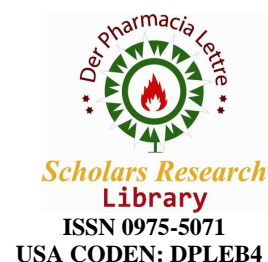




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Comparative study of antidepressant activity of methanolic extract of *Nardostachys Jatamansi* DC Rhizome on normal and sleep deprived mice

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ABSTRACT

Depression is affecting around 5% of the population. Furthermore, sleep deprivation causes depression in large number of people mainly working in night shifts like IT professionals and during exam periods for students. In the traditional systems of medicine, many plants and formulations have been used to treat depression for thousands of years. The present study was undertaken to evaluate the antidepressant activity of methanolic extract of *Nardostachys jatamansi* DC by forced swim test, tail suspension test and locomotor activity in inbred male Swiss Albino mice weighing 25-30g. The efficacy of the extract (200 and 400 mg/kg, p.o) was compared with the standard drug imipramine (10mg/kg, p.o) on normal and sleep deprived mice. Drugs were administered for 10 days in normal mice groups and the other groups were subjected to 24 hours sleep deprivation by using multiple platforms on 9th day and last dose was given 1 hour before experiment on 10th day. Duration of immobility was noted in both the models. MENJ (200 and 400 mg/kg, p.o) produced significant ($P < 0.001$) antidepressant like effect in normal and sleep deprived mice in both TST and FST and their efficacies were found to be comparable to imipramine (10 mg/kg, p.o). It did not show any significant change in locomotor functions of mice as compared to normal control. However, it significantly ($P < 0.01$) improves the locomotor activity in case of sleep deprivation which is comparable to normal control. This finding suggests that MENJ has dose dependent antidepressant activity and can also be used in patients suffering from depression due to sleep disturbances.

Keywords: Forced Swim Test, Tail Suspension Test, *Nardostachys jatamansi* DC, Depression.

INTRODUCTION

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a

depressive episode in their lifetime with suicide being one of the most common outcomes of depression [1, 2, 3]. To date, the efficacy of the drugs for depression is very limited so the need for newer, better-tolerated and more efficacious treatments is remaining high. Therefore, herbal therapies should be considered as alternative/ complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly [4]. This has been reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models.

The plant *Nardostachys jatamansi* DC of family Valerianaceae is a well known plant in the Indian medicinal system and has historically used in Ayurveda. *N. jatamansi* gives quick relieves from psychosis, maniac psychosis, syncope and hysteria [5, 6, 7], anti-parkinsonian [8], memory-enhancing [9, 48], anti-ischemic [10], anti-arrhythmic [11], hypolipidaemic [12], cardio protective [13], anti-estrogenic [14], hepatoprotective [15], anti-asthmatic [16], antifungal [17], nematicidal [18] and antibacterial activities [19].

MATERIALS AND METHODS

1.1. Collection and Authentication of Plant Material

The rhizomes of *Nardostachys jatamansi* DC of family Valerianaceae were purchased from local market of herbs in Chennai, Tamilnadu. The plant material was identified and authenticated by Dr. Sasikala Ethirajulu, Asst. Director (Pharmacognosy), Siddha Central Research Institute, Arumbakkam, Chennai-600106. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai-97.

1.2. Preparation of Methanolic Extract *Nardostachys jatamansi* DC rhizome

The rhizomes of *Nardostachys jatamansi* DC were cleaned and removed the adherent sand and dust particles. It was dried and made into a coarse powder with the help of electric grinder. About 500gm of grinded plant material was subjected to soxhlet extraction (60-70⁰C) employing methanol as solvent [20]. The solvent was evaporated at 40⁰ C to obtain a viscous mass. The dried molten mass was chocolate brown in color and was stored in refrigerator until use. The percentage yield of the extract was 6.78%.

1.3. Preliminary Phytochemical Screening

For preliminary phytochemical screening, the methanolic extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds and tannins, flavonoids, saponins, proteins and amino acids using standard procedure [21, 22].

