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CNS Activity of Methanol and Acetone Extracts of *Acorus calamus* Leaves in Mice

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Abstract: The present study was designed to evaluate CNS depression or analeptic activity of acute oral administration of methanol (ACME) and acetone (ACAE) extracts of *Acorus calamus* leaves in mice. Spontaneous locomotor activity, immobility time using forced swim test, diazepam-induced sleeping time and motor impairment assessment using rotarod were used to assess CNS depression/analeptic activity of ACME and ACAE in mice. The extracts ACME (5, 20 and 50 mg kg⁻¹, p.o.) and ACAE (20 and 50 mg kg⁻¹, p.o.) significantly decreased the spontaneous locomotor activity in dose dependent manner. The acute treatment of ACME and ACAE (5, 20 and 50 mg kg⁻¹, p.o.) significantly increased the immobility time and decreased the swimming behavior. Administration [6 h prior] of ACME (50 mg kg⁻¹, p.o.) and ACAE (20 and 50 mg kg⁻¹, p.o.) significantly potentiated the diazepam (25 mg kg⁻¹, i.p.)-induced sleeping time in mice. These extracts did not induce disturbance in motor coordination. The results of the present research provided evidences that ACME and ACAE may contain psychoactive substances that are CNS depressant in nature. The CNS depression property of these extracts can be utilized for further anticonvulsant research.

Key words: Sweet flag leaves, analeptic, CNS depression, locomotor activity, immobility, sleeping time

INTRODUCTION

Acorus calamus Linn. (Family Araceae) commonly known as sweet flag or Waan-Nam, is a well known medicinal plant used in ayurvedic medicine. It is a semi aquatic herb with creeping rhizomes and sword shaped long leaves found near marshy places, river banks and lakes throughout India and other Central Asia, Central Europe and North America (Nadkarni, 2007; Prajapati *et al.*, 2003). In India it is common in areas that surround the Himalayas. All parts of the plant contain volatile oil. The volatile oil contains terpenoids, calamine, calamenol, calamenone, eugenol, camphene, pinene and asaronaldehyde. Acorafuran is a new sesquiterpenoid found in calamus oil (Tkachev *et al.*, 2006). The rhizomes were utilized extensively by the Chinese, Indians and American Indians as well as by other cultures (Motley, 1994). Its roots and rhizomes are used in various ailments including many mental disorders, such as hysteria, insanity, insomnia, melancholia, neurasthenia, epilepsy, diarrhoea and asthma (Hazra *et al.*, 2007; Mukherjee *et al.*, 2007). The leaves extract of *Acorus calamus* were studied for anti-inflammatory activity on keratinocyte HaCaT cells (Kim *et al.*, 2009). The various pharmacological activities of roots and rhizomes extracts of *Acorus calamus* such as analgesic (Mukherjee *et al.*, 2007), anticonvulsant (Achliya *et al.*, 2005), antispasmodic (Gilani *et al.*, 2006),

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anti-inflammatory (Vohora *et al.*, 1990), antibacterial (Aqil and Ahmad, 2007), antiulcer and cytoprotective activity (Mukherjee *et al.*, 2007) was reported earlier. In most of studies the roots and rhizomes extracts of *Acorus calamus* reported for its CNS depressant activities (Mukherjee *et al.*, 2007; Hazra and Guha, 2002). Water soluble dried powder of alcoholic *Acorus calamus* extract of roots and rhizomes were studied for CNS activities. It has been reported dosage of 10, 25, 50 mg kg⁻¹, i.p. of herbal extract reduced the spontaneous locomotor activity and also amphetamine-induced hyperactivity in mice (Mukherjee *et al.*, 2007). All the reported literature on *Acorus calamus* for its pharmacological activities was done mainly by using roots and rhizomes extracts. So, the present studies are designed to evaluate on CNS depression or analeptic activity of *Acorus calamus* leaves extracts in mice.

