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ACORUS CALAMUS LINN.RHIZOMES EXTRACT FOR ANTIDEPRESSANT ACTIVITY IN MICE MODEL

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ABSTRACT:
The aim of the present study was to evaluate the antidepressant activity of aqueous-ethanolic extract of *Acorus Calamus*, by forced swimming test (FST) and tail suspension test (TST). *Acorus Calamus* rhizomes extract (ACE) in doses 75mg/kg and 150 mg/kg was administered once daily for 15 days to young male Swiss albino mice to study the immobility periods of control and treated mice. The antidepressant effect of extract was compared with standard drug Lofepramine (15 mg/kg) administered for 15 days. The possible mechanism of action was explored by co-administration Doxazosin (selective α_1-adrenergic blocking agents) with the extract for studying the plasma corticosterone levels. Pretreatment with ACE (75mg/kg and 150 mg/kg orally, for 15 days) significantly reduced the immobility time in both FST and TST, indicating antidepressant activity. The extract did not show significant effect on locomotor activity in mice. Doxazosin significantly attenuated the extract induced antidepressant effect. The doses of ACE significantly decreased corticosteroid levels, a measure for evaluating antidepressant activity as compared to control group. The results indicated the antidepressant effect of ACE normalizing the over-activity of hypothalamic-pituitary-arenal (HPA) axis system and through interaction with adrenergic and dopaminergic systems.

Keywords: *Acorus calamus extract*, Depression, Forced swim test, Tail suspension test, Over-activity of hypothalamic-pituitary-arenal (HPA) axis system.

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INTRODUCTION

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being[1]. Depressed people feel sad, anxious, empty, hopeless, worried, irritable or restless. They may lose interest in activities that once were pleasurable, experience loss of appetite or overeating, have problems concentrating, remembering details, or making decisions, and may contemplate, attempt, or commit suicide. Insomnia, excessive sleeping, fatigue, loss of energy, or aches, pains, or digestive problems that are resistant to treatment may also be present[2]. Depressed mood is not always a psychiatric disorder. It may also be a normal reaction to certain life events, a symptom of some medical conditions, or a side effect of some drugs or medical treatments. Depressed mood is also a primary or associated feature of certain psychiatric syndromes such as clinical depression. In the medical terms we can say that depression is a prevalent psychiatric disorder with estimates reaching as high as 21% of the world population[3]. Despite the fact that it is a psychiatric disorder, the World Health Organization predicts that it will be the second leading cause of death by the year 2020[4]. An estimated 7-12% of men and 20-25% of women experience a depressive episode in their lifetime[5].

Scientific studies have found that numerous brain areas show altered activity in patients suffering from depression, and this has encouraged advocates of various theories that seek to identify a biochemical origin of the disease, as opposed to theories that emphasize psychological or situational causes. Among the **theories of a biologically based cause of depression** are those involving genetics and circadian

rhythms, but the most prominent and widely researched is the monoamine hypothesis.

Biologically, depression is a state in which decrease level of neurotransmitters such as dopamine (DA), norepinephrine (NE), serotonin (5HT) is reported[6]. In addition, studies demonstrate that the etiology is also affected by elevated corticosterone[7] followed by the Lofepramine (a standard drug) in the function of the hypothalamic-pituitary-adrenal (HPA) axis system[8]. Several antidepressant agents belong to distinct classes i.e. tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) are being used to treat depressive symptoms[9] along with the burden of various adverse effects[10] and possibility of the recurrence as well, this necessitates the continue search of newer, safe and more effective antidepressants from traditional medicinal plants.

Natural source from the *p*lant extracts are one of the most attractive sources for the treatment of depression[11-13]. ***Acorus calamus*** (also called **Sweet Flag** or **Calamus**, among many common names[14] Hindi-bach) is a tall perennial wetland monocot of the *Acoraceae* family, in the genus *Acorus* is a famous plant for the treatment of the disease. The roots and rhizomes of *Acorus calamus* (AC) have been used in the treatment of various ailments in ancient Indian, Chinese and other Asian systems of medicine for hundreds of years[15-19]. It is commonly distributed throughout Europe, temperate India and the Himalayan region (Motley, 1994). Phytochemically, AC contains monoterpenes, sesquiterpenes, phenlypropanoids, flavonoids, quinine and volatile compounds including αand β-asarone[20,21]. Aqua-methanolic extract of *Acorus calamus* and its constituent α-asarone has been reported to possess anti-stress activity against noise stress[17,21,22]. On the basis of this premise, present study has been designed to evaluate the potential antidepressant activity of *Acorus calamus* extract (ACE) in behavioral models of depression.

