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Effect of rhizome extract of *Acorus calamus* on depressive condition induced by forced swimming in mice

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Abstract

The present study evaluated the anti-depressant properties of *A. calamus* rhizome in a forced swimming test (FST) of mice model. Three doses of methanol extract of rhizome (200,400 and 600 mg extract/kg b.wt) and imipramine (15 mg/kg b.wt), a positive control, were orally administered once a day for the consecutive period of 14 days in Balb/c mice. The effect of extract on immobility period was measured using forced swimming test. The levels of cortisol monoamine oxidase and neurotransmitters were analyzed using standard methods. The anti-depressant effect was observed maximum at the dose of 200 mg/kg. b.wt that caused 23.82% reduction in immobility period. The extract also significantly attenuated the FST-induced elevation of plasma cortisol, monoamine oxidase activity and returned the altered levels of neurotransmitters near to the normal levels in brain. 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. Therefore, *A.calamus* rhizome may be used as a valuable herbal supplement for the treatment of depression related conditions.

Keywords: Forced swimming test; Cortisol; Neurotransmitters; Monoamine oxidase

Introduction

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 [1]. 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 [2]. The dysfunctions of the serotonergic and neuroendocrinological systems in response to chronic stresses are one of the triggers that provoke the depressive illness [3]. Clinical studies have shown that the hyperactivity of the HPA axis (hypothalamic-pituitary-adrenal) chronically elevates the cortisol level by stimulating the hypersecretion 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. The advantages of using herbal medicines are numerous, in particular that they are more effective for long-standing health complaints that do not respond well to traditional medicine. Plants such as *Ginko biloba, Psoralia corylifolia, Hypericum reflexum, Curcuma longa, Allium macrostemon, Asparagus racemosus* have been proved to have potential antidepressant-like properties[4-6]. Further, many new plant sources are also being screened and evaluated for better management of depression throughout the world.

Acorus. calamus (Calamus or Sweet flag), an ethnomedicinally important herbal plant, belongs to the family Acoraceae[7]. Various studies have shown that the rhizome and leaves of this plant posses a range of pharmacological effects such as hepatoproective[8], hypolipidemic[9], anti-inflammatory[10-11], radio-protective[21], anti-adipogenic activity[13], anti-diabetic[14] etc. There are also reports indicating the use of this plant in treating CNS abnormalities[15-16]. GC-MS analysis has shown that the volatile oil extracted from this plant contains β-asarone as the main active constituent among others[17]. The rhizome also contains other phytochemicals such as polyphenols, flavonoids, saponins, etc. Our earlier report demonstrated the protective effect of methanol extract of *A.calamus* rhizome against hepatotoxicity and oxidative stress in rats[18]. Here, we report for the protective effect of the rhizome extract with respect to its anti-depressive properties in mice.

Materials and methods

Plant material

The rhizomes of *A. calamus* were collected in the January month of year 2011 from a botanical garden in Mysore, India and the plant material was identified and authenticated by Dr.Sudharhan, botanist, Mysore University, Mysore. The plant material was shade dried and powdered to fine particles using a mixer. 200 g of powdered plant material was extracted with methanol using orbital shaker (1:10 ratio). Extract obtained was passed through Whatman filter paper No.1 and the methanol was flash evaporated and the extract stored at -20°C.

Experimental design

For the animal experiment, male Balb/c mice weighing about 20-25 g were used. 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). Experiments, performed by an observer who was unaware of the each treatment, were carried out between 1-3 p.m. For the behavioral test, different doses of the extract and a standard drug (Imipramine) were given by gastric gavage once daily over a period of 14 days. Behavioral test was conducted 1 hour after the last treatment as described below.

Forced swimming test (FST)

The procedure was same as that described by Porsolt et al[19]. 36 mice were randomly divided into 6 groups and treated as follow: Group I served as normal control and group II received Tween-80 suspensions. Group III-V were orally administered with various doses of *A.calamus* extract (200,400 and 600 mg extract/kg of body weight) and group VI received standard antidepressant drug-imipramine (15 mg/kg bd.wt). All the groups of mice were subjected to swimming test except group I. In a cylindrical glass aquarium (20 x 12 cm diameter), animals were allowed to swim for 6 min and the duration of immobility was recorded for the last 4 min using a video tracking system. The mouse was considered immobile whenever it stopped swimming and floating in the water, with its head just above the water level. Animals were sacrificed on the last day of the experiment and bled between 1-3 pm to avoid the variation on the hormonal levels due to circadian rhythms. Blood was collected and plasma was separated in a refrigerated centrifuge at 4°C.

