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Original Article

Evaluation of Cognition Enhancing Activities of Telmisartan, Nimodipine and their Combination in REM Sleep Deprived Wistar Rats

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ABSTRACT

Background: Sleep Deprivation (SD) may lead to the failure of advanced neural functions, including decision-making, learning and memory. Studies show that nimodipine plays a role in intracellular Ca^{2+} to reduced influx of Ca^{2+} into mitochondria. Thereby, nimodipine improves the spatial cognition and elevates hippocampal acetylcholine. Telmisartan, has been proven to improve cognitive function in scopolamine induced amnesic rats. **Aims:** To evaluate the cognition enhancing activities of telmisartan and nimodipine in REM sleep deprived Wistar rats. **Materials and Methods:** SD rats were treated with telmisartan (3.6mg/kg), nimodipine (5mg/kg) and combination of both for 4 weeks. Morris water maze was done to estimate the spatial learning and memory. Brain glutathione, malondialdehyde, acetylcholinesterase, Brain Derived Neurotropic Factor (BDNF) and histopathological examinations were done. Results were analysed by ANOVA followed by *post hoc* Tukey's test. Brain samples were sectioned for histopathological examination. **Results:** Increase in oxidative stress following REM sleep deprivation was reversed in chronic study. Chronic intake of telmisartan, nimodipine and combination of both the drugs mitigated spatial learning and memory deficit in Wistar rats induced by REM sleep deprivation. In telmisartan treated group there was significant increase in BDNF levels ($p<0.05$) as compared to SD rats. The histopathological sections showed less damaged neurons in telmisartan, nimodipine and their combination group. **Conclusion:** Current study demonstrated that telmisartan, nimodipine and combination of these two drugs reversed the sleep deprivation induced cognitive impairment by reducing oxidative stress, enhancing cholinergic activity, BDNF levels and histopathological findings support the above fact. However, further studies are essential to confirm the result.

Keywords: Cognition, Nimodipine, Telmisartan, Sleep deprivation, BDNF.

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INTRODUCTION

Lack of sleep holds the first place among the neglected human basic needs in today's sprint-paced world. According to some sources, sleep is essential for maintaining normal biological processes and for promoting neuronal and synaptic plasticity, all of which are essential for cognitive function and brain health.[1] Rapid Eye Movement (REM) and Non-Rapid Eye Movement (NREM) sleep are the two stages of the sleep cycle. According to the studies, REM sleep improves hippocampal-dependent memory consolidation, restoration, making it vital for recall of events and spatial learning[2,3] and also may lead to hyperphagia, weight loss.[4] Number of pervious animal studies showed that REM sleep deprivation inflicts memory deficits: showcased by utilizing behavioral experimental models, such as Morri's water maze.[5]

It is absolutely not known that the mechanism by which how the sleep deprivation results in memory deficit. Reimund's free radical theory is the recent addition among some theories have been proposed. According to this theory sleep promotes the endogenous antioxidant mechanisms activities and decreases the production of free radicals in the brain.[6] Hence, sleep plays an important role as catalyst of antioxidants production in the brain. Further, Zepelin and Rechtschaffen believe that metabolic requirements were limited by sleep.[6] Sleep deprivation can

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therefore induce the metabolic rate and thus increase oxidative stress.

Studies earlier have reported that brain Renin-Angiotensin-System (RAS) has role in mediating cognitive functions along with learning and memory consolidation, proving the presence of a Brain RAS.[7,8] ACE inhibitors and ARBs are used in the treatment of hypertension and they reduce morbidity and mortality, and said to improve cognitive impairment in such patients.[9,10] In scopolamine induced amnesic rats, an Angiotensin Receptor blocker, telmisartan has been shown to improve cognitive impairment.[11] Spatial memory impairment due to cerebral ischemia was improved by Nimodipine.[12] Nimodipine may enter the cell and by inhibiting excessive Ca^{2+} entry into the mitochondria, it will check the intracellular Ca^{2+} ion cascade to protect neuronal cells. As a result, nimodipine alleviates cognitive impairment and increases intrinsically acetyl choline levels in hippocampus.[12,13]

In this study, sleep-deprived Wistar albino rats were used to determine how telmisartan, nimodipine, and their combination improved learning and spatial memory.

MATERIALS AND METHODS

Animal selection

For the experiment, 36 male albino Wistar rats (Rattus norvegicus), weighing 150-250 g, were employed. All rats were procured from the Central Animal Research Facility, Manipal. Three animals were housed in each polypropylene cage of size 41cm x 28cm x 14cm. Animals were maintained at temperature (22±3°C), humidity (approximately 50±10%) and light (12 hr light and 12 hr dark cycle). The Experiment was conducted as per CCEA guidelines and the rats received standard animal feed (VRK Nutritional Solutions, Pune, India). Animal bedding consists of paddy husk and it was changed and cleaned alternative days. The experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/62/2017 dated 23.09.2017).