MATERIALS AND METHOD

Animals: Male Swiss Albino mice, 90 days old and weighing around 25-35 gms were purchased from Animal house Punjab. The animals were kept in standard conditions, with free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee of PCTE Group of institute vide approved number 1493/PO/a/11/CPCSEA.

Preparation of *Acorus calamus* rhizomes extracts (ACE): Rhizomes of *Acorus calamus* Linn. were collected from the herbal garden of PCTE Institute of Pharmacy. The Rhizomes were dried in sun. The dried rhizomes were crushed into coarsely powdered form. The coarse powder was extracted with aqueous-ethanol (1:1) solvent using soxhlet extractor. The extract was distilled off and dried completely by using vacuum evaporator. The yield of extract was obtained 15-18% w/w.

Drugs and Chemicals

Lofepramine hydrochloride (sigma aldrich New Delhi), Doxazosin (Sun Pharmaceuticals, Halol), corticosterone (Hi Media Laboratories, Mumbai), chloroform, methanol (LobaChemie Pvt. Ltd., Mumbai), EDTA (Central Drug House Pvt. Ltd., New Delhi) were used in present study. Laboratory models employed for evaluating antidepressant activity.

Tail suspension test: The tail suspension test was based on the method of Steru with little modifications[23-27]. Mouse was individually suspended on the edge of table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. Testing was carried out in an isolated room with minimal background noise. The immobility was observed during 10 min. period of total duration. Animals were considered immobile only when they hung passively and completely motionless.

Forced swimming test: The studies were carried out on mice according to the method of Porsolt[24,26,28]. Mouse was individually forced to swim in a glass jar (25×12×25 cm^3) containing fresh water up to a height of 15 cm at 25^0C ($\pm 3^0$C) for 10 min. The duration of immobility was measured during the final 3 min of the total test duration of ten minutes. Immobility period was regarded as the time spent by mouse floating motionless in the water and ceased struggling, making only those movements necessary to keep its head above water.

Measurement of corticosterone levels induced by forced swimming stress (FSS): Mice were subjected to swim for 10 min. At the end of swimming session blood was collected with carotid bleeding in eppendorf tubes containing anticoagulant EDTA (5%). Plasma was

separated by centrifugation with speed 2000 rpm for 10 minutes. The supernatant 0.5 ml of plasma was collected in separating funnel to which 10 ml of chloroform was added, it was shaken for 5 min. The organic layer of chloroform was collected in beaker. The pooled organic layers were evaporated on a water bath. The residue collected was dissolved in absolute methanol and solution was filtered through a 0.22µm membrane filter. The filtrate was injected into HPLC system for analysis of corticosterone levels. Chromatographic separation was performed with water-methanol (45:55) as the mobile phase at a flow rate of 1 ml/min[29, 30].

Experimental design

The animals were divided into 10 groups and each consisting of 5 animals, as follows:

Groups for Tail Suspension Test (TST)

Group 1: Control group (Tween 80 (1.5% v/v), vehicle for ACE),

Group 2: Lofepramine (standard) (15 mg/kg),

Group 3: ACE (75 mg/kg) and

Group 4: ACE (150 mg/kg)

To all the groups, dose was administered orally for continuous 15 days. After 90 min. of the last dose on 15th day, immobility period was recorded in each group.

Groups for Forced Swimming Test (FST)

Groups 5 to 8 received the same treatment schedule as TST, except the immobility period was recorded using FST.

Groups for studying the mechanism of action of ACE

Group 9: Control Doxazosin: Tween 80 (3% v/v) was administered orally for 15 days. Doxazosin was administered on 15th day after 45 min. of last oral administration of vehicle. The animals were subjected to TST, after 45 min. of Doxazosin administration.

Group 10: ACE + Doxazosin: ACE (150 mg/kg) was administered orally for 14 days. Doxazosin was administered on 15th day after 45 min. of last oral administration of extract. The animals were subjected to TST, after 45 min. of Doxazosin administration.

RESULTS

The present study was conducted to investigate the antidepressant effect of aqueous-ethanolic extract of *Acoruscalamus* in widely used models for depression i.e. tail suspension test and forced swim test and also to explore the mechanism of ACE for its antidepressant activity.

Effect of ACE on immobility periods in TST

Figure 1, illustrates the effect of ACE at doses (75 and 150 mg/kg orally) on duration of immobility time in the TST model. Administration of ACE once daily for 15 successive days, showed significant decrease in immobility time ($p< 0.001$) as compared to control group. Standard drug, Lofepramine also showed similar profile of activity.

