“EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF ROOTS OF ACORUS CALAMUS IN ALBINO MICE”.

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ABSTRACT

Objectives: To evaluate the antidepressant activity of aqueous extract of Acorus calamus in mice. Materials and Methods: A total of 72 albino mice were included in the study. Six groups of six animals in each group were taken in each of the behavior despair models [forced swimming model (FSM) and tail suspension model (TSM)]. Three groups of mice received aqueous extract of Acorus calamus (at doses 100, 150, 200 mg/kg body weight), two groups received standard drugs (imipramine 15 mg/kg and fluoxetine 10 mg/kg) and control group received normal saline. Antidepressant activity (i.e., Immobility time) was assessed after 30 minutes of administration of drugs intraperitoneally. Data was analyzed by ANOVA statistical test (with p<0.05) followed by Tukey’s Post Hoc Analysis. Results expressed as Mean ± SEM.

Results: In FSM, test drug groups (100 mg 78.8±15.3 s, 150 mg 88.5±19.3 s, 200 mg 94.7±16.6 s) and both the standard drug groups (imipramine 91.8±8.4 s, fluoxetine 84.8±14.4 s) showed significant reduction in immobility time when compared to control group (141.3±5.6 s) (p<0.05). The immobility time of the test drug groups and standard drug groups were comparable to each other. In TSM, only imipramine group (109.0±10.6 s) and test drug 200 mg/kg group (104.7±5.7 s) showed statistically significant reduction (p<0.05) in immobility time when compared to control group (144.0±9.3 s). However, the other two test drug groups and fluoxetine group did not show a significant difference when compared to control and also with each other. Conclusion: The aqueous extract of roots of Acorus calamus has shown antidepressant activity when tested in FSM & TSM.
KEYWORDS: Depression, Acorus calamus, Imipramine, Fluoxetine, Forced swim model and Tail suspension model.

INTRODUCTION
Major depression is one of the common psychiatric disorders affecting people worldwide. Major depression is characterized by sad mood, loss of interest and pleasure, suicidal thoughts.[1] It causes significant morbidity, affecting people’s working capacity, function in relationships and social interaction.[2] It results from a combination of multiple etiological factors like genetic, biochemical, psychodynamic, and socio environmental. It also leads to serious social and educational impairments, substance misuse and obesity.[3] It is often undiagnosed and even more frequently, it is treated inadequately.[4] Overdetection and underdetection are important factors that should be considered to ensure the appropriate diagnosis and management of clinical depression.[5]

There are different modalities of treatment for depression which includes Behavioral therapy, Pharmacotherapy and Electro convulsive therapy. However Pharmacotherapy predominates over other modalities. Various drugs are available in the market for the treatment of depression. They include Tri cyclic antidepressants, MAO inhibitors, Selective serotonin re-uptake inhibitors and other atypical antidepressants. These drugs primarily act by normalizing the levels of neurotransmitters, notably serotonin and nor-epinephrine. Nearly two-thirds of the depressed patients respond to the currently available treatments but still the magnitude of improvements is still unsatisfactory.[6] Even though many anti-depressant medicines are available in the market, the currently available drugs have got side effects associated with their use like sedation, insomnia, sexual dysfunction and anti-cholinergic side effects etc. Plants are of enormous medicinal importance and have been extensively studied for their use against depression.

Herbal drugs can be quite acceptable as these drugs are believed to cause less adverse effects.[7] Acorus calamus, also called as sweet flag (English) is indigenous to South East Asia. Leaves and roots have been in ethno medicine for several medicinal properties: antispasmodic, aphrodisiac,[8] nervous ailments, dyspepsia,[9] anti-inflammatory and antioxidant[10] properties. Previous studies have shown that Methanol extract of roots of Acorus Calamus has anti-depressant activity.[11] In present study an attempt has been made to evaluate anti-depressant activity of aqueous extract of Acorus calamus.
MATERIALS AND METHODS

STUDY DESIGN: The design of this experimental study was comparative and parallel group. A total of 72 animals were used in the study. The animals were divided into six groups (n=6, in each group) in each of the behavior despair model.