1.4. Drugs and Chemicals

The standard antidepressant drug imipramine (M/s. Alkem Ltd. Mumbai) was purchased from Retail Pharmacy; Methanol was obtained from institutional store and was of analytical grade. All the drug and extract were suspended in Gum acacia (1% in water) used as vehicle and applied orally [23].

1.5. Animals

Inbred Swiss albino male mice (20-25 gm.) were obtained from the animal house of C.L.Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited, Bangalore) and drinking water was provided *ad libitum*. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Institutional Animal Ethical

Committee (IAEC) approved the protocol of the study with reference number IAEC/XXVIII/04/CLBMCP/2009-2010, Dated 17/12/2009.

1.6. Acute Toxicity Study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, neurological toxicity and mortality 14 days [24].

1.7 Experimental Design

On the 1st day of the experiment, the animals were divided randomly into eight groups of six animals in each.

Group I: Received the vehicle, 1% gum acacia (10ml/kg, p.o) and served as the control group.

Group II: Received the vehicle, 1% gum acacia (10ml/kg) and subjected for 24 hours sleep deprivation on 9th day and served as negative control.

Group III: Received MENJ (200 mg/kg, p.o)

Group IV: Received MENJ (400mg/kg, p.o)

Group V: Received imipramine (10mg/kg, p.o)

Group VI: Received MENJ (200mg/kg, p.o) and are subjected for 24 hours sleep deprivation on 9th day.

Group VII: Received MENJ (400mg/kg, p.o) and are subjected for 24 hours sleep deprivation on 9th day.

Group VIII: Received imipramine (10mg/kg, p.o) and are subjected for 24 hours sleep deprivation on 9th day.

Behavioural evaluation was carried out 60 minutes post drug/vehicle administration on 10th day. The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST [25].

1.8. Tail Suspension Test (TST)

In tail suspension test [26, 27, 28], the animals were hung by the tail on a plastic string 50 cm above the surface with the help of an adhesive tape, placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test.

1.9. Forced Swim Test (FST)

In forced swim test [27, 28, 29, 47], each animal was placed individually in a glass chamber (25 X 15 X 25 cm³) filled with water up to a height of 15 cm and maintained at 26⁰C±1⁰C. At this height of water, 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 to FST because “used water” has been shown to alter the behavior [30]. Animals were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period because each animal showed vigorous movement during initial 2 min period. The duration of the mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test. The test was conducted in a dim lighted room and each mouse was used only once in the test [31, 32].

1.10. The Locomotor Activity

The locomotor activity was measured by using an Actophotometer [33, 34]. The actophotometer consisted of a square arena (30 × 30 × 25 cm) with wire mesh bottom, in which the animal moves.

Six lights and six photocells were placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The locomotor activity was measured for a period of 10 min. technically its principle is that, a photocell is activated when the rays of light falling on the photocells are cut off by animals crossing the beam of light. As the photocell activated, a count is recorded. The photocells are connected to an electronic automatic counting device which counts the number of 'cut offs'.

1.11. Sleep Deprivation (SD) Method

This method of sleep deprivation used was an adaptation of the multiple platform method, originally developed for rats [45]. The animals which were subjected for 24 hours sleep deprivation was done by multiple platform method [35, 36, 37]. Each mice was kept on small platform (3cm diameter) each in a water tank like water maze (41 X 34 X 16:5 cm) and water is kept 1cm below the platform by giving bright light whole the night. . In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. Food and water were made available through a grid placed on top of the water tank [46]. This is based on the principle that when the mice will get sleepy and drowsiness they fall on water due to muscle relaxation and after falling on water they wake up quickly.

1.12. Statistical analysis

The mean \pm S.E.M. values were calculated for each group. The data were analyzed using Graph pad software version 5 by one-way ANOVA followed by Dunnet's multiple comparison test. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

2.1. Preliminary Phytochemical Screening

The preliminary phytochemical analysis of MENJ showed that the plant contains alkaloids, carbohydrates, sterols, saponins, phenolic compounds and tannins.

