Evaluation of antidepressant activity of Acorus calamus L. rizhome extract in mice

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Abstract

Background

Acorus calamus (AC) L. (Araceae) is an annual semi-aquatic and aromatic plant found in Europe, North America and Asia. Its rhizomes are often used by Native Americans, Americans, and Chinese as well as by other cultures. Ethnobotanical studies and documents have shown their use in various disease treatments, such as insomnia, mental disorders, diabetes mellitus, epilepsy, inflammation, asthma, neuropathic pain, and diarrhea. In this study, the antidepressant activity of methanolic and hydroalcoholic extracts of the AC rhizome part in mice was investigated.

Materials and methods

Three doses of methanolic extract of AC rhizome (MEACR) (25, 50 and 100 mg/kg b.wt), three doses of hydroalcoholic extract of AC rhizome (HAACR) (100, 200 and 400 mg/kg b.wt), and standards (imipramine, 15 mg/kg b.wt and fluoxetine, 20 mg/kg b.wt) was daily oral administration to the mice for consecutive 14 days. The extract effect on the immobility time was monitored by a tail suspension test (TST) and a forced swimming test (FST). Monoamine oxidase (MAO) levels were also analyzed using standard methods.

Results

The optimum antidepressant activity was viewed at 100 mg/kg b.wt of MEACR extract and 400 mg/kg b.wt of HAACR extract with 23.82% and 20.59% immobility period reduction, respectively. Besides, the extracts weakened the FST-induced elevation of MAO activity significantly and returned to near-normal levels of neurotransmitters in the brain. 100 mg/kg b.wt or above of MEACR extract significantly prevented the MAO-A and MAO-B activities in mice brain at a dose-dependent fashion. But, just 400 mg/kg b.wt of HAACR extract prevented the activity of MAO-A and MAO-B. Fluoxetine and imipramine showed a tendency to prevent the activity of MAO-A and MAO-B.

Conclusion

This study suggests that AC rhizome extract mediated antidepressant activity by modulating the central neurochemical and hypothalamic-pituitary-adrenal (HPA) axis in response to FST and TST-induced stress. Therefore, AC rhizome extract can be used as a valuable plant supplement to treat depressive disorders.

Background

Depression is a serious condition as characterized by the World Health Organization (WHO) as one of the most disabling disorders in the world. The global burden of disease predicts that depression is the
second-leading origin of disability in 2020 [1]. Several mental disorders of depression including loss of interest, sadness, low appetite, feelings of guilt, disturbed sleep, low concentration, and others [2]. These mental disorders transform into chronic/recurrent, essentially disturbing a personal capacity to deal with daily life activities. Depression influences approximately up to 20% of the global community [3]. The serotonergic and neuroendocrinological dysfunctions to respond to chronic stresses can produce depressive illness [4]. Clinical studies showed that the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis can raise the level of cortisol by promoting the hyper-secretion of corticotrophin-releasing factor (CRF). Furthermore, the disturbance of neurotransmission can reduce the level of the brain neurotransmitter, supporting to the depression development in susceptible people. Antidepressant drugs are commonly the first treatment line for the problem that utilizes their effect by enhancing the monoamine neurotransmitter level such as 5-hydroxytryptamine (5-HT), dopamine (DA), and norepinephrine (NE). Due to the late offensive, weak reaction, and some disadvantages of existing drugs, new materials from the natural herbal medicines are frequently searched by society as a supplementary and alternative medicine.

Acorus calamus (AC) L. (Araceae) is an annual, semi-aquatic and smelling plant discovered in several regions of Europe, Asia, and North America [5]. It is six feet tall and aromatic plant with creeping rhizomes. The AC leaves are slender, long, and sword-shaped, growing alternately from the rhizomes. Its leaves are longitudinally fissured with nodes, quite vertically suppressed and spongy internally. Its flowers are small and aromatic with a pale green spadix and its fruits are three-celled fleshy capsules [6]. All plant parts contain volatile oil having calamine, terpenoids, calamenone, calamenol, camphene, pinene, eugenol, and asaronaldehyde. Acorafuran is a sesquiterpenoid found in AC oil [7]. The rhizomes are widely utilized by the Indians, Chinese, American and other people [8]. Its rhizomes are commonly used to treat many disorders including insanity, hysteria, insomnia, diarrhea, epilepsy, and asthma [9]. Besides, the AC plant is also extensively utilized in diabetes mellitus treatment in America and Indonesia as traditional folk medicine [10]. The AC alcoholic extract containing saponins played a role in hyperlipidemia treatment [11]. The AC rhizome ethanolic extract was used as the anti-ulcer agent [12]. An important phyto-compound in the AC plant, α-asarone, possessed anti-cancerogenic activity toward the human carcinoma cells [13]. Traditionally, it also has been used to cure asthma [14]. Most studies proved that the AC rhizome possessed the most central nervous system (CNS) depressant activities [15]. β-asarone from the AC oil inhibited the differentiation of adipocytes, having the possibility for the obesity treatment and other obesity-associated insulin resistance [16]. In the last, AC had the inflammation activity of vincristine-induced painful neuropathy and chronic constriction injury led to neuropathic pain in rats [17].