Determination of neurotransmitter levels

Mouse brain monoamine neurotransmitters viz 5-HT, NA and DA levels were measured by HPLC coupled with electrochemical detection[20].The brain tissue were homogenized in 0.4 M perchloric acid containing 5 mM sodium bisuphite, 0.04 mM EDTA and then centrifuged at 15,000 rpm for 15 min at 4°C. 20 µl of the resulting supernatant was chromatographed on a C18 RP-18 column (150 mm x 4.6 mm, 5 µm). The separation was done in an isocratic elution mode at column temperature of 25°C using a mobile phase consisting of 17.6% methanol, 82.4% water containing $EDTA_2Na$ (0.0876 mM), triethylamine (1.5 mM), DL-camphorsulphonic acid (9 mM), disodium phosphate (20 mM) and citrate (9 mM) at a flow rate of 0.7 ml/min. The measurement was done at electropotentials of a glassy carbon electrode +650 mV versus Ag/AgCl reference electrode with Waters electrochemical detectors. Neurotransmitters were identified and quantified by comparing their retention times and peak areas with those of standard solutions.

Measurement of MAO-A and MAO-B activities

Mice brain mitochondrial fraction was prepared following the procedure of Schurr and Livne [21] . MAO activity was measured as per the method described by Yu et al., 2002[22]. Protein concentration was measured using kit from Agappe (Agappe Diagnostics Ltd, Kerala). The assay mixture contained 100 µl of 4 mM 5-hydroxy tryptamine and 100 µl of 2 mM β-PEA as the specific substrate for MAO-A and MAO-B plus 200 µl of mitochondrial fraction and sodium phosphate buffer (100 mM, pH 7.4) up to a final volume of 1 ml. The reaction was allowed to proceed for 20 min at 37°C and finally stopped by addition of 200 µl of 1M HCl. The reaction product was extracted with 4 ml of butylacetate and cyclohexane for MAO-A and B respectively. The upper organic phase was measured at 280 nm and 242 nm for MAO-A and MAO-B respectively using spectrophotometer.

Measurement of Cortisol level

The plasma cortisol level was estimated using EIA kit (Cayman chemical company, USA).

Locomotory activity test

For the measurement of locomotory activity naive mice were placed in an open field (60 x 30 x 30 cm) with black background. The apparatus was divided into equal areas by both parallel and perpendicular lines. In this experiment the animals received the same dose of extracts and standard as used for the forced swimming test. The experiment was recorded with a video camera and the motion activity (number of square crossed) was counted for 5 min.

Statistical analysis

Data are shown as mean ± standard deviation. Comparison between groups was analyzed by Tukey's honestly significant difference (HSD) method. The p values less than 0.05 were considered as significantly different.

Results

The effect of extract on the body weight change is presented in Table 1.

Groups	Body weight(g)	
	Day 1	Day 14
Group I (Vehicle control)	23.5 ± 2.6	30.5 ± 3.6
Group II (Stress vehicle)	22.4 ± 3.4	28.9 ± 2.6
Group III (AC, 100mg/kg)	25.4 ± 3.2	30.6 ± 2.8
Group IV (AC, 200 mg/kg)	21.8 ± 2.7	28.4 ± 3.2
Group V (AC, 400 mg/kg)	20.6 ± 3.5	27.4 ± 2.4
Group VI (Imipiramine)	24.2 ± 3.1	31.6 ± 2.8

Table 1. Effect of oral administration of methanolic extract of *A.calamus* rhizome on body weight gain of mice.

