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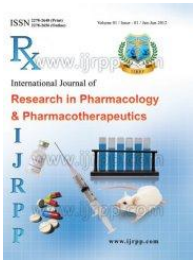

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Research Article ***Open Access***

CNS depressant activity of ethyl acetate leaf extract of *Avicennia officinalis* in mices

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ABSTRACT

The present study deals with the investigation of the ethyl acetate extracts of *Avicennia officinalis* leaves was assessed for an effect on the central nervous system (CNS) using neuropharmacological experimental models (muscle co-ordination and locomotor activity) in mice. The extract used for a dose-dependent reduction of the onset and duration of a reduction in locomotor activity. The result of ethyl acetate extract of *Avicennia officinalis* leaves (250, 500 and 1000 mg/kg, p.o.) mostly decreases the activity. These results suggest that the ethyl acetate extracts of *Avicennia officinalis* leaves possess a wide range of CNS activities, which need further investigation. These results suggest that the extract possesses CNS depressant activity.

Keywords: CNS depressant, *Avicinnia officinalis,* Diazepam.

INTRODUCTION

Use of plant products is increasing in many segments of the population[1]. At present, thousands of plant metabolites are being successfully used for the treatment of a variety of diseases. According to an estimate, 80% of the world's population relied upon plants for their medication[2]. The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products[3]. Depression is a common mental disorder that presents with depressed mood, loss of interest or pressure, feeling of guilt or low self-worth, disturbed sleep or appetites low energy and poor concentration.

Advance in modern science and technology has contributed to an enormous development in the quality of human life. Though, stress in modern life responsible for the surge in incidence of variety of psychiatric disorders. Drugs currently used in the treatment of different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, parkinsonism either refractory or have serious side effects or possess unfavorable drug-drug/drug-food interactions. Psychoneural drugs like benzodiazepines, commonly employed in anxiety, depression, epilepsy and insomnia, but possess side effects like cognitive function, physical dependence and tolerance[4, 5]. Plants have been used as medicine since time immemorial. Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects[6, 7]. The leaves of *Avicennia officinalis* were used in folk

medicine for treating ulcerative stomatitis, skin diseases, ulcers, wounds etc[8].

MATERIALS AND METHODS

Animals

Healthy adult male albino mice of Wister strain weighing 25-35 grams were selected for the study. The animals were acclimatized to standard laboratory condition with temperature 25±2^{0}C and feed with a standard animal pellet feed (Hindustan lever limited) and water libitum. The protocol was approved by an animal ethics committee constituted for the purpose of animal experimentation as per CPCSEA guidelines. (IAEC. Ref. No: 1477/PO/a/CPCSEA/2012-PH/PCOL-03).

Plant material

The fresh leaves of *Avicennia officinalis* were collected from local areas of Tirupathi, Andhra Pradesh, India and authenticated by Prof. Dr. D. S. R. RajendarSingh, Senior Reader, Department of Botany, MVS Degree College, mahabubnagar. The leaves were dried in shade the midrib was separated and the leaves were ground to get a coarse powder.

Preparation of extracts

The ethyl acetate extract (EALEAO) of leaves was prepared by using ethyl acetate, by soxhlation method. The extract was concentrated by simple evaporation at room temperature. A suspension of EAEAO in Tween 80 was prepared for oral administration.

Phytochemical study

The successive extracts were subjected to various qualitative tests to determine the presence of various phytoconstituents using reported methods[9].

Acute toxicity studies

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method). Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 50, 300, 500, 2000 and 5000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. Information suggests that mortality is unlikely at the highest starting dose level (5000 mg/kg body weight). The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. All observations are systematically recorded with individual records being maintained for each animal. Additional observations will be necessary if the animals continue to display signs of toxicity.

CNS Depressant activity

The assessment of CNS Depressant activity of the test extract was performed by using the following models.

1. Rota rod Model.
2. Forced swim test.

Rota rod model

Fresh mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 5 RPM. The mice capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into four groups (n=6). Ethyl acetate extract of *avicennia officinalis* at the dose of 250, 500 and 1000 mg/kg, respectively, were injected orally in to group 2, 3 and 4. Diazepam (5mg/kg) was given to a group 1. Each group of animals was then placed on the rod after 60mins of drug administration. The animals failed more than once to remain on the rotarod for 3 min were considered as passed the test[10].

Forced swim test

Forced swim test, the most frequently used behavioral model for screening CNS depressant like activity in rodents. The procedure was same as follows previously. Mice were individually forced to swim in the open glass chamber (25×15×25cm) containing fresh water to a height of 15 cm and maintained at 26 ± 1^0 C. At this height of water, the animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind paws or tail. Water in the chamber

was changed after subjecting each animal. Each animal showed vigorous movement during initial 2min period of the test. The duration of immobility was manually recorded during the next 4min of the total 6 minutes testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their head above water. Following a swimming session, mice were towel dried and returned to their housing conditions[10].

