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Acute anti-inflammatory activity of *Pandanus fasicularis* Lam

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ABSTRACT

Plants are widely used in the various traditional system of medicine like Ayurveda, Siddha, and Unani for their analgesic, and anti-inflammatory, antipyretic activity. *Pandanus fascicularis Lam. (Synonyms-Pandanus tectorius, Pandanus odoratissimus,* family–Pandanaceae) has been used in rheumatic fever, rheumatism and rheumatoid arthritis. The principle constituent is the Kewda oil, isolated from the inflorescences of *Pandanus fascicularis.* The chemical composition of this essential oil, obtained by hydrodistillation of staminate inflorescences of Kewda *(Pandanus fascicularis*) include more than sixty components. The major components of the hydrodistillated Kewda oil are 2-phenyl ethyl methyl ether terpene-4-ol, a –terpeniol and 2- phenyl ethyl alcohol benzyl benzoate etc. Kewda oil is traditionally used in ear ache, head ache, arthritis, debility, giddiness, laxative, rheumatism. After initial phytochemical screening of methanolic (MEPF) and aqueous extracts(AEPF) of the plant, the extracts were screened for possible acute anti-inflammatory properties in vivo models. Acute anti-inflammatory properties of both the extracts were tested in rodent models by carrageenan induced paw edema, albumin induced plantar edema, acetic acid induced vascular permeability and castor induced diarrhoea. In all these animal models MEPF and AEPF have shown significant anti-inflammatory activity.

Key words: *Pandanus fascicularis,* acute inflammation, Carrageenan, castor oil induced diarrhoea, rodents, vascular permeability.

INTRODUCTION

Use of plants and plant products as a source of medicinal agents started from ages. Plants are widely used in the various traditional system of medicine like Ayurveda, Siddha, and Unani for their analgesic, anti-inflammatory and anti-pyretic activities. Pandanus fascicularis Lam. *(Synonyms-Pandanus tectorius, Pandanus odoratissimus,* family –Pandanaceae) has been used in rheumatic fever, rheumatism and rheumatoid arthritis. Vernacular names [1, 2] of this plant are**:**

Sanskrit-ketaki, **Hindi**-keura, Kewda, Ketki, Gagandhul, **Tamil**- Tazhai, **Telugu**-Mugali, **Kannada**-tale mara, **English**-screw pine. Although India has the tradition of alternative therapies there are no procedures to test the safety and efficacy of traditional remedies and to standardize their effective cure. For these reasons it is essential to increase our efforts in the area of medicinal plant research and exploit it efficiently for the benefit of humanity.

Pandanus fascicularis grows wildly in coastal regions of India. This plant **is** a branched palm like shrub, stem supported by aerial roots, leaves glaucous-green, and 0.9-1.5 m, ensiform, long lanceolate, acuminate with three rows of prickles each on the margins and on midrib beneath. Male flowers in spikes enclosed in large, white fragrant spathes, female flowers in solitary spadix, syncarpium yellow or red [3].

Parts of the plant used in traditional medicine are leaves, flowers, roots, fruits, spadices, bracts [2,3,4] in leprosy, smallpox, syphilis, scabies, pain, and in leucoderma**.** Oil from bracts is considered as stimulant and anti spasmodic [20]. Kewda attar or water prepared by distillation of spadices is used to flavor sweets, syrups and soft drinks [2**]**.The flower is pungent, bitter; improves complexion. Fruit is useful in "vata", "kapha" and urinary discharge [3]. Root is considered as diuretic, depurative and tonic [3]**.** Juice obtained from inflorescence used for rheumatic arthritis in veterinary medicine [5].

Chemical constituents:

The principle constituent is the Kewda oil, isolated from the inflorescences of *Pandanus fascicularis.* The chemical composition of this essential oil, obtained by hydrodistillation of staminate inflorescences of Kewda *(Pandanus fascicularis*), when subjected to high resolution GC (gas chromatography) and GC-MS (gas chromatography and mass spectrometry has been shown to yield as many as 60 components, amounting 98.7% of the total oils. The major components of the hydrodis tillated Kewda oil were 2-phenyl ethyl methyl ether (37.7%), terpene-4-ol (18.6%), a –terpeniol(8.3%) and 2- phenyl ethyl alcohol(7.5%), benzyl benzoate (11%), viridine (8.8%) and gesmacrene B (8.3%) along with a small amount of benzyl salicylate, benzyl acetate, benzyl alcohol etc. Ethnobotanically Kewda oil is used in ear ache, head ache, arthritis, debility, giddiness, laxative, rheumatism, small pox and in spasms [6, 7, 8, and 9].

