Research Article

NARDOSTACYS JATAMANSI DC PROTECTS FROM THE LOSS OF MEMORY AND COGNITION DEFICITS IN SLEEP DEPRIVED ALZHEIMER'S DISEASE (AD) MICE MODEL

Habibur Rahman*, Palayyan Muralidharan
Dept. of Pharmacology, C. L. Baid Metha College of Pharmacy, OMR, Thoraipakkam, Chennai-97, India.
*Corresponding author's E-mail: habiburruh@gmail.com

ABSTRACT

Different research studies and hypothesis suggest that there is a strong linkage between Insomnia and Alzheimer's disease (AD). Statistical data revels that sleep disturbances and dementia increases with advancing age and in young and middle-aged adults who *suffer* from *insomnia* are 11 times more *likely to develop AD* and depression in their later life. The present study is designed to evaluate anti amnesic activity by studying the effect of methanolic extract of *Nardostacys jatamansi* DC (MENJ) rhizome on sleep deprived (SD) amnesic mice. Animals were pretreated with MENJ for 14 days (200 and 400 mg/kg, orally) and Piracetam (200 mg/kg, orally) as standard drug and followed by 5 days sleep deprivation using multiple platform method. Behavioral changes were evaluated against normal control and negative control animals using Elevated plus maze, passive shock avoidance, Y-maze, Morris water-maze and object recognition tests. MENJ at doses of 200 mg/kg and 400mg/kg treated groups showed a significant improvement in learning and cognition parameters in behavioral tests. These findings suggest MENJ exerts a protective effect against loss of memory and cognitive deficits due to sleep deprivation.

Keywords: Alzheimer's disease (AD), *Nardostacys jatamansi* DC, sleep deprived (SD), Morris water-maze, Y-maze.

INTRODUCTION

Alzheimer's disease (AD) is a slow progressive neurodegenerative disease of the brain that is characterized by impairment of memory and eventual disturbances in reasoning, planning, language, and perception. Many scientists believe that Alzheimer's disease results from an increase in the production or accumulation of a specific protein (β-amyloid protein) in the brain that leads to nerve cell death[1-4].

Sleep disorders affect a large part of the general population, with up to 56% of individuals reporting sleeping problems in the USA [5]. Impairment of sleep causes daytime sleepiness and mental dysfunction which leads to various health and socioeconomic issues. The prevalence of insomnia increases with age, and a remarkably strong link exists with psychiatric disorders, notably depression and dementia [6-8]. About 45% of AD patients have disruptions in their sleep and sun-downing agitation [9]. Young and middle-aged adults who *suffer* from *insomnia* are 11 times more *likely to develop Alzheimer's* and depression in their later life [10]. *Chronic* lack of sleep may promote the development of *AD* and for *people* suffering from *insomnia* and other sleep disorders increases the risk of *Alzheimer's in later life* [11, 12].

The literature evidences revels that Sleep deprivation in experimental animal can be used as Alzheimer's disease model. Sleep deprivation results in memory impairment due to decrease in the extra cellular signal-regulated kinase phosphorylation in the hippocampus of rat brain [13]. Sleep may either actively promote memory formation, or alternatively, sleep may provide optimal conditions of non-interference for consolidation. There is increasing evidence that sleep may be important or learning and memory, whereas a sleep deficit results in performance impairment both in rodents and humans [14, 15]. Numerous studies have demonstrated that sleep deprivation in laboratory animals produces memory deficits in several behavioral models, such as avoidance tasks [16,17], Morris water maze task [18-19], and radial maze task [20] and in object recognition test [21].

The plant *Nardostachys jatamansi* DC of family Valerianaceae is a well known plant in the Indian traditional medicinal system and has historically used in Ayurveda as Medhya (Brain tonic), Rasayana (Rejuvenative to the mind), Nidrajnana (Promotes sleep) and Manasrogaghna (Alleviates mental diseases) [22, 23]. *N. jatamansi* DC gives quickly relieves from psychosis, maniac psychosis, syncope and hysteria [24], anti-parkinsonism [25], memory-enhancing [26, 27], anti- cerebral ischemic [28], antiarrhythmic [29], hypolipidaemic [30], cardioprotective [31], anti-estrogenic [32], hepatoprotective [33], anti-asthmatic [34], antifungal [35], nematicidal [36], antibacterial [37] and antidepressant [38].

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The rhizomes of *Nardostachys jatamansi* DC of family Valerianceae 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.

Preparation of Methanolic Extract *Nardostachys jatamansi* DC rhizome (MENJ)

The rhizomes *of Nardostachys jatamansi* DC were cleaned and removed 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 ($60\text{-}70^0$C) employing methanol as solvent [39]. The solvent was evaporated at 40^0C 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%.

Preliminary Phytochemical Screening

For preliminary phytochemical screening; the methanolic extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and amino acids using standard procedure [40, 41].