Drugs and dosage

Preparation of Drugs

Angiotensin receptor blocker, telmisartan and calcium channel blocker, nimodipine are used in this study. Telmisartan 3.6mg/kg given orally and nimodipine 5mg/kg given intraperitonially. The dose and route administration of drugs was taken from previous studies. Telmisartan 40 mg tablet dissolved 20 mL of distilled water and nimodipine 30 mg tablet dissolved in 20 mL of distilled water.[13]

Experimental groups

Group	Drugs, Dose and Route	Duration
Group 1	REM sleep control animals.	4 weeks
Group 2	REM sleep control animals + distilled water.	4 weeks
Group 3	REM sleep deprived animals.	4 weeks
Group 4	REM sleep deprived animals with telmisartan 3.6mg/kg.p.o dissolved in distilled water.	4 weeks
Group 5	REM sleep deprived animals with nimodipine 5mg/kg,i.p dissolved in distilled water.	4 weeks
Group 6	REM sleep deprived animals with telmisartan 3.6mg/kg and nimodipine 5mg/kg dissolved in distilled water.	4 weeks

Thirty-six animals were divided into six groups equally (n=6).

Experimental Design

Prior to experimentation the animals were allowed to acclimatize to the laboratory conditions. The animals were housed under standard conditions of 12 hr light/dark cycles and was provided with a standard rat feed and water *ad libitum*.

Sleep deprivation procedure

Based on the concept of the inverted flowerpot model of sleep deprivation, we established a paradigm called the modified multiple platform model, an improved earlier version, with the aim of providing a better result. The inverted flowerpot method was associated with significant amount of inflicting stress,[14,15] which might have confounded the end results. Therefore, the approach had been altered to provide many platforms in a comparably larger tank so that a larger number of rats may be deprived of sleep at once and reduce stress.[15] This experimental model was validated. Apparatus consists of a square shaped box and 16 platforms placed inside the box 9 cm above the floor, maintaining 6 cm distance from each other. Platforms were fixed the floor using metal rods. Box was filled with water (24°C) up to 1 cm below the platforms. Animals had an access to free water and food. Animals were laid on the platform with freedom of movement. Once the rat entered the REM sleep cycle, the atonic state of the skeletal muscles caused the rats to fall into the water.

Rats were divided into 6 groups (n=6). REM sleep deprived group of rats and treatment groups animals placed over the platform of diameter 5.5 cm. REM sleep was disturbed for 18 hr/day from 11:00 am to 17:00 pm, by allowing animal to stay over platform daily, for 21 days.[16] Rats could sleep normally for rest of the 6 hr/d. Same conditions were maintained for control animals as well except the fact that control animals were placed over larger platform of diameter 12.8 cm. Then the rats were tested

for learning and memory by Morri's water maze apparatus. The rats received drugs daily for 4 weeks as shown in table under Experimental groups.

Assessment of spatial learning and memory

Morris Water Maze

The experiment was carried out in accordance with Morris R. (Morris, 1984). The device comprises of a round tank. (165 cm x 35 cm) that is kept at 25°C and filled with water. Water was made transparent by the addition of milk. There were four equally sized zones in the tank. SE, SW, NW, NE, etc.). In one of the zones that was barely submerged in water, a platform (10 cm^2) was kept. A cue was a black and white symbol board. Throughout the learning sessions, the extra maze cue and platform's location remained fixed. The water maze test was conducted in two stages.[17]

Acquisition phase (Spatial task)

Over the course of four days, each animal underwent four trials, each lasting 2 min, in which it acquired to climb a hidden platform and stay there for 20 sec in order to escape the water. Four distinct starting positions were employed (North, South, East, and West). The animals underwent a daily regimen of trials with arbitrary start positions. A preliminary study was carried out to acquaint the rat with the water maze. The time taken to reach the platform was recorded. When the animal was unable to locate the platform after 90 sec, it was guided to it.[18]

Retrieval trial

The platform was removed on the final day of the experiment. The animal was moved to a new location in the maze and directed towards the tank wall in the opposite quadrant as the original target quadrant. Following the 30 sec, the animal was removed. The target zone's time and distance travelled were measured.

Dissection and Tissue preparation

After 4 weeks of dosing, cervical dislocation was performed to sacrifice the rats and brain tissues were removed from all the rats. Each rat's brain was separated and weighed. They were immersed in phosphate buffered saline (0.1M, pH 7.4) for biochemical analysis. Brain tissues were fixed in 10% neutral buffered formalin for histopathology.

