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## Anti-Inflammatory Activity of *Pandanus odoratissimus* Extract

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**Abstract:** This study examined the anti-inflammatory activity of *Pandanus odoratissimus*. Anti-inflammatory drugs presently available for the treatment of various inflammatory disorders have diverse and undesirable side effects. In recent years, many active chemicals have been isolated from plants possessing anti-inflammatory activity. The anti-inflammatory activity was estimated by carrageenan-induced acute and formalin-induced chronic paw edema models in rats. The methanolic extract of *Pandanus odoratissimus* was given in the doses of 25, 50 and 100 mg kg<sup>-1</sup>. The plant extract at the dose of 100 mg kg<sup>-1</sup> showed significant anti-inflammatory activity peaked at 3 h where, it caused inhibition for the increase in carrageenan-induced acute (68%) and for the increase in formalin-induced chronic (64.2%) paw edema models in rats. The standard drug was used as diclofenac sodium. As a result methanolic extract of *Pandanus odoratissimus* was shown to have an anti-inflammatory activity.

**Key words:** Anti-inflammatory, *Pandanus odoratissimus*, paw edema, rats, drug

### INTRODUCTION

Many noninfectious diseases are known to be treated by herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role as therapeutic remedies in many developing countries (Khan *et al.*, 2008). The inflammation in the oral tissues may be related to the expression of inflammatory cytokines including IL-1 $\beta$ , a key mediator of various immunological and inflammatory phenomena (Dinarello, 2006). IL-1 $\beta$  can stimulate the expression of IL-6 and prostaglandin E2 (PGE2) from human gingival fibroblasts (Kida *et al.*, 2005). The use of herbal remedies for anti inflammatory and arthritis treatment has been gaining momentum in recent years (Chrubasik *et al.*, 2007). There has been some concern over the use of COX-2 inhibitors for therapeutic intervention, especially since, some of the products based on COX-2 were either withdrawn or made to carry warning by the US FDA (Naesdal and Brown, 2006). Inflammation is known to occur via a series of complex pathophysiological pathways, influenced by various mediators such as prostaglandins and leukotrienes. These mediators can cause edema such as heat, pain, disturbed tissue function, reddening and swelling (Rasadah *et al.*, 2004).

Pandan is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of wellbeing and acting as a counter to tropical lassitude. It may be chewed as a breath sweetener or used as a preservative on foods. It is also said to have healthful properties, including antiviral, anti-allergy, antiplatelet,

anti-inflammatory, antioxidant and antitumor. The medicinal action of the essential oil yielded by the screw pine's highly scented flowers to be useful in headaches, earaches and as a liniment for rheumatic pains. The distilled water made from, the flowers are used for inducing perspiration. It is also prescribed as a stimulant and an antispasmodic. The flowers themselves are powdered and included in medicines, which are either sniffed like snuff or smoked for asthma and other bronchial infections (Kirtikar and Basu, 2000). Hence, the present investigation aimed to study the antioxidant property of *Pandanus odoratissimus*.

### MATERIALS AND METHODS

**Collection of samples:** Leaves of the plants *Pandanus odoratissimus* (PO) were collected during March 2008 from Gurnitkal, Gulbarga District North Region of Karnataka, India. The samples were authenticated in Botany Department, Gulbarga University, Gulbarga. Plant materials were dried and stored in shade and were powdered to mesh as and required. PO was aliquot to equal amounts of powders by weight. This study was conducted from May 2009 to October 2009.

**Preparation of extract:** The dried material of *Pandanus odoratissimus* was taken for extraction of bioactive compounds with methanol using Soxhlet apparatus. The solvent was removed under reduced pressure and a semi-solid mass was obtained. The extract at different doses of 25, 50 and 100 mg kg<sup>-1</sup> was suspended in Tween

80 solution was used for the treatment. Positive control was maintained with commercially available anti-inflammatory drug Diclofenac sodium.

**Anti inflammatory activity:** Anti-inflammatory activity was determined by carrageenan-induced acute and formalin-induced chronic paw edema models in rats.

**Carrageenan-induced paw edema in rats:** Animals were divided into five groups comprising six animals in each group. In all groups, acute inflammation was produced by sub-plantar injection of 0.02 mL freshly prepared 1% carrageenan in normal saline in the right hind paw of rat. One group injected with carrageenan alone served as positive control. Three groups were administered with methanolic extracts of *Pandanus odoratissimus* at a concentration of 25, 50 and 100 mg kg<sup>-1</sup> b.wt. intraperitoneally and one group was administered with diclofenac (10 mg kg<sup>-1</sup> i.p.), as standard drug. The paw thickness was measured using vernier calipers before and 3 h after carrageenan injected (Ajith and Janardhanan, 2001).

