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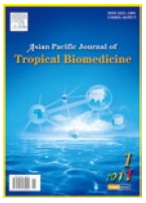

Document heading

Antidepressant-like effects of *Acorus calamus* in forced swimming and tail suspension test in mice

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ABSTRACT

Objective: To evaluate the antidepressant activity of methanolic extract of rhizomes of *Acorus calamus* (*A. calamus*). **Methods:** Tail suspension test (TST) and forced swimming test (FST) in mice were used to evaluate the antidepressant activity of methanolic extract of rhizomes of *A. calamus*. Methanolic extracts (50 and 100 mg/kg i.p.) were administered daily for 7 days. Imipramine 5 mg/kg was used as standard antidepressant agent throughout the study. **Results:** Test extracts of *A. calamus* decreased immobility periods significantly in a dose dependent manner in both TST and FST. The observed results were also comparable with known standard drug *i.e.* imipramine. The flavonoid apigenin, which selectively binds with high affinity to the central benzodiazepines receptor, possesses important anxiolytic and antidepressant activities. The review of literature reveals that the *A. calamus* contains saponin, glycosides, tannin and flavonoid. **Conclusions:** Methanolic extract of *A. calamus* rhizomes shows antidepressant activity probably through interaction with adrenergic, dopaminergic serotonergic and γ-aminobutyric acid (GABA) nergic system. Both the models have been proved to be equally valuable for demonstration of substances with a potential antidepressant activity.

1. Introduction

Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects[1,2]. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with less side effects and wider safety margin. Various plants are being used in complementary and alternative medicine for management of mood disorder.

Acorus calamus (*A. calamus*) L. also known as sweet flag is a native plant of India. It is commonly known as Bach or Ugragandha in north India. It is a semi-aquatic, perennial, aromatic herb with creeping rhizomes. It exhibits polyploidy. This plant belongs to Araceae family and has been used in the Indian and Chinese system of medicine for hundreds of years to cure disease especially the central nervous system (CNS) abnormalities[3-6]. In ayurvedic medicine it is used for the treatment of many mental disorders such as insomnia, melancholia, epilepsy, hysteria, loss of memory, remittent fevers and neurosis[7,8]. The main constituents of *A. calamus* were monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinone[9]. Recently, *A. calamus* proved high antioxidant activity[10,11]. With this background, the present study was undertaken to evaluate antidepressant-like effect of *A. calamus* rhizomes using reported experimental methods.

2. Materials and methods

2.1. Plant material and preparation of extract

The rhizomes of *A. calamus* were collected from the local market of Baramati, Pune district, Maharashtra, India in the month of October 2008 and authenticated by Head, Botany Department, SPMM, Shardanagar, Baramati, Pune district, Maharashtra. The dried rhizomes were coarse powdered and subjected to extraction with hot methanol at (64-65.5)

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℃ using Soxhlet extractor. The extract was concentrated using rotary flash evaporator. The dried extract was stored in airtight container in refrigerator below 10 ℃. Percentage yield of extract was 13.12%. The methanolic extract of *A. calamus* rhizomes (MEAC) was subjected to its antidepressant investigations.

2.2. Phytochemical evaluation

Phytochemical screening for presence of secondary metabolides in the methanolic extract was carried out by Mayer's and Dragendroff's tests (alkaloids), Shinoda's test (flavonoids), Libermann-Burchard test (terpenoid/steroids) and foam test (saponins)[12,13].

2.3. Animals

The male albino mice of (20-30 g) were used throughout the experimentation. After randomization into various groups, animals were acclimatized for a period of 10 days under standard husbandry conditions *i.e.* room temperature (27±3) ℃, relative humidity (65±10)%, 12 h light/dark cycle. All the animals were fed with rodent-pellet diet (Gold Mohr, Lipton India Ltd.) and water was allowed *ad libitum* under strict hygienic conditions. Ethical clearance for performing experiments on animals was obtained from Institutional Animals Ethics Committee (Reg. No. 12/14/ac/08/CPCSEA).

2.4. Acute toxicity study (LD_{50})

An acute toxicity of MEAC was carried out in female albino mice (20-30 g) who maintained under standard conditions. The animals were fasted over night prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies[14].