Biochemical estimation

Malondialdehyde estimation (MDA)

The Okhawa *et al.* method was used to estimate lipid peroxidation by measuring MDA levels in the brain. MDA levels in brain homogenates were determined using Thiobarbituric Acid (TBA), which produces a red compound with a peak absorbance at 532 nm that was measured using a spectrophotometer.[19]

Reduced Glutathione Estimation (GSH)

Ellman's protocol was used to calculate glutathione. Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) is reduced by -SH groups in GSH to produce a yellow compound with a peak absorbance at 412 nm that can be measured with a spectrophotometer.[20]

Acetylcholinesterase (AChE) activity

AChE activity was quantitatively measured by Ellman's method. Ellman's reagent (DTNB) reacts with thiocholine to form a yellow compound with a 412 nm absorbance. The enzyme activity was determined using a spectrophotometer to record the rate of change in absorbance at 412 nm.[21]

Brain Derived Neurotropic Factor (BDNF) levels in brain

A rat BDNF ELISA kit was used to measure the levels of BDNF in the brain. Each of the 96 wells in the kit used to hold a sample. 50 µL standard solution was added to six wells. Six samples in the respective wells are used for each group. 40 µL of special diluent and then add 10 µL of tissue homogenate samples are added. The plate was then sealed, and following a gentle shake, it was incubated for 60 min at 37°C. Extra liquid was discarded. After drying; each well was filled with diluent washing liquid, mixed properly by shaking for 30 sec. Then the washing liquid was discarded and the plate was tapped on adsorbent papers to dry. This washing steps were repeated for five times and then the plate was pat dried. Each well filled with 50 µL of chromogen solution A and 50 µL of chromogen solution B. After being gently shaken, the plate was incubated at 37°C away from light. To halt the reaction, 50 µL of stop solution was added to each well (The blue changes into yellow immediately). After adding the stop solution to the blank wells, the Optical Density (OD) at 450 nm wavelength was measured within 15 min. The concentration of the standards and the associated OD values were used to compute the standard curve linear regression equation, and the OD values of the samples were then utilised to calculate the concentration of the corresponding sample. SPSS 17 version was used to make calculations and to assess the significance.[22]

Histopathological examination

The whole brain was dissected after sacrificing the rats. Brain samples were sectioned and stained with cresyl violet stain for histopathological examination.

Procedure

Initially the blocking or embedding the tissue was done. Then the tissue was transferred from the final wax bath to a mould filled with molten paraffin wax. A microtome was used to cut thin sections of tissue blocks of 4 microns. Tissue sections were floated in a 50°-52° water bath before being placed on microscopic slides.

After passing through alcohol the slides were immersed in distilled water for 15 min and were stained for 25-30 min with 0.1% cresyl violet stain and allowed to cool at room temperature. Stained sections were again immersed in distilled water for 5 min and ascending grading of alcohol for 2 min. Finally, sections were dipped in xylene for clearing and mounted with DPX. Histopathological evaluation of hippocampus was done.

Statistical analysis

SPSS version 17 was used for analysing the data. Results were analysed by using One-way Analysis of Variance (ANOVA), followed by *post hoc* Tukey's test. Results were expressed in terms of Mean ± SEM. A *p*-value of < 0.05 was considered to be statistically significant.

RESULTS

Morris water maze results

Acquisition trial: Results

During acquisition trials of day 1 and day 2, all the group of rats were comparable with respect to time required to reach the hidden platform (latency period). On day 3, 4 sleep deprived inflicted rats showed significant ($p<0.001$) increase in latency as compared to control group. (SD + telmisartan) and SD + (telmisartan+nimodipine) group of treated rats showed significant ($p<0.01$) decrease in latency period as compared to sleep deprived rats. However, it was observed that nimodipine treated rats showed decrease in latency period ($p<0.01$) with respect to sleep deprived group only on Day 4 (Table 1).

Probe trial: Percentage of time spent in target zone

In probe trial, sleep deprived rats showed significant decrease ($p<0.001$) in percentage of time spent in target quadrant and distance travelled as compared to control rats. The Sleep Deprived (SD) rats treated with telmisartan, nimodipine and (telmisartan+ nimodipine) combination of drugs exhibited significant increase in percentage of time spent and distance travelled in target quadrant as compared to sleep deprived rats ($p< 0.01$) (Table 2).

Malondialdehyde (MDA) and reduced Glutathione (GSH) levels in brain homogenate

REM sleep-deprived rats demonstrated a significant ($p<0.01$) rise in brain MDA and a decline in GSH levels when compared to control rats. The Sleep Deprived (SD) rats treated with telmisartan, nimodipine and (telmisartan+ nimodipine) combination of drugs showed significant decrease ($p<0.05$) in brain MDA levels and increase ($p<0.05$) in brain GSH levels compared to REM sleep deprived group (Table 3).

Brain Acetyl Cholinesterase (AChE) and Brain Derived Neurotropic Factor (BDNF) estimation in brain

In contrast to animals in the control group, REM sleep-deprived rats showed a substantial rise in AChE, indicating that cholinergic activity was being compromised. Rats treated with telmisartan, nimodipine and combination of both were able to reverse the sleep deprivation induced inhibition of cholinergic activity which was evident by statistically increasing ($p<0.05$) brain levels of AChE (Table 3). There was significant ($p<0.05$) decrease in brain BDNF levels, when REM sleep deprived group compared with control group. In telmisartan treated group there was significant increase in BDNF levels ($p<0.05$) as compared to sleep deprived group; however, these values were comparable to control group. Although there was a small rise in BDNF levels in the Sleep-Deprived (SD) rats treated with the nimodipine and (telmisartan+ nimodipine), this difference was not statistically significant (Figure 1).

