Inhibition of AChE and antioxidant activities are probable mechanism of *Nardostacys jatamansi* DC in sleep deprived Alzheimer's mice model

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Abstract: 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 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 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) followed by 5 days sleep deprivation using multiple platform method. The Acetylcholinesterase (AChE) activity, glutamate, antioxidants enzymes Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GRD), Glutathione peroxidase (GPx), Lipid peroxidase assay (LPO) and ascorbic acid (Vit.C) are estimated from mice brain on 19th day. MENJ at doses of 200 mg/kg and 400mg/kg treated groups showed a significant inhibition of AChE activity and improved antioxidants enzyme levels in sleep deprived amnesic mice. These findings suggest MENJ exerts a protective effect against loss of memory and cognitive deficits due to its inhibition of AChE and protection from oxidative damage due to sleep deprivation.
Keywords: Alzheimer's disease (AD), *Nardostacys jatamansi* DC, sleep deprived (SD), Acetylcholinesterase (AChE), Antioxidant enzymes.

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive neurodegenerative disease of the brain characterized by impairment of memory and eventually 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].

Sleep disorders affect a large part of the general population, with up to 56% of individuals reporting sleeping problems in the USA[2]. Impairment of sleep causes day time 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 [3]. About 45% of AD patients have disruptions in their sleep and sun-downing agitation[4]. Young and middle-aged adults

who suffer from insomnia are 11 times more likely to develop AD and depression in their later life [5]. Chronic lack of sleep may promote the development of AD and for people suffering from insomnia and other sleep disorders increases the risk of AD in later life[6].

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 the extra cellular signal-regulated kinase phosphorylation in the hippocampus of rat brain [7]. 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[8,9]. Numerous studies have demonstrated that sleep deprivation in laboratory animals produces memory deficits in several behavioral models, such as avoidance tasks [10], Morris water maze task [11], and radial maze task[12]and in object recognition test [13].

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)[14]. *N. jatamansi* DC gives quickly relieves from psychosis, maniac psychosis, syncope and hysteria[15], anti-parkinsonism[16], memory-enhancing [17], anti- cerebral ischemic[18], anti-arrhythmic[19], cardioprotective[20], anti-estrogenic[21], hepatoprotective[22], anti-asthmatic[23], antifungal[24], nematicidal[25], antibacterial[26] and antidepressant[27]. This study was conducted to understand the mechanism of *Nardostachys jatamansi* DC in protection on loss of memory and cognition deficit in sleep deprived amnesic mice.

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 ($60\text{-}70^0C$) employing methanol as solvent[28]. The solvent was evaporated at $40^0 C$ 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%.

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 [29, 30].

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; 5,5$^/$-Dithiobis(2-nitrobenzoic acid) , Ellman's reagent , Sisco Research Laboratories Pvt. Ltd Mumbai ; Glutathione reduced , Sisco Research Laboratories Pvt. Ltd ; S-acetyl thiocholine iodide , Sisco Research Laboratories Pvt. Ltd, Mumbai ; Perchloric acid, 70% , Chemspure, Chennai ; (Tris buffer [Tris(hydroxymethane)aminoethane], Merck Specialities Private Limited, Mumbai); Butanol-HCl , Paxy Speciality Chemicals, Chennai; 2-thiobarbituric acid , Kiran light laboratories, Mumbai; Nicotinamide Adenine Dinucleotide Phosphate reduced tera sodium sat.($NADPH.Na_4$), Sisco Research Laboratories Pvt. Ltd, Mumbai; Glutathione oxidised , Sisco Research Laboratories Pvt. Ltd, Mumbai.; Methanol and all others common reagents are obtained from institutional store and were 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 [31]. 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) [32] up to 19th day, where the last dose was given 60 min prior to scarification of animal for *in-vitro* studies.

Sleep Deprivation (SD) Method

This method of sleep deprivation used was an adaptation of the multiple platform method, originally developed for rats [33]. The animals which were subjected for 5 days sleep deprivation by multiple platform method [34]. 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. A 100-W light illuminates the chamber during the period of sleep deprivation. 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.

