

Study of Antidepressant Activity of Acorus Calamus Linn. In Mice.

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Date of Submission: 20-06-2021

Date of Acceptance: 03-07-2021

ABSTRACT

The present study was aimed to evaluate the anti-depressant properties of Acorus calamus rhizome in a forced swimming test (FST) and Tail Suspension Test of mice models. Three doses of Methanol extract of rhizome (MEAC) (25, 50 and 100mg extract/kg b.wt) and three doses of Hydroalcoholic extract of rhizome (HAAC) (100, 200 and 400mg extract/kg b.wt) and Imipramine (15 mg/kg b.wt), Fluoxetine (20mg/kg b.wt) as positive controls were orally administered once a day for the consecutive period of 14 days in Balb mice. The effect of extract on immobility period was measured using forced swimming test and Tail Suspension test. The levels of monoamine oxidase were analyzed using standard methods. The anti-depressant effect was observed maximum at the dose of 100 mg/kg b.wt of MEAC and 400mg/kg b.wt of HAAC that caused 23.82% and 20.59% reduction in immobility period respectively. The extract also significantly attenuated the FST-induced elevation of monoamine oxidase activity and returned the altered levels of neurotransmitters near to the normal levels in brain. The MEAC at the dose of 100 mg/kg or above for 14 days, significantly inhibited the monoamine oxidase (MAO) A & B activity in mice whole brain at a dose-dependent manner, however, oral administration of the HAAC extract only at a dose of 400 mg/kg produced observable MAO A & B inhibitory activity in animal brain. Fluoxetine and imipramine showed tendency to inhibit MAO A and B activity in animal brain in the study. These results of the present study suggest that the extract of A. calamus rhizome has antidepressant-like activity which is mediated by modulating the central neurochemical as well as HPA (hypothalamic-pituitary-adrenal) axis in response to stress induced by FST and TST. Therefore, A. calamus rhizome may be used as a valuable herbal supplement for the treatment of depression related condition.

Keywords, Forced Swimming test, Tail Suspension Test, Mice, Acorus calamus. 2-p

I. INTRODUCTION

Depression is a serious condition, unfortunately a common one. The World Health Organization characterizes depression as one of the most disabling disorders in the world, affecting roughly one in five women and one in ten men at some point in their lifetime (NIMH, 2012). Depression is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, poor concentration etc (Anisman, et al., 1982). These problems can become chronic or recurrent, substantially impairing an individual's ability to cope with daily life. Depressive illness affects nearly 10-20% of the population worldwide (Chuang et al., 2011). The dysfunctions of the serotonergic and neuroendocrinological systems in response to chronic stresses are one of the triggers that provoke the depressive illness (Xu et al., 2008). Clinical studies have shown that the hyperactivity of the HPA axis (hypothalamic-pituitary-adrenal) chronically elevates the cortisol level by stimulating the hyper-secretion of corticotrophin-releasing factor (CRF). Moreover, the impaired neurotransmission decreases the level of neurotransmitters in the brain, contributing to the development of depression in susceptible individuals. Pharmaceutical antidepressants are generally the first line of treatment for depression that exerts their effect by increasing the levels of monoamine (5-hydroxytryptamine (5-HT), norepinephrine (NE), and dopamine (DA)). Due to the slow-onset, low response and several side effects of currently available drugs, newer natural substances from the medicinal and herbal sources are often sought by people as a complementary and alternative remedy to their pharmaceutical medications.

