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Anti-inflammatory activity of *Aponogeton natans* (Linn.) Engl. & Krause in different experimental animal models

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ABSTRACT

The present investigation was carried out to find the effect of *Aponogeton natans* (Linn.) leaf and leafstalks various extracts for its anti-inflammatory activity in different experimental animal models. The anti-inflammatory activity was evaluated using acute inflammatory models like; carrageenan induced paw edema, histamine induced paw edema and chronic inflammatory model like; cotton-pellet induced granuloma models in rats. Oral administration of the methanolic extract at the doses 200 mg/kg body weight exhibited significant anti-inflammatory activity in different models. In carrageenan and histamine induced paw edema *Aponogeton natans* (Linn.) methanol extract showed maximum inhibition of 60.89% and 53.97% respectively at late phase. In the cotton pellet induced inflammation study in rats, *Aponogeton natans* (Linn.) methanol extract 200 mg/kg showed significant decrease in wet weight and dry weight of granuloma tissue formation by 52.72% and 43.76% respectively. Hence, present investigation established pharmacological evidences that it can be used as anti-inflammatory agent. A significant percentage inhibition of paw oedema by the methanol extract as compared to control suggests its usefulness in acute and chronic anti-inflammatory models.

Key words: *Aponogeton natans*, inflammation, carrageenan, histamine, cotton pellets.

INTRODUCTION

Aponogeton natans (Linn.) Engl. & Krause belongs to aponogetonaceae family. The plant occurs in plains, in the ponds and marshy places in Asia, Australia, India and Srilanka. Leaf pastes are consumed with hot water to treat cuts & wounds [1]. Fresh tuber are ground into a paste and boiled with 200 ml of coconut oil and applied on hair before bath for three days to get rid of fungal infection [2]. A perusal of existing reports reveals that the no detailed anti-inflammatory study had been done earlier. Therefore, the present study has been planned to investigate the anti-inflammatory activity of *Aponogeton natans* (Linn.) Engl. & Krause. leaf and leafstalks various extracts in different experimental animal models of acute and chronic inflammation.

MATERIALS AND METHODS

Plant material

Fresh parts of *Aponogeton natans* (Linn.) Engl. & Krause were collected from Salipur, Cuttack, Odisha, India which was identified and authenticated by Prof. P. Jayaraman, PARC, Chennai. The voucher specimen was given the No. PARC/2009/398. The air dried powdered leaves and leafstalks was loaded into soxhlet apparatus and was subjected

to extraction for about 72 hours with petroleum ether (60-80°C), benzene, chloroform and methanol successively. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using rotary evaporator. The extracts were stored in a closed bottle and kept in refrigerator until tested. For the pharmacological tests, the extracts were dissolved in 3% Tween-80 in normal saline solution to prepare 200 mg/kg concentrations.

Drugs and Chemicals

Diclofenac sodium and carrageenan were purchased from Sigma-Aldrich, Germany. Petroleum ether, benzene, chloroform and methanol were purchased from Merck, India.

Animals

Wistar albino rats 180-200 g of either sex were used for the experimental models. The animals were obtained from the animal house of Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha. All the rats were kept in standard polypropylene rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature ($25 \pm 2^\circ\text{C}$). They were provided with commercial rat and mice feed and water given *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

Acute toxicity study

The acute oral toxicity study was carried out as per the guidelines set by organization for economic cooperation and development (OECD) revised draft guidelines 423 B received from committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The LD₅₀ cutoff dose for all extracts and 1/10th of the LD₅₀ dose is taken as a therapeutic dose [3, 4].

Carrageenan induced rat paw edema

The rats were divided into six groups (n=6). Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. Group I served as control, Group II, III, IV, V of rats were administered with pet ether, benzene, chloroform, methanol extract (200 mg/kg, p.o.) and Group VI was administered with diclofenac sodium (15 mg/kg, p.o.). The control group received vehicle (0.9% normal saline in 3% Tween 80 (2ml/kg)). 1 h after drug treatment, paw edema was induced by the injection of carrageenan. The paw volume was measured initially and then at 1, 2, 3, 4 and 5 h after the carrageenan injection by using digital plethysmometer. [5,6]

The antiinflammatory effect of *Aponogeton natans* (Linn.) extracts was calculated by the following equation:

$$\% \text{ of inhibition} = \frac{VC - VT}{VC} \times 100$$

Where VC and VT are the paw volume in control rats and treated group of rats respectively.

Histamine induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model [7].