In traditional system of medicine, herbal remedies are prescribed for the treatment of various inflammatory diseases such as different types of rheumatic diseases which are very common throughout the world. The greatest disadvantage in the potent synthetic drugs available at present lies in their side effects, toxicity and reappearance of symptoms after discontinuation. Hence search for new anti-inflammatory agents that retain the therapeutic efficacy and yet are devoid of adverse effects are justified. There is much hope of finding active anti-rheumatic agents from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective synthetic drugs.

Many plants have been shown to possess anti-inflammatory activities in animals and humans. So a plant that was traditionally used for rheumatism and no scientific proof has been claimed on its leaf yet is investigated here. The present study has been undertaken to investigate and evaluate the anti-inflammatory activities of *Pandanus fascicularis* leaves on acute inflammation in rodents. After its preliminary phytochemical screening we studied for its effect in invivo acute anti-inflammatory activity.

METHODS

Plant collection and identification: Leaves of *Pandanus fascicularis* used for the investigation were collected from the east coast road, chennai-96, in the month of April 2004. The plant was identified and authenticated by Research officer (Pharmacognosy), Central Research Institute (Siddha), Arumbakkam, Chennai-600106.

Preparation of methanolic and aqueous extracts:

The *Pandanus fascicularis* leaves were collected and coarsely powdered. The powder was then successively extracted with methanol and distilled water using soxhlet extractor. The methanol (MEPF) and aqueous (AEPF) extracts of *Pandanus fascicularis* were dried under reduced pressure using a rotary flash evaporator and they were kept under the refrigeration. The percentage yield of methanolic extract and aqueous extract was 9% and 6% respectively**.** The methanol and aqueous extracts thus obtained were used for the preliminary phytochemical screening and pharmacological studies. The extracts were administered to the animals by dissolving in normal saline.

Experimental animals:

Colony of inbred adult Wistar rats (150-200 g) and Albino mice (25-30 g) of

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either sex were used in the pharmacological studies. The animals were maintained in well-ventilated room at room temperature with natural day-night cycle in polypropylene cages. They were fed balanced rodent pellet diet obtained from Sai Durga feeds and foods, K. Kamaraj road, Banglore-560042 and tap water *ad-libitum* through out the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) - Reference No: IAEC-X-3 / CLBMCP / 2004-2005.

Preliminary phytochemical screening [9]:
The methanol and aqueous extracts of the leaves of *Pandanus fascicularis* was subjected to preliminary phytochemical screening and they were tested for the presence of alkaloids, carbohydrates, proteins, steroids, sterols, phenols, tannins, terpenes, flavonoids, gums and mucilages, saponins and glycosides.

***In vivo* acute anti-inflammatory methods:**
Wistar albino rats (150-200 g) and mice (25-30 g) of either sex were housed under uniform environmental conditions. They were divided into four groups of six animals each and the following regimen of treatment was instituted.

Group I: Animals received normal saline (10 ml/kg, p.o)
GroupII: Animals received MEPF (200 mg/kg, p.o) in 0.9% saline
GroupIII: Animals received AEPF (200 mg/kg p.o) in 0.9 % saline
GroupIV: Animals received indomethacin (10 mg/kg, p.o) in 0.9 % (w/v) saline

Carrageenan induced paw edema in rats [10]:
Acute inflammation was tested on edema induced by carrageenan in rats. The animals were fasted and divided in to four groups. All the animals received their respective doses of test drugs 1 hr prior to the administration of the phlogistic agent. After 1 hour, 0.1 ml of 1% solution of freshly prepared carrageenan in normal saline was injected in to the plantar surface of the right hind paw of the rats. The paw volume was measured before and each hour afterwards for a period of 6 hours using mercury displacement plethysmograph. The difference between the left and right paw volumes indicated the degree of inflammation and percentage of inhibition of paw volume by the standard and the test drugs was calculated.

Albumin induced edema [11]:
Acute inflammation was induced by the injection of 0.1 ml of fresh egg albumin in to the sub plantar surface of the right hind paw of rats which had been fasted for 12 hr. Edema was assessed for 3 hours at 30 min intervals after administration of phlogistic agent, in terms of an increase in paw volume of the albumin injected paw compared to the non-injected paw.

Acetic acid induced vascular permeability in mice [12]:
The mice were injected with 0.25% solution of Evans blue intravenously after 30 min of oral administration of the drug. Fifteen minutes later mice were injected intraperitoneally (1 ml/100 gm body weight) with freshly prepared 0.6% of acetic acid (v/v) in normal saline. After 30 minutes of acetic acid injection mice of all the groups were sacrificed, their peritoneal cavities washed with 10 ml of heparinised sterile normal saline and centrifuged for 10 minutes (3000 rpm). Absorbance of the supernatant was measured at 610 nm using a spectrophotometer.