Data shows that there is no difference in body weight gain among all the groups after 14 days of oral administration. Figure 1

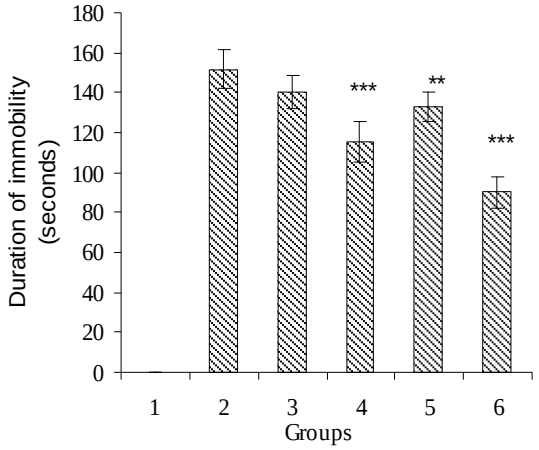

Figure 1.Effect of methanolic extract of *A.calamus* rhizome on immobility period of mice in FST. Group I (normal control), Group II (Stress control), Group III (AC, 100 mg/kg), Group IV (AC, 200 mg/kg), Group V (AC, 400mg/kg), Group VI (Imipiramine)

shows that the administration of extract significantly shortened the immobility time in FST at the dose of 200 and 400 mg/kg b.wt compared to stress control. The maximum antidepressant effect was obtained at 200 mg which resulted in 23.82% immobility reduction, while the 400 mg dose caused only 12.52% reduction. On the other hand, the extract at lower dose of 100 mg failed to alter the duration of immobility significantly. Overall, the effect was in an inverted U-shaped dose-dependant fashion. The standard antidepressant (imipiramine) produced a marked reduction in immobility time by 40.61%.

Swim stress procedure evoked a significant 1.8 fold increase (Figure 2)

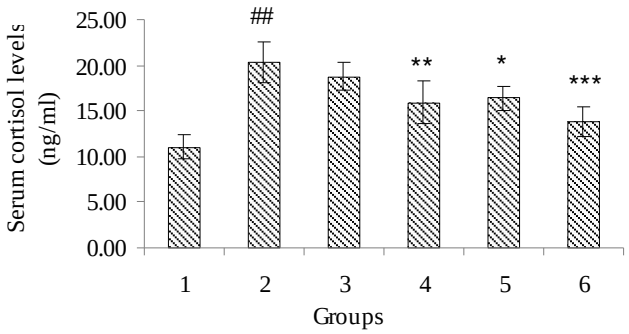

Figure 2.Effect of methanolic extract of *A.calamus* rhizome on serum cortisol level mice in FST. Group I (normal control), Group II (Stress control), Group III (AC, 100 mg/kg), Group IV (AC, 200 mg/kg), Group V (AC, 400 mg/kg), Group VI (Imipiramine)

in plasma cortisol level (20.32 ± 2.26 ng/ml) compared to unstressed control (11.05 ± 1.3). This effect was slightly decreased at 100 mg dose whereas doses at 200 and 400 mg produced a marked reduction to 15.92 ± 2.3 and 16.45 ± 2.3 ng/ml respectively that are comparable to the standard group value of 13.88 ± 1.6 ng/ml. Figure 3

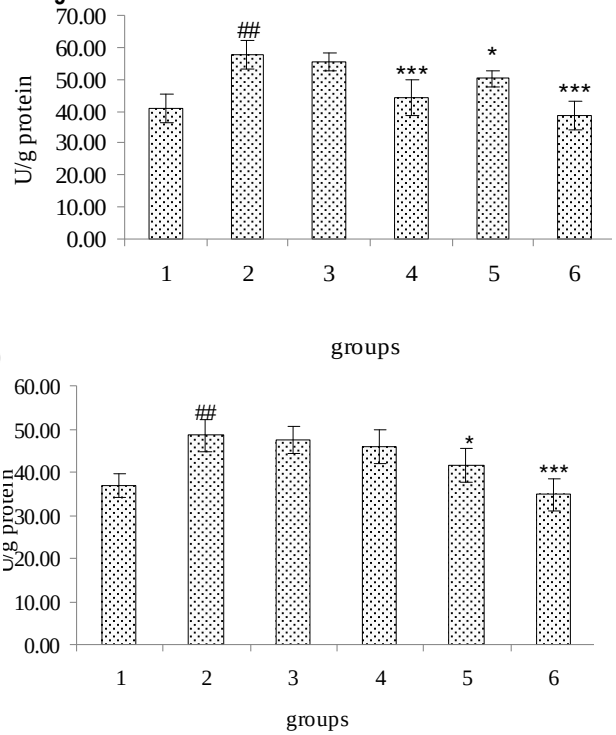

Figure 3.Effect of methanolic extract of *A.calamus* rhizome on moanoamine oxidase A (a) and B (b) activity in mice brain homogenate. Group I (normal control), Group II (Stress control), Group III (AC, 100 mg/kg), Group IV (AC, 200 mg/kg), Group V (AC, 400 mg/kg), Group VI (Imipiramine)

