

PHARMACOLOGICAL EVALUATION OF ANTI DEPRESSANT ACTIVITY OF ALANGIUM SALVIIFOLIUM (L. F.) WANGERIN LEAVES IN ANIMAL MODELS

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ABSTRACT

The objective was to investigate the antidepressant activity of methanolic extract of leaves of Alangium Salviifolium (L. F.) Wangerin in mice. To study the effect of Alangium Salviifolium (L. F.) Wangerin on anti depressant activity of brain. The results from the present study confirm the antidepressant activity of hibiscus, since it reduced the immobility in both FST and TST. In the present study, Alangium Salviifolium (L. F.) Wangerin is significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pre treatment with hibiscus, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of Alangium Salviifolium (L. F.) Wangerin might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of Alangium Salviifolium (L. F.) Wangerin is, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the anti depressant activity.

Keywords: Depression, Alangium Salviifolium (L. F.) Wangerin etc.

1. 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. Major depressive disorder is a complex and frequent psychiatric condition that poses significant challenges to both the patients who experience it and the physicians who treat them. The goal of therapy is for patients to achieve remission, which requires identifying and measuring symptoms at the outset and throughout treatment to document both response and resistance to treatment. ^[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 salviifolium (L. f.) Wangerin (ankolemara) is one of the most valuable plants in traditional system of the medicine from ancient time..Alangium salviifolium (L. f.) Wangerin is a small shrub or deciduous tree that 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 phyto-chemicals like alkaloids (ipecac and benzopyridoquinolizidine), flavonoids, triterpenoids, saponins, tannins, phenolic glycosides, volatile oil, alangine, lamarckinine, salviifosides A-C, salicin, kaempferol, and kaempferol 3-O-b-D-glucopyranoside.

As per traditional claim, the plant possess 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 . Therefore, the present study was carried out to evaluate antidepressant activity of ethanolic extract of leaves of Alangium salviifolium (L. f.) Wangerin by stress induced depression by forced swim test model and tail suspension test model in Swiss albino mice.

2. MATERIALS AND METHODOLOGY

Thiobarbituric acid and DTNB reagent (Hi Media Laboratories Ltd., Mumbai), Trichloro acetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneal by dissolving in normal saline.

2.1 Extraction of Plant Material

The cold extraction process was done with the help of ethanol. About 45-60gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750ml of ethanol. The container with its contents were sealed and kept for period of 7days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool. The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccators for 7 days.

2.2 Selection of Animals

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 20-25gms were selected. Animals are housed 4 per cage in temperature controlled ($27^{\circ}\text{C}\pm3^{\circ}\text{C}$) room with light/dark cycle in a ratio of 12:12hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

2.3 Acute toxicity studies

Acute toxicity studies will be performed for Ethanolic extract according to the acute toxic classic method as per OECD guidelines. Male mice were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract will be administered orally at the dose of 300mg/kg and observed for 14days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72h.

2.4 in vivo models of depression employed in the study

Forced swimming test (FST)

The procedure was described by Porsoltetal. (1978) was used. Swimming sessions were conducted by placing mice in individual glass cylinders (45cm high \times 20cm in diameter) containing ($25\pm2^{\circ}\text{C}$) water 38cm deep, so mice could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 12:00 and 19:00h, an initial 15 min pre test followed 24 h later by a 6 min test.

Doses were given once daily for 7days. On the 7th day mice were subjected to 15min pretest. After 15min, in the water the mice were removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They were again placed in the cylinder 24h later and the total duration of immobility was measured during a 6min test. Floating behavior during this 6min period had been found to be reproducible in different groups of mice. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The total immobility time for the period of 6min was recorded with the help of stop watch.

Tail suspension test (TST)

Doses are given once daily for 7days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were suspended on the edge of a table 50cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Immobility time was recorded during a 6min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively.

5-HTP induced head twitches in mice

Doses were given once daily for 7 days. On the 7th day, 1 hr after the administration of the test and standard drugs, mice were treated with 5-HTP (100mg/kg i.p.) and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21min), (23–25min), (27–29min) after 5-HTP administration and number of head twitches were scored live by a blind observer.

Clonidine-induced aggression in mice

The method of Morpurgo (1968) was used. Mice were divided into 5 groups of 8 each (n=8), each group contain 4 pairs of mice, two pairs from each sex (each pair contained same sex of mice). Doses were given once daily for 7 days. On the 7th day, Clonidine was given 1h after the administration of the test and standard drugs. The animals were then caged in bell shaped glass jar with a floor area of approximate 16cm². The biting/fighting episodes were recorded live by a blind observer over a period of 30min, in each pair.

