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Evaluation of Antidepressant Activity of Ethanolic Extract of *Alangium Salvifolium* Leaves in Swiss Albino Mice

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ABSTRACT

To evaluate the *in vivo* antidepressant activity of Ethanolic extract of *Alangium salvifolium* leaves (EASL) in Swiss albino mice. Ethanolic extract of *Alangium salvifolium* (EASL) leaveswas prepared by a continuous method using Soxhlet apparatus. The extract was subjected to phytochemical screening followed by acute oral toxicity studies in mice. EASL in the doses of 100 and 200 mg/kg body weight was administered to test groups 1 and 2 respectively. Imipramine hydrochloride 15mg/kg body weight was administered to Standard group by oral route. Test group 3 received 100mg/kg (p.o) of EASL + 10mg/kg (p.o) of Imipramine. Control group received Normal saline 10ml/kg body weight. Antidepressant activity was identified by using modified Forced Swimming Test (FST) and Tail Suspension Test (TST). Period of immobility was observed in both the models which was indicative of anti depressant activity. Standard statistical methods were used to evaluate the results. The results showed significant dose dependent antidepressant effect of EASL in Swiss albino mice for both the models in all the test groups (Test group I, II and III). EASL possess significant antidepressant activity. However, further investigations are required to determine its active constituents and molecular level of target mechanism of the extract for further use in humans.

Keywords: Antidepressant, *Alangium salvifolium,* Forced Swimming test, Tail suspension test.

INTRODUCTION

Depression is a type of serious neurological disorder, characterized by disturbances in sleep and appetite as well as deficit in cognition and energy[1]. Depression can be potentially life threatening condition that has affected millions of people across the globe and can occur at any age groups from childhood to later life. It is known to exert a huge burden upon the society. The distinctive symptoms exhibit as a triad forms that include: low or depressed mood, "***anhedonia***" (reduced ability to experience natural rewards), and low energy or fatigue[2].

The clinical course of Major Depression (MD: formerly unipolar depression) is categorised by one or more major depressive episodes that can be categorized as: without a history of manic, mixed, or hypo-manic episodes. Thoughts of guilt, insomnia or hyposomnia and suicidal ideation or acts are more common[3]. The life time prevalence of depression is between 10-20% in general population worldwide, with a female to male ratio

about 5:2. Typically, the course of the disease is recurrent, and most patients recover from depressive episodes. However, a substantial proportion of patients become chronic and after 5 or 10 years of potential follow up, about 12% and 7% of them respectively are still depressed[4].

Mood disorder are the second primary cause for disability adjusted life years worldwide and the leading cause of years lived with disability in all the age groups in the world. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms are observed and there are numerous side effects caused by antidepressants[5].

Alangium salvifolium (ankolemara) is one of the most valuable plants in traditional system of the medicine from ancient time. The plant *A. salvifolium* is small shrub or deciduous tree may or may not be armed. Leaves are alternative, usually unequal, 12.5-17cm long, 2.5-7 .0 cm broad, oblong lanceolate or oblong- oval, acute or rounded, prominent beneath and obtuse at apex with 3-6 pairs of oblique veins with white or yellowish-white colour and fragrance. It is known to contain various phytochemicals like alkaloids (ipecac and benzopyridoquinolizidine), flavonoids, triterpinoids, saponins, tannins, phenolic glycosides, volatile oil, alangine, lamarckinine, salviifosides A-C, salicin, kaempferol, and kaempferol 3-O-b-D-glucopyranoside[6].

As per traditional claim, the plant possess a different pharmacological activities like anti-cancer, anti-oxidant, anti-bacterial, anti-fungal, anti-inflammatory, and anti-fertility. It is also used in the treatment of anxiety and mood disorders[6]. Therefore, the present investigation was carried out to evaluate antidepressant activity of ethanolic extract of leaves of *A. Salvifolium* by stress induced depression by forced swim test model and tail suspension test model in Swiss albino mice.

MATERIAL AND METHODS

The experiment was carried out after obtaining due clearance from Institutional animal ethics committee of JSS Medical College, Mysuru.

Animals

Swiss albino mice weighing 25-30g, of either sex were procured from the central animal facility of the Institute and maintained under the standard conditions: room temperature (25±3) °C, humidity 45%–55%, 12 /12hr light/dark cycle. They were fed with commercially available mouse pellet diet and water was allowed ad libitum.