Histopathological examination

Normal neurons were identified as Hippocampal CA3 neurons (soma) with a lightly stained nucleus, clear cytoplasm, and a healthy cell membrane. Damaged / degenerated cells were identified as flame-shaped hippocampal CA3 neurons (soma) with pyknosed cell bodies (karyopyknosis), homogeneous cytoplasm, and intense basophilic appearance (Figures 2 and 4) Histopathological changes were observed in CA1, CA3 and dentate gyrus sections of all the group of rats. The majority of neurons in the CA3, CA1, and dentate gyrus of control group rats were healthy, with pale and round nuclei, well-defined nuclear boundaries, and prominent nucleoli. No degenerative features

Table 1: Effect of telmisartan and nimodipine on REM sleep deprivation induced alteration in latency in Morris Water Maze (MWM).

Groups	Day - 1	Day - 2	Day - 3	Day - 4
Control	100.49±6.10	80.11±6.67	43.39±2.22	17.66±1.68
Control +Distilled Water	99.87±5.60	78.97±2.99	41.89±1.94	16.61±1.55
Sleep deprived (SD)	100.96±6.33	84.61±3.46	56.24±3.44[a]	49.39±2.40[a]
SD+Telmisartan	95.43±2.57	71.91±2.23	38.67±1.15*	26.57±0.93*
SD +Nimodipine	98.18±2.13	74.65±1.36	49.45±1.86	30.43±0.79[#]
SD + Telmisartan + Nimodipine	96.75 ± 2.33	76.18±2.43	36.18±2.09[$]	28.89±2.97[$]

[a] $p<0.001$ vs control; * $p<0.01$ vs SD; [#] $p< 0.05$ vs SD; [$] $p< 0.01$ Telmisartan +Nimodipine vs SD.

Table 2: Effect of telmisartan and nimodipine on REM sleep deprived; alterations in percentage of time spent and percentage of distance travelled is measured in target zone of Morris Water Maze.

Group	Percentage of time spent in target zone (%) (Mean ±SEM)	Percentage of distance travelled in target zone (%) (mean±SEM)
Control	55.67±3.99	46.83±3.34
Control +DW	58.00±1.61	43.33±2.84
Sleep Deprived (SD)	17.33±3.49[a]	20.50±3.77[a]
SD + Telmisartan	53.50±2.20*	44.17±7.24*
SD + Nimodipine	47.33±1.87[#]	43.00±3.44[#]
SD+Telmisartan+ Nimodipine	49 ±1.02[$]	42 ±1.98[$]

[a] $p< 0.001$ SD vs control; * $p< 0.01$ telmisartan vs SD; [#] $p< 0.01$ nimodipine vs SD; $ $p< 0.01$ telmisartan+nimodipine vs SD.

Table 3: Effect of telmisartan, nimodipine and combination of both on Brain MDA, GSH and acetylcholine esterase activity.

Groups	MDA (nmol/g tissue) (Mean±SEM)	GSH (micro mol/min/g tissue) (Mean±SEM)	AChase activity (micromol/L/g tissue)
Control	12.7 ± 0.35	2.35±0.24	2.04± 0.36
Sleep deprived + distilled water	12.4± 1.24	2.29±0.24	2.12± 0.52
Sleep deprived	24.5±1.81[a]	1.41±0.08[α]	4.52±0.38[A]
Sleep deprived + telmisartan	14.2±0.72*	2.16±0.15[β]	2.62±0.36[B]
Sleep deprived + nimodipine	16.5±2.45[#]	2.19±0.18[γ]	2.74±0.26[C]
Sleep deprived + telmisartan + nimodipine	14.8±1.98[$]	2.09±0.14[δ]	2.36±0.28[D]

[a] $p<0.01$ SD vs control, *$p<0.05$ telmisartan vs SD, [#] $p<0.05$ nimodipine vs SD, [$] $p< 0.01$ telmisartan +nimodipine vs SD.[α] $p<0.01$ SD vs control, [β] $p<0.05$ telmisatan vs SD, [γ] $p<0.05$ nimodipine vs SD, [δ] $p< 0.01$ telmisartan +nimodipine vs SD.[A] $p<0.01$ REM vs control, [B] $p<0.03$ telmisartan vs SD, [C] $p<0.05$ nimodipine vs SD, [D] $p< 0.01$ telmisartan+nimodipine vs SD. One-way ANOVA followed by *post hoc* Tukey's test.

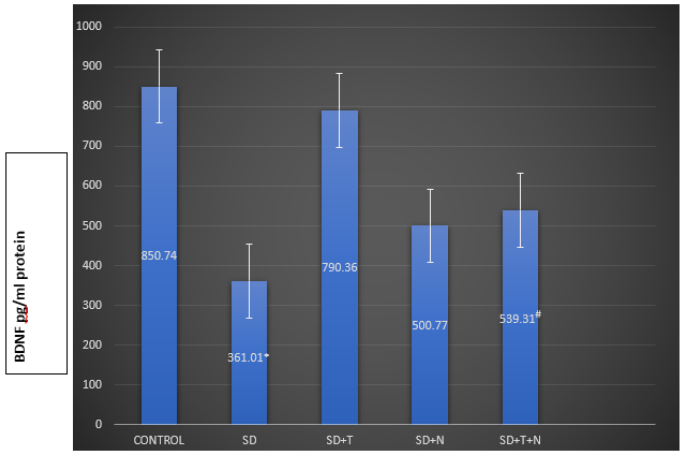

Figure 1: BDNF levels in brain tissue.