Castor oil induced diarrhea[13]:
One hr after drug administration castor oil (20 ml/kg) was administered orally to all the groups of animals. Animals were examined for the presence or absence of characteristic diarrheal droppings on a white paper placed on the floor of the cages every hour for 4 hrs. Absence of diarrheal droppings was recorded as a positive result indicating possible inhibition of biosynthesis of prostaglandins.

Statistical tests:
For the in vivo experimental methods the statistical differences between absolute data of control and treated groups were tested by one way ANOVA (Analysis of variance) followed by Dunnet's test. The differences were considered significant at $p<0.05$. Results were presented as mean ± SEM.

RESULTS

Preliminary phytochemical screening:
The results of preliminary phytochemical screening of the methanol extract of leaves of *pandanus fascicularis* showed the presence of alkaloids, carbohydrates, phenols, steroids, sterols, proteins and glycosides. The aqueous extract showed the presence of alkaloids, carbohydrates.

Effect of MEPF and AEPF on carrageenan induced paw edema is shown in table 1. Both the extracts showed significant anti-inflammatory activity ($p<0.001$) when compared to control, but less activity when compared to indomethacin ($P< 0.001$). The activity of methanolic extract is better than the aqueous extract (Table1).

Table 1.Effect of MEPF and AEPF on carrageenan induced paw edema

Group	Dose (p.o)	Paw edema volume (ml) 1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Group – I [Control]	10 ml/kg	0.20±0.004	0.24± 0.008	0.34±0.001	0.36 ± 0.01	0.4 ± 0.01	0.41± 0.01
Group – II [MPEF]	200mg/kg,	0.19± 0.004c (6.14%)	0.22±0.008b (10.56%)	0.21± 0.001a (37.24%)	0.19 ± 0.008a (47.1%)	0.17 ±0.006a (56.6%)	0.14± 0.013a (64.64%)
Group – III [AEPF]	200mg/kg,	0.2± 0.001NS (2.96%)	0.23± 0.004NS (6.49%)	0.22± 0.009a (33.5%)	0.21 ±0.008a (40.0%)	0.21 ± 0.008a (47.5%)	0.20±0.009a (50.1%)
Group – IV [Indomethacin]	10 mg/kg,	0.19± 0.004b (7.76%)	0.20± 0.006a (15.98%)	0.18± 0.008a (47.05%)	0.16±0.005a (55.5%)	0.13±0.008a (67.5%)	0.12±0.008a (68.5%)

Values are mean ± SEM of 6 animals per group; Percentage inhibition in parenthesis; NS- non significant; --a p < 0.001, b p<0.01, c p< 0.05; comparison group II, III, IV Vs group I

Albumin induced edema: Both MEPF and AEPF showed significant anti inflammatory activity ($p<0.001$) when compared to control but the effect of indomethacin was more significant when compared to both extracts. (Table 3).The anti-inflammatory activity of 200mg each of MEPF and AEPF was 0.833 and 0.588 times that of 10mg of indomethacin. MEPF is more effective than AEPF.(Table 2).

Table 2. Effect of MEPF and AEPF on albumin induced inflammation in rats

Group	Dose (p.o)	Paw edema volume (ml) 30 Minutes	60 Minutes	90 Minutes	120 minutes	150 Minutes	180 Minutes
Group – I [Control]	10 ml/kg	0.32± 0.009	0.38 ± 0.015	0.43 ±0.019	0.44 ± 0.011	0.4 ± 0.008	0.36± 0.011
Group – II [MPEF]	200 mg/kg,	0.26 ± 0.012a	0.22 ± 0.011[b]	0.18 ± 0.005[a]	0.16 ± 0.011[a]	0.14 ± 0.007[a]	0.12± 0.009[a]
Group – III [AEPF]	200 mg/kg,	0.28 ± 0.009[NS]	0.26 ± 0.008[a]	0.22 ± 0.006[a]	0.20 ± 0.011[a]	0.18 ± 0.009[a]	0.17 ± 0.008[a]
Group – IV [Indomethacin]	10 mg/kg,	0.18 ± 0.008a	0.16 ± 0.008[a]	0.15 ± 0.006[a]	0.14 ± 0.005[a]	0.12 ±0.005[a]	0.10 ±0.042[a]

Values are Mean±SEM of 6 animals/group. Comparison groups II, III, IV Vs I. NS=Not significant, a=p<0.001.