MATERIALS AND METHODS

Plant Material

Acorus calamus is an aromatic plant which is mainly found in wet and marshy places. The fresh and matured leaves of *Acorus calamus* were collected from well-grown plants at Kodaikanal of Tamilnadu, India and authenticated by Dr. G.V.S Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu. A voucher specimen (BSI/SC/5/23/06-07/Tech. 1304) has been kept in our museum for future reference. After due authentication, fresh matured leaves were collected, cleaned thoroughly with distilled water and the leaves were dried under shade. The shade dried leaves were pulverized in a mechanical grinder to obtain coarse powder.

Animals

Albino mice (Swiss 20-25 g, either sex) were used. They were allowed food and water *ad libitum* up to the experimentation. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room. All observations were made between 9.00-17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India.

Drugs

Diazepam (Ranbaxy Laboratories Ltd., India), carboxymethylcellulose (Lobachemie Pvt. Ltd., India). Methanol and acetone ($\geq 99.9\%$ v/v) used were of analytical-reagent grade and obtained from E. Merck (Mumbai, India).

Preparation of *Acorus calamus* Leaves Extracts

The leaves were dried, ground and soaked in methanol and acetone, at room temperature. The dried leaves were soaked in a particular solvent for 3 days, each day the treated solvent being recovered and replaced with fresh solvents were then pooled together. The extracts were finally obtained by steam distillation followed by evaporation at 37°C of the remaining solvent. The yield of methanol, acetone extract of *Acorus calamus* leaves extract was found to be 12.2% w/w, 10.8% w/w g, respectively. The samples were uniquely coded and stored at 10°C till further use. The extracts were suspended in 1% w/v carboxy methyl cellulose solution and administered orally (p.o.).

Phytochemical Analysis

Phytochemical investigations of methanol and acetone extracts of *Acorus calamus* leaves for identification of active principles such as carbohydrates, alkaloids, proteins, volatile oils, triterpenes,

flavonoids, saponins, phenols, resins and tannins was carried out using the methods previously described by Evans (2002). The presence of all of above active principles except carbohydrates and proteins was ascertained for both methanol and acetone extracts of *Acorus calamus* leaves.

Methods

Effect of *Acorus calamus* Leaves Extracts on Spontaneous Locomotor Activity

The photocell activity cage was utilized to determine the degree of depression. The actions of plant extract on spontaneous locomotor activity were measured automatically by using actophotometer (Medicraft actophotometer, model No. 600-40, India). The units of the activity counts were arbitrary and based on the beam breaks by movement of the mice. The different groups of mice were treated with methanol and acetone extracts (5, 20 and 50 mg kg⁻¹, p.o.), respectively. The spontaneous locomotor activity was measured at 0, 1, 3 and 6 h intervals by placing animals in a novel cage in the infrared apparatus. Six mice were used for each treatment group. The treatments were randomized throughout the day, between 09:00 and 17:00 h, to control for diurnal variations in activity.

Effect of *Acorus calamus* Leaves Extracts on Behavioral Despair Swim Test

The behavioral despair test has been used as a test of depression like behavior. This test is sensitive and specific to all major classes of antidepressant drugs including tricyclic antidepressants, serotonin specific reuptake inhibitors and monoamine oxidase inhibitors. The method used was essentially similar to that described by Porsolt *et al.* (1977). The animals were forced to swim individually for 15 min, in glass cylinder (30 cm high, 22.5 cm in diameter) containing 15 cm water at room temperature. The animals were individually trained in 15 min sessions. This constituted the pre-test session. Twenty-four hours later, the animals were treated either with a drug (test group) or vehicle (control group) and each animal was again forced to swim in similar environment for a period of 6 min in a test session and duration of immobility time for each mouse was recorded. The mouse was judged to be immobile when it ceased struggling and remained floating motionless in water making only those movements necessary to keep its head above water. Reduction in the duration of immobility by a drug was considered as it having antidepressant like effect. Each experimental group consisted of 6 mice and was chosen by means of completely randomized schedule.