* $p<0.05$ Sleep Deprivation vs control, # $p<0.05$ Sleep Deprivation +telmisartan vs SD.

were seen (Figures 1A, 2A, 3A and 4A). The sections from the sleep-deprived group revealed many damaged neurons in the CA3, CA1, and dentate gyrus, which were darkly (basophilic) stained and had shrunken and fragmented nuclei. Vacuoles are seen in hippocampus neutrophils. Degenerative changes ranging from mild to severe were observed. Brain section of Sleep-Deprived (SD) rats treated with telmisartan, nimodipine and those with combination of these drugs protected from neuronal damage compared to sleep deprived group (Figures 3, 4 and 5).

Neuronal counting

Photograph of the CA1, CA3, Dentate gyrus area were taken and the number of Normal healthy neurons out of 100 neurons were counted with the help of image J software.

Healthy neurons-cells with well-defined nuclear boundary, pale and round nucleus with prominent nucleoli.

Damaged neurons-Darkly stained with shrunken and fragmented nuclei.

Groups	CA1	CA3	DG
Control 1	94	84	95
Control 2	95	86	93
SD 1	72	54	77
SD 2	89	05	09
SD+ N 1	90	79	82
SD+ N 2	90	68	80

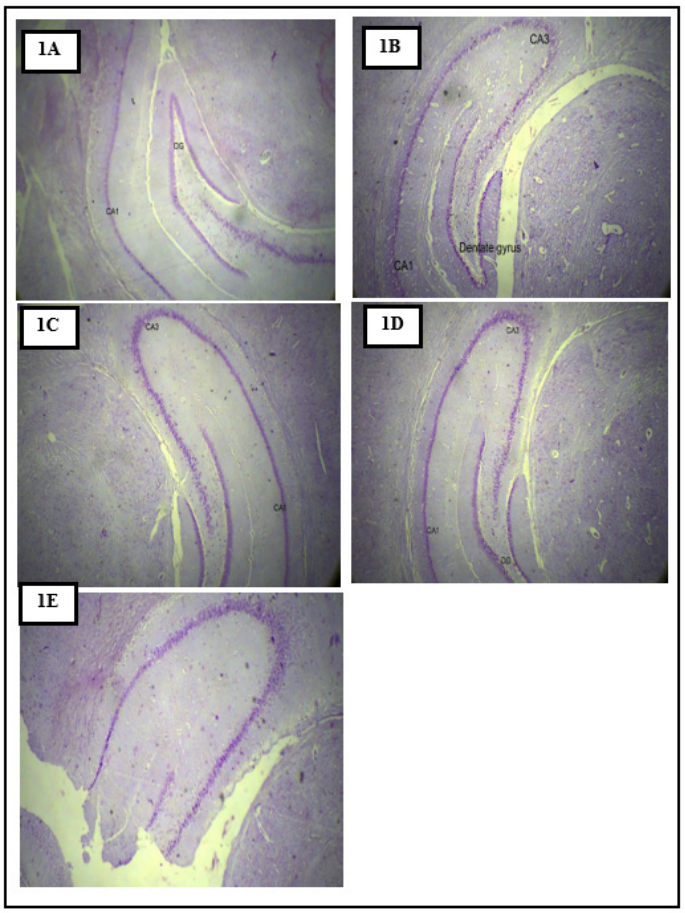

Figure 2: Histopathological findings Cresyl violet stained sections of hippocampus of brain samples of all the groups.

1A-control, 1B-sleepdeprived, 1C-SD+telmisartan, 1D-SD+nimodipine, 1E – SD + telmisartan + nimodipine.