Grouping

Animals were randomly divided into 5 groups of 6 each and received drugs as follows:

Group 1: Control group is treated with normal saline (10ml/kg)

Group 2: Standard group treated with drug Imipramine (15mg/kg p.o)[7]

Group 3: Test group-1- ESAL (100mg/kg p.o)

Group 4: Test group -2-ESAL (200mg/kg p.o)

Group 5: -Test group-3-ESAL (100mg/kg p.o) +Imipramine (10mg/kg p.o)

Chemicals

Ethanol, Normal saline, Imipramine (Sun pharmaceuticals)

Plant materials and Preparation of Drug Solution

Alangium salvifolium leaves were collected from Bannari Hill (Dimbam), Coimbatore district, Tamilnadu state, and it was authenticated by Dr Mrutyunjaya (Asst. Prof, Dept. of Pharmacognosy, JSS Pharmacy College, Mysore). The leaves were subjected to wash with 70% of alcohol and made into coarse powder after a shade dry for 1 week. About 500grams of this powder was subjected to soxhlet extraction for 12 h using ethanol as a solvent under suitable temperature. The extract was further concentrated using vacuum extractor for complete removal of the ethanol (absolute, e”99.5%). The concentrated ethanolic extract of *Alangium salvifolium* leaves (EASL) was used to evaluate the antidepressant activity. Stock solution was freshly prepared by using solvent as Normal saline before dosing from which the different doses were administered by selecting the appropriate concentration. Before starting the actual experiment phytochemical screening of the ethanolic extract and acute oral toxicity study was carried out.

Forced swim test

All the groups of animals were subjected to forced swim test after administering the respective drug solutions. On day 0, in training session, mice were forced to swim individually in a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm) containing fresh water up to 15 cm maintained at 25°C for 15 minutes and the animals were observed for 6 minutes. In this test, after a brief spell of vigorous activity, animals show a posture of immobility which was characterized by floating motionless in the water making only those movements necessary to keep the head above the water. This immobility reflects the state of depression. Each mouse was subjected to this procedure 24h prior and 1h after administration of respective drugs for 5 minutes in the test session, and the duration of immobility during last 4 minutes was recorded. Actual test recordings were done on 1st, 7th and 14th day of treatment. After recording of mobility-immobility time, the each mouse were removed, wiped with dry cloth and allowed to dry before being returned to their home cages[8, 9, 10].

Tail suspension test

All the groups of animals were subjected to this test by suspending them on a string held by a metal stand, by an adhesive tape placed 1 cm from the tip of the tail and the string was 58 cm above the table top. The duration of mobility-immobility of the mice was recorded for a period of last 4 minutes during a period of 5 minutes observation. Mice were considered immobile when they hang passively and completely motionless. During the experiment, each animal under test was both acoustically and visually isolated from other animals. Mice were considered immobile when they hang passively and completely motionless. Readings were taken on 1st, 7th and 14th day of treatment.

Statistical Analysis

The results were computed using GRAPH PRISM PAD version 7 software, one way ANOVA test followed by Post-hoc Tukey's multiple comparison tests were applied for analysis. Observations were expressed as mean ±SD/SEM. The differences between means were considered to be significant at $p<0.05$(95% confidence individuals)

RESULTS

Phytochemical screening test

The freshly prepared extract of the leaves of *Alangium salvifolium* was subjected to

Table 1: Result of chemical group tests of the Ethanolic extract of *Alangium salvifolium* leaves

Extract	Carbohydrates	Tannins	Flavonoid	Saponin	Phenols	Steroids	Alkaloids	Glycosides
A.Salvifolium	++	++	++	-	++	+	+++	+++

EE- Ethanolic extract; (+): Present; (-): Absent; (+++); Reaction intensity is high; (++): Reaction intensity is medium; (+): Reaction intensity is normal;

Table 2: Shows Acute Toxicity Studies on Ethanolic Extract of *AlangiumSalvifolium* leaves

Group	Dose (mg/kg)	No of animals	Dose differences (a)	Mortality (b)
1	50	6		No
2	100	6	50	No
3	500	6	400	No
4	1000	6	500	No
5	1500	6	500	No
6	2000	6	500	No

phytochemical screening tests for the detection of various active constituents. The extract showed the presence of alkaloids, tannins, steroids, phenolic and flavonoids, carbohydrates, and glycosides in crude extract of *Alangiumsalvifolium* leaves as depicted in Table 1.

Acute Oral Toxicity Study

The acute toxicity study aims in establishing the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species. The extract of *Alangiumsalvifolium* was safe up to the dose of 2000mg/kg (p.o) body weight. Behaviour of the animals was closely observed for the first 3 h then at an interval of every 4 h during the next 48h. The extract did not cause mortality in the mice during 48h observation **but** some behavioural changes were noted.There was no significant difference in food and water intake among the animal groups studied. Then the results of the LD_{50} study performed on mice were expressed using Karber's method. The results obtained were expressed in the table no. 2. From the table no. 2, it can be concluded that there is no mortality and toxicity symptoms for the ethanolic extract. So the dose was optimized up to 2000mg/kg[11]. (Table 2).