2.2. Acute toxicity Study

Acute oral toxicity studies revealed the non-toxic nature of MENJ. There was no morbidity observed or any profound toxic reactions found at a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

2.3. Tail suspension Test (TST)

Results were given in table 1. A significant ($P < 0.001$ for 200mg/kg, MENJ and $P < 0.01$ for 400mg/kg MENJ) decrease in the duration of immobility was seen with the standard drug imipramine ($P < 0.001$) and MENJ in all the tested doses as compared to the normal mice. 200 mg/kg of MENJ has a decrease in duration of immobility in sleep deprived mice when compared with imipramine 10 mg/kg more significantly results are seen ($p < 0.001$) for all doses of MENJ and imipramine in sleep deprived mice.

2.4. Forced Swim Test (FST)

Results were given in table 2. A significant ($P < 0.001$) decrease in the duration of immobility was seen with MENJ in all the tested doses when compared with the normal and sleep deprived mice. The standard drug imipramine ($P < 0.01$) and 200 mg/kg and 400 mg/kg of MENJ has a significant decrease in the duration of immobility in sleep deprived mice.

2.5. Effect on Locomotor activity

Results were given in table 3. The 200mg/kg and 400 mg/kg MENJ showed non significant effect on locomotor activity in normal control and normal drug treated animals but it shows significant effect on negative control and sleep deprived (SD) animals. Methanolic extract of *Nardostachys jatamansi* DC has significantly ($P < 0.01$) improved the locomotor activity in case of sleep deprivation which is comparable to normal control. Where standard antidepressant drug imipramine (10mg/kg, p.o) has more significantly ($P < 0.001$) improved the locomotor activity in mice.

In the present study, methanolic extract (200 and 400 mg/kg, p.o) administered for 10 successive days to mice, produced significant antidepressant like effect in normal and sleep deprived mice in both TST and FST and their efficacies were found to be comparable to imipramine (10 mg/kg, po). TST and FST are two of the most commonly used behavioral tests in rodents for evaluating drugs having antidepressant-like activity [38, 39, 40]. These tests are quite sensitive and relatively specific to all major classes of antidepressants.

The antidepressant-like effect of methanolic extract of *N. jatamansi* seems not to be associated with any motor effects since it did not show any significant change in locomotor functions of mice when compared with normal control. It confirms the assumption that the antidepressant-like effect of the extract is specific and not the false positive. However, it has significant effect on sleep deprived mice. That may be related to suppression of neurotransmitter level mainly serotonin, melatonin and increased acetylcholinestase activity due to sleep deprivation [41, 42, 43, 44].

Moreover, Methanolic extract of *Nardostachys jatamansi* DC has significantly improved the locomotor activity in case of sleep deprivation which is comparable to normal control. Effect of MENJ (200&400 mg/kg, p.o) is comparable to improve locomotor activity due to sleep deprivation to the standard antidepressant drug imipramine (10mg/kg/p.o).

Table 1: Effect of MENJ on immobility time in the Tail Suspension Test (TST) using mice

Group	Drug Treatment	Duration of Immobility period Mean (Sec) \pm SEM
I	Vehicle control 1% gum acacia (10ml/kg, p.o)	172.7 \pm 4.38
II	Negative control; Vehicle, 1% gum acacia (10ml/kg, p.o) + SD	218.8 \pm 5.42
III	MENJ(200mg/kg,p.o)	117.7 \pm 5.43, ^a ***
IV	MENJ (400mg/kg, p.o)	138.7 \pm 6.64, ^a **
V	Imipramine(10mg/kg, p.o)	124.0 \pm 6.19, ^a ***
VI	MENJ(200mg/kg,p.o) + SD	150.7 \pm 5.27, ^b ***
VII	MENJ (400mg/kg, p.o) + SD	168.5 \pm 9.20, ^b ***
VIII	Imipramine(10mg/kg, p.o)+SD	178.7 \pm 7.58, ^b ***

Values represented in (Mean \pm S.E.M. (n=6), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ^a P compared vs. Group I and ^b P compared vs. Group II).