Acorus calamus (L.) is a perennial, semiaquatic and smelly plant found in the northern temperate and subtropical regions of Asia, North America, and Europe. It is six feet tall, aromatic herb with creeping rhizomes. The leaves are long, slender, sword-shaped and simple, arising alternately from the horizontal rhizomes. These are longitudinally fissured with nodes, somewhat vertically compressed and spongy internally. Flowers are small and fragrant with pale green spadix, fruits are three-celled fleshy capsule (Nadkarni, 2007). All parts of the plant contain volatile oil having terpenoids, calamine, calamenol, calamenone, eugenol, camphene, pinene and asaronaldehyde. Acorafuran is a sesquiterpenoid found in calamus oil (Tkachev, 2006). The rhizomes are utilized extensively by the Chinese, Indians and Americans as well as by other cultures (Pandy, 2009). Its roots and rhizomes are used in treatment of various ailments including mental disorders, such as hysteria, insanity, insomnia, epilepsy, diarrhoea and asthma (Mukherjee, 2007). The plant is a native of eastern countries and indigenous to the marshes of the mountains of India. It is cultivated throughout India in the marshy tracts of Kashmir, Shirmour (Himachal Pradesh), Manipur, and in Nagahills and in the Koratageretaluka of Karnataka state in peninsular India. The second fortnight of June is the best time for planting. It is hardly found to grow in tropical and subtropical climates. The harvesting/propagation period starts in the month of December, where the lower leaves turn yellow and dry indicating their maturity. *Acorus calamus* is widely used in the treatment of diabetes in the traditional folk medicine of America and Indonesia (M et al., 2010). The alcoholic extract of *A. Calamus* contains saponins which play a role in hyperlipidemia. The ethanolic extract of *acorus* rhizome is used as the antiulcer agent (Raja et al., 2009). α -asarone, an important phytoconstituent of the plant has been found to possess anticarcinogenic activity against the human carcinoma cells (Neumeister et al., 2001). Traditionally it has been used in asthma (Nalamwari et al., 2009). Most studies have proven that the roots and rhizome of the plant possess the most CNS depressant activities and antidiarrheal (Sigmaaldrich.com). β -asarone which is isolated from the calamus oil has been found to inhibit the differentiation of adipocytes possess the potential for the treatment of obesity and other obesity-associated insulin resistance (Lee et al., 2011). *Acorus calamus* has shown the

inflammatory activity in the tested rat model of vincristine induced painful neuropathy and chronic constriction injury induced neuropathic pain in rats (Muthuraman et al., 2015).

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or multiple exposures in a short period of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the toxinogen. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a toxinogen over a longer time period (months or years). It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g., factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, in vitro testing methods and inference from data on similar substances (Walum, 1998).

Photochemical compounds from various plants are good sources of antioxidants and free radical scavengers. Medicinal plants especially those rich in various secondary metabolites including but not limited to polyphenols and flavonoids are capable of eliminating free radicals. Both in-vitro and in-vivo experiments have shown the ability of these plant derived compounds in neutralizing free radicals. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Buettner, 1993).

II. MATERIALS AND METHODS

2.1.1 Collection and air drying of plant material

The whole plant of *Acorus calamus* was collected from the Lolab forest area of district Kupwara, Jammu & Kashmir, India where they occur widely. It was authenticated by the curator, department of Taxonomy, University of Kashmir, Srinagar under voucher specimen No. 2436-KASH Herbarium, University of Kashmir, 1/07/2016. A sample specimen of collected

material was deposited in herbarium for future reference. The rhizome parts were allowed to dry under shade (30°C) for 10- 20 days.

2.1.2 Preparation of extracts

After collection, the roots were cut off from the plant and dried in shade for 20 days. The dried powder material (200 g) of the root of *Acorus calamus* was soaked in each methanol (100%) and methanol: water (50:50) %, at room temperature. The dried root powder was soaked in a particular solvent for 3 days, each day the treated solvent being recovered and replaced with fresh solvents were then pooled together (Gupta et al., 2007). The extract was concentrated using rotatory flash evaporator. The dried extract was stored in airtight container in refrigerator below 10°C. The extracts obtained were weighed and their percentage yield was 17.56% of methanol and 11.50% of hydro-alcoholic.