Drugs and Chemicals

Piracetam (NOOTROPIL® Tab, UCB India Pvt. Ltd., Mumbai) was used as standard neuroprotective drug for amnesia and drug was purchased from Retail Pharmacy from Chennai; Methanol was obtained from institutional store and was of analytical grade.

Animals

Inbred Swiss albino male mice (20-25 gm.) of 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. The female mice were not considered because their changes in the concentration of estrogen and progesterone may influence in the cognitive behavior of the animal[42]. Institutional Animal Ethical Committee (IAEC) approved the protocol of the study with reference number IAEC/XXIX/04/CLBMCP/2009-2010, Dated 20/04/2010.

Experimental Design

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

Group I: Normal control; Vehicle (1% gum acacia).

Group II: Negative control; Vehicle (1% gum acacia) and subjected for 5 days sleep deprivation from 15th day to 19th day.

Group III: Pretreatment with MENJ (200 mg/kg, p. o) for 14 days and 5 days sleep deprivation from 15th day to 19th day.

Group IV: Pretreatment with MENJ (400 mg/kg, p. o) for 14 days and 5 days sleep deprivation from 15th day to 19th day.

GROUP V: Pretreatment with Piracetam (200 mg/kg, p.o) for 14 days and 5 days sleep deprivation from 15th day to 19th day.

All the treatment groups are pretreated with respective drug and dosage for 14 days and followed by 5 days sleep deprivation. During sleep deprivation all the groups received respective drugs and normal control group received vehicle. The extract (MENJ 200mg/kg, MENJ 400mg/kg), standard drug (Piracetam 200 mg/kg) and vehicle (Gum acacia 1% in water) were given orally by using intra-gastric catheter at dose (10ml/kg) [25] up to 19th day, where the last dose was given 60 min prior to behavioral Trainings and tests.

Elevated Plus Maze

The apparatus consists of two open arms (35 X 6 cm) and two enclosed arms (35 X 6 X 15 cm). The arm was connected together with a central square of 5 X 5 cm. The maze was elevated to a height of 100 cm. The maze was placed inside a light and sound attenuated room. Mice were placed individually at the end of an open arm of elevated plus maze (EPM) facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms Transfer Latency (TL) was recorded [43].

Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day. On the 19th day, 90 min after the treatment of last dose first trial is given and after 24 hr TL was noted for second time (i.e. on 20th day)[44, 45]. The inflexion ratio was calculated by the formula [46].

$$IR = (L_0 - L_1)/L_0,$$

Where, L_0 is the initial transfer latency (TL) in Sec on first time,

L_1 is the transfer latency (TL) in Sec on 2nd time.

Decrease IR indicates the induction of amnesia, and increased IR indicates in improvement in cognition and memory impairment.

Passive Shock Avoidance Test

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 X 27 X 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart) with a wooden platform (10 X 7 X 1.7 cm) in the centre of the grid floor. Electric shock (20 V, AC) was delivered to the grid floor[47, 48]. During Training session, each mouse was gently placed on the wooden platform set in the centre of the grid floor, when the mouse stepped down and placed all its paw on the grid floor,

shocks were delivered for 15 sec and the Step-Down Latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range of 2-15 seconds during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. During second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 seconds and subjected to retention test.

On the 19th day, 90 min after the treatment of last dose training was given and memory retention was examined after 24 h (i.e. on 20th day) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds [27, 49].

Y Maze Test

Immediate working memory performance was assessed by recording spontaneous alternation behavior in a single session in a Y-maze made up of black painted wood. Each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top and converged in an equilateral triangular central area. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The series of arm entries was recorded visually. Entry was considered to be completed when the hind paws of the mouse had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B and C) on overlapping triplet sets [50]. The percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as an 'alternation' to estimate short-term memory. Percentage alternation was calculated as the ratio of actual to possible alternation (defined as the total number of arm entries minus two), multiplied by 100 as shown

% Alternation = {(No. of alternations) / (Total arm entries – 2)} X 100.

On the 19th day, 90 min after the treatment of last dose arm entries was recorded visually and percentage alteration was calculated [51].