LD_{50} = higher dose -Σ (a x b) /n
n = No. of animals in each group

Table 3: Effects of EASL on immobility time in mouse forced swimming test (FST)

Group no.	Treatment	Immobility Time (s) Day 1	Day 7	Day 14
I	Vehicle Control (10ml/kg)	158.5±3.87	152.7±3.89	148.5±3.73
II	Imipramine (15mg/kg)	96.5± 9.0****	80±4.46****	72.5±3.72****
III	EASL (100mg/kg)	144.2±0.94^{ns}	136.7±1.45****	135.7±1.70****
IV	EASL (200 mg/kg)	135.2±1.07****	130.7±0.80****	127.8±0.40****
V	EASL +Imipramine (100mg/kg + 10mg/kg)	121.6±2.49****	129.67±4.41****	109.67±0.42****

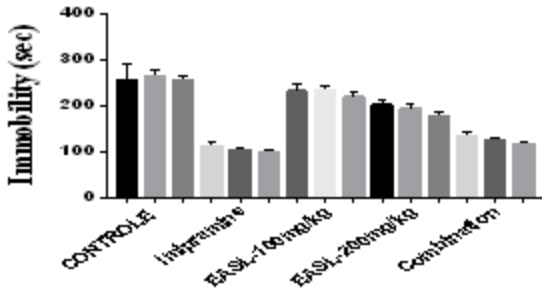
Values are expressed as mean ± SEM. Comparison between control v/s all the other groups. Statistical test done by one-way ANOVA followed by Post-hoc Tukey's multiple comparison test, *p<0.05, **p<0.01; *** p<0.001; ****p<0.0001.

Table 4: Effects of EASL on immobility time in mouse Tail suspension test (TST)

Group no.	Treatment	Immobility Time (s) Day 1	Day 7	Day 14
I	Vehicle Control (10ml/kg)	257±13.91	264.8.5±4.43	257.8±2.15
II	Imipramine (15mg/kg)	112.3±2.525****	103.8±2.04****	100±1.155
III	EASL (100mg/kg)	232 .3±5.391****	234.8±2.921*	218.8 ±4.922****
IV	EASL (200 mg/kg)	201.7±3.451****	194.2±3.962****	178.3± 3.593****
V	EASL +Imipramine (100mg/kg + 10mg/kg)	135.8±1.815****	126.7± 0.9888****	116.5±1.607****

Values are expressed as mean ± SEM. Comparison between control v/s all the other groups. Statistical test done by one-way ANOVA followed by Post-hoc Tukey's multiple comparison test, *p<0.05, **p<0.01; *** p<0.001; ****p<0.0001.

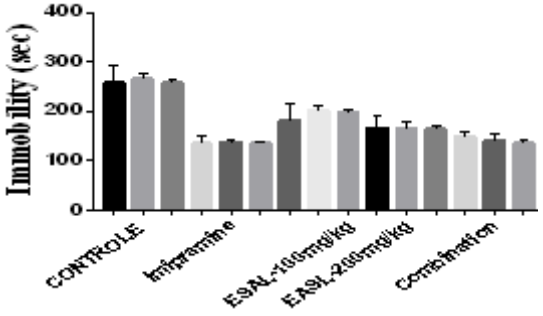
Effect of EASL on immobility in the FST using mice



Results are expressed as mean ± SEM (n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Post-hoc Tukey's multiple comparison test, *p<0.05, **p<0.01; *** p<0.001; ****p<0.0001.

Fig. 1: Effects of the ethanolic extract of *A. Salvifolium* on the immobility time in the mouse Forced swim test

Effect of EASL on immobility in the TST using mice



Results are expressed as mean ± SEM (n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Post-hoc Tukey's multiple comparison test, *p<0.05, **p<0.01; *** p<0.001; ****p<0.0001.

Fig. 2: Effects of the ethanolic extract of *A. Salvifolium* on the immobility time in the mouse Tail suspension test

LD_{50} = 2000 – 0
= 2000 mg / kg
ED_{50} = LD_{50}/ 10
= 2000 / 10
= 200 mg / kg.