Estimation of Acetylcholineserase (AChE) activity [35]

The mice were decapitated; brains are removed quickly and placed in ice-cold saline. Frontal cortex, hippocampus and septum are quickly dissected out on a Petri dish chilled on crushed ice. Weigh quantity (50 mg) of tissue was homogenized in 0.1M Phosphate buffer (pH 8) (5ml). 0.4ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100μl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20μl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in the absorbance per minute is thus determined. The enzyme activity is calculated using the following formula…

Achetylcholinestease activity (M/ml) =

$$\frac{A/min \ X \ V_t}{\varepsilon \ X \ b \ X \ V_s}$$

A/min= Change in absorbance per min
ε= 1.361 $X10^4$ M-1cm-1
b= pathlenth (1 cm)
V_t= Total volume in assay
V_s= sample volume in assay

The final reading of enzyme activity is expressed as μ moles/minute/mg tissue after correction of assay and sample dilution.

$$\mu \ moles/minute/mg \ tissue = \frac{\mu \ moles \ /ml \ sample}{mg \ tissue/ \ ml \ sample \ dilution}$$

Estimation of Glutamate [36]

The level of Glutamate was estimated by multiple development paper chromatography method as described by Raju *et al.* 1.0 ml of the supernatant from brain homogenate was evaporated to dryness at 70°C in an oven and the residue is reconstituted in 100 ml of distilled water. Standard solutions of glutamate and GABA at a concentration of 2mM along with the

sample are spotted on Whatman No. 1 chromatography paper using a micropipette. It was placed on a chamber containing butanol: acetic acid: water (12: 3: 5 v/v) as solvent. When the solvent front reached the top of the paper, it was removed and dried. A second run is performed similarly, after which the papers are dried sprayed with ninhydrin reagent and placed in an oven at 100°C for 4 minutes. The portions which carry glutamate corresponding with the standard are cut and eluted with 0.005% $CuSo_4$ in 75% ethanol. Their absorbance is read against blank at 515 nm in spectrophotometer. The glutamate level is calculated by using the following formula.

$$A = \frac{\text{Unknown OD X Standard in mg (3µg) X 1000}}{\text{Standard OD X Volume spotted (10µl) X W}}$$

Where,

A = glutamate content in µmoles/gram wet weight tissue

1000 = Conversion factor for gram wet weight tissue

W = weight of the tissue in gram

Preparation of Brain homogenate for antioxidant enzyme estimation

The animals were sacrificed by cervical dislocation, and the whole brain were dissected out, blotted free of blood, transferred to trays maintained at ice-cold conditions by rinsing with ice-cold physiological saline. 50 mg of the brain tissue (Hippocampal region) was weighed and homogenate was prepared in 5 ml tris hydrochloric acid buffer (0.5 M; pH 7.4) at 4°C. The homogenate was then centrifuged for 10 minutes at 10,000 rpm and the resultant supernatant was used for the biochemical determinations antioxidant enzymes.

Estimation of Superoxide dismutase (SOD) [37]

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500 µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100 µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec. Parallel blank and standard were run for determination SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation. The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer. The enzyme activity is calculated by following equation.

% Inhibition=

$$\frac{\Delta A_{480nm}/\text{min Uninhibited} - \Delta A_{480nm}/\text{min inhibited}}{\Delta A_{480nm}/\text{min Uninhibited} - \Delta A_{480nm}/\text{min Blank}} \text{X } 100$$

$$\text{Units/ml enzyme} = \frac{\text{\% Inhibition X } V_t}{(50\%) \text{ X } V_s}$$

The final value is expressed in unit/mg of tissue after correction of assay and sample dilution.

Estimation of Glutathione reductase (GRD) [38]

Glutathione reductase was assayed by the method of Stahl *et al*. The reaction mixture containing 1 ml phosphate buffer, 0.5 ml EDTA, 0.5 ml GSSG and 0.2 ml of NADPH was made up to 3 ml with distilled water. After the addition of 0.1 ml of tissue homogenate, the change in optical density at 340 nm was monitored for 2 minutes at 30 seconds interval. One unit of the enzyme activity was expressed as nmoles of NADPH oxidized / min/ mg protein. The enzyme activity is calculated by following equation.

$$\text{Enzyme activity (M/min/ml)} = \frac{A_{340/min} \text{ X } Vt}{\varepsilon \text{ X d X Vs}}$$

Where,

$\varepsilon = 6.22 \text{ X } 10^6 \text{ M}^{-1}\text{cm}^{-1}$

d=1cm

Vt= Total volume

Vs= Sample volume

The final value is expressed in nmoles of NADPH oxidized / min/ mg tissue after correction of assay and sample dilution.

