

ANALGESIC AND ANTICONVULSANT EFFECTS OF *ACORUS CALAMUS* ROOTS IN MICE

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ABSTRACT: *Acorus calamus* has been used for a long time in traditional medicine as a remedy for pain, convulsion, inflammation, and ulcer. In the present work, the analgesic effects of methanolic extract of *Acorus calamus* roots (MEAC) have been evaluated using acetic acid induced Writhing response and Rat caudal immersion method. Whereas the anticonvulsant effect were investigated by utilizing pentylenetetrazol induced convulsion methods. MEAC administered orally at the doses of 100 and 200 mg/kg, exhibited protective effect against the pain models in mice. Also the methanolic extract of *Acorus calamus* roots significantly increased the latency period in seizures induced by PTZ in mice. These obtained results indicate the analgesic as well as anticonvulsant effect *Acorus calamus* roots.

KEY WORDS: *Acorus calamus* roots, Methanol extract, Analgesic, Anticonvulsant activity

INTRODUCTION

Drugs acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects. From the vast array of materia medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering¹.

Acorus calamus Linn. (Araceae) is commonly known as “bath” or “ugragandha” in North India. The roots and rhizomes of this plant have been used in the Indian systems of medicine for hundreds of years. While the drug is used in Ayurvedic medicine on a regular basis for the treatment of insomnia, melancholia, neurosis, epilepsy, hysteria, loss of memory and remittent fevers^{2,3,4,5}.

The plant has been extensively investigated and a number of chemical constituents from the rhizomes, leave and roots of the plant have previously reported which includes β - Asarone, α - Asarone, elemicine, cis-isoelemicine, cis and trans isoeugenol

and their methyl ethers, camphene, P-cymene, b-gurjunene, a-selinene, b-cadinene, camphor, terpinen-4-ol, a-terpineol and a-calacorene, acorone, acenone, acoragermacrone, 2-deca -4,7 dienol, shyobunones, linalool and preisocalamendiol are also present². Acoradin, galangin, 2, 4, 5- trimethoxy benzaldehyde, 2,5- dimethoxybenzoquinone, calamendiol, spathulenol and sitosterol have been isolated from *Acorus calamus*^{6,7}. In the present study, we investigated the Analgesic and anticonvulsant effects of methanol extracts of *Acorus calamus* roots in rat and mice.

MATERIALS AND METHODS

Plant material

The roots of *Acorus calamus* were procured from the local market, rajkot, India and authenticated by G.K.S Moorthy Botanical survey of India, Coimbatore. The roots were dried under shade and pulverized to coarse powder. The powder was passed through 40-mesh sieve and exhaustively extracted with 90% v/v methanol in a soxhlet apparatus. The extract was evaporated under reduced pressure until all the solvent had been removed. The dried methanol extract (MEAC) was stored in the refrigerator and a weighed

amount was dissolved in propylene glycol for the present investigation.

Phytochemical investigation of the extracts

In order to detect the various constituents present in the Methanolic extract of *A. calamus* were subjected to Phytochemical screenings^{8,9}.

Animals

Wistar albino mice of either sex (20–25 g) were used for the study. They were housed in a quite temperature of $25 \pm 1^{\circ}\text{C}$ and relative humidity of 45–55%. A 12:12 light/dark cycle was maintained during the experiment. They were given free access to food and water, except during the test period. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All experiments were performed at the same time of the day and during the light period. Each group consists of a 6 animals/dose and the experimental protocols were approved by institutional animal ethics committee (IAEC) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

Toxicological study

The acute toxicity study was done as per the OECD guidelines (407). MEAC were administered orally in different doses, where 24 h toxicity was recorded to identify the toxic doses. The doses of the test compounds were then fixed on the basis of their acute toxicity as 100 mg/kg and 200 mg/kg for evaluation.

Analgesic study

Writhing test

Animals were divided in 4 groups of 6 mice each. Group 1 served as negative control and was treated with a mixture of 3% of DMSO and 3% of Tween 20 (1 mL/100 g body weight). The second group received indomethacin (10 mg/kg) and was used as positive controls. The remaining groups received the plant extracts at the doses of 100–200 mg/kg. One hour after oral administration of these substances, each animal was injected intraperitoneally with 0.6% acetic acid, in a volume of 0.1 mL/10 g body weight. After acetic acid injection, the number of stretchings or writhing responses per animal was recorded during a subsequent 30 min¹⁰.

Rat caudal immersion method

Animals were divided into 4 groups of 6 mice each. Group 1 served as negative control and was treated with a mixture of 3% of DMSO and 3% of Tween 20 (1 mL/100 g body weight). The second group received indomethacin (10 mg/kg) and was used as positive controls. The remaining groups received the plant extracts at the doses of 100–200 mg/kg. p.o. The reaction time for withdrawal of tail was recorded after 60 min from the administration of test

compounds. It was determined by immersing the tail up to the caudal portion (5 cm from the tip) in hot water ($55 \pm 0.5^{\circ}$) and by noting the time taken to withdraw the tail clearly out of water¹¹.