shows a significant ($p<0.001$) increase in activities of MAO-A (57.48 ± 4.5 U/g protein) and MAO-B (48.47 ± 3.87 U/g) in whole brain tissues of mice that is exposed to swim stress compared to unstressed control. The treatment with extract caused a marked reduction in the elevated level of MAO-A at 200 and 400 mg doses whereas MAO-B was inhibited significantly only by higher dose. The effect was observed to be insignificant at the lower dose. The standard drug (Imipiramine) could block the activities of both the MAO significantly.

The effect of *A.calamus* extract on whole brain neurotransmitter levels in unstressed and stressed mice is presented in Table 2.

Table 2.Effect of oral administration of methanolic extract of *A.calamus* rhizome on brain monoamine neurotransmitters level in FST exposed mice.

	Neurotransmitter levels(ng/g tissue)				
Groups	NA	DA	5-HT	5-HIAA	5-HIAA/ 5-HT
Normal control	311.7 ± 21.1	1043.8±65.3	854.9±50.8	247.1±16.8	0.290±.0.03
Stress control	214.1 ±24.1##	890.7±34.8##	594.4±37.1##	209.9±11.5#	0.354±0.01#
AC (100 mg)	220.3±20.2	935.1±47.2	641.4±50.4	222.3±10.1	0.348±0.02
AC (200 mg)	271.4±24.5**	978.6±65.5	752.1±37.7***	225.9±17.6	0.301±0.03*
AC (400 mg)	258.3±17.8*	988.1±38.2*	690.4±41.1**	218.4±22.7	0.317±0.03
Imipiramine	293.5±17.6***	1011.2±61.1*	784.7±28.9***	240.3±15.5*	0.307±0.02*

# $P<0.05$,## $P<0.001$ compared to normal control
*$P<0.05$,**$P<0.01$, ,**$P<0.001$ compared to stressed control

Swim stress markedly reduced the levels of noradrenaline, dopamine, 5-HT and 5-HIAA. The treatment with extract significantly increased the noradrenaline level in a U-shaped trend dose dependently and the 200 mg dose produced a maximum effect while 100 mg extract failed to do so. The decreased level of dopamine was only attenuated significantly at the higher dose of 400 mg whereas the other doses failed to increase the dopamine content. The dose dependant effect of extract on 5-HT level was similar to that of NA. FST test also indicated a tendency toward an increase in 5-HT/HIAA ratio (0.354 ± 0.01) compared to unswimmed control (0.290 ±.0.03). Pretreatment of mice with extract produced a pronounced effect by decreasing the ratio of 5-HT/HIAA (0.301 ± 0.03) only at 200 mg dose and was comparable to that of standard drug.

In a separate experiment, the oral administration of the extract at all the three doses for 14 days period did not produce any overt behavioral change or motor dysfunction compared to control as shown in Figure 4.

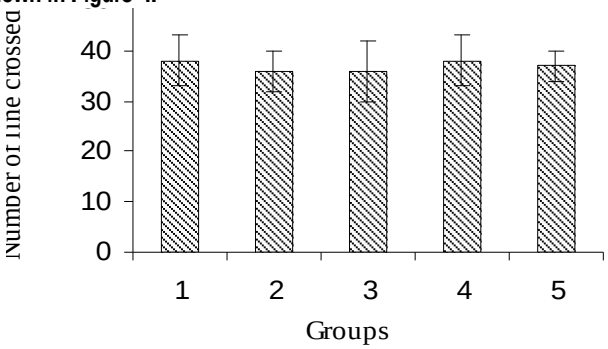

Figure 4.Effect of methanolic extract of *A.calamus* rhizome on locomotor activity of mice. Group I (normal control), Group II (Stress control), Group III (AC, 100 mg/kg), Group IV (AC, 200 mg/kg), Group V (AC, 400 mg/kg), Group VI (Imipiramine)

Discussion

In the present study, we have evaluated the antidepressant activity of methanol extract of *A.calamus* rhizome (MEA) in mice by forced swimming test. It is a behavioral 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 [23-24] . The present study also showed that the MEA administered for 14 days could significantly reduce the immobility time in FST at the medium and higher doses used compared to stress control in a U-shaped dose-dependant fashion and such an activity curve has also been reported for several herbal medicines like *Apocynum venetum*, St.Jhon's wort extract [25-26] albeit the reason for this response is obscure. These results suggest that MEA may have an anti-depressant effect only in certain dose range.