Cotton pellet induced granuloma in rats

The effect of Pet ether, benzene, chloroform and methanol extract of *Aponogeton natans* (Linn.) leaf and leafstalk extracts on the chronic phases of inflammation was assessed in the cotton pellet induced granuloma rat model. Autoclaved cotton pellets weighing 10 ± 1 mg each were implanted subcutaneously. One on each side of the abdomen of the animal, through a small ventral incision of rats anesthetized with ether. Group I served as control, Group II, III, IV, V of rats were administered with pet ether, benzene, chloroform, methanol extract (200 mg/kg, p.o.) and Group VI was administered with diclofenac (15 mg/kg, p.o.). The control group received vehicle (0.9% normal saline in 3% Tween 80 (2ml/kg)). On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. The results

are expressed as mg granulation tissue formed. The antiproliferative effect of different extracts was compared with control [8].

The antiinflammatory effect of *Aponogeton natans* (Linn.) extracts was calculated by the following equation:

$$\% \text{ of inhibition} = \frac{WC - WT}{WC} \times 100$$

Where WC and WT are difference in pellet weight of the drug control group and treated group of rats respectively.

Statistical analysis:

The data were expressed as mean±SEM. The statistical significance was determined by using the ANOVA followed by Dunnett's t test. Values of $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The present study establishes the antiinflammatory activity of *Aponogeton natans* (Linn.) methanol extract of leaves and leafstalks in different experimental animal models. Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer and vascular diseases. Inflammatory reactions are not only the response of living tissues to injury and infection, but also are relevant to disease developments, such as asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [9]. *Aponogeton natans* (Linn.) methanol extract of leaves and leafstalks showed significant anti-inflammatory effects in various animal models. Our results revealed that administration of methanol extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

Oral Acute Toxicity Study

In this assay, neither deaths nor symptoms associated with toxicity such as convulsion, ataxy, diarrhoea or increased diuresis occurred during the 72 hour observation period. These results indicate the effectiveness and relative safety of all extracts for the treatment of conditions associated with inflammation.

Carrageenan induced rat paw edema

The results of anti-inflammatory activity of different extracts of *Aponogeton natans* (Linn.) on carrageenan induced paw edema is shown in Table 1. Carrageenan-induced oedema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever [10, 11]. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins. The methanol extract and diclofenac sodium showed inhibitions at both early and late phase. The maximum inhibition of methanol extract was at 4th and 5th hr (60.89% and 60.27%, $P < 0.01$). The standard diclofenac sodium also showed maximum inhibition at 3rd, 4th and 5th hr (61.81 %, 62.82% and 63.69%, $P < 0.01$). The chloroform extract showed inhibition at 3rd and 4th hr (45.45% and 45.51%, $P < 0.05$). In this model, among the various extracts methanol extract of 200mg/kg showed significant inhibition of edema formation when compared to vehicle treated control group.

Histamine induced rat paw edema

The results of anti-inflammatory activity of different extracts of *Aponogeton natans* (Linn.) on histamine induced paw edema is shown in Table 2. Histamine induced paw edema is said to occur in earlier stage of the vascular reaction in the chemically induced inflammation. In this, swelling occurs primarily due to action of histamine. Generally histamine is released following the mast cell degranulation by number of inflammatory mediators including substances P interleukin-1. This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxy eicosatetraenoic-acid from endothelial cell leading to hyperalgesia and other pro-

inflammatory effects. Methanol extract and diclofenac sodium showed significant inhibition against histamine induced oedema .

The methanol extract and diclofenac sodium showed inhibitions at both early and late phase. The maximum inhibition of methanol extract was at 3rd, 4th and 5th hr (52.43% 53.97% and 53.01%, P < 0.01). The standard diclofenac sodium also showed maximum inhibition at 3rd, 4th and 5th hr (55.13 %, 55.68% and 56.02%, P < 0.01). The chloroform extract showed inhibition at 3rd and 4th hr (40.54% and 36.93%, P<0.05). In this model, among the various extracts methanol extract of 200mg/kg showed significant inhibition of edema formation when compared to vehicle treated control group.

Cotton pellets granuloma

The results of anti-inflammatory activity of different extracts of *Aponogeton natans* (Linn.) in cotton pellet method is shown in Table 3. The cotton-pellet model is based on the foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets of compressed cotton. The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulomatous tissues. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides. In the cotton pellet induced inflammation studies in rats, methanol extract 200mg/kg and the standard drug diclofenac sodium showed significant decrease in wet weight of granuloma tissue formation i.e 52.72% and 59.01%, P<0.01 respectively . Further, methanol extract 200mg/kg and the standard drug diclofenac sodium showed significant decrease in the dry weight of granuloma tissue formation i.e 43.76% and 51.51%, P<0.01 respectively when compared to vehicle treated control group.