RESULTS AND DISCUSSION

Preparation of extracts

Extracts of *Avicennia officinalis* leaves using ethanol, methanol, petroleum ether, diethyl ether and ethyl acetate were prepared by using soxhlation method. The dried and purified extracts were weighed and stored in air tight container. The percentage yields of various extracts were calculated as 2, 1.5, 1, 1, and 3 % respectively.

Preliminary Phytochemical Analysis

Qualitative phytochemical studies were performed on extracts using suitable chemicals and reagents to confirm the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, proteins, amino acids, phenolic compounds, flavonoids, triterpinoids, and phytosterols. The results of qualitative phytochemical studies indicate that the maximum number of chemical constituents was present in the ethyl acetate extract when compared to the other extracts (Table-1) and hence, the ethyl acetate extract was selected for further pharmacological screening.

Acute toxicity studies

The purified and completely died yield of EALEAO was subjected for the acute toxicity study to determine the therapeutic dose using albino mice in a controlled environment. Acute toxicity studies were performed according to the OECD 423 guidelines. The extract was administered through oral route to different groups of mice using oral feeding needle (22guage). No deviation from normal behavior pattern was observed. But only a few animals showed mild behavioral changes like dyspnoea and mild writhingsin higher dose. Observation was done continuously for 14 days and mortality was not observed in any of the drug treated group, hence it was confirmed that the test drug EALEAO is practically nontoxic in normal mice and fall under the category of class V drug, according to Anupama and Handa. 1990. $1/10^{th}$ of dose was considered as therapeutic dose and to identify the dose dependent action the 50% and 200% of therapeutic dose was considered as minimum and maximum dose for further pharmacological evaluation in animal model.

CNS Depressant activity

1. Rota rod Model - In this present study, the standard and test drug treated group animal's shows decrease in time spent on the rod when compared with control group animals.
2. Forced swim test- In this study, the standard drug produces the 84.65 and test drug treated group showed 69.03, 60.20, 60.76 % inhibition, respectively, then results indicate that the test drug at all three doses posses the CNS Depressant activity.

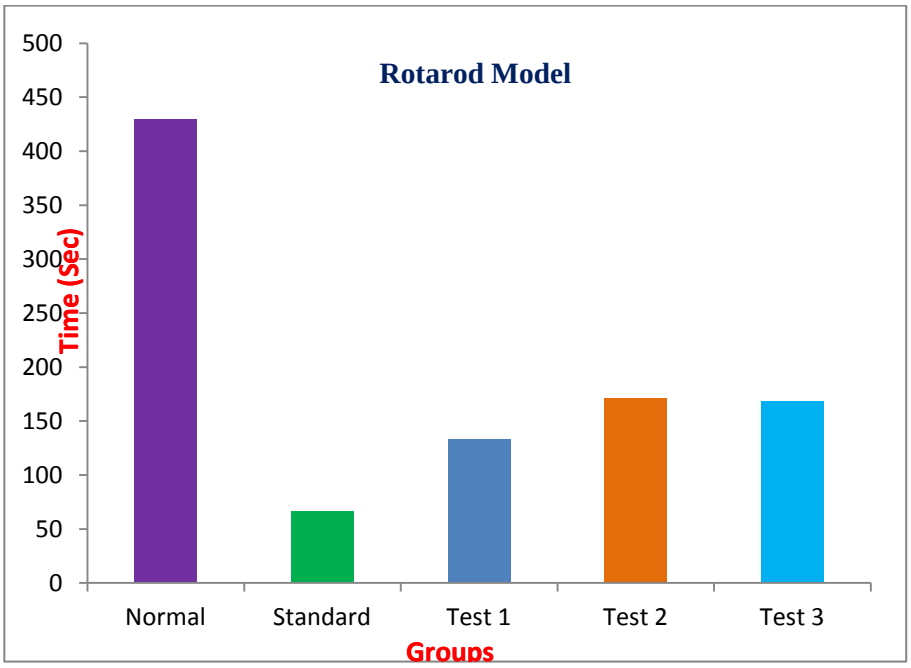
Table 1: Phyotochemical Analysis of leaf extract of *Avicennia officinalis*

Phytoconstituets	Ethanol	Methanol	Di ethyl ether	Pet. Ether	Ethyl acetate
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Saponins	-	-	-	-	-
Carbohydrates	+	+	-	-	+
Tannins	-	-	-	-	+
Flavonoids	+	+	+	+	-
Steroids	-	-	-	-	+
Triterpinoids	+	+	+	+	+
Lignins	-	-	-	-	-
Proteins	-	-	-	-	+
Amino acids	-	-	-	-	+

"+" indicates the presence.
"-"Indicates the absence.

