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Evaluation of CNS Depressant Activity of Different Plant parts of Aegle marmelos Linn.

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Abstract

The present study was carried out with the water-soluble portion of the ethanol extracts of flowers, barks, seeds and leaves of Linn. Aegle marmelos The ethanol extracts of the plant parts were obtained by soxhlet extraction. After performing the gross behavioral study, the CNS depressant activity was evaluated by observing the prolongation of sleeping time induced by pentobarbital sodium in mice. Attempts have been made to explore the possible mechanism behind this activity by determining their effect on brain monoamine neurotransmitters like dopamine and serotonin. The gross behavioral study showed that ethanol extracts of the leaves, flowers and seeds possess significant CNS depressant activity. The leaves, flowers, seeds and barks (600 mg/kg) showed significant and dose-dependent prolongation of onset and duration of sleep and so found to cause decrease dopamine and increase serotonin level. From which it can be concluded that the CNS depressant activity of the ethanol extracts of seeds, leaves and flowers may be due to the decrease in dopamine and increase in serotonin level.

Keywords: *Aegle marmelos*L., behavioral study, CNS depressant activity, histamine, serotonin

Introduction

Aegle marmelos L. (Family: Rutaceae) commonly known as Bael in Hindi is an essential food plant of India. Traditionally the fruit was used to treat diabetes, respiratory problem, inflammation, dysentery and diarrhea. The fruits of *Aegle marmelos* are rich in flavonoids, terpenoids, carotenoids and coumarins. The major bioactive constituents include imperatorin (54), aegelin, lupeol, eugenol (7), cineol, citronellal etc. The CYP450-CO assay to evaluate the inhibitory potential of the fruit extract and the standard phytoconstituent imperatorin revealed dose dependent inhibition of the *Aegle marmelos* extract. The fruit extract and imperatorin showed lesser inhibition ($P < 0.001$) than the standard ketoconazole. The 96-well fluorogenic assay showed lowest inhibition for *Aegle marmelos* extract against CYP2D6 (159.73 ± 1.43 µg/mL) and highest inhibition against CYP1A2 (128.49 ± 1.27 µg/mL). The higher IC50 values of the *Aegle marmelos* extract than the positive controls showed less or moderate interaction in drug metabolism

EXPERIMENTAL:

Procurement of plant

Aerial parts of Aegle marmelos were obtained from regional parts of Vivekanand College of Pharmacy, Bhopal. Obtained plant sample were collected, shade dried and send it to the saifia college bhopal for authentification.

Authentification of plant

Authentification of plant was done at saifia college bhopal; they have reported that given sample of plant is DURVA belongs to Cynodon dactylon (L.) Pers, Family-Poaceae.[6]

Preparation of the ethanol extract

Fig – **Soxhlet extraction** at Laboratory of vivekanand college of pharmacy

Shade dried, powdered, sieved (40 mesh size) plant materials will be extracted first with petroleum ether (40-60OC) and then with ethanol. The ethanol extract will be evaporated to dryness. The trace amount of ethanol which might be present within the solid mass of extracts will be removed under vacuum.[7]

