

ANTI-INFLAMMATORY EFFECTS OF THE SAPONINS OBTAINED FROM THE LEAVES OF *ACORUS CALAMUS*

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Summary

Acorus calamus (family araceae) is a popular traditional medicinal plant. In Ayurvedic medicine, it is used for the treatment of insomnia, neurosis, epilepsy, hysteria and loss of memory. Saponins, the steroid or triterpenoid glycosides are common component of a large number of nutritionally important plants. They show several biological effects including anti-inflammatory properties. Phytochemical investigations revealed presence of alkaloids, saponins, triterpene steroids and tannins in *Acorus calamus*. The present study was intended to evaluate the anti-inflammatory activity of the purified saponins obtained from methanolic and water extracts of *Acorus calamus* leaves. The carrageenan induced paw oedema in wistar rats was treated using different doses of the extracts (75, 150, 300 mg/kg, p.o.). The extracts exhibited significant ($P < 0.01$) anti-inflammatory activity, supporting its use as traditional medicine. The study established anti-inflammatory activity of the leaves of *Acorus calamus*.

Key words: *Acorus calamus*, anti-inflammatory activity, saponin, paw oedema

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Introduction

Acorus calamus Linn. also known as sweet flag, is a native plant of India which was introduced in Europe in the 16th century as a medicinal plant. The plant thus became a common littoral species of the European wetlands, which are characteristic by high trophic status (1). It possesses grass-like or sword-shaped, long slender leaves that fan out from a pinkish base and grow up to 1.5m in length. Many ethnomedicinal and ethnobotanical uses of the rhizome of plant have been reported. *A. calamus* Linn. has also been used traditionally in China and India for its beneficial effects on memory disorders (2). It also exhibits antispasmodic, carminative and antihelmintic properties.

The plant is also used for the treatment of epilepsy and mental ailments (3). Saponins belong to vast group of glycosides, widely distributed in higher plants. They are believed to form the main constituents of many plant drugs and folk medicines and considered responsible for numerous pharmacological properties. Many plant saponins were found to produce inhibition of inflammation in the mice (4). Phytochemical investigations revealed presence of alkaloids, saponins, triterpene steroids and tannins in the plant (5). The current study was aimed at isolating saponin from *Acorus calamus* leaves and using a mechanistic pharmacological approach to validate their anti-inflammatory effect.

Material and Methods

Plant material

The leaves of *Acorus calamus* were collected from the High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar, Uttarakhand, India in the month of Mar 2010 and were taxonomically authenticated by the Dr. Alok Lehri, Scientist E-1, Central Instrumental Facility, National Botanical Research Institute, Lucknow, India. The leaves were air dried for 20 days, crushed into coarse powder with a grinder and passed through 40-mesh sieve. They were stored in a well closed container.

Preparation of crude extract

The powdered leaves (200g) each were macerated separately in methanol and water at room temperature for seven days with occasional shaking. Subsequently the extracts were filtered through filter paper. This maceration procedure was repeated thrice and the combined filtrate was evaporated using rotary evaporator under reduced pressure at $\leq 50^{\circ}\text{C}$ temperature. After evaporation the dark brown crude extracts were obtained with an yield approximately 12.23g for methanolic extract and 16.56 g for water extract. The crude extracts were stored in a desiccator. Fresh solutions of the extracts were prepared for each study (6). A known quantity of the crude methanolic extract (11g) was dispersed in distilled water (100ml). Further, this dispersion was transferred to separating funnel and extracted three times with 20 ml of *n*-hexane. The mixture was shaken vigorously for 5 minutes, allowing the air to escape out. It was kept for about 30 min to separate out two layers (7). The upper layer of *n*-hexane was collected and the aqueous layer was further extracted three times with 20 ml of ethyl acetate and *n*-butanol, respectively. Each fraction was evaporated to dryness under reduced pressure. The yield of *n*-hexane, ethyl acetate and *n*-butanol extracts was found to be 1.20g, 2.43g and 3.13g respectively. The remaining aqueous layer was discarded. Same procedure was repeated with crude water extract (15g). In this case yield for obtained from *n*-hexane, ethyl acetate and *n*-butanol extracts was found to be 2.11g, 3.23g and 4.35g respectively. The *n*-butanol fractions of both methanolic and water extracts were refluxed with 6 M HCl for 6 h. The hydrolysates were extracted with chloroform and evaporated to dryness. The average yield was found to be 2.01g and 2.26 g respectively (5).

Phytochemical Analysis

The chemical constituents of the *Acorus calamus* leaves extract were analysed using the method suggested by Harbone (8). The results are presented in table 1.

Chemical and Drugs

Carrageenan, disodium chromoglycate and methanol were procured from Sigma-Aldrich, U.S.A., Great Chemical Industry Co., China, Rankem, India respectively.