Herein, the antidepressant activity of methanolic and hydroalcoholic extracts of AC rhizome (MEACR and HAACR) was carried out to explore more about its use as herbal medicine on the mice. The methanolic and hydroalcoholic extracts of AC rhizome were utilized to compare their effects on the immobility period using a tail suspension test (TST) and forced swimming test (FST). Monoamine oxidase (MAO) levels were also analyzed using standard methods.
Materials And Methods

Collection and air drying of plant material

The whole AC plant was taken from the Lolab Valley area of Kupwara district, Jammu and Kashmir, India. It was verified by the curator in the Department of Taxonomy, University of Kashmir, under voucher specimen number of 2436-KASH Herbarium. A sample specimen of collected material was stored in the herbarium for future reference. The AC rhizome parts were dried under shade at 30˚C for 20 days.

Preparation of extracts

The powder of dried AC rhizome plant (200 g) was soaked in methanol (100%) and methanol: water (50%:50%) at room temperature for 3 days. Each day, the treated solvent being recovered and replaced with new fresh solvents were then pooled together [18]. Then, the AC extracts were concentrated using rotary evaporator. The dried AC extracts were stored in an airtight container in a refrigerator at 4˚C. The dried AC extracts were weighed and their percentage yield was 17.56% with methanol solvent and 11.50% with hydroalcoholic solvent.

Animal experimental design

The 45 male BALB/c mice (20–25 g) were employed. They were grouped into nine different groups (n = 5). The study was conducted for consecutive 14 days. These mice owned free access to food and water for their life under normative conditions. The mice were kept based on the instruction of the National Institute of Nutrition, India after being agreed by the Institutional Animal Ethics Committee. MEACR extract with the dose levels of 25, 50, and 100 mg/kg b.wt/day and HAACR with the dose levels of 100, 200, and 400 mg/kg b.wt/day were given to the mice groups as displayed in Table 1. Fluoxetine and imipramine were utilized as the standards for two groups. Mice in Group 1 received a vehicle only in a single dose once for 14 days as a control group. On the 14th day, 1 hour after dosing, the blood sample was collected and mice were then sacrificed directly after the behavioral test for various biochemical estimations. The data acquired from the behavioral paradigm and the biochemical evaluations were stated as Mean ± SEM values. The statistical analysis was carried out by using one-way ANOVA.
Table 1
The protocol of the drug and extract treatments for mice oral administration.

| Group | Treatment                                      | Dose       |
|-------|-----------------------------------------------|------------|
| 1     | Normal control-vehicle only, a single dose of 2% *Gum acacia* | 10 mg/kg b.wt |
| 2     | Imipramine with 200 mg *Gum acacia*           | 15 mg/kg b.wt |
| 3     | Fluoxetine with 200 mg *Gum acacia*           | 20 mg/kg b.wt |
| 4     | MEACR-25 with 10 mL olive oil                 | 25 mg/kg b.wt |
| 5     | MEACR-50 with 10 mL olive oil                 | 50 mg/kg b.wt |
| 6     | MEACR-100 with 10 mL olive oil                | 100 mg/kg b.wt |
| 7     | HAACR-100 with 200 mg *Gum acacia*           | 100 mg/kg b.wt |
| 8     | HAACR-200 with 200 mg *Gum acacia*           | 200 mg/kg b.wt |
| 9     | HAACR-400 with 200 mg *Gum acacia*           | 400 mg/kg b.wt |

**TST and FST testings**

The TST was performed based on Steru's test method with little modifications [19–20]. Every mouse was suspended on the table border with the assistance of adhesive tape located about 1 cm from the tail tip. The experiment was performed in an isolated room with less noise. The immobility period was monitored for 10 min of total duration. The mice were assumed immobile at the time when they suspended passively and quiet. Meanwhile, the FST was performed on the mice based on the Porsolt's test method [21–22]. Each mouse was induced to swim in a glass jar with a size of 25 × 12 × 25 cm³ containing water up to a 15 cm height for 10 min at 30°C. The immobility period was monitored during 6 min of 10 min in total duration. The immobility period was considered as the time spent by the mouse when it floated motionless in the water and stopped the struggle. It moved only if obligatory to maintain its head above the water surface.