2.1.3 Experimental Design

For the animal experiment, male Balb/c mice weighing about 20-25g were used. A total of 45 mice were employed in the present study. They were divided into nine different groups (n=5) and the experimental study was conducted for a period of 14 days. These mice had free access to laboratory feed and tap water under standard laboratory conditions. The animals used in the present study were maintained in accordance with the guidelines of National Institute of Nutrition, India and approved by Institutional Animal Ethics Committee (IAEC). All the groups were administered with different extracts except mice of Group I, (received vehicle only) in a single dose-only once for 14 days. Methanolic extract (MEAC) of the rhizomes of *Acorus calamus* at three dose levels of 25, 50, 100 mg/kg/day were given to other three groups of mice's respectively as per the following protocol (A 3). Fluoxetine and Imipramine were used as standards given to next two groups. Hydroalcoholic extract (HEAC) of *Acorus calamus* at doses of 100, 200, 400 mg/kg was given to remaining three groups. On 14th day 60 minutes after dosing the blood sample was collected and mice were then sacrificed immediately after the behavioural test for various biochemical estimations.

2.1.3 (a) Tail suspension test:

The tail suspension test was based on the method of Steru with little modifications (Set al., 2005; Steru et al., 1985) Mouse was individually suspended on the edge of A, 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.

2.1.3 (b) Forced swim test:

The studies were carried out on mice according to the method of Porsolt (Porsolt RD et al., 1977) Mouse was individually forced to swim in a glass jar (25×12×25 cm³) containing fresh water up to a height of 15 cm at (30C) for 10 min. The duration of immobility was measured during the final 6 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.

2.1.4 Statistical analysis

The data obtained from the behavioural paradigm and the biochemical evaluations was expressed as MEAN ± SEM for each group. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Student's 't' test.

III. RESULTS

3.1.1 (a) TAIL SUSPENSION TEST

Both MEAC and HAAC extracts of the rhizome parts of *Acorus Calamus* has showed dose-dependent decrease in Immobility period in mice during Tail suspension test, in A.2 and graphically represented in B.1 At the dose of 100mg/kg b.w/day, of MEAC extract when administered to Group VI showed a very highly significant decrease in immobility period (2.03±0.20mint/sec) compared to the dose 25,50 mg/kg b.wt of MEAC. Also at the dose of 400mg/kg b.w/day of HAAC extract of the rhizome parts of *Acorus Calamus* administered to Group IX for 14 days showed a highly-significant decrease in immobility period (1.52±0.22mint/sec), compared to the dose 100,200 mg/kg b.w of HAAC.

3.1.2 (b) FORCED SWIM TEST

The extracts of the rhizome parts of *Acorus calamus* (MEAC & HAAC) has showed dose-dependent decrease in Immobility period (6 minutes) in mice after administered extracts for 14-days during Force swim test, represented in A .3 and B.2 which was compared with the control

Group I which received vehicle for 14 days. **100mg/kg b.w/day**, of **MEAC** extract of rhizome when administered to Group VI showed a very highly significant decrease in immobility period (**1.35±0.26mint/sec**) compared to the Group IV & Group V Also dose of **400mg/kg b.w/day**, of **HAAC** extract administered to Group IX showed a highly-significant decrease in immobility period (**1.14±0.26 mint/sec**), when compared to the Group I (**4.2±0.17 mint/sec**).

3.2.1 (a) MEASUREMENT OF MAO-A LEVELS.

Extracts of the rhizome parts of *Acorus calamus* (**MEAC & HAAC**) has showed dose-dependent decrease in **Mano amine oxidase-A (MAO-A)** in **A 4** and graphically represented through **B.3** in the brain homogenates of mice. **400mg/kg b.w/day**, of **HAAC** extract of *Acorus calamus* administered to Group VI showed a highly-significant decrease in Mano

amine oxidase-A levels (**0.27±0.01µg/ml**), Also the dose of **100mg/kg b.w/day**, of **MEAC** extract of rhizome parts when administered to Group IX showed a very highly significant decrease in Mano amine oxidase-A level (**0.26±0.0µg/ml**) compared to the Group I (**0.76±0.02µg/ml**) Group II (**0.29±0.02 µg/ml**).