Acetic acid induced vascular permeability: The effect of MEPF and AEPF was shown in the table. Both the extracts reduced the intensity of peritoneal inflammation by 44.57% and 34.85% respectively when compared to control as observed in the reduction of Evans blue dye leakage induced by acetic acid in rats ($p< 0.001$) (Table 3)

Table 3. Effect of MEPF and AEPF on acetic acid induced vascular permeability

Group	Dose (p.o)	Dye concentration (µg)	Percentage inhibition
Group – I[Control]	10 ml/kg	46.3 ± 1.68	—
Group – II[MEPF]	200 mg/kg,	25.66 ± 0.98[a]	44.57
Group – III[AEPF]	200 mg/kg,	30.16 ± 0.65[a]	34.85
Group – IV[Indomethacin]	10 mg/kg,	22.16 ± 1.10[a]	52.13

Castor oil produced characteristic semi solid diarrhoeal droppings in all the animals of the control group at all time intervals. At the first hour both MEPF and AEPF at a dose of 200 mg/kg delayed and prevented diarrhoea 66.08% and 57.28%, respectively ($p<0.001$) when compared to control. Indomethacin showed 87.3% protection against diarrhoea. At the second hour interval both the extracts and indomethacin showed 68.81%, 61.02% and 74.01% inhibition, respectively. Both the extracts and indomethacin delayed diarrhoea and afforded significant protection ($p<0.001$) at the third and fourth hour intervals when compared to control (Table 4).

Table 4. Effect of MEPF and AEPF on castor oil induced diarrhoea

Group	Dose[p.o.]	Percentage protection from diarrhoea 1 hr	2 hr	3 hr	4 hr
Group – I[Control]	10 ml/kg	0	0	0	0
Group – II[MEPF]	200mg/kg	66.08 ±4.14*	68.81± 2.84	64.81 ±2.78	50.54 ±2.84
Group – III[AEPF]	200 mg/kg	57.28 ±4.01*	61.02 ±2.61	60.41 ±1.70	42.67 ±3.37
Group – IV[Indomethacin]	10 mg/kg	87.3 ±3.27	74.01 ±3.85	65.91 ±2.02	53.79± 3.25

*Values are mean ± SEM of 6 animals per group; Comparison groups II, III, IV Vs group I. *p<0.001*

DISCUSSION

Indigenous drug system can be a source of a variety of new drugs, which can provide relief to pain, fever and inflammation but their claimed reputation has

to be verified on a scientific basis. The present investigation revealed that plant *Pandanus fascicularis* has a significant anti-inflammatory activity in acute models of inflammation.

Carrageenan induced edema of the rat foot is used widely as a working model of inflammation in the search for new anti-inflammatory drugs. This method the basis for the discovery of new anti-inflammatory drug indomethacin [14].

Carrageenans are algal extracts of sulphated polysaccharides used to induce tissue edema in experimental animals [14]. Carrageenan caused visible redness and pronounced swelling that was well developed in 4 hrs and persisted for more than 48 hrs.

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory agents as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. [14, 15] In the rat paw edema model, carrageenan induces edema formation in 3 distinct phases according to the mediators involved. The initial phase occurring during the first hour after sub plantar injection of carrageenan into rat hind paw is mediated by histamine and serotonin. After that increased vascular permeability in the 2nd phase is mediated by kinin release up to 2.5 hrs. The mediators involved in the 3rd phase, from 2.5-6 hours are prostaglandins [16]

All NSAIDS are effective in inhibiting edema formation especially in the late phase in which prostaglandins are involved. In this study indomethacin showed maximum inhibitory effect on edema formation at 6th hr, this corresponds to the period of prostaglandins phase. MEPF and AEPF also inhibited edema formation with maximum activity at the same period as indomethacin when compared to control.

Sub plantar injection of fresh egg albumin produced marked, sustained, time related and progressive increase in the rat paw volume [17]. Maximum swelling was observed approximately 2 hrs after albumin injection. Both the extracts and indomethacin showed significant activity.

Acetic acid induced vascular permeability indicates acute phase of inflammation where there is increased vascular permeability and migration of leukocytes in to the inflamed area occurs [18]. Decreased concentration of dye indicates reduction in permeability.

Castor oil produced characteristic semisolid diarrhoeal droppings in all the animals of the control group at all time intervals. Ricinolic acid, an active constituent of castor oil was believed to irritate the gastric mucosa and stimulate intestinal contractions. The primary action has been shown to be decreased intestinal absorption of water and electrolytes and enhanced secretion by a detergent like action on the mucosa. Structural damage to the villous tips has also been observed. Peristalsis is increased secondarily [19]. Fluid accumulation in the lumen of the intestinal tract is a generally recognized effect of Ricinolic acid. This accumulation together with the histological evidence of damage to mucosal cell layers would define Ricinolic acid as an irritant which induces inflammation of the intestine upon oral administration. Inhibition of castor oil induced diarrhoea is related to inhibition of prostaglandin biosynthesis [20, 21].

Thus the results of the present study confirm the folklore and traditional claims suggested for *Pandanus fascicualris*. The anti-inflammatory effect of *Pandanus fascicualris* indicated a likelihood of intervention with PG synthesis, as PGs have been established as a common mediator in all these responses. However, this possibility remains to be investigated in detail. Moreover, the active constituents responsible for these pharmacological activities are also remaining to be identified.

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