Morris Water Maze Test

The Morris water maze test is preformed to evaluate spatial working and reference memory [18, 52]. In this model the animals are placed into a large circular pool of water and they can escape onto a hidden platform. The platform is hidden by its placement just below the water surface and by opaque water. Therefore the platform offers no local cues to guide escape behavior. The animal can escape from swimming by climbing onto the platform and with time the animal apparently learns the spatial location of the platform from any starting position at the circumference of the pool. Morris water maze consists of a large circular tank made of black opaque polyvinyl chloride or hard board coated with fiberglass and resin and then surface painted white (1.8-2.0 meter in diameter and 0.4-0.6 meter high). The pool is filled up to a height of 30 cm with water maintained at around 25^0C and rendered opaque by addition of a small quantity of milk or nontoxic white color. The pool is provided with filling and draining facilities and is mounted at waist level. The tank is hypothetically divided into four equal quadrants and a platform ($11cm^2$) of 29 cm height is located in the centre of one of these four quadrants. The platform remains fixed in the position during the training session. Each animal is subjected to four consecutive trials for four days (from 15th to 18th day) during which they are allowed to escape on to the hidden platform and allowed to remain there for 20 sec. Escape latency time to locate the hidden platform in water maze is noted as an index of acquisition or learning. In case the animal is unable to locate the hidden platform within 120 sec, it is gently guided by hand to the platform and allowed to remain there for 20 sec. On the 19th day, 60 min after last dose, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval and measured [53].

Object Recognition Test

The apparatus comprises of a wooden box (70 X 60 X 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trail. The objects to be discriminated were placed at diagonally opposite corners of the box and were in two different shapes: pyramid of 8 cm side and cylinder of 8 cm height. On day 0, animals were allowed to explore the box without any object for 2 minutes. On first trail (T1), two identical objects were presented in two opposite corners of the box, and the time taken by each mouse to complete 20 seconds exploration was measured. Exploration meant directing the nose at a distance less than 2 cm to an object and / or touching with the nose. During the second trail (T2, 90 minutes after T1), a new object replaced one of the objects present in T1, and mice were left in the box for 5 minutes. The time spent for exploring new (N) and familiar (F) objects were recorded separately. Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (F or N) and the position of the two objects during T2 and cleaning them carefully [54]. On the 19th day, 60 min after last dose first trial was given and which was followed by 24 hr acquisition period and then final reading are taken [21].

Sleep Deprivation (SD) Method

This method of sleep deprivation used was an adaptation of the multiple platform method, originally developed for rats [55]. The animals which were subjected for 5 days sleep deprivation by multiple platform method [56]. 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 [57]. A 100-W light illuminates the chamber during the period of sleep deprivation [58]. 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.

Statistical analysis

The mean ± 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 t test. $P < 0.05$ (95% confidence limit) was considered to be statistically significant.

RESULTS AND DISCUSSION

Elevated Plus Maze

The results are given in Table 1 and plotted graph is shown in Fig 1. The Inflexion ratio (IR) of the Group II (sleep deprived) animals were significantly decreased in comparison with the Group I (normal control) animals ($p < 0.01$). MENJ (200 & 400 mg/kg) dose dependently increased IR in Group III & Group IV significantly ($p < 0.01$)and is comparable with Piracetam (200mg/kg) Group V compared with group II. Decrease IR (sleep deprived, Group II) indicates the induction of amnesia, and increased IR (Treatment groups) indicates protection from memory loss due to sleep deprivation and improved in cognition and memory impairment.

Step down Passive Shock avoidance test

The results are given in Table 2 and plotted graph is shown in Fig 2. The short term memory (STM) of the animals of Group II was found to be reduced in comparison with Group I animals in terms of Step down Latency ($p < 0.001$). Groups III, IV and V animal treated with MENJ (200 and 400 mg/kg) and Piracetam (200 mg/kg) restored the reduced STM to near normal animals. MENJ 400 mg/kg showed significant ($p < 0.01$)

improvement in comparison with Group II animals. The increase in SDL indicates protection from sleep deprived memory loss.

Y maze task

The results are given in Table 3 and plotted graph is shown in Fig 3. The percentage of alteration was reduced in Group II (sleep deprived) when compared with Group I animals significantly ($P < 0.001$). Groups III, IV and V animal treated with MENJ (200 and 400 mg/kg) and Piracetam (200 mg/kg) showed significant increased percentage alteration that indicates protection from loss of spatial working memory due to sleep deprivation and the decrease in percentage alteration indicates decrease of spatial working memory.

Morris water maze task

The results are given in Table 4 and plotted graph is shown in Fig 4. The escape latency of Group II (sleep deprived) animals were increased in comparison with Group I (normal control) animals significantly ($p < 0.001$). Groups III, IV and V animal treated with MENJ (200 and 400 mg/kg) and Piracetam (200 mg/kg) showed significant decrease in latency to escape onto the hidden platform in comparison with the Group II animals ($p < 0.001$) indicates protection from loss of memory and non-spatial working memory due to sleep deprivation.

Object recognition task

The results are given in Table 5 and plotted graph is shown in Fig 5(a) and Fig 5(b). Time needed for exploring a novel as well as familiar object was increased in Group II (sleep deprived) animals in comparison with Group I animals significantly ($p < 0.001$). Groups III, IV and V animal treated with MENJ (200 and 400 mg/kg) and Piracetam (200 mg/kg) showed significant decrease in both familiar and new object in comparison to Group II. The decrease in exploration time indicates protection from loss in learning and memory retention of object due to sleep deprivation.