Table 2: CNS Depressant activity of *Avicinna officinalis* on mice by rotarod

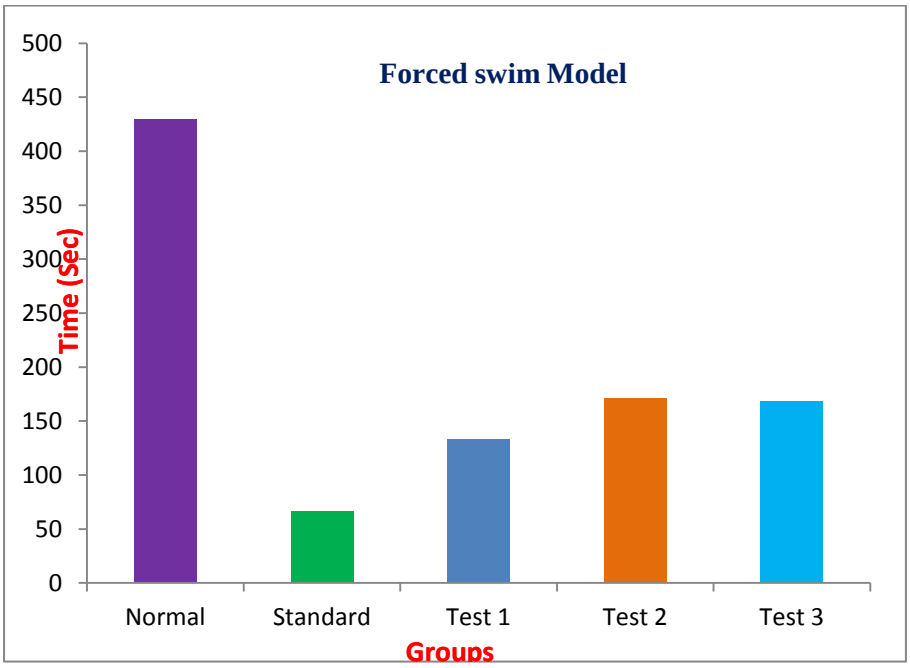
S.No	Group & Drug Treatment	Time spent in Rotarod	% Inhibition
1	Normal	675±11.19	-
2	Standard (Diazepam 5 mg/kg)	93.83±3.48***	86.09
3	T_1 (EALEAO 250mg/kg)	149.2±8.78***$^{@@@}$	77.89
4	T_2 (EALEAO 500mg/kg)	175.8±9.34***$^{@@@}$	73.95
5	T_3 (EALEAO 1000mg/kg)	158.3±5.33***$^{@@@}$	76.54



All values are expressed as mean ± SEM for 6 rats in each group.
Comparisons made between
***p<0.001, **p<0.01, *p<0.05; Negative control, Standard, T1, T2, T3 Vs Normal.
@@@p<0.001, @@p<0.01, @p<0.05; Standard T1, T2, T3 Vs Negative control, One way ANOVA followed by Tukey test.

Table 3: CNS Depressant activity of *Avicinna officinalis* on mice by forced swim test

S.No	Group & Drug Treatment	Time of swim	% Inhibition
1	Normal	430.2±25.07	-
2	Standard (Diazepam 5 mg/kg)	66.0±11.22	86.65
3	T_1 (EALEAO 250mg/kg)	133.2±6.46	69.03
4	T_2 (EALEAO 500mg/kg)	171.2±15.85	60.20
5	T_3 (EALEAO 1000mg/kg)	168.3±16.09	60.76



All values are expressed as mean ± SEM for 6 rats in each group.
Comparisons made between
***p<0.001, **p<0.01, *p<0.05; Negative control, Standard, T1, T2, T3 Vs Normal.
@@@p<0.001, @@p<0.01, @p<0.05; Standard T1, T2, T3 Vs Negative control, One way ANOVA followed by Tukey test.

CONCLUSION

In the present study, the ethyl acetate extract of *Avicinna officinalis* CNS has been evaluated. The result indicated that the extract significantly decreased the loco motor activity as shown by the results of the rotarod and forced swim tests. The locomotor activity is a measure of the level of excitability of the CNS[11] and any decrease of this activity may be closely related to sedation resulting from depression of the central nervous system[12]. All three doses of the crude extract were produced a significant increase in the sedative effect induced by the diazepam, in a dose dependent manner, thus suggesting a profound sedative activity.

The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Literature review of the plant reveals that *avicennia officinalis* contains flovonoids, saponins, steroids, tannins and terpinoids[13, 14].

Many flavonoids and neuroactive steroids were found to be ligands for the gamma aminobutyric acid type A ($GABA_A$) receptor in the central nervous system (CNS); which lead to the hypothesis that they act as benzodiazepines-like molecules[15]. This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion[16].

Hence we have concluded that the *Avicennia officinalis* plant leaf extract was found to be significantly increases the sedative action. The ethanolic extract showed CNS depressant activity may be due to the presence of phenolic constituents.

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