There are numerous reports also indicating that swimming stress potentially activates HPA axis system leading to the elevation of corticosterole release factor (CRF) and corticosterone levels in plasma. This increased level of biomarkers is an indicator of stress

response mechanism. The role of corticosterone has earlier been demonstrated in a study where the administration of corticosterone caused an increase in immobility period [27]. Our data also demonstrate that swim stress elevated the plasma cortisol level and this effect was maximally reduced at 200 mg dose used. It is clear from the present study that the possible explanation for the antidepressant effect seen following MEA treatment could involve preventing the activation of HPA axis and that is similar to the earlier reports.

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 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 swim stresses mice. This trend is in good agreement with the earlier published reports [28-29] . The bioactive compounds present in the MEA 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 [30].

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 compared to unswimmed control which is in concordance with the previously published reports[31] . The treatment with extract at the medium dose significantly increased the noradrenaline as well as level 5-HT level. This may be due to the inhibitory effect of the phytoconstituents present in the MEA extract as mentioned earlier on MAO activities that prevented the neurotransmitters turnover.

Psychostimulants such as caffeine or amphetamine and other drugs sometimes exert a false positive effect for anti-depression activity via increased locomotory activity. This effect can be excluded by carrying out an open-field test in parallel with FST as a check [32] . The results of the present study did not produce any overt behavioral change or motor dysfunction following MEA administration. Therefore, the reduction in immobility time as observed through the forced swimming test can be attributed to an inherent antidepressant-like effect of the extract and not due to stimulation of locomotor activity.

Earlier reports have investigated the attenuating effect of *A.calamus* rhizome oil and leaf extracts [33], and here, we have studied the polyphenols-rich methanol extract towards anti-depressive effects. Various phytochemicals such as polyphenolic acids, flavonoids, alkaloids, saponins, xanthones of several medicinal plants have been reported as antidepressants through the inhibition of MAO activity [34-38]. Recent reports have shown that polyphenols such as rutin, trans-resveratral, proanthocyanidin, naringenin, rosmarinic acid and caffeic acid have poptential antidepressive properties [39-42]. It is likely that the anti-depressant effect of the *A.calamus* could be attributed to the presence of polyphenols and other active constituents present in the extract.

Conclusion

Taken together, our results clearly demonstrates 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.

Acknowledgements

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References

[1]. Anisman, H, Zacharko RM. Depression. The predisposing influence of stress. *Behav Brain Sci* 1982; 5: 89-137.

[2]. Chuang C, Shi Y, You H, Lo Y, Pa T. Antidepressant effect of GABA-rich monascus-fermented product on forced swimming rat model. *J Agric Food Chem* 2011; 59: 3027-3034.

[3]. Xu Q, Pan Y, Li Y, Mo S, Jiang F, Qiao C, Xu H, Lu X, Kong L, Kung H. Anti-depressant-like effects of psoralen isolated from the seeds of *Psoralea corylifolia* in the mouse forced swimming test. *Biol Pharm Bull* 2008; 31: 1109-1114.

[4]. Sánchez-Mateo CC, Prado CXB, Rabanal RM. Antidepressant activity of some *Hypericum reflexum* L. fil extracts in the forced swimming test in mice. *J Ethnopharmacol* 2007; 112: 115-121.

[5]. Rojas P, Serrano-García N, Medina-Campos ON, Pedraza-Chaverri J, Ögren SO, Rojas C. Antidepressant-like effect of a *Ginkgo biloba* extract (EGb761) in the mouse forced swimming test: Role of oxidative stress. *Neurochem (International)* 2011; 59: 628-636.

[6]. Xia X, Cheng G, Pan Y, Xia ZH, Kong LD. Behavior, neurochemical and neuroendocrine effects of the ethanolic extract from *Curcuma langa* L in the mouse forced swimming test. *J Ethanopharmacol* 2007 ; 110: 356-363.

[7]. Yende SR, Harle UN, Rajgure DT, Tuse TA, Vyawahare NS. Pharmacological profile of *Acorus calamus*: An Overview. *Pharmacog Reviews* 2009; 2: 22-26.