Effect of ACE on immobility periods in FST

ACE (75 and150 mg/kg orally) administered once daily for 15 days, significantly decreased the period of immobility as compared to control ($p< 0.001$), as shown in Figure 2. Treatment of ACE did not show dose-dependent effect on duration of immobility. However, the effect of ACE at the dose 150 mg/kg appeared to be more potent than that of ACE at the dose of 75 mg/kg after 15 days treatment in study. The effect of ACE was comparable to that of Lofepramine (15 mg/kg) administered for 15 successive days in FST.

Effect of combination of ACE with Doxazosin on immobility period in TST

The results presented in Figure 3, show that and Doxazosin alone significantly increased the immobility period ($p< 0.001$) as compared to control group. Pretreatment of animals with ACE (150 mg/kg) for 15 successive days, significantly blocked the increase in immobility time ($p< 0.001$) induced by Doxazosin in TST.

Effect of ACE on plasma corticosterone in mice

The effect of swim stress, Lofepramine and ACE treatment on plasma levels of corticosterone in mice is shown in Figure 4. Administration of ACE at doses (75 and 150 mg/k.) for 15 days, significantly ($p< 0.001$) decreased stress induced corticosterone levels as compared to control group.

Table 1. Effect of aqueous-ethanolic extract of *Acorus calamus* on immobility period in tail suspension test.

Group No	Treatment for 15 days	Dose (kg-)	Immobility time (sec.)(mean ± SEM)
1.1	Control (vehicle treated)	10 ml	192.4 ± 1.9
1.2	Lofepramine	15 mg	121.0 ± 2.6*
1.3	ACE	75 mg	132.4 ± 2.8*
1.4	ACE	150 mg	121.6 ± 1.5*

*$p < 0.001$ when compared with control group, $n = 5$

Table 2. Effect of aqueous-ethanolic extract of *Acorus calamus* on immobility period in forced swim test.

Group No	Treatment for 15 days	Dose (kg-)	Immobility time (sec.) (mean ± SEM)
2.1	Control(vehicle treated)	10 ml	162.4 ± 2.5
2.2	Lofepramine	15 mg	68.6 ± 1.8*
2.3	ACE	75 mg	92.4 ± 1.9*
2.4	ACE	150 mg	75.2 ± 0.5*

*$p < 0.001$ when compared with control group, $n = 5$

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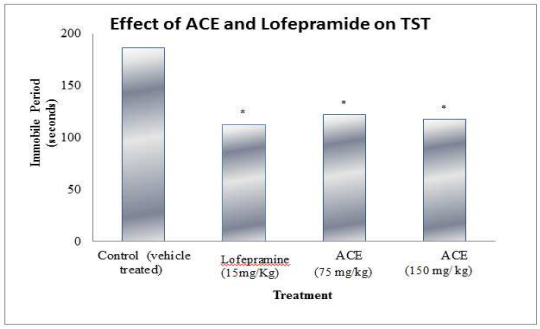

Fig. 1. Effect of ACE and lofepramine on immobility period in tail suspension test.

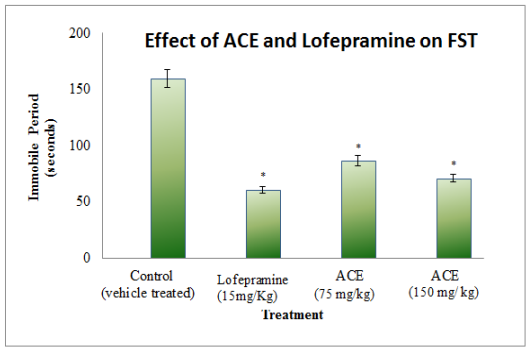

Fig. 2. Effect of ACE and Imipramine on immobility period in forced swim test.

Table 3. Effect of aqueous-ethanolic extract of *Acorus calamus* with Doxazosin on immobility period in tail suspension test.

Group No	Treatment for 15 days	Dose (kg-)	Immobility time (sec.) (mean ± SEM)
1	Control (vehicle treated)	10 ml	188.4 ± 2.5
9	Vehicle	10 ml + Doxazosin	214.0 ± 2.8[a]
10	ACE	150 mg + Doxazosin	172.4 ± 2.9[b]

[a]$p < 0.001$ when vehicle + Doxazosin compared to control,
[b]$p < 0.001$ when ACE + Doxazosin compared to vehicle + Doxazosin, $n = 5$

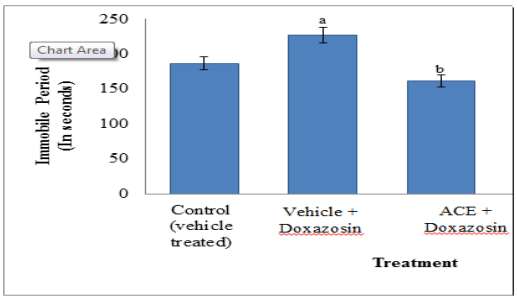

Fig. 3. Effect of alone Doxazosin and ACE + Doxazosin on tail suspension test.