L-DOPA induced hyperactivity and aggressive behavior in mice (LHA)

Doses were given once daily for 7 days. On the 7th day, L-DOPA was given 1h after the administration of the test and standard drugs. Stages of activity and aggressive behavior were recorded live every 10min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumping, queaking, and aggressive fighting. The scores were graded in the following manner:

0—No effect; 1—Piloerection, slight salivation, slight increase in motor activity; 2—Piloerection, salivation, marked increase in motor activity and irritability; 3—Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting.

STATISTICAL ANALYSIS

Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall P-value was found statistically significant (P<0.05)

3. RESULTS AND DISCUSSION

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino mice were administered Alangium Salviifolium (L. F.) Wangerin and doses were selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD₅₀ value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose anti depressant investigations.

3.1. Forced Swim Test (FST)

The results (Table. 1) showed that both EEASW (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the EEASW (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different (p<0.001) from the vehicle treated group.

Table. 1: Forced Swim Test (FST)

Group no.	Treatment (dose in mg/kg)	Immobility period (sec) Mean ± SEM
I	Control (0.3% CMC) + FST	135.1±5.1
II	EEASW (100 mg/kg, p.o.) + FST	122.5±8.5
III	EEASW (200 mg/kg, p.o.) + FST	96.3±2.7*
IV	EEASW (400 mg/kg, p.o.) + FST	80.1±5.2*
V	Imipramine (15 mg/kg, i.p.) + FST	73.2±8.1*

3.2 Tail Suspension Test (TST)

The results (Table. 2) showed that both EEASW (100, 200, 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the EEASW (100, 200 and 400 mg/kg) and IMP treated groups were significantly different (p<0.001) from the vehicle treated group.

Table. 2: Tail Suspension Test (TST)

Group no.	Treatment (dose in mg/kg)	Immobility period (sec)
I	Control (0.3% CMC) + TST	137.1±8.1

II	EEASW (100 mg/kg, p.o.) + TST	113.2±02.1 ^a
III	EEASW (200 mg/kg, p.o.) + TST	96.2±7.2 ^a
IV	EEASW (400 mg/kg, p.o.) + TST	82.1±5.2 ^a
V	Imipramine (15 mg/kg, i.p.) + TST	62.5±1.4 ^a

3.3. 5-HTP induced head twitches in mice

Table 3. illustrates the effect of *Alangium Salviifolium* (L. F.) Wangerin and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *Alangium Salviifolium* (L. F.) Wangerin (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *Alangium Salviifolium* (L. F.) Wangerin was significantly higher than IMP (p<0.001).

Table. 3: 5-HTP induced head twitches in mice

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	11.9±2.1
II	EEASW (100 mg/kg, p.o.)	28.5±1.2 ^a
III	EEASW (200 mg/kg, p.o.)	26.1±3.2 ^b
IV	EEASW (400 mg/kg, p.o.)	35.5±2.1 ^b
V	Imipramine (15 mg/kg, i.p.)	23.1±1.1 ^b

3.4. L-DOPA induced hyperactivity and aggressive behavior in mice

The effect of *Alangium Salviifolium* (L. F.) Wangerin and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *Alangium Salviifolium* (L. F.) Wangerin (100, 200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group.

Table. 4: 5-HTP induced head twitches in mice

Group o.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	EEASW (100 mg/kg, p.o.)	2.1 ± 0.2 ^a
III	EEASW (200 mg/kg, p.o.)	2.3 ± 0.2 ^a
IV	EEASW (400 mg/kg, p.o.)	2.2 ± 0.2 ^a
V	Lorazepam (2.5 mg/kg, i.p.)	2.2 ± 0.2 ^a

3.5. Clonidine induced aggression in mice

Table. 5. indicates the effect of *Alangium Salviifolium* (L. F.) Wangerin (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *Alangium Salviifolium* (L. F.) Wangerin (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control.

Table. 5: Clonidine induced aggression in mice

Group no.	Treatment (dose in mg/kg)	% Response (MEAN ± SEM)	
		Latency to 1 st attack	Fighting response
I	Control (0.3% CMC)	101.2 ± 8.2	98.9 ± 5.1
II	EEASW (100 mg/kg, p.o.)	120.2 ± 10.2 ^a	86.2 ± 1.5 ^a
III	EEASW (200 mg/kg, p.o.)	132.3 ± 15.1 ^b	65.2± 3.4 ^b
IV	EEASW (400 mg/kg, p.o.)	132.1 ± 8.9 ^b	63.2 ± 4.1 ^b
V	Lorazepam (2.5 mg/kg, i.p.)	141.2 ± 6.1 ^b	41.3± 2.5 ^b

4. CONCLUSION

Alangium Salviifolium (L. F.) Wangerin significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with hibiscus, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of Alangium Salviifolium (L. F.) Wangerin might be a part of the mechanism for its antidepressant activity.

Results from behavioral experiments indicate that the antidepressant activity of Alangium Salviifolium (L. F.) Wangerin, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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