Table 1: Inflexion ratio on Elevated plus Maze

Groups	Treatment	Inflexion ratio (IR); (Mean ± SEM)
I	Normal Control, Vehicle	0.641 ± 0.005
II	Negative Control, Vehicle; SD	$0.126 \pm 0.013^{a***}$
III	MENJ 200 mg/kg; SD	$0.342 \pm 0.011^{b***}$
IV	MENJ 400 mg/kg; SD	$0.448 \pm 0.012^{b***}$
V	Piracetam 200 mg/kg; SD	$0.403 \pm 0.004^{b***}$

Values represented in (Mean ± S.E.M, n=6), [ns] Non Significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; a-compared Group I vs. Group II, b-compared Group II vs. Group III, IV & V.

Table 2: Step down Latency (SDL on Step down Passive Shock avoidance test

Groups	Treatment	Step down Latency in Sec;(Mean ± SEM)
I	Normal Control, Vehicle	50.14 ± 2.43
II	Negative Control, Vehicle; SD	$29.17 \pm 2.10^{a***}$
III	MENJ 200 mg/kg; SD	34.67 ± 2.52^{ns}
IV	MENJ 400 mg/kg; SD	$41.33 \pm 2.49^{b**}$
V	Piracetam 200 mg/kg; SD	$45.00 \pm 2.36^{b***}$

Values represented in (Mean ± S.E.M, n=6), [ns] Non Significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; a-compared Group I vs. Group II, b-compared Group II vs. Group III, IV & V.

Table 3: Percentage alteration on Y maze task

Groups	Treatment	Percentage alteration; (Mean ± SEM)
I	Normal Control, Vehicle	57.50 ± 2.89
II	Negative Control, Vehicle; SD	34.00 ± 1.50^{a}***
III	MENJ 200 mg/kg; SD	45.00 ± 2.68^{b}*
IV	MENJ 400 mg/kg; SD	48.83 ± 3.10^{b}**
V	Piracetam 200 mg/kg; SD	50.83 ± 2.73^{b}***

Values represented in (Mean ± S.E.M, n=6), [ns] Non Significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; a-compared Group I vs. Group II, b-compared Group II vs. Group III, IV & V.
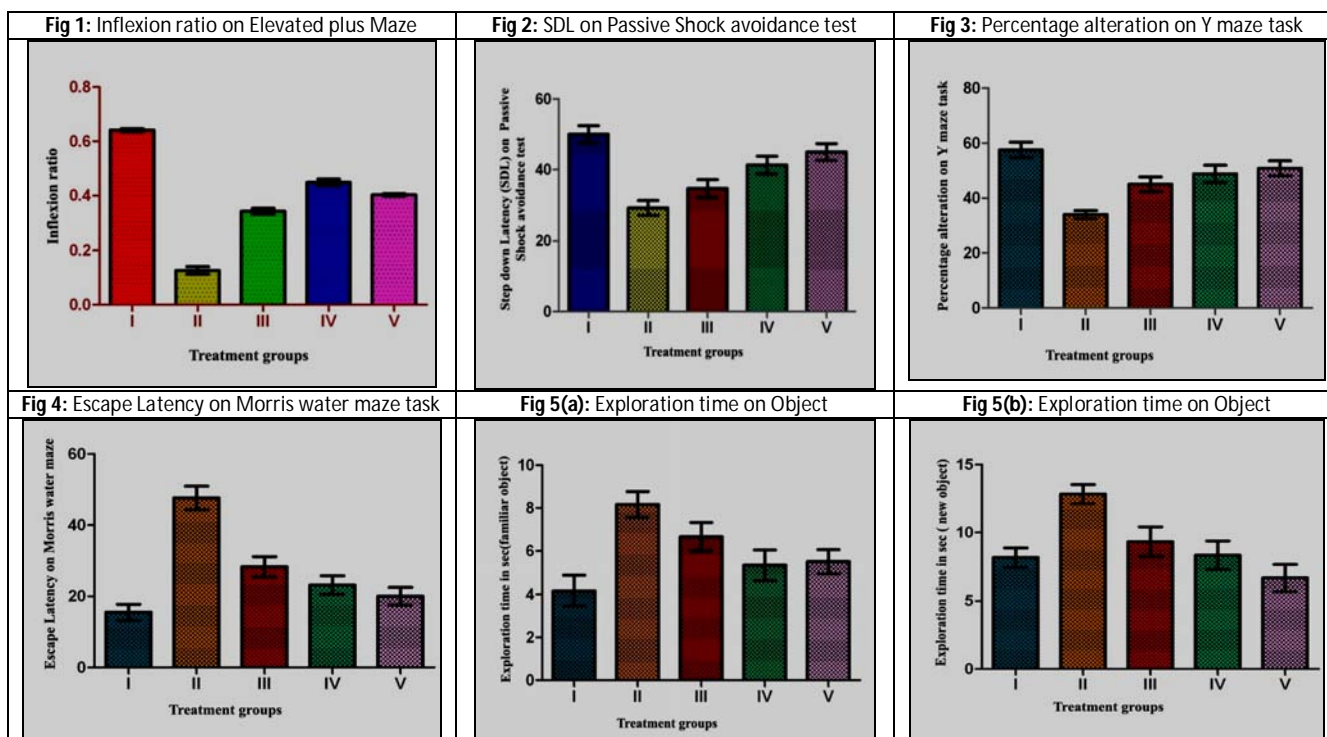
Table 4: Escape Latency on Morris water maze task