**MAO-A and MAO-B level measurements**

The fraction of mice brain mitochondria was processed based on the previous procedure [23]. MAO activity was evaluated based on Yu's method [24]. The concentration of protein was calculated by the Bradford method [25]. The assay mixture included 100 µL of 4 mM 5-HT and 100 µL of 2 mM phenylethylamine (PEA) as the substrate for MAO-A and MAO-B mixing with 200 µL of mitochondrial fraction and 100 mM sodium phosphate buffer at pH 7.4 up to 1 mL for the final volume. The reaction was kept for 20 min at 37°C and 200 µL of 1 M HCl was added to discontinue the reaction process. 4 mL
of butylacetate and cyclohexane were used to extract the product for MAO-A and MAO-B, respectively. The upper layer of the organic phase was recorded at 280 nm and 242 nm wavelengths for MAO-A and MAO-B using a spectrophotometer, respectively.

Results

The effect of AC extracts on the TST testing

Both MEACR and HAACR extracts showed a dose-dependent decrease of the immobility period in mice during the TST testing as shown in Table 2. The result was also graphically represented in Fig. 2. 100 mg/kg b.w/day of MEACR extract exhibited a very significant reduction of immobility period (2.03 ± 0.20 mint/sec) when it was administered to the mice in Group 6, compared to 25 mg/kg b.w/day and 50 mg/kg b.w/day of MEACR extracts. Furthermore, 400 mg/kg b.w/day of HAACR extract exhibited a high significant reduction of immobility period (1.52 ± 0.22 mint/sec) when it was administered to the mice in Group 9 for 14 days, compared to 100 mg/kg b.w/day and 200 mg/kg b.w/day of HAACR extracts.
Table 2
The effects of MEACR and HAACR extracts on the TST.

| Group        | Mouse No. | Mean   | Stdev | SEM  | Mean ± SEM       |
|--------------|-----------|--------|-------|------|------------------|
| 1 (control)  |           | 4.45   | 3.50  | 3.44 | 4.20             |
| 2 (imipramine) | 1.25 | 2.55   | 1.47  | 2.03 | 2.48             |
| 3 (fluoxetine) | 1.11 | 2.15   | 1.06  | 0.54 | 0.41             |
| 4 (MEACR25)  | 3.25      | 3.11   | 4.15  | 4.05 | 4.12             |
| 5 (MEACR50)  | 3.19      | 2.48   | 4.32  | 3.56 | 3.21             |
| 6 (MEACR100) | 2.03      | 1.58   | 2.45  | 2.03 | 1.24             |
| 7 (HAACR100) | 3.20      | 3.18   | 2.22  | 4.25 | 2.31             |
| 8 (HAACR200) | 1.16      | 2.19   | 2.12  | 1.36 | 3.25             |
| 9 (HAACR400) | 1.29      | 1.47   | 1.52  | 2.08 | 2.49             |

Percent inhibition expressed as mean ± SEM. p < 0.0001, considered as extremely significant.

The effect of AC extracts on the FST testing

The MEACR and HAACR extracts showed a dose-dependent decrease of immobility period (6 min) in mice after being administered the extracts for 14 days during the FST testing as represented in Table 3 and Fig. 3, compared with the mice in Group 1 (control) which was received vehicle for 14 days. 100 mg/kg b.w/day of MEACR extract exhibited a very significant reduction in the immobility period (1.35 ± 0.26 min/sec) when it was administered to the mice in Group 6, compared with the mice in Group 4 and Group 5. Also, 400 mg/kg b.w/day of HAACR extract administered to the mice in Group 9 showed a highly
significant decrease in immobility period \((1.14 \pm 0.26 \text{ mint/sec})\), compared to the mice in Group 1 \((4.20 \pm 0.17 \text{ mint/sec})\).