Table 1. Effect of different extracts of *Aponogeton natans* (Linn.) Engl. & Krause on carrageenan induced edema in rats

Groups	Treatment	Dose	Mean paw volume (ml±S.E.M)				
			1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr
Groups I	3% Tween 80	2ml /kg	0.96±.454	1.21±.483	1.65±.634	1.56±.234	1.46±.227
Groups II	Pet-ether extract	200mg/kg	0.65±.314 (32.29)	0.85±.493 (29.75)	1.18±.407 (28.48)	1.03±.187 (33.97)	1.01±.195 (30.82)
Groups III	Benzene extract	200mg/kg	0.66±.307 (31.25)	0.78±.360 (35.53)	1.15±.568 (28.48)	1.10±.194 (29.48)	1.03±.201 (29.45)
Groups IV	Chloroform extract	200mg/kg	0.51±.285 (46.87)	0.76±.225 (37.19)	0.9±.316* (45.45)	0.85±.114* (45.51)	0.85±.102 (41.78)
Groups V	Methanol extract	200mg/kg	0.47±.264* (51.04)	0.63±.242* (47.93)	0.68±.354* (58.78)	0.61±.113** (60.89)	0.58±.113** (60.27)
Groups VI	Diclofenac sodium	15mg/kg	0.46±.242* (52.08)	0.53±.206* (56.18)	0.63±.326** (61.81)	0.58±.151** (62.82)	0.53±.145** (63.69)

Data are the mean ±SEM values for six rats in each group using the ANOVA followed by Dunnet's t test.*p < 0.05, **p < 0.01 as compared to the control.; % of inhibition is given in parentheses.

Table 2. Effect of different extracts of *Aponogeton natans* (Linn.) Engl. & Krause on histamine induced paw edema in rats

Groups	Treatment	Dose	Mean paw volume (ml±S.E.M)				
			1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr
Groups I	3% Tween 80	2ml /kg	1.16±.185	1.41±.212	1.85±.240	1.76±.214	1.66±.206
Groups II	Pet-ether extract	200mg/kg	0.85±.128 (26.72)	1.05±.201 (25.53)	1.36±.183 (26.48)	1.23±.190 (30.11)	1.21±.200 (27.10)
Groups III	Benzene extract	200mg/kg	0.86±.149 (25.86)	0.98±.157 (30.49)	1.35±.211 (27.02)	1.25±.185 (28.97)	1.23±.207 (25.90)
Groups IV	Chloroform extract	200mg/kg	0.71±.179 (38.79)	0.95±.095 (32.62)	1.1±.115* (40.54)	1.11±.157* (36.93)	1.08±.070 (34.93)
Groups V	Methanol extract	200mg/kg	0.58±.110* (50.00)	0.81±.110* (42.55)	0.88±.137** (52.43)	0.81±.101** (53.97)	0.78±.101** (53.01)
Groups VI	Diclofenac sodium	15mg/kg	0.55±.114* (52.58)	0.73±.105* (48.22)	0.83±.1406** (55.13)	0.78±.144** (55.68)	0.73±.125** (56.02)

Data are the mean ±SEM values for six rats in each group using the ANOVA followed by Dunnet's t test.*p < 0.05, **p < 0.01 as compared to the control.

% of inhibition is given in parentheses.

Table 3. Effect of different extracts of *Aponogeton natans* (Linn.) Engl. & Krause on cotton pellets-induced granuloma in rats

Groups	Treatment	Dose	Weight of wet cotton pellet granuloma (mg±S.E.M)	Weight of dry cotton pellet granuloma(mg±S.E.M)
Groups I	3% Tween 80	2ml /kg	187.06±12.22	44.78±3.88
Groups II	Pet-ether extract	200mg/kg	169.21±13.45 (9.54)	40.61±4.41 (9.31)
Groups III	Benzene extract	200mg/kg	163.93±8.40 (12.36)	37.28±3.46 (16.74)
Groups IV	Chloroform extract	200mg/kg	157.23±14.36 (15.94)	31.76±4.15 (29.07)
Groups V	Methanol extract	200mg/kg	88.43±9.23** (52.72)	25.18±3.91** (43.76)
Groups VI	Diclofenac sodium	15mg/kg	76.25±6.30** (59.01)	21.71±3.68** (51.51)

Data are the mean ± SEM values for six rats in each group using the ANOVA followed by Dunnet's t test.* $p < 0.05$, ** $p < 0.01$ as compared to the control.

% of inhibition is given in parentheses.

CONCLUSION

In conclusion, this study has shown that the *Aponogeton natans* (Linn.) Engl. & Krause methanol extract of leaf and leaf stalks possesses significant anti-inflammatory effects in both acute and chronic phases of inflammation that may be mediated through inhibition of cell mediators such as bradykinin, and prostaglandins. These results support the traditional use of this plant in some painful and inflammatory conditions.

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