Increase in paw thickness was calculated using the formula Pt-Po, where as Pt is the thickness of paw at time t (i.e., 3 h, after carrageenan injection) and Po is the paw thickness at 0 time. Percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = (C-T/C) \times 100 \quad (1)$$

where, C is the increase in paw thickness of the control and T is that of treatments.

**Formalin-induced paw edema in rats:** Animals were divided into five groups comprising six animals in each group. In all groups, chronic inflammation was produced by a single sub-plantar injection of 0.02 mL freshly prepared 2% formalin in the right hind paw of rat (Ajith and Janardhanan, 2001). Three groups were administered with methanolic extracts of *Pandanus odoratissimus* at a concentration of 25, 50 and 100 mg kg<sup>-1</sup> b.wt. intraperitoneally and one group was administered with diclofenac (10 mg kg<sup>-1</sup> b.wt.) intraperitoneally 30 min prior to formalin injection and one group injected with formalin alone served as control. The administration of the extracts (25, 50 and 100 mg kg<sup>-1</sup> b.wt.) and diclofenac was continued once daily for six consecutive days. The paw thickness measured using vernier calipers before and 6 days after formalin injection.

Increase in paw thickness was calculated using the formula Pt-Po, where as Pt is the thickness of paw at

time t (6 days after formalin injection) and Po is the paw thickness at 0 time. Percent inhibition was calculated using the formula which is mentioned in Eq. 1.

**Effect of methanolic extracts of *Pandanus odoratissimus* on croton oil induce edema in rat:** Corton oil was isolated from the seeds of croton tiglium according to the method (Berenblum, 1941). Croton oil contains 12-O-Tetra Decanoyl Phorbol-13-Acetate (TPA) an inducer of inflammation. The back of 50 rats was shaved using surgical clippers before two days of experiment. Animals with complete hair growth arrest were grouped into 4 groups of 10 animals each and treated as follows. Methanolic extract of *Pandanus odoratissimus* (10 mg in 0.2 mL of acetone) was applied topically to the shaved area of dorsal skin 30 min before application of croton oil (0.2 mL 50% croton oil in acetone). After 24 h, the extract and croton oil application was repeated on the same area. The group treated with 0.2 mL of croton oil in acetone alone as kept as control. One hour after the second treatment of croton oil animals were sacrificed and the skin punches were obtained with 8 mm diameter cork borer. The skin punches were weighed immediately in an analytic balance the percent inhibition was calculated after comparing with the control group (Lakshmi *et al.*, 2002).

**Statistical analysis:** The significance of difference between means was determined by student's t-test values of p<0.05 were considered.

## RESULTS AND DISCUSSION

**Carrageenan-induced paw edema in rats:** The methanolic extract of *Pandanus odoratissimus* is significantly reduced the carrageenan-induced paw edema. The reductions of edema by the extracts were in a dose dependent manner. The concentrations of 25, 50 and 100 mg kg<sup>-1</sup> body weight inhibited the inflammation by 35, 50 and 68%, respectively. The inhibition of inflammation at 100 mg kg<sup>-1</sup> b.wt. was equivalent to the standard reference drug diclofenac (Table 1).

**Formalin-induced paw edema:** The methanolic extract of *Pandanus odoratissimus* is significantly reduced formalin induced paw edema. The reduction of edema by the *Pandanus odoratissimus* extracts was in a dose dependent manner. *Pandanus odoratissimus* at concentration at 25, 50 and 100 mg kg<sup>-1</sup> b.wt. inhibited the inflammation by 42.2, 52.3 and 64.2%, respectively. The inhibition of inflammation at 100 mg kg<sup>-1</sup> b.wt. was equivalent to the standard reference drug diclofenac (Table 2).

Table 1: Effect of methanolic extract of *Pandanus odoratissimus* on carrageenan induced acute inflammation

Treatments	Dose (mg kg <sup>-1</sup> )	Increase in paw thickness after 3 h	Inhibition (%)
Control		0.120±0.028	
Diclofenac sodium (standard)	25	0.64±0.003	58.50
MEPO	25	0.072±0.002	35.00
	50	0.056±0.008	50.00
	100	0.048±0.014	67.00

Table 2: Effect of methanolic extract of *Pandanus odoratissimus* on formalin induced chronic inflammation

Treatments	Dose (mg kg <sup>-1</sup> )	Increase in paw thickness after 3 h	Inhibition (%)
Control		0.125±0.037	
Diclofenac sodium (standard)	25	0.120±0.026	57.7
MEPO	25	0.120±0.026	42.2
	50	0.103±0.029	52.3
	100	0.083±0.025	64.2

**Croton oil induced skin edema:** Topical application of croton oil has been used to screen for topically applied anti-inflammatory steroids and nonsteroid agents and promotes events of inflammatory processes such as oedema, cell infiltration and proliferation, with the production of arachidonic acid metabolites, cytokines and other proinflammatory mediators. The topical application of the methanolic extract of *Pandanus odoratissimus* was capable to prevent important events of inflammation such as skin punch caused by topical administration of croton oil. Topical application of the extract prior to croton oil the skin punch weight was significantly reduced compared to the control group (Fig. 1).