### Table 3
The effects of MEACR and HAACR extracts of the AC rhizome part on FST.

| Group       | No. of mouse | Mean ± SEM | Stdev | SEM | Mean ± SEM |
|-------------|--------------|------------|-------|-----|------------|
| 1 (control) | 1            | 4.20       | 0.39  | 0.17| 4.20 ± 0.17 |
|             | 2            | 1.44 ± 0.06|
| 2 (imipramine) | 3     | 1.20 ± 0.04|
|             | 4            | 3.36 ± 0.19|
| 3 (fluoxetine) | 5       | 1.35 ± 0.26|
| 4 (MEACR25) | 6            | 2.36 ± 0.03|
| 5 (MEACR50) | 7            | 1.57 ± 0.19|
| 6 (MEACR100)| 8            | 1.14 ± 0.26|

Percent inhibition expressed as mean ± SEM. \(p < 0.0001\), considered as extremely significant.

### Measurement of MAO-A levels

The MEACR and HAACR extracts showed dose-dependent decrease in monoamine oxidase-A (MAO-A) in the mice brain homogenates as shown in Table 4 and graphically represented in Fig. 4. 400 mg/kg b.w/day of HAACR extract administered to the mice in Group 9 showed a very significant decrease in MAO-A level \((0.28 \pm 0.01 \mu g/mL)\). Also, 100 mg/kg b.w/day of MEACR extract administered to the mice in
Group 6 showed a highly significant decrease in MAO-A level (0.26 ± 0.01 µg/mL) compared to the mice in Group 1 (0.77 ± 0.02 µg/mL) and Group 3 (0.30 ± 0.02 µg/mL).

### Table 4

The effects of MEACR and HAACR extracts of the AC rhizome part on the MAO-A level.

| Group | No. of mouse | Mean ± SEM | Stdev | SEM |  |
|-------|--------------|------------|-------|-----|-----|
| 1 (control) | 1 | 0.77 ± 0.02 | 0.05 | 0.02 | 0.77 ± 0.02 |
| 2 (imipramine) | 2 | 0.38 ± 0.03 | 0.07 | 0.03 | 0.38 ± 0.03 |
| 3 (fluoxetine) | 3 | 0.30 ± 0.02 | 0.05 | 0.02 | 0.30 ± 0.02 |
| 4 (MEACR25) | 4 | 0.39 ± 0.02 | 0.05 | 0.02 | 0.39 ± 0.02 |
| 5 (MEACR50) | 5 | 0.31 ± 0.01 | 0.03 | 0.01 | 0.31 ± 0.01 |
| 6 (MEACR100) | 6 | 0.26 ± 0.01 | 0.01 | 0.01 | 0.26 ± 0.01 |
| 7 (HAACR100) | 7 | 0.54 ± 0.01 | 0.03 | 0.01 | 0.54 ± 0.01 |
| 8 (HAACR200) | 8 | 0.51 ± 0.03 | 0.08 | 0.03 | 0.51 ± 0.03 |
| 9 (HAACR400) | 9 | 0.28 ± 0.01 | 0.03 | 0.01 | 0.28 ± 0.01 |

Percent inhibition expressed as mean ± SEM. p < 0.0001, considered as extremely significant.

### Measurement of MAO-B levels

Both MEACR and HAACR extracts showed a less dose-dependent decrease in monoamine oxidase-B (MAO-B) than MAO-A as represented in Table 5 and graphically represented in Fig. 5. No such effect was seen on MAO-B except for 400 mg/kg b.w/day of HAACR extract and 50 mg/kg b.w/day or 100 mg/kg
b.w/day of MEACR extract. The mice in Group 6 exhibited a significant reduction in the MAO-B level compared to the control and standard groups.

| Group No. | No. of mouse | Mean | Stdev | SEM | Mean ± SEM |
|-----------|--------------|------|-------|-----|------------|
| 1 (control) | 1 (control) | 0.89 | 0.86 | 0.89 | 0.86 ± 0.02 |
| 2 (imipramine) | 2 (imipramine) | 0.42 | 0.44 | 0.45 | 0.43 ± 0.01 |
| 3 (fluoxetine) | 3 (fluoxetine) | 0.39 | 0.38 | 0.39 | 0.38 ± 0.01 |
| 4 (MEACR25) | 4 (MEACR25) | 0.55 | 0.57 | 0.49 | 0.54 ± 0.01 |
| 5 (MEACR50) | 5 (MEACR50) | 0.46 | 0.42 | 0.39 | 0.42 ± 0.01 |
| 6 (MEACR100) | 6 (MEACR100) | 0.40 | 0.37 | 0.34 | 0.34 ± 0.02 |
| 7 (HAACR100) | 7 (HAACR100) | 0.59 | 0.59 | 0.58 | 0.59 ± 0.02 |
| 8 (HAACR200) | 8 (HAACR200) | 0.54 | 0.53 | 0.51 | 0.53 ± 0.01 |
| 9 (HAACR400) | 9 (HAACR400) | 0.48 | 0.45 | 0.45 | 0.45 ± 0.01 |