3.2 .2 (b) MEASUREMENT OF MAO-B LEVELS.

Both extracts (**MEAC and HAAC**) of the rhizome parts of *Acorus calamus* has showed less dose-dependent decrease on Mano amine oxidase-B (**MAO-B**) as compared to (**MAO-A**) in **A.5** and graphically by **B.4** No such effect was seen on **MAO-B** except the dose of **400mg/kg b.w/day**, of **HAAC** and **100mg/kg b.w/day**, of **MEAC** extract of the rhizome parts of *Acorus calamus* administered to Group VI showed a significant decrease in Mano amine oxidase-B levels when compared to the control and standard group.

A 1: Treatment Schedule		
GROUP	TREATMENT	DOSE
I	Normal Control-Vehicle only Daily single dose of 2% Acacia	10mg/kg
II	Methanolic extract of <i>Acorus calamus</i> 25mg with 10ml olive oil(MEAC-25))	25mg/kg
III	Methanolic extract of <i>Acorus calamus</i> 50mg with 10ml olive oil(MEAC -50))	50mg/kg
IV	Methanolic extract of <i>Acorus calamus</i> 100mg with 10ml olive oil(MEAC-100)	100mg/kg
V	Standard: Fluoxetine 20mg with 200mg Gum acacia(Std1)	20mg/kg
VI	Standard: Imipramine 15mg/kg with 200mg Gum acacia (Std2)	15mg/kg
VII	Hydroalcoholic extract of <i>Acorus calamus</i> 100mg/kg with 200mg Gum acacia(HAAC-100)	100mg/kg
VIII	Hydroalcoholic extract of <i>Acorus calamus</i> 200mg/kg with 200mg Gum acacia(HAAC-200)	200mg/kg
IX	Hydroalcoholic extract of <i>Acorus calamus</i> 400mg/kg with 200mg Gum acacia(HAAC-400)	400mg/kg:

A.2: Effects of Methanolic (MEAC) and Hydroalcoholic (HAAC) extracts of the Rhizome parts of Acorus Calamuson TAIL SUSPENSION TEST

S.No	Group(i)	Group(ii)	Group(iii)	Group(iv)	Group(v)	Group(vi)	Group(vii)	Group(viii)	Group(ix)
Mice	Control	Imipramine	Fluoxetine	MEAC25	MEC50	MEAC100	HAAC100	HAAC200	HAAC400
1	4.45	1.25	1.11	3.25	3.19	2.03	3.2	1.16	1.29
2	3.5	2.55	2.15	3.11	2.48	1.58	3.18	2.19	1.47
3	3.44	1.47	1.06	4.15	4.32	2.45	2.22	2.12	1.52
4	4.2	2.03	0.54	4.05	3.56	2.03	4.25	1.36	2.08
5	4.17	2.48	0.41	4.12	3.21	1.24	2.31	3.25	2.49
Mean	4.17	2.03	1.06	4.05	3.21	2.03	3.18	2.12	1.52
Standard deviation	0.453729	0.584705	0.686171	0.511253	0.668409	0.465972	0.823754	0.825669	0.49935
SEM	0.202914	0.261488	0.306865	0.228639	0.298921	0.208389	0.368394	0.369251	0.223316
Mean±SEM	4.17±0.20	2.03±0.26	1.06±0.30	4.05±0.22	3.21±0.29	2.03±0.20	3.18±0.36	2.12±0.36	1.52±0.22

Each value represents the mean. N=5, *Percent inhibition expressed as mean± SEM Experimental group<0.0001, considered extremely significant

A.3: Effects of Methanolic (MEAC) and Hydroalcoholic (HAAC) extracts of the Rhizome parts of Acorus Calamus on FORCE SWIM TEST.