Table 2: Effect of MENJ on immobility time in the Forced swim test (FST) using mice

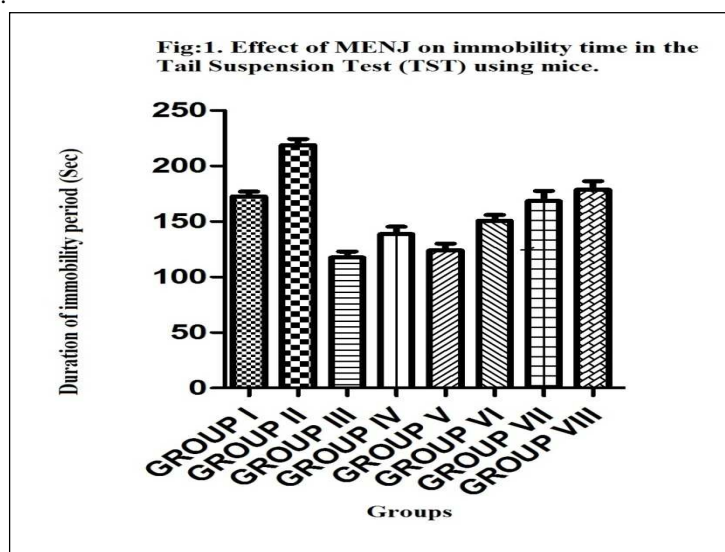
Group	Drug Treatment	Duration of Immobility period Mean (Sec) ± SEM
I	Normal control; Vehicle, 1% gum acacia (10ml/kg, p.o)	163.3 ± 5.45
II	Negative control; Vehicle, 1% gum acacia (10ml/kg, p.o) + SD	211.2 ± 6.30
III	MENJ(200mg/kg,p.o)	99.0 ± 6.37, ^a ***
IV	MENJ (400mg/kg, p.o)	108.2 ± 6.36, ^a ***
V	Imipramine(10mg/kg, p.o)	105.3 ± 6.97, ^a ***
VI	MENJ(200mg/kg,p.o) + SD	149.3 ± 6.64, ^b ***
VII	MENJ (400mg/kg, p.o) + SD	164.8 ± 7.69, ^b ***
VIII	Imipramine(10mg/kg, p.o) +SD	176.3 ± 7.29, ^b **