Groups	Treatment	Escape Latency in Sec;(Mean ± SEM)
I	Normal Control, Vehicle	15.50 ± 2.26
II	Negative Control, Vehicle; SD	47.67 ± 3.32^{a}***
III	MENJ 200 mg/kg; SD	28.33 ± 2.84^{b}***
IV	MENJ 400 mg/kg; SD	23.17 ± 2.58^{b}***
V	Piracetam 200 mg/kg; SD	20.00 ± 2.50^{b}***

Values represented in (Mean ± S.E.M, n=6), [ns] Non Significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; a-compared Group I vs. Group II, b-compared Group II vs. Group III, IV & V.

Table 5: Exploration time on Object recognition task

Groups	Treatment	Exploration time in sec(familiar object); (Mean ± SEM)	Exploration time in sec (new object); (Mean ±SEM)
I	Normal Control, Vehicle	4.16 ± 0.70	8.16 ± 0.70
II	Negative Control, Vehicle; SD	8.16 ± 0.60^{a}***	12.83 ± 70^{a}**
III	MENJ 200 mg/kg; SD	6.66 ± 0.66^{bns}	9.33 ± 1.08^{b}*
IV	MENJ 400 mg/kg; SD	5.33 ± 0.71^{b}*	8.33 ± 1.05^{b}**
V	Piracetam 200 mg/kg; SD	5.50 ± 0.56^{b}*	6.66 ± 0.98^{b}***

Values represented in (Mean ± S.E.M, n=6), [ns] Non Significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; a-compared Group I vs. Group II, b-compared Group II vs. Group III, IV & V.

Fig 1: Inflexion ratio on Elevated plus Maze

Fig 2: SDL on Passive Shock avoidance test

Fig 3: Percentage alteration on Y maze task

Fig 4: Escape Latency on Morris water maze task

Fig 5(a): Exploration time on Object

Fig 5(b): Exploration time on Object

Sleep loss is a common feature of many sleep disorders in humans, and for this reason analyses of behavioral and neurochemical effects seen in animal models of sleep deprivation are of considerable interest. Different research had shown the important of sleep for cognition and memory. The mechanisms underlying learning and memory deficits following sleep deprivation are not understood clearly at present. It is found that the synthesis and secretion of melatonin and other neurotransmitter occur mainly in sleep cycle. Currently Alzheimer's patients responding well with melatonin treatment and shows lots of improvement in cognition and memory [59]. Melatonin was recently reported to be an effective free radical scavenger and antioxidant. Melatonin has been shown prophylactically to reduce amyloid β protein toxicity of Alzheimer's disease, to reduce oxidative damage in several models of Parkinson's disease [60]. Melatonin production declines so drastically with age, probably explains many of the sleep disturbances seen in the elderly and be a cause of Alzheimer's disease [60]. Melatonin also reduces the hyperphosphorylation of tau protein, which leads to the neurofibrillary tangles of Alzheimer's disease [61]. Different research studies suggest that sleep deprivation would reduce the antioxidant defenses [62]. Sleep might involve the elimination of toxic compounds (e.g. free radicals) and the replenishment of energy stores. Increase oxidative stress in hippocampus reported to be responsible for the passive avoidance deficit induced in mice by sleep deprivation. Indeed, the repeated treatment with three different antioxidant agents revert the deficit showed in the test session in sleep-deprived mice [63]. Increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases.

In our study, we observed that MENJ (200 and 400mg//kg) has shown significant protection from loss of memory and cognition impairment due to sleep deprivation. Earlier, pharmacological investigation on *Nardostachys jatamansi* DC suggests about its antioxidant effects [64], Acetyl cholinesterase inhibitory activity[26], enhances the function of γ-amino

Butyric acid (GABA) [65] and other neuroprotective activities where as Piracetam (a GABA derivative) showed it neuroprotection by modulating GABAminergic neurotransmission mainly [66]. So, MENJ may protects from the loss of memory and cognition deficits in sleep deprived AD mice model due to these mention mechanism. However, further studies are required to know the exact mechanism involved for its neuro protective activity.