Effect of *Acorus calamus* Leaves Extracts on Diazepam-Induced Sleeping Time in Mice

Diazepam (25 mg kg⁻¹, i.p.) was administered to the control group. To the other group ACME and ACAE (5, 20 and 50 mg kg⁻¹, p.o.) was administered 6 h prior to the diazepam injection (25 mg kg⁻¹, i.p.). The time interval between the loss and regaining of righting reflex (falls asleep) was measured as sleeping time. Note the time of recovery from sleep as the animal turns to recover its normal posture. Calculate the duration of action of diazepam *per se* and in combination with *Acorus calamus* leaves extracts was noted (Pal and Nandi, 2005).

Effect of *Acorus calamus* Leaves Extracts on Motor Impairment Assessment

The rotarod test was used to determine the effect of the extracts on motor coordination. This test used a custom built apparatus, Medicraft Rota Rod, Model No.519/E-2C, Medicraft electro medicals (P) Ltd., India. The instrument (a horizontal rotation device) was set at a rate of 25 rpm. Mice were placed on the rod and those that were able to remain on the rod longer than 3 min were selected for the study. The animals were then evaluated for motor coordination at an interval of 30, 60, 180 and 360 min after oral administration of the diazepam (4 mg kg⁻¹), ACME and ACAE (5, 20

and 50 mg kg⁻¹, p.o.). If the animals failed more than once to remain on the rotating rod for 3 min then it was considered to be positive. The time each animal falls off from the rod was noted. A control group was also used (Dunham and Myia, 1957).

Statistical Analysis

The effect of *Acorus calamus* leaves extracts on spontaneous motor activity, behavioral despair swim test, diazepam-induced sleeping time in mice and motor impairment assessment were expressed as Mean±SEM. The experimental data were analyzed by one way analysis (ANOVA) followed by Dunnett's test. p<0.05 was regarded as statistically significant.

RESULTS

Effect of *Acorus calamus* Leaves Extracts on Spontaneous Locomotor Activity

The effect of leaves extracts of *Acorus calamus* on locomotor activity was measured at 0, 1, 3 and 6 h. ACME and ACAE (5, 20 and 50 mg kg⁻¹, p.o.) significantly decreased the spontaneous locomotor activity in dose dependent manner in mice is shown in Fig. 1. The maximum reduction in locomotor activity of the extracts was found at 6 h (Fig. 1).

Effect of *Acorus calamus* Leaves Extracts on Behavioral Despair Swim Test

The acute treatment of ACME and ACAE significantly increased the immobility time and decreased the swimming behavior in mice is shown in Table 1. ACME and ACAE (5, 20 and 50 mg kg⁻¹, p.o.) at 6 h showed maximum CNS depressant activity in mice (Table 1).

Effect of *Acorus calamus* Leaves Extracts on Diazepam-Induced Sleeping Time in Mice

Administration (6 h prior) of ACME (50 mg kg⁻¹, p.o.) and ACAE (20 and 50 mg kg⁻¹, p.o.) significantly enhanced the diazepam (25 mg kg⁻¹, i.p.) induced sleeping time. The sleeping time of mice treated with diazepam *per se* and in combination with extracts is shown in Table 2.

Effect of *Acorus calamus* Leaves Extracts on Motor Impairment Assessment

The fall-off time of mice administrated with methanol and acetone leaves extracts (ACME and ACAE) at doses 5, 20 and 50 mg kg⁻¹, p.o., did not differ from vehicle treated. Therefore the extracts did not induce disturbance in motor coordination (data not shown).