Forced swim test

The results of acute model of FST with mice are displayed in Table-3 & Graph-1. In this test, animals of all the test groups showed significant results**.**The EASL extract (100 & 200mg/ kg body weight) treated groups exhibited significant delay in the onset of immobility and also significantly reduced time of immobility in the forced swimming test after 14 day of treatment. Post-hoc Tukey's multiple comparison tests analysis demonstrated that the test treatment significantly reduced the immobility time in comparison to the control group (p<0.0001). Combination of EASL extract (100mg/kg b wt) and imipramine in the reduced dose (10mg/kg b wt.) i.e. Test group-3 showed significantly reduced immobility and increase in the normal behaviour of mice in water filled apparatus and also exhibited antidepressant activity comparable to the standard drug Imipramine (10mg/kg b wt.) i.e. the standard group. On day-1 of the test the immobility time was 144.2, 135.2, 98.6 seconds in test groups 1, 2 and 3. The results were statistically significant in test groups 2 and 3 when compared to control in which the immobility time was 158.5 seconds. However with subsequent drug administration the immobility time was significantly reduced in all test groups to 136.7, 130.7 and 95.67 seconds (test groups 1, 2 and 3 respectively) when compared to control group which was 152.7 seconds on day 7 of the test. On day 14 also reduction in immobility time was significant in all test groups at 135.7, 127.8 and 83.67 seconds (test groups 1, 2 and 3 respectively) when compared to the control group which was 148.5 seconds. However, the results of the standard drugs were significantly better on all the test days at 96.5, 80 and 72.5 seconds on day 1, 7 and 14 respectively.

Tail suspension test

The results of the antidepressant effect of Ethanolic extract of *A. Salvifolium* arepresented in Table-4 and figure-2. The extract showed slight reduction in immobility on 1 day treatment, but significantly reduced the immobility time after 7 and 14 days of treatment. Combined group showed almost nearly same significant reduction in immobility time comparable to standard Imipramine**.** On day 1 of the test the immobility time in test groups

1, 2 and 3 was 232.3, 201.7 and 135.8 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 257 seconds. On day 7 the of the test the immobility time in test groups 1, 2 and 3 was 234.8, 194.8 and 126.7 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 264 seconds. Similarly on day 14 of the test the immobility time in test groups 1, 2 and 3 was 218.8, 178.3 and 116.5 seconds respectively which was statistically significant when compared to the control group in which the immobility time was 257 seconds. The standard drug was far superior in reducing the immobility time on all days of the test at 112.3, 103.8 and 100 seconds respectively on days 1, 7 and 14 of the test.

DISCUSSION

The present study revealed the significant anti-depressant effect of ethanolic extract of *A.Salvifolium* leaves in experimentally induced depression by Forced swim test and Tail suspension test models. The ethanolic extract of *A. Salvifolium* leaves significantly decreased the immobility time in dose dependent manner which is an indicator of antidepressant activity.

Alangium salvifolium is known to contain natural phytonutrients such as alkaloids (ipecac and benzopyridoquinolizidine), Glycosides, Steroids, flavonoids, Amino acids which may be responsible for improving the vital neurotransmitters involved in memorization, information and processing that may be helpful in depression. The action of triterpinoids and saponins may have resulted in the enhancement of nerve impulse transmission[11]. Literature review of the plant reveals that A. *Salvifolium* also contains Flavonoids & Tannin[14]. Different types of neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; which indicates that they act as a benzodiazepine like molecules[15].

The anti-depressant effect may be attributed to the active compounds in the extract that act on GABA/benzodiazepine receptor complex as well as by stimulating glucocorticoid production and its release in the adrenal cortex[12.13].

EASL extract reduced the immobility period during the forced swimming and tail suspension test in comparison with control and exhibited a dose dependent antidepressant activity. The characteristic behaviour evaluated in these test, termed immobility, has been considered to reflect behavioural despair similar to that seen in the human depression, and hence any reduction in this parameter reflects antidepressant activity. There is a significant correlation between the clinical efficacy of antidepressant drugs and their potency in FST which was not found in any other model. Interestingly, our data indicate that higher doses of plant extracts were more effective than smaller doses both in forced swim test and tail suspension tests.

The major inhibitory neurotransmitter in central nervous system is Gamma amino butyric acid (GABA). Different type of antidepressants, muscle relaxants, sedative- hypnotic drugs exhibit their action through GABA-ergic inhibition in the CNS that leads to either decrease in the firing rates of critical neurons in the brain or direct activation of GABA receptors by the extracts[15]. This result indicates the significantly decreased immobility period in FST and TST by EASL. A probable mechanism being our plant extracts acting through GABAergic and/or glutamatergic transmission, cytokine or steroidal alterations cannot be ruled out.

Though the EASL extract have a modest effect when compared to standard it can serve as an add-on drug to current regimens or may be used along with current regimens in lower dose. The reduction in dose of these Standard drugs is always a welcome change and may help in reducing the adverse effect profile which becomes obvious at higher doses. Further isolation and identification of the bioactive ingredient responsible for anti depressant activity is necessary.

CONCLUSION

The present study has showed antidepressant activity of EASL in all classic models such as forced swimming test (FST) and tail suspension test (TST) comparable to the standard drug Imipramine hydrochloride. However, further

studies are needed to elicit its exact mechanism of action and to identify the active ingredient as a potent and efficacious antidepressant agent.

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