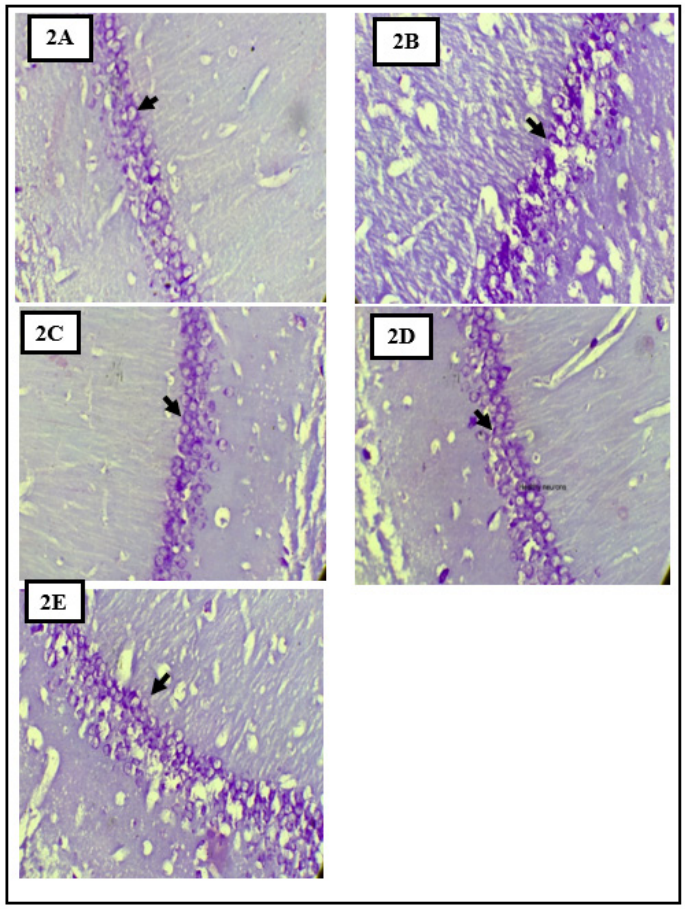

Figure 3: Cresyl violet stained sections of CA1 of the hippocampus.

2A- control, 2B-sleep deprived 2C-SD+telmisartan, 2D-SD+nimodipine, 2E-SD+telmisartan+nimodipine.

Groups	CA1	CA3	DG
SD + T 1	82	61	91
SD + T2	92	59	86
SD+N+T 1	85	55	73
SD+N+T2	89	74	85

SD-sleep depivation, N1- nimodipine slide1, N2-nimodipine slide 2, T1-telmisartan slide 1, T2-telmisartan slide2

DISCUSSION

In the present study, telmisartan, nimodipine and combination of both mitigated the memory impairment caused by chronic REM Sleep deprivation (18 hr/day) for 21 days with chronic dosing. Spatial learning and memory were assessed using Morris water maze test. The data showed that spatial and learning memory were impaired by 21 days REM Sleep deprivation. Malondialdehyde (MDA) and reduced GSH were measured to assess oxidative stress in brain. The level of cholinergic activity was evaluated by measuring acetylcholinesterase activity. Structural changes in the brain were studied by histopathological examination of brain.

In current study, sleep deprivation was induced in rats using modified multiple platform model.[23] The principle is the same as in the inverted flower pot model, with muscle tone loss during REM sleep. The model results in a significant reduction of 90% to 95% in Rapid Eye Movement (REM) sleep, which has been confirmed by Machado *et al.*, 2004 and Medeiros al., 1998 using electroencephalographic recording to monitor sleep deprivation.[24,25] Our data showed that chronic REM sleep deprivation (18 hr/day) for 21 days impaired spatial learning and memory. Numerous studies have demonstrated inverse relationship between REM sleep and cognition, and REM sleep deprivation inflicts cognitive distortion. Previous research has shown that chronic sleep deprivation using the multiple platform method for 18 hr per day for up to 21 days impairs both the acquisition rate in the Morris water maze and the ability to recall the platform position in the subsequent probe test.[26]

The modified multiple platform model used in this study has some advantages over the other models of sleep deprivation. For instance, it is possible to deprive several animals at once, without having to laboriously monitor their electrophysiological sleep features. Additionally, it removes the immobilization and

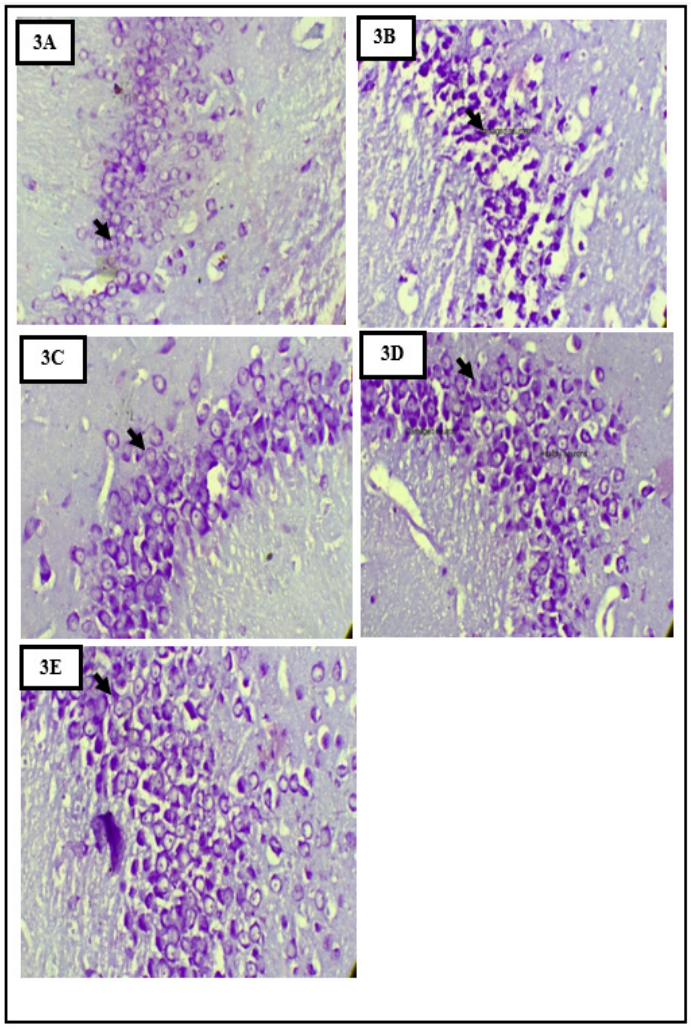

Figure 4: Cresyl violet stained sections of CA3 of the hippocampus.