Anticonvulsant study

Pentylenetetrazol-induced seizures

Wistar albino mice of either sex (20–30 g) were used for the anticonvulsant activity. Animals were divided into four groups of six mice each. Group 1 served as control (saline 10 ml/kg) and second groups is treated with carbamazepine (50mg/kg). remaining two groups were given different doses of the extract (100 and 200 mg/kg .p.o), 60 min before the subcutaneous injection of pentylenetetrazole (PTZ). Onset of convulsions and Duration of convulsion were recorded. These two parameters were compared with control animals, in order to assess the anticonvulsant activity¹².

STATISTICAL ANALYSIS

The results were expressed as mean \pm S.E.M. All statistical comparisons were made by means of Student's *t*-test, and a $P < 0.05$ was regarded as significant.

RESULTS

Phytochemical investigation of the extracts

Phytochemical screening revealed the presence of saponins, alkaloids, tannins, sugars and gums and mucilages.

Analgesic activity

Writhing test

The oral administration of the MEAC extract at the doses of 100 and 200 mg/kg significantly reduced the writhing reaction induced by acetic acid. Indomethacin inhibited pain sensation by 16.48 from 37.90 and the percentage of protection is 59.9%. Whereas the Methanolic extract of *Acorus calamus* at the dose of 100 and 200mg/kg showed 38.09 and 45.02% respectively. (Table 1)

Rat caudal immersion method

In this method, the Methanolic extract of *Acorus calamus* roots at the dose of 100 and 200mg/kg exhibited significant analgesic activity ($***P < 0.001$) which was confirmed by increased tail withdrawal time of MEAC treated animals when compared to control groups. (Table 2)

Anticonvulsive activity

Pentylenetetrazol-induced seizures

Single dose, subcutaneous administration of pentylenetetrazole (PTZ; 90 mg/kg) caused clonic convulsions as well as lethality in mice. Pretreatment of the mice with the plant extract caused a dose-dependent protection against PTZ-induced convulsions as shown in Table 3.

DISCUSSION

The results of the present study indicate that methanol extract of *Acorus calamus* roots possesses analgesic and anticonvulsant activity in mice. The results of our study shows that extracts from the roots of *Acorus calamus* have an analgesic effect against writhing response induced by acetic acid. Intraperitoneal injection of acetic acid produced pains through activation of chemo sensitive nociceptors¹³ or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin^{14,15}. Thus, analgesic activity of opioid agonist, opioid partial agonist and non-steroidal anti-inflammatory agents can be determined by writhing test. Since *Acorus calamus* root extract were active in this type of pain, they may belong to at least one of these classes of analgesics.

It is now accepted that many anti-epileptic drugs can have an analgesic effect in human neuropathic pain^{16,17}. Considering this, the extract of

Acorus calamus root was tested for its anticonvulsant activity. The extract significantly increased the latency period and reduced the duration of seizures induced by PTZ. Two mechanisms have been proposed for the mode of PTZ-induced convulsion. It is proposed that PTZ induces convulsion by either inhibiting gamma amino butyric acid (GABA) pathway in CNS¹⁸ or by increasing the central noradrenergic activity¹⁹. The effect of extract in this model can therefore suggest its involvement in GABA-ergic or noradrenergic pathways and its efficacy against generalized Tonic-clonic and partial seizures in mice.

CONCLUSION

In conclusion, the extracts from *Acorus calamus* root seem to possess central analgesic properties as well as anticonvulsant effects, which may be mediated by the Potentiation of the activity of GABA.

Table1 :Effect of oral administration of Methanolic extract of *Acorus calamus* roots on pain induced by intraperitoneal injection of acetic acid in mice.

Treatment	Dose (mg/kg)	Writhing Response	% Inhibition
Control	1 mL/100 g	37.90±0.48	0.00
Indomethacin	10	16.48±0.89***	59.9
MEAC	100	23.48±0.15***	38.09
MEAC	200	21.37±0.79***	45.02

Values are Mean ± S.E.M n=6.

** $P < 0.01$, *** $P < 0.001$, significant difference compared to control.

Table 2 : Effect of oral administration of Methanolic extract of *Acorus calamus* roots on pain induced by caudal immersion method

Treatment	Dose (mg/kg)	Caudal immersion reaction time (in sec)
Control	1 mL/100 g	3.40±0.48
Indamethacin	10	12.49±0.80***
MEAC	100	6.10±0.47**
MEAC	200	8.59±0.39***

Values are Mean ±S.E.M n=6.

** $P < 0.01$, *** $P < 0.001$, significant difference compared to control.

Table 3: The effect of Methanolic extract of *Acorus calamus* roots on pentylenetetrazole induced seizure in Mice

Groups	Treatment	Dose (mg/kg p.o.)	Onset of convulsion (Sec)	Duration of convulsion (Min)
1	PTZ + saline	(90+ 10ml/kg)	67.20±1.25	463.48±11.39
2	PTZ +Carbamazepine	50	79.20±1.56	205.37±17.49
3	PTZ +MEAC	90 +100	72.39±2.49	210.59±13.49*
4	PTZ + MEAC	90+200	91.20±2.55***	163.44±17.04**

Values are Mean ±S.E.M n=6.

** $P < 0.01$, significant difference compared to control.

*** $P < 0.001$, significant difference compared to control.

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