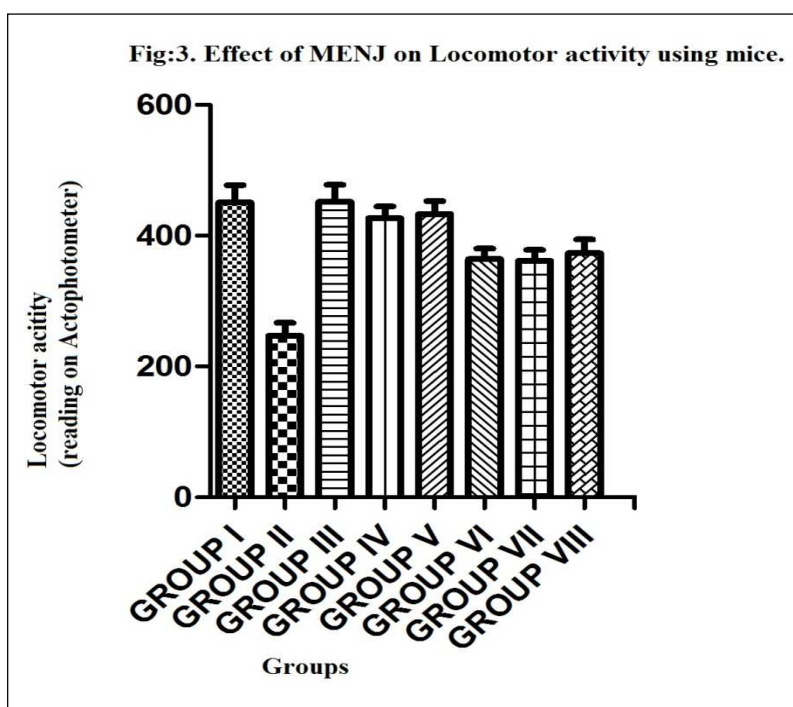
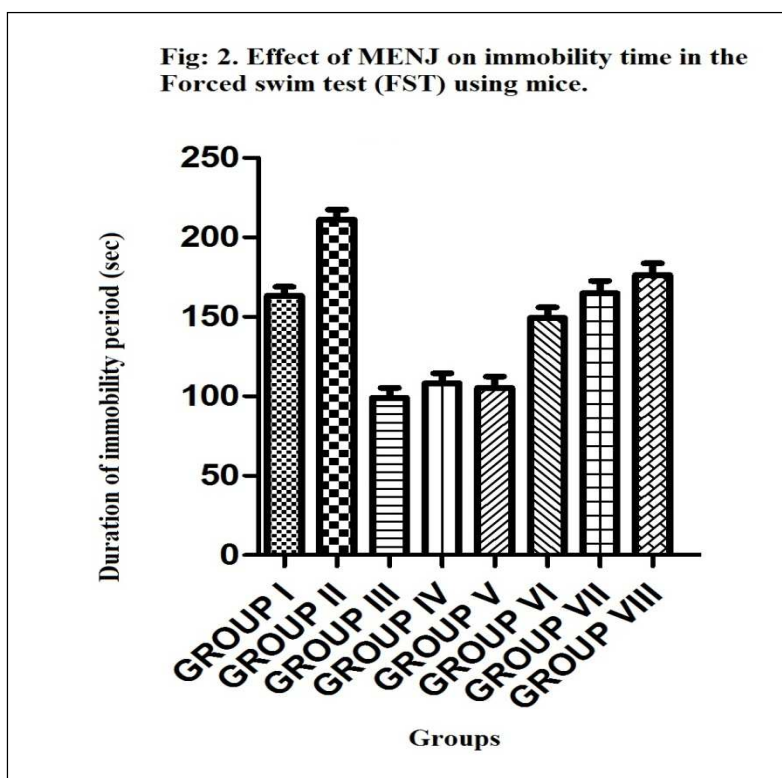
Values represented in (Mean ± S.E.M. (n=6), *P<0.05, **P<0.01, ***P<0.001, ^a P compared vs. Group I and ^b P compared vs. Group II).

Table 3: Effect of MENJ on Locomotor activity using mice

Group	Drug Treatment	Reading of Locomotor activity
I	Normal control; Vehicle, 1% gum acacia (10ml/kg, p.o)	450.8 ± 26.49
II	Negative control; Vehicle, 1% gum acacia (10ml/kg, p.o) + SD	247.2 ± 19.44
III	MENJ(200mg/kg,p.o)	451.8 ± 26.05, ^a
IV	MENJ (400mg/kg, p.o)	426.7 ± 18.10, ^a
V	Imipramine(10mg/kg, p.o)	433.0 ± 19.61, ^a
VI	MENJ(200mg/kg,p.o) + SD	364.3 ± 16.03, ^b **
VII	MENJ (400mg/kg, p.o) + SD	361.7 ± 16.66, ^b **
VIII	Imipramine(10mg/kg, p.o)+SD	373.2 ± 21.30, ^b ***

Values represented in (Mean ± S.E.M. (n=6), *P<0.05, **P<0.01, ***P<0.001, ^a P compared vs. Group I and ^b P compared vs. Group II).





CONCLUSION

It can be concluded that methanolic extract of *Nardostachys jatamansi* DC, has dose dependent antidepressant activity and can also be used in patients suffering from depression due to sleep disturbances. Which also improve the locomotor activity in sleep deprived mice. So, *Nardostachys jatamansi* DC will be an important plant to carry research for antidepressant

activity and can be a drug of choice for people who suffers from acute sleep deprivation mainly IT professionals, night time working people and sleep deprivation during exam period for students. However further studies are required to know the exact mechanism of action of MENJ as antidepressant.

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