Estimation of Glutathione peroxidase (GPx) [39]

Glutathione peroxidase is estimated by Wood method. 3-ml cuvette containing 2.0 ml of phosphate buffer(75 mmol/L, pH 7.0) , 50µl of (60mmol/L) glutathione reductase solution, 50µL of (0.12 mol/L) NaN3, 0.1 ml of (0.15mmol/L) Na2 EDTA ,100µL of (3.0 mmol/L) NADPH, and 100µL of tissue supernatant was added. Water was added to make a total volume of 2.9 ml. The reaction was started by the addition of 100µL of (7.5 mmol/L) H_2O_2, and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbance at 340 nm at 1-min interval for 5 min.

$$\text{Enzyme activity (M/min/ml)} = \frac{A_{340/min} \text{ X Vt}}{\varepsilon \text{ X d X Vs}}$$

$\varepsilon = 6.22 \times 10^{6} \text{ M}^{-1}\text{cm}^{-1}$

d=1cm

Vt= Total volume

Vs= Sample volume

The final value is expressed in nmoles of NADPH oxidized / min/ mg tissue after correction of assay and sample dilution.

Estimation of Catalase Activity[40]

Catalase activity was measured by the method of Aebi. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0ml of freshly prepared 30 mM H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second. The reaction occurs immediately after the addition of H2O2. Solutions are mixed well and the first absorbance (A1) is read after 15 seconds (t1) and the second absorbance (A2) after 30 seconds (t2).The absorbance is read at wave length 240 nm.

Calculation:

$$K = \frac{Vt}{Vs} \times \frac{2.3}{\Delta t} \times \text{Log} \frac{A1}{A2} \times 60$$

Where, K= Rate constant of the reaction.

Δ t= (t2 – t1) =15 seconds.

A1= absorbance after 15 seconds.

A2= absorbance after 30 seconds.

Vt = total assay volume

Vs = volume of the sample in assay

The final value is expressed in unit/ mg tissue after correction of assay and sample dilution.

Estimation of Lipid peroxidase (LPO)[41]

The level of Lipid peroxides was estimated by Thiobarbituric acid reaction method described by Ohkawa *et al.* To 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60 minutes. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine

mixture were added and shaken vigourously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm. Enzyme activity is calculated by,

The concentration of MDA =
(M)

$$\frac{\text{Absorbance at 532 nm}}{\text{L x } \mathrm{E}} \text{x D}$$

L: light path (1 cm).

Є: extinction coefficient 1.56 x 105 M-1.Cm-1.

D: dilution factor

The level of lipid peroxides was expressed as nmoles of MDA released/ g wet tissue after correction of assay and sample dilution.

Estimation of ascorbic acid (vit.c)[42]

The level of vitamin C was determined by the method of Omaye *et al.* To 0.5 ml of homogenate, 0.5 ml of water and 1.0 ml of TCA were added, mixed thoroughly and centrifuged. To 1.0 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37°C for 3 hours. Then 1.5 ml of sulphuric acid was added, mixed well and the solutions were allowed to stand at room temperature for another 30 minutes. The colour developed was read at 520 nm in a UV spectrophotometer.

Calculation:

Amount of ascorbic acid (μg/mg of tissue) =

$$\frac{\text{Abs}_{Tesr} \text{ X Std. dilution}}{\text{Abs}_{Std} \text{ X test dilution}} \text{ X1000}$$

Abs $_{Tesr}$= Test sample absorbance at 520 nm

Abs $_{Std}$ = Standard sample absorbance at 520 nm.

The final values are expressed as μg/ g wet tissue after correction of assay and sample dilution.

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.