S.No	Group(i)	Group(ii)	Group(iii)	Group(iv)	Group(v)	Group(vi)	Group(vii)	Group(viii)	Group(ix)
MICE	Control	Imipramine	Fluoxetine	MEAC25	MEC50	MEAC100	HAA C100	HAA C200	HAA C400
1	4.45	1.25	1.11	4.25	1.55	1.11	2.25	2.1	1.17
2	4.25	1.5	1.1	3.33	2.11	1.56	2.45	1.32	1.45
3	3.44	1.44	1.27	3.21	1.15	1.35	2.33	1.17	0.3
4	4.2	1.3	1.2	3.54	2.27	1.45	2.4	2.05	1.14
5	4.17	1.55	1.25	3.36	1.46	0.11	2.36	1.57	0.12
Mean	4.2	1.44	1.2	3.36	1.55	1.35	2.36	1.57	1.14
Standard deviation	0.385837	0.128725	0.078294	0.415175	0.467782	0.586413	0.075299	0.420678	0.587563

SEM	0.17255 1	0.05756 7	0.03501 4	0.1856 72	0.20919 8	0.2622 52	0.033 675	0.188 133	0.262 766
Mean +SEM	4.2±0.17	1.44±0.0 5	1.2±0.03	3.36±0 .18	1.55±0.2 0	1.35±0 .26	2.36± 0.03	1.57± 0.18	1.14± 0.26

Each value represents the mean. N=5, *Percent inhibition expressed as mean± SEM Experimental group.p<0.0001, considered extremely significant.

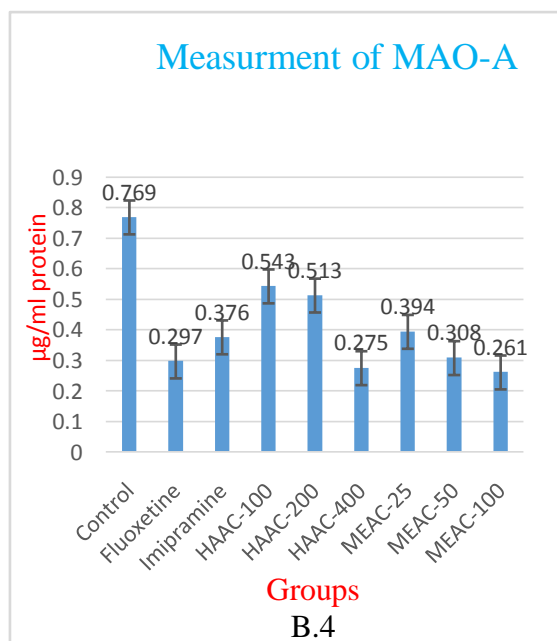
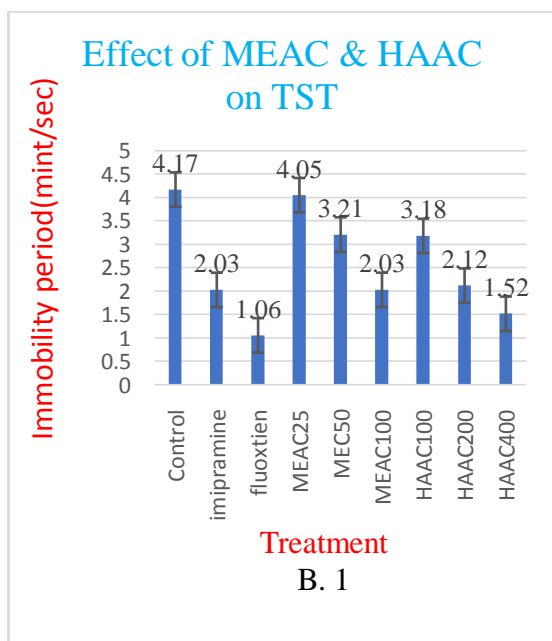
A.4: Effects of Methanolic (MEAC) and Hydroalcoholic (HAAC) extracts of the Rhizome parts of Acorus Calamus MAO-A level									
S.N O	Group(i)	Group(ii)	Group(ii)	Group (iv)	Group(v)	Group (vi)	Group (vii)	Group(viii)	Group(ix)
Mice	Control	Fluoxetine	Imipramine	HAAC 100	HAAC200	HAAC 400	MEAC-25	MEAC-50	MEAC 100
1	0.769	0.245	0.348	0.564	0.513	0.275	0.394	0.305	0.261
2	0.846	0.297	0.395	0.552	0.518	0.281	0.425	0.312	0.274
3	0.728	0.288	0.487	0.543	0.457	0.277	0.319	0.239	0.28
4	0.817	0.378	0.376	0.492	0.398	0.241	0.421	0.308	0.261
5	0.746	0.349	0.298	0.517	0.606	0.209	0.332	0.322	0.247
Mean	0.769	0.297	0.376	0.543	0.513	0.275	0.394	0.308	0.261
Standard deviation	0.04924	0.05247 2	0.06970 4	0.0289 7	0.07741	0.0310 6	0.049 777	0.03316 2	0.0128 57
SEM	0.02202	0.02346 6	0.03117 3	0.0129 5	0.03461 9	0.0138 9	0.022 261	0.01483	0.0057 5
Mean+SEM	0.76±00 2	0.29±0. 02	0.37±0. 02	0.54±0 .1	0.51±0. 03	0.27±0 .1	0.32± 0.0	0.30±0. 01	0.26±0 .0