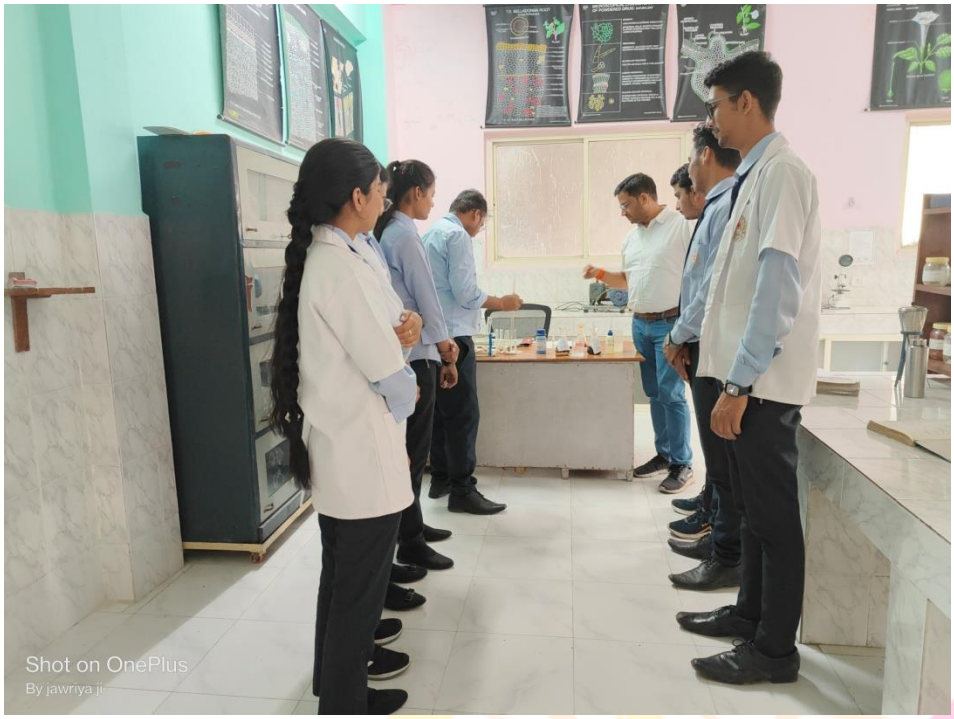

Fig –Confirmation test of Alkaloids by dr. Vivekanand Katare (Principal)

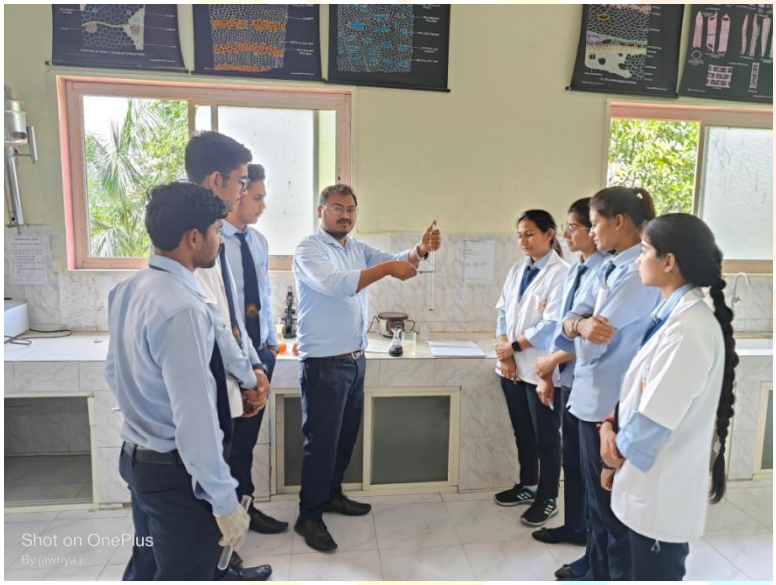

Fig. Quantitative analysis (CHEMICAL TEST) of extract

Drugs

Pentylenetetrazole (PTZ, 60.0 mg/kg, Sigma) was used as the convulsant drug; sodium Phenobarbital inj. (PB, 30.0 mg/kg) as the sleep-inducer drug. Diazepam inj. (DZP, 1.0 mg/kg) was used as a positive control in the plus-maze, Phenobarbital-induced hypnosis and Pentylenetetrazole-induced seizure tests. Imipramine hydrochloride (IMI, 15 mg/kg) was used as a positive control in the forced swimming test (FST). Propylene glycol solution was used as a vehicle. All treatments were administrated intraperitoneal (i.p.) pathway.

Animal

mice (30–35 g) were obtained from Serum Research Institute, Pune and maintained in our animal house under controlled temperature (23–25 ◦C) and 12 h light–dark cycle (lights on 07:00 h), with free access to food and water. All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience . The experimental protocol was approved by the Institutional Research Committee. The minimum number of animals and duration of observation required to obtain consistent data were employed.

Elevated plus-maze test

The elevated plus-maze (EPM) test has been widely validated for measuring anxiolytic and anxiogenic-like activities in rodents[8]. This apparatus was made of Plexiglas and consisted of two open arms (30 cm×5 cm) and two closed arms (30 cm×5 cm) with 25 cm walls. The arms extended from a central platform (5 cm×5 cm). The maze was elevated 38.5 cm from the room's floor. The mice were treated, 30 min before the test, with different doses of -HEAM (50, 75, and 100 mg/kg, i.p.; *n* = 8), DZP (positive control, i.p.; *n* = 6) and Propylene glycol 50% (control group, i.p.; *n* = 6). Each animal was placed at the center of the maze, facing one of the enclosed arms. The number of entries and the time spent in enclosed and open arms were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. Total exploratory activity (number of entries) and other ethologically derived measures (grooming, rearing, stretched attend postures and head dipping) were also registered. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