[8]. Palani S, Raja S, Praveen Kuma R, Parameswaran P, Senthil Kumar B. Therapeutic efficacy of *Acorus calamus* on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. *Acta Pharmaceutica Sciencia* 2010; 52: 89-100.

[9]. Parab RS, Mengi SA. Hypolipidemic activity of *Acorus calamus* L. in rats. *Fitoterapia* 2002; 73: 451-455.

[10]. Muthuraman A, Singh N, Jaggi AS. Protective effect of *Acorus calamus* L. in rat model of vincristine induced painful neuropathy: An evidence of anti-inflammatory and anti-oxidative activity. *Food Chem Toxicol* 2011; 49: 2557-2563.

[11]. Kim H, Han T, Lee S. Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells. *J Ethnopharmacol* 2009; 122: 149-156.

[12]. Sandeep D, Nair CKK. Protection of DNA and membrane from γ-radiation induced damage by the extract of *Acorus calamus* Linn.: An *in vitro* study. *Enl Toxicol Pharmacol* 2010; 29: 302-307.

[13]. Lee M, Chen Y, Tsai J, Wang S, Watanabe T and Tsai Y. Inhibitory effect of β-asarone, a component of *Acorus calamus* essential oil, on inhibition of adipogenesis in 3T3-L1 cells. *Food Chem* 2011; 126: 1: 1-7.

[14]. Si M, Lou J, Zhou C, Shen J, Wu H, Yang B, He Q, Wu H. Insulin releasing and alpha-glucosidase inhibitory activity of ethyl acetate fraction of *Acorus calamus in vitro* and *in vivo*. *J Ethnopharmacol* 2010; 128: 154-159.

[15]. Vohora, SB, Shah, SA, and Dandiya, PC. Central nervous system studies on an ethanol extract of *Acorus calamus* rhizomes. *J Ethnopharmacol* 1990; 28: 53-62.

[16]. Shukla PK, Khanna V. Neuroptotective effect of *Acorus calamus* against middle cerebral artery occlusion-induced ischaemia in rats. *Human Exp Toxicol* 2006; 5: 187-194.

[17]. Mazza G. Gas chromatographic and mass spectrometric studies of the constituents of the rhizome of calamus : I. The volatile constituents of the essential oil. *J Chromatography A*, 1985; 328: 179-194.

[18]. Ilaiyaraja N, Farhath Khanum. Amelioration of alcohol induced hepatotoxicity and oxidative stress in rats by *Acorus calamus*. *J Diet Suppl* 2011; 8: 331-345.

[19]. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice. A primary screening test for antidepressant. *Arch Int Pharmacodyn Ther* 1977; 229: 327-336.

[20]. Alburges ME, Narang N, Wamsley JK. A sensitive and rapid HPLC-ECD method for the simultaneous analysis of norepinephrine, dopamine, serotonin and their primary metabolites in brain tissue. *Biomed Chromatogr* 1993; 7: 306-310.

[21]. Schurr A, Livne A. Different inhibition of mitochondrial monoamine oxidase from brain by hashish components. *Biochem Pharmacol* 1976; 25: 1201-1203.

[22]. Yu ZF, Kong LD, Chen Y. Antidepressant activity of aqueous extracts of *Curcuma longa*. *J Ethanopharmacol* 2002; 83: 161-165.

[23]. Lucki, I.The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* 1997; 8: 523-532.

[24]. Dhingra D, Sharma A. Evaluation of antidepressant-like activity of glycyrrhizin in mice. *Ind J Pharmacol* 2005; 37: 390-394.

[25]. Butterweck V, Nishibe S, Sasaki T, Uchida M. Antidepressant effects of *Apocynum ventum* leaves in a forced swimming test. *Biol Pharm Bull* 2001; 20: 848-851.

[26]. Nakazaw T, Yasuda T, Ueda J, Ohsawa K. Antidepressanat-like effects of apigenin and 2, 4, 5-trimethoxycinnamic acid from *Perilla frutescens* in the forced swimming test. *Biol Pharm Bull* 2003; 26: 474-480.

[27]. Hill MN, Brotto LA, Lee TT and Gorzalka B. Corticosterone attenuate the antidepressant-like effects elicited by melatonin in the forced swim test in both male and female rats. *Prog.Neuropsycopharmacol Biol Psychiatry* 2003; 27: 905-911.