The methanolic extract of *Pandanus odoratissimus* was also effective in inhibiting the croton oil induced lipid peroxidation in rats skin (Fig. 2). The content of lipoxygenation product MDA in carrageenan induced inflamed paws of rats and marked lessened the activity of NOS and the content of in exudates of carrageenan induced paw edema in rats. Topical application of the extract 30 min prior to croton oil could significantly inhibit the lipid peroxidation compared to control group. The malondialdehyde level as an indicator of lipid peroxidation was elevated in the control group compared to treatments. The extracts at a concentration of 10 mg showed the maximum effect.

The results of the present investigations revealed that methanolic extract of *Pandanus odoratissimus* possess significant anti-inflammatory activity against carrageenan induced acute and formalin induced chronic inflammatory model in rats in a dose dependent manner. Several inflammatory mediators such as kinins, PGs and serotonin account for the edema formation caused by sub-plantar formalin or carrageenan injection. This increased synthesis of PGs is due to increase of cyclooxygenase-2 (Subbaramaiah *et al.*, 1997).

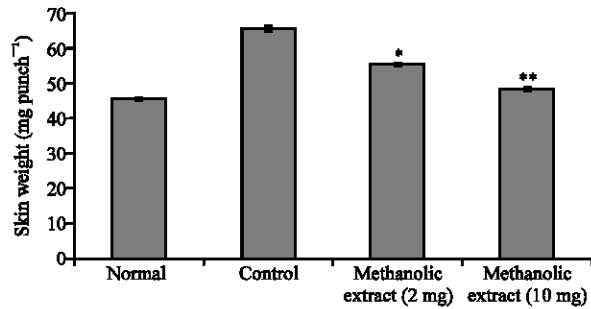


Fig. 1: Effect of methanolic extract of *Pandanus odoratissimus* on corton oil induced rat skin edema. Values are Mean±SD, n = 6 animals. Any two values having a common letter are not significantly different at 5% level. LSD = 3.402

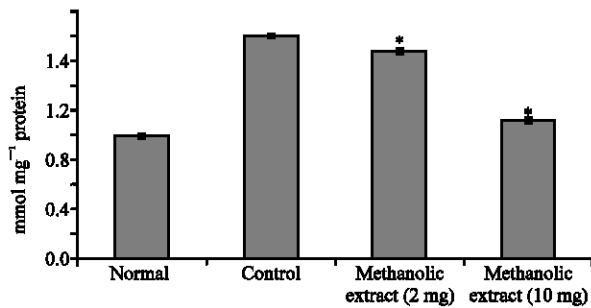


Fig. 2: Effect of methanolic extract of *Pandanus odoratissimus* on lipid peroxidation inhibition in corton oil induced rat. Values are Mean±SD, n = 6 animals. Any two values having a common letter are not significantly different at 5% level. LSD = 0.1289

The methanolic extract of *Pandanus odoratissimus* showed activity of antioxidant, reducing power and scavenging activity (Londonkar and Kamble, 2009).

The methanolic extract of *Pandanus odoratissimus* doses (25, 50 and 100 mg kg<sup>-1</sup>) exhibited significant (p<0.05) anti-inflammatory effect as compared to saline control in the carrageenan induced paw edema. The activity was comparable to the standard drug diclofenac. The plant extract at the dose of 100 mg kg<sup>-1</sup> showed significant anti-inflammatory activity peaked at 3 h where, it caused 68% inhibition of the increase in carrageenan-induced acute and 64.2% inhibition of the increase in formalin-induced chronic paw edema models in rats.

The anti-inflammatory activity of *Pandanus odoratissimus* suggests its potential ability to modulate inflammation, which is increasingly implicated in coronary artery disease and cancer. Chemical substances, which counter platelet aggregation, have a protective role in thromboembolic disorders.

## CONCLUSION

*Pandanus odoratissimus* showed anti-inflammatory property, similar to those observed for non-steroidal anti-inflammatory drugs, such as diclofenac. It is also suggested that the mechanism of action of *Pandanus odoratissimus* might be associated with the inhibition of histamine, serotonin and prostaglandins synthesis. However, further studies are needed to isolate and characterize anti-inflammatory chemical constituents present in methanolic extract of plant.

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