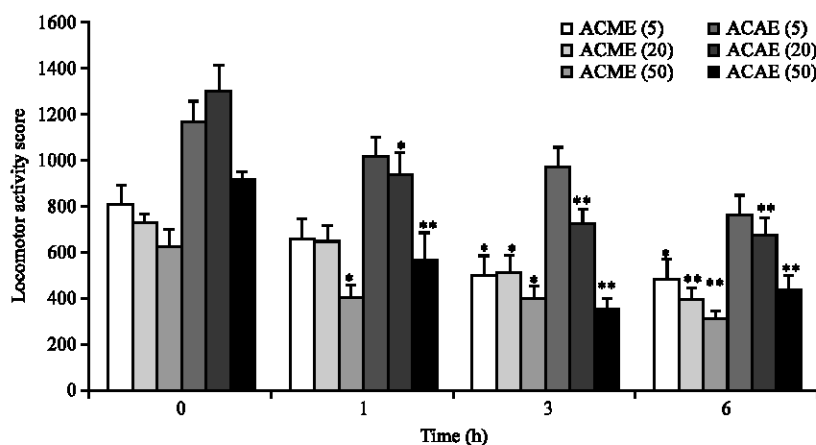


Fig. 1: Effect of methanol extracts (ACME) and acetone extracts (ACAE) of *Acorus calamus* leaves on spontaneous locomotor activity in mice. Values are Mean±SEM of 6 animals a group. *p<0.05, **p<0.01 as compared with 0 h of respective dosage group

Table 1: Effect of methanol extracts (ACME) and acetone extracts (ACAE) of *Acorus calamus* leaves on immobility in mice

Treatment (mg kg ⁻¹ , p.o.)	Duration of immobility (s)	ANOVA values
Control	191.67±7.51	F (3,20) = 5.015 p = 0.0094
ACME(5) [1 h prior]	177.00±15.90	
ACME(5) [3 h prior]	232.83±18.52	
ACME(5) [6 h prior]	253.17±18.61*	
Control	196.83±13.59	F (3,20) = 10.359 p = 0.0003
ACME(20) [1 h prior]	193.17±14.13	
ACME(20) [3 h prior]	232.33±13.50	
ACME(20) [6 h prior]	285.17±11.71**	
Control	226.33±10.55	F (3,20) = 19.459 p = 0.0001
ACME(50) [1 h prior]	252.50±7.78	
ACME(50) [3 h prior]	292.50±8.26**	
ACME(50) [6 h prior]	307.67±6.60**	
Control	166.33±5.12	F (3,20) = 5.459 p = 0.0066
ACAE(5) [1 h prior]	209.50±18.14	
ACAE(5) [3 h prior]	241.83±20.49*	
ACAE(5) [6 h prior]	266.67±24.54**	
Control	205.33±11.72	F (3,20) = 4.940 p = 0.0100
ACAE(20) [1 h prior]	255.17±20.88	
ACAE(20) [3 h prior]	266.00±15.30*	
ACAE(20) [6 h prior]	284.67±11.13**	
Control	219.50±8.08	F (3,20) = 9.515 p = 0.0004
ACAE(50) [1 h prior]	253.86±6.72*	
ACAE(50) [3 h prior]	276.00±10.09**	
ACAE(50) [6 h prior]	280.50±10.69**	

Values are Mean±SEM of 6 animals in each group. *p<0.05, **p<0.01 (one-way ANOVA followed by Dunnett's test as compared to control group)

Table 2: Effect of methanol extracts (ACME, 6 h prior, p.o.) and acetone extracts (ACAE, 6 h prior, p.o.) of *Acorus calamus* leaves on diazepam (DZM, i.p.) - induced sleeping time in mice

Treatment (mg kg ⁻¹)	Duration of sleep (min)	ANOVA values
Vehicle+DZM (25)	91.69±2.97	F (6,35) = 7.983 p<0.001
ACME (5)+DZM (25)	80.50±3.43	
ACME (20)+DZM (25)	72.00±19.86	
ACME (50)+DZM (25)	152.67±11.30*	
ACAE (5)+DZM (25)	98.00±16.46	
ACAE (20)+DZM (25)	153.50±20.61*	
ACAE (50)+DZM (25)	165.67±11.18**	

Values are Mean±SEM of 6 animals in each group. *p<0.05, **p<0.01 (one-way ANOVA followed by Dunnett's test as compared to Vehicle group)

DISCUSSION

The methanol and acetone extracts of *Acorus calamus* leaves (ACME and ACAE) were studied for CNS activities in mice. The results of the present work provided evidences that ACME and ACAE may contain psychoactive substances that are depressant in nature. The extract was found to produce alteration in general behavioral pattern, significant reduction of spontaneous motor activity, increase in the immobility time in behavior despair swim test, potentiation of the diazepam-induced sleeping time and in addition, the results showed that the extracts did not induce disturbances in motor co-ordination. It is generally believed that locomotor activation results from brain activation, which manifests as an excitation of central neurons and as an increase in cerebral metabolism. While different neurochemical metabolisms are involved in brain activation, dopamine (DA) appears to play an essential role (Salamone *et al.*, 2005; LeMoal and Simson, 1991).