[28]. Pan Y, Kong L, Xia X, Zhang W, Xia Z, Jiang F. Antideprssant-like effect of icariin and its possible mechanism in mice. *Pharmaco Biochem Behav* 2005; 82: 686-694.

[29]. Chen Y, Kong L, Xia X, Kung H, Zhang L. Behavioral and biochemical studies of total furocoumarins from seeds of *Psoralea corylifolia* in the forced swimming test in mice *J.Ethanopharmacol* 2005; 96: 451-459.

[30]. Leem SA, Homh SS, Han XX, Hwang JS, Oh GJ, Lee KS, Lee MK, Hwang BY and Ro JS. Piperine from the fruits of *Piper longum* with inhibitory effects on monoamine oxidase and antidepressant-like

activity. *Chem Pharm Bull* 2005; 53: 832-835.

[31]. Connor TJ, Kelliher P, Shen Y, Harkin A, Kelly JP, Leonard BE. Effect of subchronic antidepressant treatments on behavioral, neurochemical, and endocrine changes in forced-swimming test. *Pharmacol Biochem Behav* 2000; 65: 591-7.

[32]. Yi L, Li C, Zhan X, Cui C, Xiao F, Zhou L, Y Xie Y. Involvement of monoaminergic system in the antidepressant-like effect of the flavonoid naringenin in mice *Prog Neuropsychopharmacol Biol Psychiatry* 2010; 34: 1223-1228.

[33]. Pandy V, Subhash H. CNS activity of methanol and acetone extracts of *Acorus calamus* leaves. *J Pharmacol Toxicol* 2009; 4: 79-86.

[34]. Hwang JH, Hong SS, Han XH, Hwang JS, Lee D, Lee H, Yun YP, Kim Y, Ro,JS, Hwang BY. Prenylated xanthones from the root bark of *Cudrania tricuspidata*. *J Nat Prod* 2007; 70:1207-1209.

[35]. Lee SJ, Chung HY, Lee IK, Oh SU and Yoo ID. Phenolics with inhibitory activity on mouse brain monoamine oxidase (MAO) from whole parts of *Atemisia vulgaris* L (Mugwort). *Food Sci Biotechnol* 2000; 9: 179-182.

[36]. Jeong SH, HanXH, Hong SS, Hwang JS, Hwang JH, Lee D, Lee MK, Ro JS, Hwang BY. Monoamine oxidase inhibitory coumarins from the aerial parts of *Dictamnus albus*. *Arch Pharm Res* 2006; 29: 1119-1124.

[37]. Zhou Y, Shen Y, Zhang C, Su J, Liu R, Zhang W. Triterpene saponins from *Bacopa monnieri* and their antidepressant effects in two mince models. *J Nat Prod* 2007; 70:652-655.

[38]. Yun B, Lee I, Ryoo I, Yoo I. Coumarins with monoamine oxidase inhibitory activity and antioxidative coumarino-lignans from *Hibiscus syriacus*. *J Nat Prod* 2001; 64 : 1238-1240.

[39]. Xu Y, Li S, Chen R, Li G, Barish PA, You W, Chen L, Lin M, Ku B, Pan J, Ogle WO. Antidepressant-like effect of low molecular proanthocyanidin in mice: Involvement of monoaminergic.system. *Pharm Biochem Behav* 2010a; 94: 447-453.

[40]. Machado DG, Bettio, LEB, Cunha MP, Santos ARS, Pizzolatti MG, Brighente IMC, Rodrigues ALS. Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: Evidence for the involvement of the serotonergic and noradrenergic systems. *Eur J Pharmacol* 2008; 587: 1-3, 10: 163-168.

[41]. Xu Y, Wang Z, You W, Zhang X, Li S, Barish P, Vernon MM, Du X, Li G, Pan J, William WO. Antidepressant-like effect of *trans*-resveratrol: Involvement of serotonin and noradrenaline system. *Eur Neuropsychopharmacol* 2010b; 20: 405-413.

[42]. Takeda H, Tsuji M, Inazu M, Egashira T, Matsumiya T. Rosmarinic acid and caffeic acid produce antidepressive-like effect in the forced swimming test in mice. *Eur J Pharmacol* 2002; 449: 3, 261-267..