Percent inhibition stated as mean ± SEM. p < 0.0001, considered as extremely significant.

Table 5
The effects of MEACR and HAACR extracts on the MAO-B level

**Discussion**

Herein, the antidepressant activity of AC rhizome extracts was measured in mice on TST and FST testings. TST and FST are the behavioral tests for evaluating the medicines or herbals for their antidepressant activity [26]. The immobility of mice is reflected in a state of despair or lowered mood,
describing to the depression in humans [27]. The mice have surrendered the expectation of running away from the limited area. It has been informed that the antidepressant medicines can reduce the immobility period in the animal model [28]. The MEACR extract and HAACR extract administered to the mice at medium and higher doses could degrade significantly the immobility period based on the FST and TST testings compared to the stress control in U-shaped dose-dependent manner that has been declared for some herbal medicines. These results suggest that MEACR and HAACR extracts may own good antidepressant activity only in medium and higher doses.

Furthermore, MAO-A is a prominent metabolism enzyme of a large range of monoamine neurotransmitters, including 5-HT, NE, and DA. There are significant reports representing that MAO is a potential object for the healing of anxiety and depression [16]. The abnormally enhanced MAO-A activity can effect in lowered levels of monoamine neurotransmitters, leading to depressive illness [29]. MAO-A is a highly significant enzyme in the metabolism of the primary neurotransmitter monoamines compared to the MAO-B [30]. Then, the inhibitors of MAO-A have been approved to medicate the depression. It was informed that the FST stress significantly increases the MAO-A and MAO-B activities which decrease the neurotransmitter levels in the mice brain [31]. The neurotransmitters like 5-HT and NE are specifically decreased by MAO-A, while DA is decreased by both MAO-A and MAO-B [32]. The AC rhizome extracts with dose-dependent decreased the MAO-A activity in the brain homogenates of mice. Thus, the presence of neurotransmitter levels is organized by MAO activities and looks to present a significant part in various psychiatric and neurological disorders.

This study exhibited the reduction of enhanced activities of MAO-A and MAO-B stress of mice. This tendency is in harmony with the previous report [33]. The bioactive compounds presenting in the MEACR and HAACR extracts at medium and higher doses might cause a reduction in the elevated MAO-A level while MAO-B was prevented significantly just at a higher dose of the extracts. The insignificant effect was viewed at the lower doses. In the FST testings, the brain neurotransmitter levels have been informed as a major element in interceding the immobility period reduction [34]. The catabolic rate of neurotransmitters can be analyzed by evaluating the native transmitters and their metabolites as an outcome of MAO activities [35]. This comparison is an indicator of the catabolic rate of neurotransmitters. The data provided in the present study proved that the FST stress grossly degraded the neurotransmitter levels. FST testing also pointed out a trend towards enhancement in the ratio of 5-HT/HIAA. A highly significant enhancement in the MAO-A levels of the mice in Group 1 (control) (0.77 ± 0.02 µg/mL) administered with a vehicle, compared to the mice in Group 3 (0.30 ± 0.02 µg/mL) which were received 20 mg/kg b.wt/day of fluoxetine. As fluoxetine increases the 5-HT levels being an SSRI drug, whereas the MAO-A acts on the substrate of 5-HTP during the depression [36]. Oral administration of the HAACR-400, MEACR-25, MEACR-50, and MEACR-100 extracts significantly prevented MAO-A activity in a dose-dependent fashion, giving 50%, 70.25%, 70.75%, and 80% inhibition, respectively. The estimation of a decrease in MAO-A levels after FST testing in mice brain confirms the potential of AC rhizome extracts at different dose levels as antidepressant-like activity comparable to those of the standard compounds, such as fluoxetine. Moreover, as fluoxetine increases the 5-HTP levels being an SSRI drug, whereas the MAO-B act on the substrate of PEA levels during the depression [37]. Fluoxetine has less effect on MAO-B, after
Pargyline, clorgyline, iproniazid, fluoxetine [38]. No significant inhibition was exhibited to prevent MAO-B activity in a dose-dependent fashion, just the MEACR-100 extract showed the highest inhibition. Estimation of a decrease in MAO-B levels after FST testing in mice brain confirms the antidepressant-like activity potential of AC rhizome extracts.