Table-1: Effect of MENJ on AChE Activity and Glutamate level

Groups	Treatment	AChE activity (µmoles/min/mg wet tissue)	Glutamate level (µmoles/g wet tissue)
I	Normal Control, Vehicle	16.38± 0.85	70.88± 2.18
II	Negative Control, Vehicle; SD	26.06± 1.89[a]***	89.07± 2.77[a]***
III	MENJ 200 mg/kg; SD	21.26± 0.97[b]*	78.97± 1.84[b]*
IV	MENJ 400 mg/kg; SD	20.69± 0.74[b]**	77.23± 2.11[b]**
V	Piracetam 200 mg/kg; SD	17.41± 0.73[b]***	90.37± 2.01[b ns]

Table-2: Effect of MENJ on SOD, CAT and GRD

Groups	Treatment	SOD (unit/mg wet tissue)	Catalase (unit/mg wet tissue)	GRD (unit/mg wet tissue)
I	Normal Control, Vehicle	6.74± 0.51	4.98± 0.23	2.56± 0.29
II	Negative Control, Vehicle; SD	3.19± 0.37[a]***	2.81± 0.37[a]***	1.39± 0.18[a]**
III	MENJ 200 mg/kg; SD	4.85± 0.46[b]*	4.06± 0.32[b]*	1.62± 0.17[bns]
IV	MENJ 400 mg/kg; SD	5.06± 0.41[b]*	4.37± 0.27[b]**	2.20± 0.10[b]*
V	Piracetam 200 mg/kg; SD	3.46± 0.31[b ns]	3.34± 0.32[ns]	1.59± 0.16[bns]

Table-3: Effect of MENJ on GPX, LPO and Vit.C

Groups	Treatment	GPx (unit/mg wet tissue)	LPO (unit/mg wet tissue)	Vit.C(unit/mg wet tissue)
I	Normal Control, Vehicle	3.85± 0.44	138.0± 4.10	125.7± 2.36
II	Negative Control, Vehicle; SD	2.24± 0.27[a]*	95.60± 3.06[a]***	90.76± 3.39[a]***
III	MENJ 200 mg/kg; SD	2.52± 0.24[bns]	105.00± 2.58[bns]	97.30± 1.80[bns]
IV	MENJ 400 mg/kg; SD	3.71± 0.48[b]*	111.3± 2.68[b]**	98.40± 2.10[bns]
V	Piracetam 200 mg/kg; SD	2.36± 0.27[bns]	99.43± 1.60[bns]	92.56± 2.14[bns]

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.
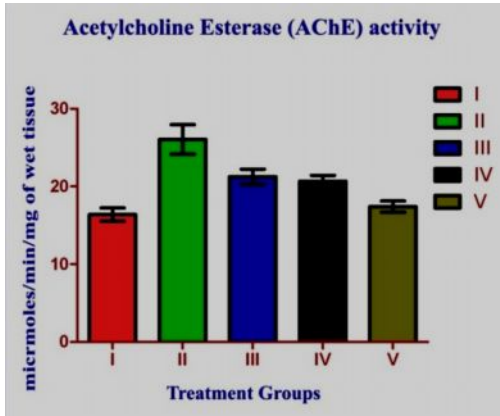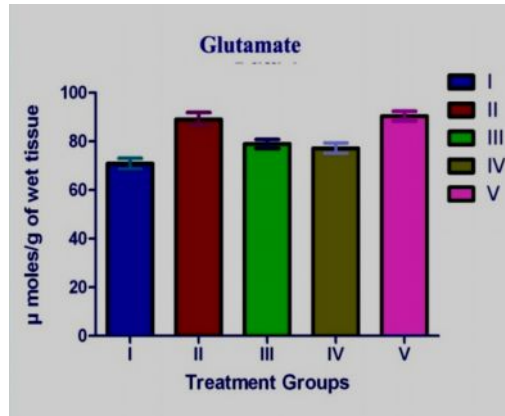
Fig-1: Effect of MENJ on AChE Activity

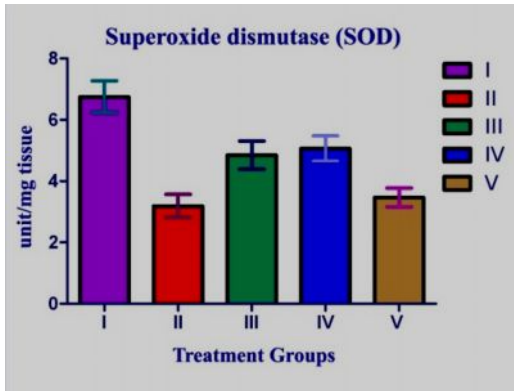**Fig-2 Effect of MENJ on Glutamate level**
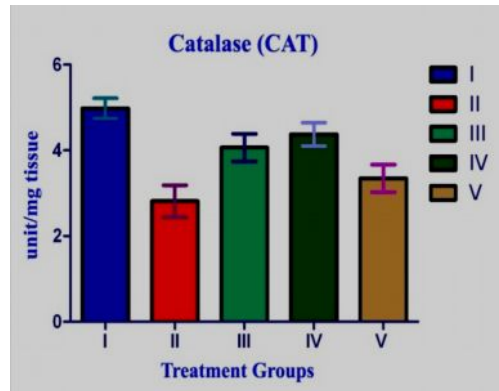