Forced swimming test

This test is the most widely used and recognized pharmacological model for assessing antidepressant activity[9]. The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear Plexiglas cylinder (20 cm high×12 cm diameter) filled to 15 cm depth with water (24±1 ◦C). The mice were treated with different doses of HEAM (50, 75 and 100 mg/kg, i.p.; *n* = 6), IMI (positive control, *n* = 6) and PG 2.5% (control group, *n* = 6). In the pre-test session, every animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. During the test session the following behavioral responses were recorded by a trained observer: climbing behavior, which is defined as upward directed movements of the forepaws along the side of the swimming chamber; swimming behavior, defined as movement throughout the swimming chamber, which includes crossing into another quadrant; and immobility time, when the mice made no further attempts to escape, and makes only movements to keep its head above the water.

Phenobarbital-induced hypnosis

Sodium phenobarbital (a sub-hypnotic dose, 30.0 mg/kg) was injected i.p. 30 min after administration of HEAM. The mice were treated with different doses of HEAM (50, 75 and 100 mg/kg, i.p.; *n* = 6), the control group (*n*=6) was treated with PG solution and positive control group (*n*=6) was administrated with DZP 2.0 mg/kg in the same conditions. The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex[10].

Rotarod

The method of Dunham and Miya (1957) was used. The animals were placed with the four paws on a 2.5-cm diameter bar, 25 cm above the floor, which was turning at 12 rpm. For each animal, the number of falls (up to three falls) and the time of permanence on the bar for 1 min were registered.

FIG- dosing of herbal extract

Table-1 Effect of HEAM on elevated plus maze test in mice

Groups	No. of entries(n)		Time spent (s)	
	Open arms	Closed arms	Open arms	Closed arms
Vehicle(50% PG)	6.66±0.33	6.83±0.30	113.66±1.94	135.33±0.66
DZP (2mg/kg)	9.5±0.42*	6.5±0.42	170.33±1.78*	87.33±0.80*
HEAM(50mg/kg)	8.16±0.30	7.5±0.22	93.16±1.66*	128.5±0.76
HEAM(75mg/kg)	4.83±0.30**	6.16±0.30	72.83±0.79**	150.83±1.35**
HEAM(100mg/kg)	3.16±0.30***	5.66±0.33	56.5±0.76***	183.33±1.22***

Each value represents mean ± SEM for 6 no of animals,*, **, ***p≤0.001 vs. control(ANNOVA and student's-Newman-Keul's test)

Table -2 Effects of *HEAM* on the forced swimming test in mice

Groups	Immobility time (sec)
Vehicle(50% PG)	76.5 ± 5.49
IMP (10mg/kg)	18.1 ± 2.74 ***
HEAM(50mg/kg)	93.5 ± 9.15
HEAM(75mg/kg)	106.2 ± 12.25 **
HEAM(100mg/kg)	125.9 ± 10.11 ***

Each values represent mean ± S.E.M. **, ***$P > 0.05$ vs. controls (ANOVA and Student's–Newman–Keuls test as the *post hoc* test).

Table-3 Phenobarbital induced hypnosis

Groups	Latency (sec)	Duration (sec)
Vehicle(50% PG)	0	0
DZP(2mg/kg)	276.5 ± 12.79**	2240.33 ± 26.29**
HEAM(50mg/kg)	483.83 ± 21.16*	1921.16 ± 25.16*
HEAM(75mg/kg)	328.66 ± 13.17**	2009.16 ± 10.04**
HEAM(100mg/kg)	219.16 ± 9.13**	2621.33 ± 69.91**

Each value is presented as mean ±S.E.M. (*) $p < 0.05$, (**) $p < 0.01$ as compared with the control group. (ANOVA one-way following by Dunnet test); $n = 6$ mice per group. Lat = latency, Dur = duration, DZP = diazepam, vehicle-50%PG

Fig. 1- Effect produced by different doses (50, 75, 100 mg/kg, i.p.) of HEAM on the latency and duration of hypnosis induced by sodium pentobarbital (30 mg/kg, sub-hypnotic dose). The results are presented as mean ±S.E.M. (*) $p < 0.05$, (**) $p < 0.01$ as compared with the control group. (ANOVA one-way following by Dunnet test); $n = 6$ mice per group. Lat = latency, Dur = duration, DZP = diazepam, vehicle-50%PG.