ANIMALS: A total of 72 Albino mice of either sex weighing 20g – 35g from our breeding stock were used in this study. The animals were maintained at 24 ± 2 °C with 12:12 h light and dark cycle. There was free access to food and water. The animals was acclimatized for a period of 7 days before the study. Experiments was carried out between 09:00 and 11:00 hr. This study was conducted in the department of Pharmacology, JJM Medical College, Davangere, Karnataka (India) after prior approval from Institutional Animal Ethics Committee (IAEC).


INCLUSION CRITERIA
1. Albino Mice of either Sex.
2. Age 3-4 months and weight 20g - 35g.
3. Healthy with normal behavior and activity.

EXCLUSION CRITERIA
1. Mice <20g and >35g and age <3 months and >4 months.
2. Animals previously used in other experiments in the past 4 weeks.
3. Pregnant Mice.

The antidepressant activity on the experimental animal (albino mice) was assessed by two behavior despair models i.e. Forced swimming model and Tail suspension model. The antidepressant activity (i.e. Immobility time, in seconds) was assessed after 30 min of administration of drugs intraperitoneally.

GROUPING OF ANIMALS IN EACH OF THE MODELS
Group 1: Received 10 ml/kg normal saline (control group).
Group 2: Received 15 mg/kg Imipramine (standard 1)\(^{[12]}\)
Group 3: Received 10 mg/kg Fluoxetine (standard 2)\(^{[13]}\)
Group 4: Received 100 mg/kg aqueous extract of Acorus Calamus (test 1)
**Group 5:** Received 150 mg/kg aqueous extract of Acorus Calamus (test 2)

**Group 6:** Received 200 mg/kg aqueous extract of Acorus Calamus (test 3)

**FORCED SWIMMING MODEL**
In this model, the experimental animals, albino mice were forced to swim in water containing plastic cylinder for a period of 6 minutes. The water was maintained at room temperature and the depth of it was 20 cm and the cylinder containing water measured 30 x 30 cm. During the 6 minutes test, the animal exhibits vigorous activity for initial 2 minutes after which it assumes a typical immobile posture. The mouse was considered immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water.

The total duration of immobility is recorded during next 4 minutes of total 6 minute test. The changes in immobility duration was studied before and 30 min after administering drugs in separate group of animals. This model to test for antidepressant activity was originally developed by Porsolt et al. Following swimming sessions, the animals were dried with towel and placed in a cylinder heated under 60 W bulb for 15 minutes before returning to home cages.

**TAIL SUSPENSION MODEL**
In this model, animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals will try to escape by making vigorous movements but when unable to escape become immobile. The animal is considered immobile when it does not show any movement of body and hangs passively.

The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility will be recorded during last 4 minutes of total 6 minute test. The changes in immobility duration will be studied before and 30 min after administering drugs in separate group of animals. The method is similar to that described by Steru et al.
STATISTICAL ANALYSIS
All the values are presented as Mean ± Standard Error of Mean (SEM). The comparison between the groups were done by analysis of variance (ANOVA) followed by post hoc Tukey’s test in case of significant results. For all the tests a ‘p’ value of less than 0.05 was considered to be statistically significant.

RESULTS
FORCED SWIMMING MODEL
There was a significant reduction in immobility time in both the standard drug groups Imipramine (91.8±8.4 s) and Fluoxetine (84.8±14.4 s) when compared to the control group (141.3±5.6 s) (p<0.05). All the three doses of test drug groups (100 mg/kg=78.8±15.3 s; 150 mg/kg=88.5±19.3 s; 200 mg/kg=94.7±16.6 s) also showed significant reduction in immobility time when compared to the control group. The immobility time of all the test drug groups and both the standard drug groups did not significantly differ from each other [Table 1 and Figure 1].