Table 4. Effect of aqueous-ethanolic extract of *Acorus calamus* on corticosterone levels in forced swim test.

Group No	Treatment for 15 days	Dose (kg-)	Conc. of corticosterone (ppm) (mean ± SEM)
2.1	Control (vehicle treated)	10 ml	36.1 ± 0.7
2.2	Lofepramine	15 mg	7.5 ± 0.6*
2.3	ACE	75 mg	19.3 ± 0.9*
2.4	ACE	150 mg	9.7 ± 0.9*

*$p < 0.001$ when compared with control group, $n = 5$

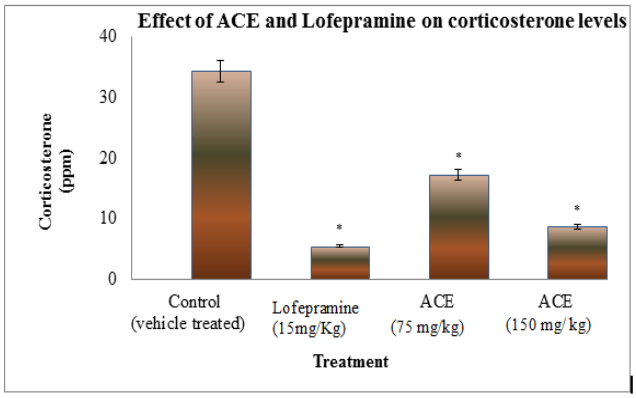

Fig. 4.Effect of ACE and Lofepramine on corticosterone levels in forced swim test.

DISCUSSION

The results of present study demonstrates that oral administration of aqueous-ethanolic extract of *Acorus calamus* rhizomes showed significant antidepressant activity in both tail suspension test (TST) and forced swim test (FST) where reduction in immobility period was observed. Various adrenergic (particularly α_1-adrenoceptor) antagonists reversed the anti-immobility action of ACE. Furthermore, ACE attenuated the elevated plasma corticosterone levels induced by FST. The activity was comparable to the standard drug Lofepramine.

The behavioral despair models of depression (FST and TST) are considered to be screening tool for antidepressant agents with good reliability and predictive validity. These models are quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclic antidepressants, monoamine oxidase inhibitors and atypical antidepressants[27, 28]. These models are based on the observations that rats or mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually ceases to struggle, surrendering themselves (despair or helplessness) to the experimental conditions. This state is considered to be as the state of depression[28-31] and characteristic observation termed as immobility (no movement). An antidepressant agent

reduces the immobility time and increases mobility such as swimming and climbing in case of FST. The results revealed that chronic treatment of ACE significantly reduced the immobility time in both TST and FST models, suggesting antidepressant effect. According to the results, antidepressant effect of ACE was significantly reversed by the treatment of animals with Doxazosin (α_1-adrenoceptor antagonist) when tested in TST. This suggests that mechanism involved in antidepressant-like effect of ACE might be due to its interaction with dopamine D_2-receptors and α_1-adrenoceptors, thereby increasing the levels of dopamine and noradrenaline.

In depression, increased release of corticotropin releasing factor (CRF) and cortisol are observed followed by the dysregulation of hypothalamic-pituitary-adrenal (HPA) axis system[32]. It is well known that stressors have a different impact on the various aspects of the stress response, depending on their exact nature (*e.g.* physical *vs.* psychological aspects and escapable *vs.* inescapable) and severity. These findings strongly supports that hormonal alterations induced by stressors in experimental animals are reminiscent of those observed in depressed patients[33]. In the present study, FSS in mice was used as stressor to induce alterations in the HPA axis system. Swim stress for 10 min. caused an increase in corticosterone levels in control group. Treatment with ACE for 15 days effectively prevented the abnormal rise in corticosterone, this indicates that ACE reverses the effects of chronic stress on behavior, the HPA axis system.

Thus, the present investigation demonstrated that *Acorus calamus* had potential against depression. The mechanisms by which ACE produced antidepressant effect may be mediated by normalizing the overactivity of hypothalamic-pituitary-adrenal (HPA) axis system and due to its involvement with adrenergic and dopaminergic systems, thereby increasing the levels of biogenic amines. The antidepressant activity was comparable to standard drug Lofepramine.

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