Table-4 Effect of HEAM on Rota-rod test in mice

Groups	Time of permanence (s)
Vehicle(50% PG)	175.53±1.38
DZP (2mg/kg)	37.16±0.87*

HEAM(50mg/kg)	156.66±2.07
HEAM(75mg/kg)	75.5±0.99**
HEAM(100mg/kg)	40.33±0.71***

Each value represents mean ± SEM for 6 no of animals.*, **, ***p ≤ 0.001 vs. control(ANNOVA and student's- Newman-Keul's test)

Statistics analysis

Data were analyzed by ANOVA for one-way and ANOVA and Student's–Newman–Keuls test as the *post hoc* test

RESULTS:

EPM test

As shown in Table 1, HEAM (75 and 100mg/kg, p.o.) decreased the NEOA (n◦ of entries in the open arms) and the TPOA (time of permanence in the open arms). Neither dose of HEAM increased significantly the number of entries in the open arms, indicating an absence of anxiolytic effect. Diazepam (2 mg/kg, i.p.) treatment increased significantly the number of entries and the time of permanence in the open arms in 70.1% and 33.2% as compared with controls.

Forced swimming test

The animals treated with all doses of extract showed in Table 2,All animals treated with HEAM showed no antidepressant behavior at all the doses ($p > 0.05$), The animals treated with imipramine (10 mg/kg, i.p.) as expected, decreased the immobility time in 76.3% as compared with control.

Phenobarbital-induced hypnosis

Since the Phenobarbital doses were sub-hypnotic the mice that received vehicle showed no changes in their behavior. Contrary, the animals treated with DZP as well as all animals treated with HEAM at 75mg/kg and 100mg/kg doses evidenced a potentiation of the Phenobarbital hypnotic effect (Fig. 1). Hypnotic effect and the time to fall asleep did not show difference when compared to DZP group ($p > 0.01$).

Rotarod test

This test was performed to investigate whether the fractions were acting via the neuromuscular junction to mediate the observed effect rather than acting centrally. Alteration was observed on rotarod test after the treatment with both 75mg/kg and 100mg/kg doses of ethanol extract of *C. dactylon* L., as like diazepam (2 mg/kg, i.p.) decreased the time of permanence on the bar in this test as compared to controls showing myorelaxant properties as expected (Table 4).

DISCUSSION:

In this work, the effects of different doses of ethanol extract of aerial parts of Cynodon dactylon *were* studied in several behavioral animal models, as like as rotarod, elevated plus maze, barbiturate-induced sleeping time and forced swimming tests to evaluate possible central activity. We decided to use i.p. administration of drugs because this pathway allows faster viability of the ethanol extract of aerial parts of Cynodon dactylon than oral pathway. The tests cited above are classical animal models for screening of activities on central nervous system and providing information about psychomotor performance, myorelaxant, anxiety, and depressant activities. The acute treatment with the extract of aerial parts of *C. dactylon* roots did not present antianxiety effects in animal models of anxiety, but it seems to have interesting effect in depression models.

Fig -2- Effect produced by different doses (50, 75, 100 mg/kg, i.p.) of HEAM on fall of time by rota-rod method

The elevated plus maze (EPM) test is the most popular test to search for new benzodiazepine-like anxiolytic agents[12]. In this study, the HEAM did not alter the performance of mice in the EPM test, suggesting that the extract, at 75 and 100 mg/kg doses used, did not interfere with anxiolytic activity. Diazepam, as expected, reduced the mouse's natural aversion to the open arms, and promoted the maze exploration thereof.

It is known that decrease in sleep latency and increase in sleeping time is classically related to central nervous system depressant drugs[10]. It was found that the i.p. administration of the plant extract induced sedative effects in mice. Our results showed that the HEAM in both doses decreased the sleeping latency time and increased the duration of sleep, suggesting a potentiation of Phenobarbital-induced sleeping time. However this test is not specific because compounds that interfere with biotransformation of Phenobarbital by cytochrome P-450 complex can show the same effects on central nervous system depressant drugs[13].

