides, tannins and phenolic compounds. The result shows that the extract at dose of 200 mg/kg and 400 mg/kg has significant reduction in the carrageenan induced paw edema ($P < 0.001$) in a dose dependent manner when compared to control. The standard drug, indomethacin (10 mg/kg, i.p.) was more potent than the extract. The higher dose of the extract (400 mg/kg) exhibited better anti-inflammatory activity than the lower dose of the extract (200 mg/kg). In cotton pellet induced granuloma model of inflammation, the results show a marked protection in granuloma by markedly reducing the weight of the cotton pellet at a dose of 200 mg/kg and 400 mg/kg when compared to control ($P < 0.001$). The higher dose of

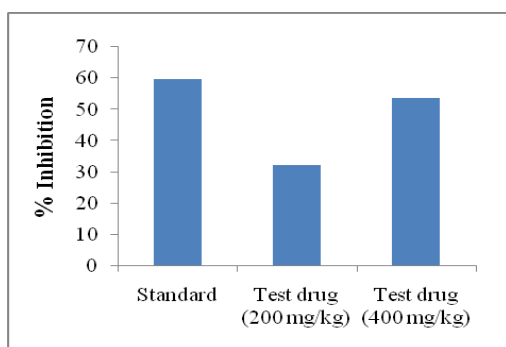
the extract (400 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of indomethacin and better than the effect of the lower dose of the extract (200 mg/kg).

DISCUSSION AND CONCLUSION

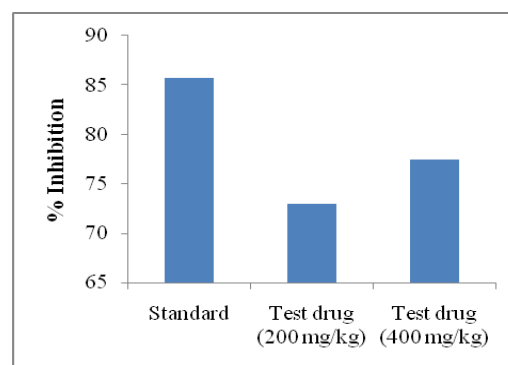
The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by an injection of an irritant agent (Chakraborty *et al.*, 2004). This edema depends on the participation of kinins and polymorphonuclear leucocytes with their pro-inflammatory factors including prostaglandins (Damas *et al.*, 1986). Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which have frequently been used to assess the anti-edematous effect of natural products (Panthong *et al.*,

2003). Development of edema in the paw of rat after carrageenan injection is a biphasic event (Vinegar *et al.*, 1969). Initial phase observed during the first hour is attributed to the release of histamine and serotonin (Crunkhon *et al.*, 1971). The second phase of edema is due to the release of prostaglandins, protease and lysosome. Carrageenan-induced edema is characterized by the presence of PGs and other compounds of slow reaction (Spector *et al.*, 1963). COX-2 is an inducible isoform found in activated inflammatory cells that generates prostanoid mediators of inflammation (Sawatzky *et al.*, 2005). The result of the present study indicates that *K. pinnata* (200mg/kg and 400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation. The higher dose of the extract (400 mg/kg) exhibited the anti-inflammatory effect better than the effect of the lower dose of the extract (200 mg/kg). The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation

(Winter *et al.*, 1957). The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. In the present study, administration of *K. pinnata* extract (200 mg/kg and 400 mg/kg, p.o.) was found to inhibit the weight of cotton pellet in a dose dependent manner and the higher dose of the extract (400 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of indomethacin and better than the effect of the lower dose of the extract (200 mg/kg). In preview of this, results of present study demonstrated that leaf extract of *Kigelia pinnata* has potential to inhibit the chronic inflammation. Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory effect of *Kigelia pinnata* in inflammatory diseases. So it is hoped that these studies will stimulate further efforts towards the development of new and urgently needed medications for the treatment of inflammatory diseases.



Graph - 2.1 Wet weight of cotton



Graph - 2.2 Dry weight of cotton

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