CONCLUSION

N. jatamansi DC is important plant of Indian Traditional Systems of Medicine. The plant is major ingredient of Ayurvedic formulations for the treatment of central nervous disorders. From the above observations we can conclude that methanolic extract of *N. jatamansi* DC possesses protective activity from the loss of memory and cognition deficits at both the dose level which is comparable with the standards. In, conclusion, it can be useful drugs for the treatment for AD patients along with suffering from sleep disorders.

REFERENCES

1. Kontush A, Amyloid-β: An antioxidant that becomes a pro-oxidant and critically contributes to Alzheimer's disease. Free Radical Biology & Medicine, 2001; 31(9):1120-1131.
2. Nitta A, Itoh A, Hasegawa T, Nabeshima T. β-amyloid protein induced Alzheimer's disease animal model. Neurosci Lett, 1994; 170:63-66.
3. Yanker B, Duffy Y, Kirschner D, Neurotropic and neuro toxic effects of Amyloid-β protein: Reversal by tachykinin neuro peptides. Science, 1990; 250:279-282.
4. Yan Z, Feng J., Alzheimer's disease: interactions between cholinergic functions and β-amyloid., Curr Alzheimer Res, 2004; 1:241-248.
5. Leger D, Poursain B, Neubauer D, Uchiyama M, An international survey of sleeping problems in the general population. Curr.Med. Res. Opin., 2008; 24, 307–317.
6. Costa e Silva J.A. Sleep disorders in psychiatry. Metabolism, 2006, 55, S40–44.
7. Lustberg L., Reynolds C F, Depression and insomnia: questions of cause and effect. Sleep Med. Rev. 2000, 4, 253–262.
8. Zammit G.K, The prevalence, morbidities, and treatments of insomnia. *CNS* Neurol. Disord. Drug Targets, 2007, 6, 3–16.
9. Cardinali DP, Brusco LI, Liberczuk C, Furio AM, The use of melatonin in Alzheimer's disease, Neuro Endocrinol Lett, 2002 Apr; 23 Suppl 1:20-3.
10. Vecchierini MF, Sleep disturbances in Alzheimer's disease and other dementias, Psychol Neuropsychiatr Vieil, 2010 Mar; 8(1):15-23.
11. McCurry SM, Sleep Dysfunction in Alzheimer's Disease and Other Dementias. Ancoli-Israel S. Curr Treat Options Neurol. 2003 May, 5(3):262-273.
12. Bliwise DL, Sleep disorders in Alzheimer's disease and other dementias, Clin Cornerstone. 2004, 6: suppl. 1A: S16-28.
13. Zhiwei Guan, Xuwen Peng, Jidong Fang, Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus, Brain Research, 2004, 1018, 38–47.
14. Stickgold R, Sleep-dependent memory consolidation. Nature, 2005, 437, 1272–1278.
15. Walker MP, Issue's surrounding sleep-dependent memory consolidation and plasticity. Cellular and Molecular Life Sciences, 2004, 61, 3009–3015.

16. Harris PF, Overstreet DH., Orbach J, Disruption of passive avoidance memory by REM sleep deprivation, methodological and pharmacological considerations. Pharmacology, Biochemistry & Behavior, 1982, 17, 1119–1122.

17. Smith C, Kelly G, Paradoxical sleep deprivation applied two days after end of training retards learning. Physiology & Behavior, 1998, 43, 213–216.

18. Smith CD, Rose GM, Evidence for a paradoxical sleep window for place learning in the Morris water maze. Physiology & Behavior, 1996, 59, 93–97.

19. Youngblood BD, Smagin GN, Elkins PD, Ryan DH., Harris RB, The effects of paradoxical sleep deprivation on spatial learning and brain 5-HT metabolism. Physiology & Behavior, 1999, 67, 643–649.

20. Smith C, Conway JM., Rose GM., Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiology of Learning and Memory, 1998, 69, 211–217.

21. Svitlana Palchykova, Raphaelle Winsky Sommerer, Peter Meerlo, Roland Durr, Irene Tobler, Sleep deprivation impairs object recognition in mice, Neurobiology of Learning and Memory, 2006, 85, 263–271.

22. Pandey VN, Medico- ethno botanical exploration in Sikkim Himalaya, Central Council for research in Ayurveda & Siddha, First edition, pp137-189, 1991.

23. Sharma P.C, Yelne N.B, Dennis T.J, Data base on Medicinal Plants used in Ayuveda , Vol-1,CCRAS, New Delhi, 2001

24. Hamied K A, Bakshi V M ,Aghara L P, Pharmacological, investigation of Nardostachys jatamansi roots, Sci Ind Res, 1962, 21C , 100

25. Ahmad M, Yousuf S, Khan Badruzzaman, Hoda N, Ahmad MA, Ishrat T , Agarwal AK, Islam F, Attenuation by Nardostachys jatamansi of 6-hydroxydopamine-induced parkinsonism in rats: behavioral, neurochemical, and immunohistochemical studies. Pharmacol and Biochem Behav, 2006, 83:150-60.