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The most widely used animal model for antidepressant screening is the forced swimming test. Although the relationship between immobility (a posture thought to reflect a state of "behavior despair" in which animals have given up the hope of scape) and depression remains controversial[14], it is well demonstrated that drugs with antidepressant activity reduce the time during which the animals remain immobile[15]. In our results, a significantly increase in the immobility time for mice for all the doses of HEAM was observed. In this way, the overall results seem to be predictive for depressant properties of the fractions.

Two doses of C. *dactylon* (i.e. 75 and 100mg/kg) alter motor coordination in the rotarod test, as like diazepam (2 mg/kg), which decreased the time of permanence on the bar, suggesting that the actions of this plant, probably, may be exerted through peripheral neuromuscular blockage.

In conclusion, we showed that acute treatment with ethanol extract of aerial parts of Cynodon dactylon potentiated the barbiturate induced sleeping time and presents depressant effect as demonstrated in the EPM and forced swimming tests. Data obtained from rotarod method suggest that, suggesting that the actions of this plant, probably, may be exerted through peripheral neuromuscular blockage. The overall results confirm the popular use of this plant. Further studies are necessary to elucidate the pharmacological action

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Nyctanthes arbortristis Linn. (Family Oleaceae), commonly known as *Harsingar* or Night Jasmine, is a common wild hardy large shrub or small tree[1,2]. *Nyctanthes arbortristis* L. is used by the rural people of Orissa in India to cure various ailments along with its use in Ayurveda, Sidha and Unani systems of medicines. It's claimed traditional uses have been proved on scientific basis using *in vitro* and *in vivo* experiments[3–7]. It has been established that its leaves possess hypnotic and tranquillizing activities[3,4] and its flowers possess sedative activity[5]. The present study is aimed at the CNS depressant activity of the ethanol extracts of different parts (flowers, barks, seeds and leaves) of *Nyctanthes arbortristis* L. along with an attempt explore the responsible mechanism.

The flowers, barks, seeds and leaves of *Nyctanthes arbortristis* L. were collected from the garden of BIT, Mesra, Ranchi and forests of Orissa. The herbarium of the plant (CNH/I-I (20)/2005-Tech-II/254) was authenticated as *Nyctanthes arbortristis* L. from Botanical Survey of India, Kolkata. After drying properly, the leaves, barks and seeds were powdered coarsely and then were extracted successively with petroleum ether, chloroform and ethanol (90%)[6,7], whereas its fresh flowers with ethanol (50%)[7]. The ethanol extracts were evaporated to dryness, having yield values 14%, 12.5%, 26.5% and 13% w/w, respectively. The water-soluble portions of the extracts were subjected to the pharmacological screening.

Adult male swiss mice weighing between 20-30 g, obtained from the animal house of BIT, Mesra, Ranchi, were used for this investigation. The Institutional Animals Ethics Committee (Registration No. 62/02/ac/CPCSEA) approved the experiments. Up and down or staircase method was followed for the estimation of acute toxicity of the water-soluble portion of the ethanol extracts of different parts of *Nyctanthes arbortristis* L. The dose was increased from 400 mg/kg to 2.0 g/kg, through intraperitoneal route of administration[8,9].

The gross behavioral study was performed 30 min after the administration of the extracts to get maximum information about the effect of the extracts on the central nervous system of mice[9]. The control group of animals was only treated with pentobarbital sodium (45 mg/kg). Each extracts and the reference compound were injected 30 min before pentobarbital sodium administration. The time taken for the loss of righting reflex was noted in all cases. The onset of sleep was recorded by noting the time of loss of righting reflex of mice and duration of sleep by noting time difference between loss of righting reflex and recovery time[10].

The monoamine neurotransmitters in brain were estimated following the method described by Shellenderger *et al*.[11] The animals were sacrificed half an hour after the administration of the extracts by cervical dislocation. After chilling the head of mice in chilling mixture of ice and $CaCl_2$, their brains were taken out and weighed[11,12].

For the estimation of dopamine, brains were homogenized with dry *n*-butanol at 0°. Clear supernatant solutions were extracted with 0.1 M phosphate buffer. To the phosphate buffer extract 4% EDTA, Iodine solution, alkaline sulfite and 5N acetic acid were added and then heated. After cooling, the intensities of fluorescence were determined by the help of photofluorometer[11,12].

For the estimation of serotonin, brains were homogenized with 0.1N HCl at 0° a