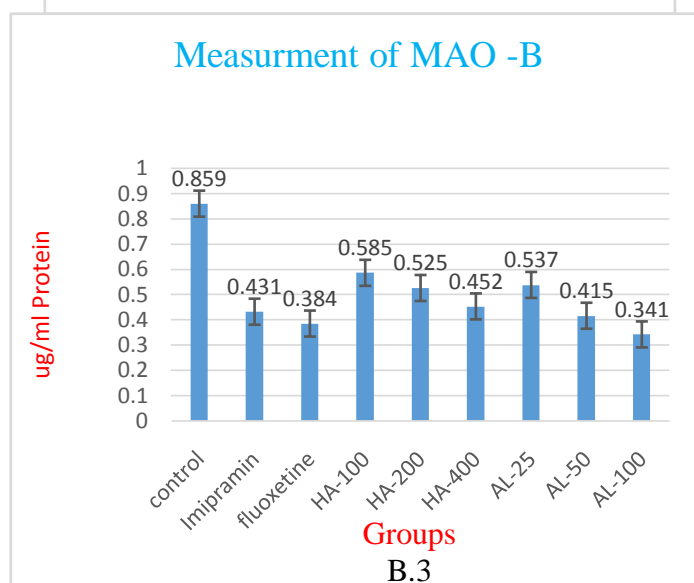
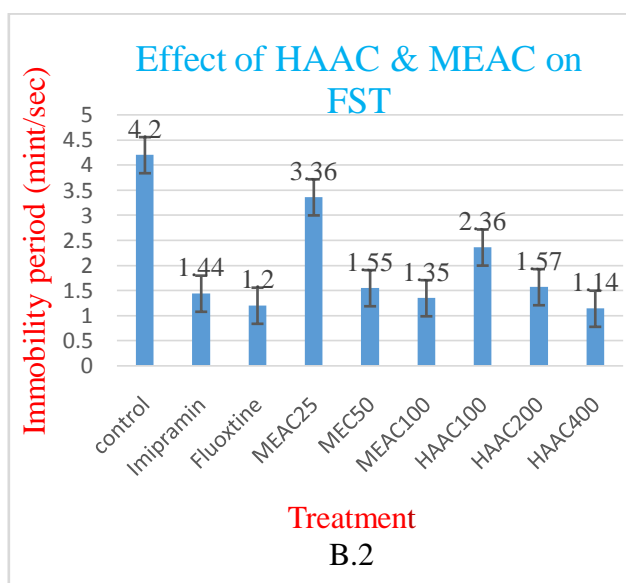
Each value represents the mean. N=5, *Percent inhibition expressed as mean± SEM Experimental group.p<0.0001, considered extremely significant.