26. Vinutha JP, Acetyl cholinesterase inhibitory activity of methanolic and successive water extracts of Nardostachys jatamansi, Ind J Pharmacol 2007; 23:127-131.

27. Joshi H, Parle M. Nardostachys jatamansi improves learning and memory in mice. J Med Food, 2006; 9:113-8.

28. Salim S, Ahmad M, Zafar K S, Ahmad A S, Islam F, Protective effect of Nardostachys jatamansi in rat cerebral ischemia, Pharmacol Biochem Behav. 2003, 74, 481.

29. Arora R B, Madan B R, Antiarrhythmic activity of Nardostachys jatamansi (an Indian indigenous drug), Indian J Med Res, 1956, 44, 259.

30. Subashini R, Ragavendran B, Gnanapragasam A, Yogeeta SK, Devaki T. Biochemical study on the protective potential of Nardostachys jatamansi extract on lipid profile and lipid metabolizing enzymes in doxorubicin intoxicated rats. Pharmazie, 2007; 62:382-7.

31. Subashini R, Yogeeta S, Gnanapragasam A ,Devaki T, Protective effect of Nardostachys jatamansi on oxidative injury and cellular abnormalities during doxorubicin-induced cardiac damage in rats, J Pharm Pharmacol, 2006, 58, 257.

32. Agrawal SS, Sharma RC, Arora RB, Antioestrogenic activity of Jatamansone Semicarbazone, Indian J Exp Biol, 1973,11, 583.

33. Ali S, Ansari K A, Jafry M A, Kabeer H, Diwakar G, Nardostachys jatamansi protects against liver damage induced by thioacetamide in rats, J Ethnopharmacol, 2000,71, 359.

34. Gupta SS, Patel CB, Mathur VS, Effect of Nardostachys jatamansi fumes and aerosols in histamine-induced bronchial asthma in guinea pigs, J Indian Med Assoc, 1961, 37, 223.

35. Mishra D, Chaturvedi R V, Tripathy S C, The fungitoxic, effect of the essential oils of the herb of Nardostachys jatamansi DC, Trop Agric, 1995, 72, 48.

36. Saxena DB, Goswami BK, Tomar SS, Nematicidal, activity of some essential oils against, Meloidogyne incogntia, Indian Perfumer, 1987, 3,150.

37. Kumar V P, Chauhan N S, Padh H, Rajani M, Search for antibacterial and antifungal agents from selected Indian, medicinal plants, J Ethnopharmacol, 2006, 107,182.

38. Habibur Rahman, Muralidharan P, Comparative study of antidepressant activity of methanolic extract of Nardostachys Jatamansi DC Rhizome on normal and sleep deprived mice, Der Pharmacia Lettre, 2010, 2(5): 441-449.

39. Watoo Phrompittayarat, Waraporn Putalun, Hiroyuki Tanaka, Kanchalee Jetiyanon, Sakehai Wittaya-areekul, Kornkanok Ingkanian. Comparison of various extraction methods of bacopa monnieri. Naresun University Journel, 2007; 15(1), 29-34.

40. Kokate CK, Practical Pharmacognosy, 5th Edn, Vallabh Prakasham, 1991, 107-121.

41. Jayaraman J, Laboratory Manual in Biochemistry, 1st Edn, New age international Pvt. Ltd. 1981, 51.

42. Harburger LL, Bennett JC, Frick KM, Effect of estrogen and progesterone on spatial memory consolidation in aged females, Neurobiol Aging, 2007; 28:602-10.

43. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of memory in mice: effects of noortropics, scopolamine and electroconvulsive shock. Psychopharmacology.1990; 101:27-33.

44. Hanumanthachar Joshi, Milind Parle, Ingiber officinale: Evaluation of Its Nootropic Effect in Mice, Afr. J. Trad. Cam, 2006, 3 (1): 64 – 74.

45. N Venkato Rao, Basabaraj Pujara, S K Nimbal, S M Shantakumar, S Satyanarayan, Noortropic activity of

tuber extract of *Pueraria tuberosa* (roxb), Indian Journal of Experimental Biology, 2008, 46, 591-598.

46. Jaiswal A K, Bhattacharya S K, Effect of Shilajit on memory, anxiety and brain monoamines in rats, Indian journal of Pharmacology, 1992, 24, 12.