3A- control, 3B-sleep deprived 3C-SD+telmisartan, 3D-SD+nimodipine, 3E-SD+telmisartan+nimodipine.

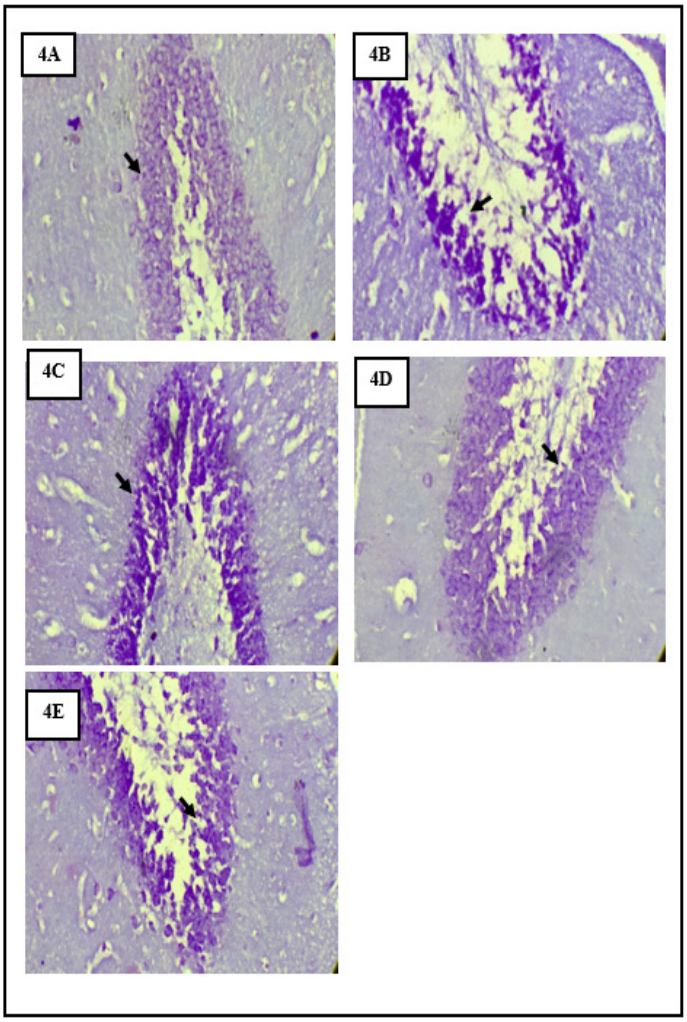

Figure 5: Cresyl violet stained sections of Dentate gyrus of the hippocampus.

4A- control, 4B-sleep deprived 4C-SD+telmisartan, 4D-SD+nimodipine, 4E-SD+telmisartan+nimodipine.

isolation stress seen in the single platform model. However, it can be still affected by confounding factors, namely, stress and anxiety. It is notable that all models of sleep deprivation affected both REM and NREM phases of sleep to different degrees.[25]

Sleep appears to reduce metabolic needs. As a result, lack of sleep may raise metabolic rate, which in turn may increase oxidative stress. The current study shows an increase in brain MDA and decrease in total GSH following REM sleep deprivation suggesting free radical generation. Our data corroborate with previous reports that sleep deprivation induces hippocampal oxidative stress, which reflects on neuronal excitability, molecular signalling, and cognitive functions.[27,28] Mallick *et al.*[29] discovered that a lack of REM sleep reduces membrane fluidity in the rat brain. D'Almeida *et al.* discovered that the thalamus and hypothalamus are more vulnerable to free radical damage after sleep deprivation, as evidenced by a decrease in GSH levels in these regions.[30] Increase in hippocampal oxidative stress is reflected by decreased levels of glutathione and increased lipid peroxidation proposed as sensitive indices of pro-oxidants[31-33] and the study showed the oxidative damages observed in hippocampus, can contribute to the impairment of learning function.[34]

In our study, on treatment with telmisartan, nimodipine and the combination of these two drugs post REM sleep deprivation for 21 days, rats brain tissue showed decrease in MDA and increased in GSH levels. These results were in accordance with previous studies, where in telmisartan and nimodipine were evident in reducing oxidative stress.[29] The antioxidant activity observed with telmisartan can be explained by that Reactive Oxygen Species (ROS) are involved in many of the Angiotensin II signalling pathways and blockade of this pathway by a RAS blocker may be involved in inhibiting the generation of reactive oxygen species. Peripheral administration of telmisartan can penetrate the blood brain barrier in a dose-dependent manner and inhibit the centrally mediated effects of angiotensin II.[11] The effect of nimodipine on oxidative stress caused by traumatic brain injury is unclear.