A.5: Effects of Methanolic (MEAC) and Hydroalcoholic (HAAC) extracts of the Rhizome parts of Acorus Calamus MAO-B level.									
S.N O	Group(i)	Group(ii)	Group(iii)	Group(iv)	Group(v)	Group (vi)	Group(vii)	Group(viii)	Group (ix)
Mice	Control	Imipramine	Fluoxetine	HHAC 100	HAAC-200	HAAC-400	MEAC-25	MEAC-50	MEAC-100
1	0.887	0.416	0.394	0.589	0.541	0.476	0.548	0.455	0.398
2	0.859	0.442	0.375	0.592	0.525	0.452	0.568	0.415	0.365

3	0.892	0.454	0.388	0.576	0.514	0.448	0.496	0.394	0.341
4	0.797	0.431	0.335	0.585	0.539	0.392	0.492	0.428	0.269
5	0.858	0.427	0.384	0.497	0.495	0.468	0.537	0.408	0.319
Mean	0.859	0.431	0.384	0.585	0.525	0.452	0.537	0.415	0.341
Standard deviation	0.037806	0.014543	0.02351	0.040034	0.019032	0.032912	0.033169	0.023098	0.048629
SEM	0.016907	0.006504	0.010514	0.017904	0.008511	0.014719	0.014834	0.01033	0.021748
Mean±SEM	0.85±0.01	0.43±0.00	0.38±0.01	0.58±0.01	0.52±0.00	0.45±0.01	0.53±0.01	0.41±0.01	0.34±0.02

Each value represents the mean. N=5, *Percent inhibition expressed as mean± SEM Experimental group.p<0.0001, considered extremely significant





IV. DISCUSSION

In the present study, we have evaluated the antidepressant activity of *A. calamus* rhizome (MEAC & HAAC) in mice by Tail suspension test and forced swimming test. It is a behavioural test for screening the drugs or the plant material for its antidepressant like effect. When subjected to unavoidable stress such as FST, the rodents' display of immobility is thought to reflect a state of despair or lowered mood, which reflects depressive illness in humans. It is also assumed that the animals have given up the hope of escaping from the restricted area. It has been reported that the antidepressant drugs have the ability to reduce this immobility period in animal model. The present

study also showed that the MEAC administered for 14 days could significantly reduce the immobility time in FST & TST at the medium and higher doses used compared to stress control in U-shaped dose-dependent fashion and such an activity curve has also been reported for several herbal medicines. These results suggest that MEAC & HAAC may have an anti-depressant effect only in certain dose range.

As the extracts of the rhizome parts of *Acorus Calamus* has showed dose-dependent decrease in **Mano amine oxidase-A (MAO-A)** in **A 4** and graphically represented through **B.3** in the brain homogenates of mice. It has been reported that the stress of FST significantly elevate the brain