2.5. Experimental protocol

Mice were randomly divided into four groups with five animals in each group. Group I received only vehicle (i.p.) and served as control; group II received standard antipressant drug *i.e.* imipramine (5 mg/kg i.p.); group III and IV received 50 and 100 mg/kg i.p. of MEAC, respectively.

2.6. Evaluation of antidepressant activity

2.6.1. Tail suspension test (TST)

The total duration of immobility by tail suspension was measured according to the method of Steru *et al*. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min test for animals of all groups[15,16].

2.6.2. Forced swimming test (FST)

The FST is the most widely used pharmacological *in vivo* model for assessing antidepressant activity. The development of immobility when the mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The cylindrical container (diameter 10 cm, height 25 cm), filled to a 19 cm depth with water at (25±1) ℃. The duration of immobility during 6 min test was scored as described previously. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water[16,17].

2.7. Statistical analysis

Results were expressed as mean ± SEM and the experimental groups were compared with control group. The statistical analysis was done using ANOVA followed by Turkey Karmmer Multiple Comparison Test. P values of 0.05, 0.01 and 0.001 were considered as statistically significant.

3. Results

3.1. Phytochemical evaluation

Preliminary phytochemical screening of extract revealed the presence of various bioactive components of which flavonoids, steroids and terpenoids were the most prominent.

3.2. Acute toxicity

The MEAC was studied for acute toxicity at a dose of 2 000 mg/kg i.p. in female albino mice. It caused the mortality in mice so dose was reduced to 300 mg/kg and no mortality was observed at this dose. Hence 500 mg/kg was considered as LD_{50} cut off value. So the doses selected for extract as per the OECD guidelines No. 420 (Annexure-2d) fixed dose method were 50 mg/kg (1/10th of 2 000 mg/kg) and 100 mg/kg (1/5th of 2 000 mg/kg).

3.3. Evaluation of antidepressant activity

In the present study, the immobility showed by the mice in TST and FST in a duration of 6-min was considered as a study parameter. Imipramine significantly decreased the immobility time in the TST and FST. MEAC (50 and 100 mg/kg) elicited the effect by reducing the immobility time. The obtained results were found significant and in dose dependent fashion. The results of TST and FST were represented in Table 1.

Table 1
Effect of MEAC on TST and FST in mice (mean±SEM) (n=6).

Groups	Mean immobility (sec)		Reduction in immobility (%)	
	TST	FST	TST	FST
Group I	181.20±8.24	106.80±11.64	–	–
Group II	83.50±9.57***	62.50±9.24***	53.91	41.47
Group III	132.00±11.46**	87.00±12.72**	27.15	18.54
Group IV	124.00±5.64***	76.00±7.46***	31.57	24.84

** P<0.01, *** P <0.001 as compared with control.

4. Discussion

Herbal medicines derived from plant extract are being increasingly utilized to a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing various plants used in Indian traditional system of medicine. Despite the widely popular use of *A. calamus* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In the present study the methanolic extract of *A. calamus* was evaluated for the antidepressant activity in which the TST and FST in mice were employed. These tests are quite sensitive and relatively specific to all major classes of antidepressant including tricyclics 5-HT reuptake inhibitors and MAO inhibitors[18]. Immobility when rodents are suspended by their tail during TST and when they are placed in an inescapable cylinder of water during FST reflects the cessation of their persistent escape-directed behavior. Conventional drugs reliably decrease the duration of immobility in animals during these tests. This decrease in duration of immobility is considered to be a good predictive value in the evaluation of potential antidepressant agents[19]. The successive 7 days treatment of extract produces the significant antidepressant effect in both model when compared with vehicle treated control. The efficacies of the extract were found to be comparable to the standard TCA-imipramine. An interesting finding of the present study is that, the anti-immobility effect of MEAC in TST and FST exhibited a significant and dose dependent effect. The flavonoid apigenin[20], which selectively binds with high affinity to the central benzodiazepines receptor, possesses important anxiolytic[21,22] and antidepressant activities[23]. The literature reveals that the main constituents of *A. calamus*are are monoterpenes, sesquiterpenes, phenlypropanoids, flavonoids, and quinine[9]. Hence MEAC showed antidepressant activity probably through interaction with adrenergic, dopaminergic serotonergic and GABA nergic system. Our study demonstrated that, MEAC exerts an antidepressant-like effect employing TST and FST in mice. Hence, MEAC may be explored further for management of mental depression.

Conflict of interest statement

We declare that we have no conflict of interest.

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