**Conclusion**

In this study, both MEACR and HAACR extracts revealed dose-dependent antidepressant activity in mice, and the higher dose of both extracts was found to have the highest effect in depression. The extracts of HAACR and MEACR significantly inhibited the activity of MAO-A *in vivo* in the mice brain in a dose-dependent manner. But, just 400 mg/kg b.wt of HAACR extract and 50 mg/kg b.wt or 100 mg/kg b.wt of MEACR extracts inhibited MAO-B activity. These results pointed out that the antidepressant activity of AC rhizome extracts in mice of immobility testings may be correlated to the inhibition of MAO activity, particularly to MAO-A activity. The oral administration of MEACR and HAACR extracts to the mice had antidepressant activity, possibly by regulating the central neurochemical axis and HPA, in response to FST-induced stress. Consequently, this work recommends the application of AC rhizome extracts as a meaningful botanical supplement for medicating the depression. In the coming study, the elaborate study is required to entirely clarify the action mechanisms of the bioactive compounds presenting in the AC rhizome extracts at the cellular level.

**Abbreviations**

AC: Acorus calamus; MEACR: methanolic extract of AC rhizome; HAACR: hydroalcoholic extract of AC rhizome; TST: tail suspension test; FST: forced swimming test; MAO: Monoamine oxidase; HPA: hypothalamic-pituitary-adrenal; WHO: World Health Organization; CRF: corticotrophin-releasing factor; HT: hydroxytryptamine; DA: dopamine; NE: norepinephrine; CNS: central nervous system.

**Declarations**

**Availability of data and materials**

The datasets used and/or analyzed during the current study would be available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate:**
Ethical letter (Sar/Kup/27) obtained from the office of the Sarpanch Block Ramhall, District Kupwara, Jammu and Kashmir, India

**Competing interests**

The authors declare that they have no competing interests.

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**authors contributions**

SY – Conceptualization, resources, formal analysis, investigation, writing-original draft

SMH– Methodology, project administration, and formal analysis

AR – Conceptualization, writing – review and editing

MZ – Formal analysis, writing – review and editing

MMH – Writing, review and editing

GGH – Writing, review and editing

MM – review and editing

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**Figures**

**Figure 1**

The AC plant and its rizhome

The AC plant and its rizhome
Figure 2

The effects of MEACR and HAACR extracts on the TST
Figure 3

The effects of MEACR and HAACR extracts of AC rhizome part on FST
Figure 4

Effects of MEACR and HAACR extracts of the AC rhizome part on the MAO-A level
Figure 5

The effects of MEACR and HAACR extracts on the MAO-B level

| Condition   | MEACR25 | MEACR50 | MEACR100 | HAACR100 | HAACR200 | HAACR400 |
|-------------|---------|---------|-----------|-----------|----------|----------|
| Control     | 0.86    | 0.43    | 0.38      | 0.54      | 0.42     | 0.34     |
| Imipramine  |         |         |           |           |          |          |
| Fluoxetine  |         |         |           |           |          |          |
| MEACR25     |         |         |           |           |          |          |
| MEACR50     |         |         |           |           |          |          |
| MEACR100    |         |         |           |           |          |          |
| HAACR100    |         |         |           |           |          |          |
| HAACR200    |         |         |           |           |          |          |
| HAACR400    |         |         |           |           |          |          |

**Mean**

| Control     | 0.86    |
| Imipramine  | 0.43    |
| Fluoxetine  | 0.38    |
| MEACR25     | 0.54    |
| MEACR50     | 0.42    |
| MEACR100    | 0.34    |
| HAACR100    | 0.59    |
| HAACR200    | 0.53    |
| HAACR400    | 0.45    |
**Figure 6**

Acorus calamus L.
Rhizome Extract

**In vivo antidepressant activity**

- TST
- MAO
- FST

**MEACR (100 mg/kg b.wt)**
**HAACR (400 mg/kg b.wt)**

Significant Reduction in Immobility Period and MAO Levels