Fig-3: Effect of MENJ on SOD
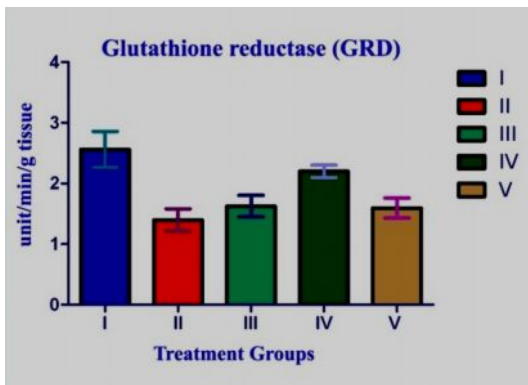

Fig-4: Effect of MENJ on CAT

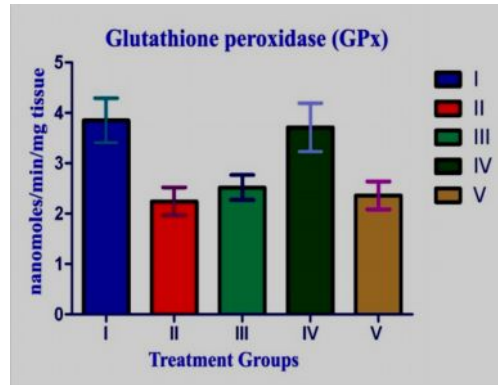**Fig-5: Effect of MENJ on GRD**

Fig-6: Effect of MENJ on GPx

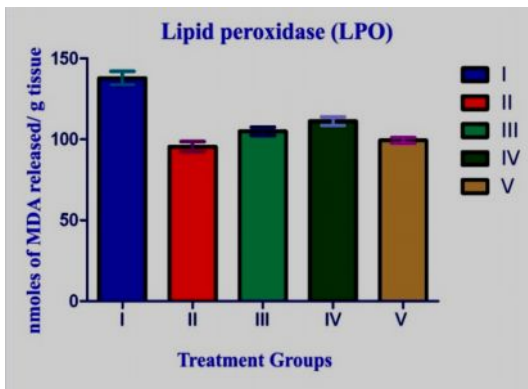**Fig-7: Effect of MENJ on LPO**

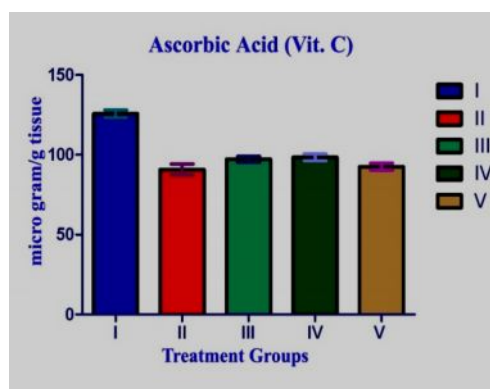**Fig-8: Effect of MENJ on Vit.C**

RESULTS

Effect of MENJ on AChE Activity

Results are shown in **Table-1 and Fig-1.** Sleep deprivation (Group-II) significantly (P<0.001) increased the AChE activity when compared with normal mice (Group-I).In the treated groups there was a significant dose dependent reduction in enzyme levels on both 200 and 400 mg/kg of MENJ treated mice (p<0.05 and p<0.01 for Group-III and Group-IV respectively) compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) also showed significant reduction (p<0.001) in AChE activity.

Effect of MENJ on Glutamate level

Results are shown in **Table-1 and Fig-2.** Sleep deprivation (Group-II) significantly (P<0.001) increased the brain glutamate level when compared with normal mice (Group-I). In the treated groups there was a significant dose dependent reduction in glutamate levels on both 200 and 400 mg/kg of MENJ treated mice (p<0.05 and p<0.01 for Group-III and Group-IV respectively) compared with sleep deprived mice (Group-II). But Standard drug piracetam at dose 200 mg/kg (Group-V) showed increase in glutamate level non-significantly.