Animals

Wistar rats (170-200 g.) of either sex were obtained from animal house of Central Drug Research Institute, Lucknow India. The animals were kept in standardized environmental conditions ($28\pm 1^{\circ}\text{C}$; $65\pm 5\%$ relative humidity; 12:12 h dark/light cycle) and allowed food and water ad libitum.

Anti-Inflammatory Activity

Carrageenan-Induced Paw Oedema in Rats

Anti-inflammatory activity was assessed by the method described by Winter et al. (9). Sixteen rats were divided into four groups (four animals in each group). Group A & B rats received water extract and methanolic extract, at three different doses of 75, 150 and 300 mg/kg p. o. respectively. Group C rats were administered with disodiumchromoglycate (5 mg/kg body weight p.o.) as positive control. Group D (control) received vehicle (10 ml/kg, p.o.) only. After forty five minute, freshly prepared 0.1ml Carrageenan solution (1% w/v in normal saline) was injected in the sub- plantar region of the left hind paw and the increase in paw volume was measured at interval of 1, 2, 3 and 4 hr with the help of volume differential meter. Inhibition of the paw oedema due to test drug and the positive control group was calculated and compared with the control. The formula to calculate percent inhibition was: $\% I = 1 - (dt/dc) \times 100$, where “dt” is the difference in paw volume in the drug-treated group and “dc” is the difference in paw volume in control group and “I” stands for inhibition of inflammation.

Statistical Analysis

The results were expressed as the mean \pm standard error of mean (S.E.M) and the statistical significance was established by the student's *t*-test. The P-values less than 0.001 imply significance (10).

Results

Anti-inflammatory activity

Results of anti-inflammatory activity are summarized in table 2. The results indicate that n-butanol fraction of the methanol and water extract of *Acorus calamus* leaves exhibited anti-inflammatory effect in the carrageenan-induced rat paw oedema. Test showed that administration of n-butanol fraction of both methanol and water extract at three different doses (75, 150 and 300 mg/kg, p.o.) inhibited the oedema in the first hr. However at the end of third hr methanolic extract ($P < 0.01$) and water extract ($P < 0.01$) significantly reduced the paw volume, which is comparable with control group. Both methanolic and water extract showed dose dependent activities (Fig.1). After 3 hr, the methanolic extract (300 mg/kg, p.o.) and water extract (300 mg/kg, p.o.) reduced inflammation by about 66.7% and 47.7% respectively, in comparison to the standard which reduced the inflammation by around 76.2%.

Table 1: Phytochemical screening of methanolic and water extract of *Acorus calamus* leaves

Chemical compounds	Test reagent	Results
Alkaloids	Meyer's reagent	++
Flavonoids	Magnesium turning	+
Saponins	Frothing test	+++
Triterpenoids	Sulphuric acid reagent	++
Tanins	Ferric chloride reagent	++
Carbohydrate	Fehling's reagent	-
Cardiac glycosides	Keller-Killani test	++

Key: -= negative; + = weakly positive; ++ = moderately positive; +++ = strongly positive.

Table 2: Effect of the methanol extract and water extract of *Acorus calamus* leaves on carrageenan induced paw oedema in rats.

Group	Dose (mg/kg, p.o.)	Oedema rate percentage (mean \pm S.E.M.) / % Inhibition			
		1 hr	2hr	3hr	4hr
Control	-	0.375 \pm 0.170 (NI)	0.45 \pm 0.086* (NI)	0.5 \pm 0.085* (NI)	0.475 \pm 0.062* (NI)
Standard (Disodiumchromoglycate)	5	0.25 \pm 0.064 (40)	0.2 \pm 0.057 (55.6)	0.125 \pm 0.025* (76.2)	0.25 \pm 0.129 (47.4)
Water extract	75	0.35 \pm 0.095 (6.7)	0.425 \pm 0.11 (10.6)	0.425 \pm 0.047 (19.1)	0.45 \pm 0.028* (5.3)
	150	0.325 \pm 0.025* (13.4)	0.375 \pm 0.085 (16.7)	0.375 \pm 0.085 (28.6)	0.425 \pm 0.094 (10.6)
	300	0.3 \pm 0.070 (20)	0.3 \pm 0.070 (33.4)	0.275 \pm 0.047* (47.7)	0.40 \pm 0.057* (15.8)
Methanolic extract	75	0.325 \pm 0.047* (13.4)	0.35 \pm 0.10 (22.3)	0.325 \pm 0.047* (38.1)	0.425 \pm 0.085* (10.6)
	150	0.3 \pm 0.070 (20)	0.30 \pm 0.057* (33.4)	0.25 \pm 0.129 (52.4)	0.375 \pm 0.025* (21.1)
	300	0.25 \pm 0.057* (33.4)	0.225 \pm 0.62 (50)	0.175 \pm 0.025* (66.7)	0.35 \pm 0.095 (26.4)

Values are mean \pm SE, * $P < 0.01$ compared with control, student's t -test.