TAIL SUSPENSION MODEL
There was a significant reduction in immobility time in only one of the two standard drugs group Imipramine (109.0±10.6 s) and one of the three test drug group 200 mg/kg (104.7±5.7 s) when compared to control group (144.0±9.3 s) (p<0.05). However, the other two test drug groups (100 mg/kg=132±6.4 s; 150 mg/kg=118.8±7.1 s) and fluoxetine group (124.7±9.2 s) did not show a significant difference when compared to control and also with each other [Table 2 and figure 2].

TABLE 1: Forced Swimming Model (FSM).

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Drug</th>
<th>Immobility time (in Sec) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>141.3 ± 5.7</td>
</tr>
<tr>
<td>2</td>
<td>Std 1 (Imipramine 15 mg/kg)</td>
<td>91.8 ± 8.4*</td>
</tr>
<tr>
<td>3</td>
<td>Std 2 (Fluoxetine 10 mg/kg)</td>
<td>84.8 ± 14.4*</td>
</tr>
<tr>
<td>4</td>
<td>Test1 (Acorus Calamus 100 mg/kg)</td>
<td>78.8± 15.3*</td>
</tr>
<tr>
<td>5</td>
<td>Test2 (Acorus Calamus 150 mg/kg)</td>
<td>88.5 ± 19.3*</td>
</tr>
<tr>
<td>6</td>
<td>Test3 (Acorus Calamus 200 mg/kg)</td>
<td>94.7 ± 16.6*</td>
</tr>
</tbody>
</table>

* p< 0.05 , significant.
FIGURE 1: Immobility time in Forced Swimming Model

TABLE 2: Tail Suspension (TSM) Model.

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Drug</th>
<th>Immobility time (in Sec) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
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<tr>
<td>3</td>
<td>Std 2 (Fluoxetine 10 mg/kg)</td>
<td>124.7 ± 9.2</td>
</tr>
<tr>
<td>4</td>
<td>Test1 (Acorus Calamus 100 mg/kg)</td>
<td>132.0 ± 6.4</td>
</tr>
<tr>
<td>5</td>
<td>Test2 (Acorus Calamus 150 mg/kg)</td>
<td>118.8 ± 7.1</td>
</tr>
<tr>
<td>6</td>
<td>Test3 (Acorus Calamus 200 mg/kg)</td>
<td>104.7 ± 5.7*</td>
</tr>
</tbody>
</table>

* p <0.05, significant.

FIGURE 2. Immobility time in Tail Suspension Model
DISCUSSION

In the present study, we have evaluated the antidepressant activity of aqueous extract of acorus calamus rhizome in mice by two models for antidepressant activity i.e., forced swimming model (FSM) and tail suspension model (TSM). It is a behavioral test for screening the drugs or any plant material for its antidepressant like activity. When animals are subjected to unavoidable and unescapable stress such as FST and TSM, the rodent’s display of immobility is thought to reflect a state of despair or lowered mood, which reflects depressive illness in humans. It has been reported that the antidepressant drugs have the ability to reduce this immobility period in animal model.\[14\]

In our study, the immobility time was significantly reduced in both the standard drug groups in comparison with control group in FSM and only with Imipramine drug group in TSM. The study drug Acorus calamus has showed significant reduction in immobility time with all the three doses (100,150 & 200 mg/kg) with maximum reduction seen with 100mg dose when compared to control group in FSM but only with 200 mg/kg drug group in TSM. Various in vivo and in- vitro studies have shown that increased MAO activity as well as exposure to oxidative stress are associated with the pathogenesis of depression. Aqueous extract of acorus calamus is found to have various phytochemicals like phenols, flavonoids etc which have antioxidant properties and also are known to inhibit MAO activity. This may probably attribute to antidepressant activity of aqueous extract of acorus calamus.\[15, 16\] In our study, in TSM, Fluoxetine group and test groups in 150 & 200 mg dose did not show a significant reduction in immobility time. This may be because some strains of mice may be resistant to TSM.\[17\]

CONCLUSION

The aqueous extract of roots of Acorus calamus has shown antidepressant activity in our present study.

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