Effect of MENJ on Super Oxide Dismutase (SOD)

Results are shown in **Table-2 and Fig-3.** Sleep deprivation (Group-II) significantly (P<0.001) reduced the Super Oxide Dismutase (SOD) level when compared with normal mice (Group-I). In the treated groups (Group-III and Group-IV) there was a significant increase (p<0.05) on both 200 and 400 mg/kg of MENJ treated mice compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) showed slight increase non-significantly.

Effect of MENJ on Catalase (CAT)

Results are shown in **Table-2 and Fig-4.** Sleep deprivation (Group-II) significantly (P<0.001) reduced the Catalase (CAT) level when compared with normal mice (Group-I). In the treated groups (Group-III and Group-IV) there was a significant increase (p<0.05 and p<0.01 respectively) on both 200 and 400 mg/kg of MENJ treated mice compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) showed slight increase non-significantly.

Effect of MENJ on Glutathione Reductase (GRD)

Results are shown in **Table-2 and Fig-5.** Sleep deprivation (Group-II) significantly (P<0.01) reduced the Glutathione reductase (GRD) level when compared with normal mice (Group-I). In the treated groups (Group-III and Group-IV) there was a significant increase (ns and p<0.05 respectively) on both 200 and 400 mg/kg of MENJ treated mice compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) showed slight increase non-significantly.

Effect of MENJ on Glutathione Peroxidase (GPx)

Results are shown in **Table-3 and Fig-6.** Sleep deprivation (Group-II) significantly (P<0.05) reduced the Glutathione peroxidase (GPx) level when compared with normal mice (Group-I). In the treated groups (Group-III and Group-IV) there was a significant increase (ns and p<0.05 respectively) on both 200 and 400 mg/kg of MENJ treated mice compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) showed slight increase non-significantly.

Effect of MENJ on MDA level of Lipid Peroxidase Assay

Results are shown in **Table-3 and Fig-7**. Sleep deprivation (Group-II) significantly (P<0.001) reduced the Lipid peroxidase level when compared with normal mice (Group-I). In the treated groups (Group-III and Group-IV) there was a significant increase (ns and p<0.01 respectively) on both 200 and 400 mg/kg of MENJ treated mice compared with sleep deprived mice (Group-II). Standard drug piracetam at dose 200 mg/kg (Group-V) showed slight increase non-significantly.

Effect of MENJ on Ascorbic acid (Vit.C)

Results are shown in **Table-3 and Fig-8.** Sleep deprivation (Group-II) significantly (P<0.05) reduced the Glutathione peroxidase (GSHPx) level when compared with normal mice (Group-I). In the treated groups including standard (Group-III, Group-IV, group-V) showed non significant increase in ascorbic acid.

DISCUSSION

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. It is found that the synthesis and secretion of melatonin and other neurotransmitter occur mainly in sleep cycle. 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. Different research studies suggest that sleep deprivation would reduce the antioxidant defenses [43]. 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 [44]. 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. Pharmacological investigation on *Nardostachys jatamansi* DC showed its antioxidant effects. At both dose levels MENJ showed decrease in Acetyl cholinesterase activity compared to sleep deprived animal. It suggests about its mechanism of

memory enhancing by increasing cholinergic level. Where it, showed decrease in glutamate level, as Piracetam showed increase in glutamate level suggesting its activity on glutaminergic receptors. MENJ showed increase in antioxidant enzymes significantly but piracetam had non significant improve in antioxidant enzymes. It suggests about indirect action on antioxidant enzymes and may not have its antioxidant activity.

CONCLUSION

Alzheimer's disease (AD) is multi-pathogenic neurodegenerative disorder. *Nardostachys jatamansi* DC showed its neuroprotective activity by different mechanism like inhibition of AChE activity and also have antioxidant properties as synthetic drug piracetam had showed improvement of memory due to inhibition of AChE activity but it has no antioxidant activity. Therefore, by giving great emphasis on investigation in herbal drug like novel Ayurvedic drug Jatamansi, can be used for the treatment of Alzheimer's disease (AD).

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