Values in parenthesis represent percent inhibition of oedema, NI = No inhibition

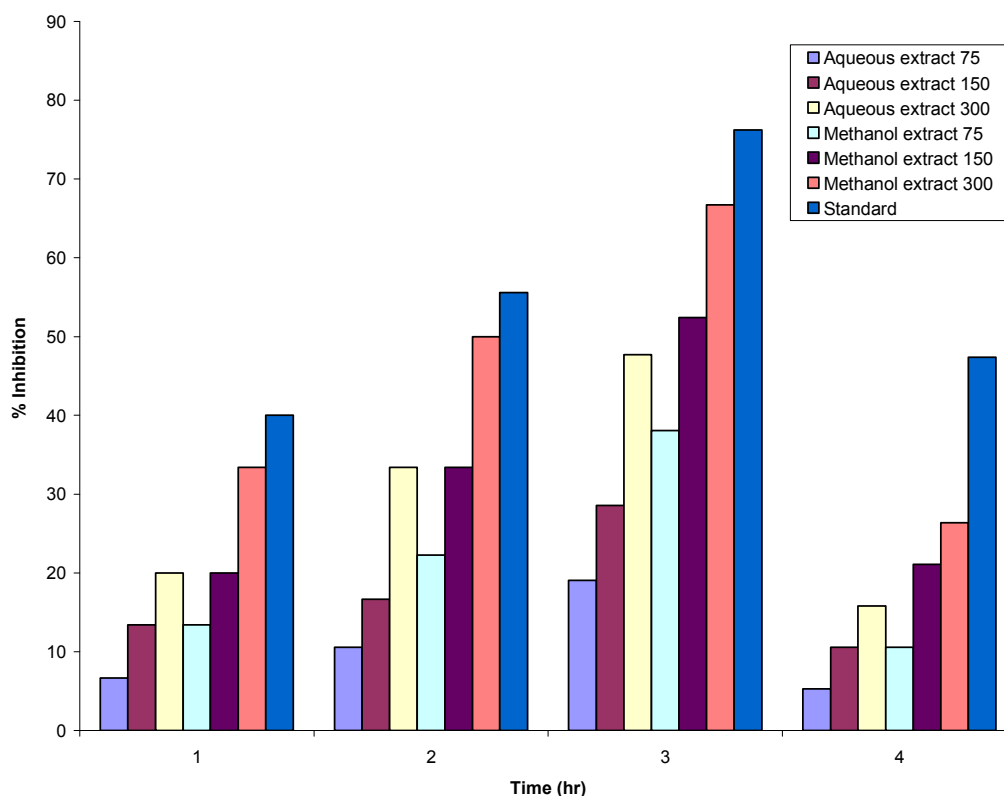


Fig 1. Effects of methanolic, water extract and disodiumchromoglycate on carrageenan-induced paw oedema in rats

Discussion

Carrageenan-induced rat paw oedema is a suitable experimental model to evaluate the anti-oedematous effect of natural products and is believed to be biphasic; the first phase (1 h) involves the release of serotonin and histamine and second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products, and the continuity between the two phases is provided by kinins (11).

There are a number of reports indicating of anti-inflammatory properties of saponins. The saponins produced a dose-dependent oedema reduction. The anti-inflammatory effect of the saponins was stronger in normal rats than adrenalectomized rats. The content of ascorbic acid in rat adrenals was decreased by Saponins. Thus in addition to the direct anti-inflammatory effect, the saponins may act indirectly via the pituitary adrenocortical system. (4).

The methanolic and aqueous extracts of leaves at doses (75, 150 & 300mg/kg, p.o.) exhibited significant ($P < 0.01$) anti-inflammatory effect as compared to saline control in the carrageenan induced paw oedema (Table 1). The activity was comparable to the standard at different doses. Both methanolic and aqueous extracts showed dose dependent activity (Fig1). The results revealed that administration of both methanolic and aqueous extracts inhibited the oedema even in the first hour. However maximum effect was observed at the end of the third hour. The significantly high anti-inflammatory activity of both methanolic and aqueous extracts may be due to inhibition of mediators of the inflammation such as

histamine, serotonin released during the first phase of inflammation and prostaglandins and bradykinins which are released during the second phase of inflammation. The qualitative phytochemical screening of *Acorus calamus* revealed the presence of alkaloids, flavonoids, tannins and saponins (Table 1). The anti-inflammatory activity of both the extracts of *Acorus calamus* leaves could be attributed to the high amount of saponins and tannins present in the plant (12). This activity was related to dose and these results corroborate the traditional use of the plant in inflammatory conditions.

Acknowledgements

The authors are thankful to Director, CDRI Lucknow for providing facilities to evaluate anti-inflammatory activity and also grateful to Dr. Alok Lehri, Scientist E-1, Central Instrumental Facility at National Botanical Research Institute, Lucknow, for the authentication of plant material used in this study.

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