47. Francisco Paulino Dubiela, Maria Gabriela Menezes de Oliveira, Karin Di Monteiro Moreira Jose ,N. Nobrega Sergio Tufika ,Debora Cristina Hipolide T, Learning deficits induced by sleep deprivation and recovery are not associated with altered [^{3}-H] muscimol and [^{3}-H] flunitrazepam binding. Brain Research, 2005, 1037, 157– 163.

48. Hiramatsu M, Kaori Inowe. Improvement by low doses of nociceptin on scopolamine induced impairment of learning and /or memory. Eur J Pharmacol. 2000; 395: 149-156.

49. Hanumanthachar Joshi, Milind Parle, Brahmi rasayana improves learning and memory in mice. Evid. Based Complement. Alter. Med, 2006, 3(1): 79-85.

50. Sarter M, Bodewitz G, Stephens DN. Attenuation of Scopolamine induced impairment of spontaneous alteration behaviour by the antagonist but not inverse agonist and antagonist β-carboline. Psychopharmacology 1988; 94; 491-495.

51. Se Jin Park, Dong Hyun Kim , Kyun Lee, Won Yong Jung , Dong Hyun Park, Jong Min Kim,Kang Ro Lee , Kyung Tae Lee , Chan Young Shin, Jae Hoon Cheong, The ameliorating effect of the extract of the flower of Prunella vulgaris var. lilacina on drug-induced memory impairments in mice, Food and Chemical Toxicology, 2010, ScienceDirect.

52. Morris R, Development of a water maze procedure for studying spatial learning in the rat. J Neuroscience Methods, 1984, 11:47-60.

53. Min Hye Yang, Kee Dong Yoon, Young –Won Chin, Ju Hyun Park, Seung Hyun Kim, Jin Woong Kim, Neuroprotective effects of *Dioscorea opposite* on scopolamine induced memory impairement *in vivo* behavioral tests and *in vitro* assays. J Ethnopharmacol, 2009, 121: 130-134.

54. Kakad VD, Mohan M, Kasture VS, Kasture SB. Effect of *Vitis vinifera* on memory and behaviour mediated by monoamines. J Nat Remedies. 2008; 8(2): 164-172.

55. Nunes, Jr.G.P, Tufik, S.,Validation of the modified multiple platform method, MPM, of paradoxical sleep deprivation in rats. Sleep Research, 1994, 23, 419.

56. Ricardo Borges Machado, De bora C. Hipolide, Ana Amelia Benedito-Silva, Sergio Tufik Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery, Brain Research, 2004, 1004, 45– 51.

57. Silva R.H., Abi Tio V.C., Takatsu A.L, Kameda S.R, et al, Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice, Neuropharmacology, 2004, 46, 895–903.

58. Silva R.H, Chehia A.B, Kameda S.R et al, Effects of pre- or post-training paradoxical sleep deprivation two animal models of learning and memory in mice, Neurobiology of Learning and Memory, 2004, 82, 90–98.

59. Furio AM, LI Brusco, Daniel P, Cardinali, Possible Therapeutic Value of Melatonin in Mild Cognitive Impairment: A Retrospective Study, Journal of Pineal Research, 2007, 43:4, 404-9. 10 Jul. 2008.

60. Pierpaoli,W , Melatonin and Aging, Journal Of Anti-Aging Medicine; 1999, 2(4):343-348.

61. Wang J, Melatonin also reduces the hyperphosphorylation of tau protein, which leads to the neurofibrillary tangles of Alzheimer's disease, Acta Pharmacologica Sinica; 2006, 27 (1):41-49.

62. Reimund E., The free radical flux theory of sleep. Medical Hypotheses, 1994, 43, 231–233.

63. Maquet P, The role of sleep in learning and memory. Science, 2001,294, 1048–1051.

64. Nazmun Lyle, Dipankar Battacharyya, Tapas K sur, Santanu Munshi, Shurita Paul, Suparna Chatterjee, Antony Gomes, Stress modulating antioxidant effect of Nardostachys jatamansi, Indian journal of Biochemistry and Biophysics, 2009. 46:93-98.

65. Prabhu VM, Karanth KS, Rao A, Vidya PM, Sudhakar K. Effect of Nardostachys jatamansi on biogenic amines and inhibitory amino acids in the rat brain. Planta Med, 1994; 60:114–7.

66. Eckert G.P. et al, Piracetam reverses hippocampal membrane alterations in Alzheimer's disease, Journal of Neural Transmission, 1999, Volume 106, Numbers 7-8, 757-761.

About Corresponding Author: Mr. Habibur Rahman

Mr. Habibur Rahman graduated from Tripura Central University, Agartala and Post graduated from the Tamilnadu Dr. M. G. R. Medical University, Chennai. At post graduation level taken specialization in pharmacology, completed master thesis in "Evaluation of anti-Alzheimer's disease (AD) activity of *Nardostacys jatamansi* DC (family: valerianaceae) by sleep deprived amnesia produced in Swiss albino mice".