Water soluble dried powder of alcoholic *Acorus calamus* extract of roots and rhizomes were studied for CNS activities. Dosages of 10, 25 and 50 mg kg⁻¹, i.p., of roots and rhizomes extracts reduced the spontaneous locomotor activity and also amphetamine-induced hyperactivity in mice

(Panchal *et al.*, 1989). The present study report is consistent with Panchal *et al.* (1989) results on spontaneous locomotor activity. ACME (5, 20 and 50 mg kg⁻¹, p.o.) and ACAE (20, 50 mg kg⁻¹, p.o.) significantly decreased the spontaneous motor activity. The results on spontaneous locomotor activity demonstrated the CNS depressant activity of ACME and ACAE. These findings were further confirmed in behavioral despair swim test.

The test models of depression (behavioral despair swim test and tail suspension test) were based on the observation that rats or mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves (despair or helplessness) to the experimental conditions. This state considered to be as the state of depression (Kulkarni and Mehta, 1985; Porsolt *et al.*, 1978). The characteristic behavior scored in this test is termed as immobility, swimming and climbing. Depressant drug increases immobility time, decreases swimming and climbing behavior, depending on the concentration, type and time of administration of drug. In the present study, the acute oral treatment of ACME and ACAE significantly increased the immobility time and decreased the swimming behavior. The maximum CNS depression effect was observed at 6 h.

The *Acorus calamus* leaves extracts enhanced the diazepam-induced sleeping time in mice. The average sleeping time due to diazepam (25 mg kg⁻¹, i.p.) per se was found to be 91.6 min. ACME (50 mg kg⁻¹, p.o.) and ACAE (20, 50 mg kg⁻¹, p.o.), significantly augmented the diazepam-induced sleeping time in mice. These results also demonstrated a CNS depressant property of *Acorus calamus* leaves extracts (ACME and ACAE). The hypnotic potentiation actions of Indian *Acorus* oil have been studied in its roots and rhizomes (Seto and Keup, 1969; Dhalla and Bhattacharya, 1968; Malhotra *et al.*, 1962; Dandiya *et al.*, 1959a, b). The present studies confirmed the CNS depressant property of *Acorus calamus* leaves extracts which is consistent with reports on roots and rhizomes (Hazra and Guha, 2002; Mukherjee *et al.*, 2007; Panchal *et al.*, 1989). Phytochemical investigations revealed methanol and acetone extracts of *Acorus calamus* leaves contains triterpenoids, flavonoids, saponins and tannins. A number of scientific reports indicated that triterpenoids produced CNS depressant action (Chattopadhyay *et al.*, 2003). Therefore, the presence of triterpenoids in methanol and acetone extracts of *Acorus calamus* leaves might be responsible for the CNS depressant activity. The rotarod method developed by Dunham and Miya (1957) and used to test the neurotoxicity of a drug and was also used to determine the forced coordinated motor ability of the animals. The extract had no effect on the motor coordination. All the animals stayed on the rotarod for longer than 180 sec, suggesting that the extracts (ACME and ACAE) are devoid of neurotoxicity at these doses (5, 20 and 50 mg kg⁻¹) used in the study. However, further investigation is underway to determine the exact phytoconstituents that are responsible for CNS depressant activity of methanol and acetone extracts of *Acorus calamus* leaves.

CONCLUSION

The results of the present study suggest that the methanol and acetone extract of *Acorus calamus* leaves extract may possess CNS depressant activity. The CNS depressant property of these leaves extracts can be utilized for its anticonvulsant research.

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