activities of MAO- A and B in mice. Neurotransmitters such as serotonin and noradrenaline are preferentially degraded by MAO-A, while dopamine is degraded equally by both the species of MAO. Thus, the availability of neurotransmitters level is regulated by the MAO enzyme activities and appears to play important role in several neurological and psychiatric disorders. The stress of FST exposure increases activities of MAO which consequently decrease the neurotransmitters level in brains of mice. The present study also shows that there is an increase in activities of MAO-A and MAO-B stress mice. This trend is in good agreement with the earlier published reports (Chen et al., 2005). The bioactive compounds present in the MEAC & HAAC might have caused a marked reduction in the elevated level of MAO-A by medium and higher doses used whereas MAO-B was inhibited significantly only by higher dose. The effect was observed to be insignificant at the lower dose. There are substantial reports indicating that MAO is a potential target for the treatment of depression and anxiety (Lee et al., 2011). In swim stress experiments, the neurotransmitter levels in the brain have been reported as a key factor in mediating the reduction of immobility period. The rate of catabolism of neurotransmitters can be analysed by measuring the original transmitters and their metabolites as a consequence of MAO activities. This ratio is an index for the catabolic rate of neurotransmitters. The data presented in this study demonstrated that the swim stress markedly reduced the levels of neurotransmitters. FST test also indicated a tendency toward an increase in 5-HT/HIAA ratio A very highly significant increase in the Monoamine oxidase-A levels of **Control Group I (0.76±0.02µg/ml)** administered with vehicle, when compared to the Group II (**0.29±0.02 µg/ml**) which receive 20mg/kg/day of Fluoxetine. As fluoxetine increases the 5HTP levels being **SSRI** drug, whereas the MAO-A act on the substrate **5-Hydroxy Tryptamine (5HTP)** during depression, Oral administration of the extract at the doses of (HAAC-400, and MEAC-25,50,100)mg/kg significantly inhibited MAO A activity in a dose-dependent manner, providing 50% of HAAC and 70.25, 70.75 and 80% inhibition of MEAC extract of *Acorus Calamus*. Estimation of decrease in Monoamine oxidase-A levels after forced swim test in mice brain confirms the antidepressant potential of *Acorus calamus* at different dose levels.

The current study affirms the **antidepressant potential** of crude extracts of

rhizomes of *Acorus Calamus*, with results comparable to those of the standard compounds **Fluoxetine**. As fluoxetine increases the 5HTP levels being SSRI drug, whereas the **MAO-B** act on substrate **Phenethylamine** levels during depression, Fluoxetine has least effect on MAO-B, after pargyline > clorgyline > ipronazide > fluoxetine. No significant inhibition was exhibited to inhibit MAO-B activity in dose dependent manner, only the methanolic extract shows inhibition was seen. Estimation of decrease in Monoamine oxidase-B levels after forced swim test in mice brain confirms the antidepressant potential of *Acorus calamus*.

V. CONCLUSION

Both the **Methanolic and Hydroalcoholic** extract of the rhizomal part of *Acorus calamus* has revealed a dose dependent **Antidepressant Potential** in mice and the higher dose of both the extracts was found to possess maximum effect in depression.

MAO is an important enzyme in the metabolism of a wide range of monoamine neurotransmitters, including noradrenaline, dopamine, and 5-hydroxytryptamine. MAO-A is more important than MAO-B in the metabolism of the major neurotransmitter monoamines. MAO-A inhibitors have been accepted to treat depression. In the present investigation, we have demonstrated that the Hydroalcoholic and Methanolic extract of *Acorus calamus* significantly inhibited in vivo MAO-A activity in mice whole brain in a dose-dependent manner, however, only the extract at a dose up to 400mg/kg of HAAC and 50,100mg/kg of MEAC exhibited to have the MAO-B inhibitory activity. These findings suggested that antidepressant effects of *Acorus Calamus* in animal models of immobility tests may be related to the inhibitory activity of MAO especially to that of MAO A. Taken together, our results clearly demonstrate that the oral administration of methanol extract of rhizome of *A. calamus* possesses an antidepressant-like activity, probably by modulating the central neurochemical as well as HPA axis in response to stress induced by FST. Therefore, our findings suggest the use of *A. calamus* rhizome as a valuable botanical supplement for treating depression related conditions. Further, detailed investigations are needed to fully elucidate the mechanism of action at cellular level for the bioactive constituents present in the extract.

ACKNOWLEDGEMENT

The authors would like to thank department of Pharmaceutical science university of Kashmir, India and Department of PK, PD Toxicology CSIR LAB-IIIIM Jammu, India.

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