Impairment in learning and memory observed in patients with AD are partly caused by modulation within the cholinergic system. Cholinergic transmission involves the activity of choline acetyltransferase enzyme which is involved in ACh synthesis and is terminated mainly by acetylcholine hydrolysis via the acetylcholinesterase enzyme. It is believed that the activity of AChE could affect the underlying processes in Alzheimer's disease.[35] Thus, in our study, we evaluated the effects of telmisartan, nimodipine and combination of both on AChE activity and correlated these findings with their cognition improvement. Telmisartan 3.60 mg/kg, nimodipine 5mg/kg and the combination of both these two drugs significantly inhibited the AChE activity within the hippocampus of rats and showed a similar level of inhibition compared to control group.

It has been established that Brain Derived Neurotropic Factor (BDNF) and nerve growth factor involve in synaptic plasticity and neuronal survival. It is believed that REM sleep deprivation is related to neurotrophic factor content in rat brain.[36] Telmisartan has a protective role in increasing cognition via upregulation of hippocampal BDNF levels in hypertensive rats.[37] It has been reported that, nimodipine has neuroprotective effect on the motor neuron survival in various rat model.[36] In our study, BDNF levels in sleep deprived group were significantly reduced as compared to control group. Only telmisartan group showed significant increase in BDNF levels in brain compared to sleep deprived group. Nimodipine group and the group treated with both telmisartan and nimodipine showed increased BDNF levels than sleep deprived, though it was not significant.

Stress affects the morphology of the hippocampus, and increased corticosterone levels suppress cell proliferation and neurogenesis in rodents, resulting in cell loss in the CA1 and CA3 sections of the hippocampus, according to previous research. Furthermore, repeated restraint stress can cause apical dendritic atrophy in CA3 pyramidal neurons. By constructing the correct route during the learning phase, neurons in the hippocampal CA1 and CA3 areas play an important role in identifying the hidden platform in the MWM learning test. The hippocampal CA1 neurons are active in the acquisition of spatial learning and memory.[37]

In the current study, histopathological examination revealed that the majority of neurons in the CA3, CA1, and dentate gyrus were healthy, with pale and round nuclei, well-defined nuclear boundaries, and prominent nucleoli in the control group. Many damaged neurons in CA3, CA1, and dentate gyrus were darkly (basophilic) stained in the sleep-deprived group, with shrunken and fragmented nuclei. Vacuoles are visible in hippocampal neutrophils. In drug treated group all the sections showed reduced damaged neurons compared to SD group. Neuronal counting was also done and reduced number of neurons were observed in sleep deprived group with respect to control group. Treatment groups showed a greater number of neurons as compared to sleep deprived group.

LIMITATIONS

a) In this sleep deprivation model, even control procedure also induces small amount of sleep deprivation.

b) EEG findings and cortisol levels in brain were not analysed.

c) Mechanism by which nimodipine improve cognition was not elucidated in a lucid way.

CONCLUSION

In the current study, oxidative stress was linked to memory deficits caused by sleep deprivation. Brain section of rats treated with telmisartan, nimodipine and those treated with both of these drugs showed less damage of neurons compared to sleep deprived group. The findings show that telmisartan and nimodipine have significant cognitive-enhancing activity, which could be attributed to antioxidant properties or acetylcholinesterase inhibition. However, other putative mechanisms need to be investigated.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this manuscript.

ABBREVIATIONS

SD: Sleep deprivation; **BDNF:** Brain derived neurotropic facto; **ANOVA:** Analysis of variance; **REM:** Rapid eye movement; **NREM:** Non-rapid eye movement; **RAS:** Renin-Angiotensin-System; **ACE:** Angiotensin converting enzyme; **ARB:** Angiotensin receptor blocker; **CCSEA:** Committee for Control and Supervision of Experiments on Animals; **IAEC:** Institute Animal Ethics Committee; **MDA:** Malondialdehyde estimation; **GSH:** Reduced glutathione estimation; **ACE:** Acetylcholinesterase; **SEM:** Standard Error Mean; **MWM:** Morris Water Maze.

SUMMARY

In today's fast-paced world, sleep deprivation ranks first among neglected human basic needs. Sleep Deprivation (SD) may impair advanced neural functions such as decision-making, learning, and memory.

Nimodipine boosts hippocampal acetylcholine and improves spatial cognition. Telmisartan has been shown to improve cognitive function in amnesic rats given scopolamine.

Chronic administration of telmisartan, nimodipine, or a combination of the two drugs improved spatial learning and memory deficits in Wistar rats caused by REM sleep deprivation. When compared to SD rats, the telmisartan group had a significant increase in BDNF levels ($p<0.05$). Telmisartan, nimodipine, and their combination groups had less damaged neurons in histopathological sections.

The current study found that telmisartan, nimodipine, and the combination of these two drugs reversed sleep deprivation-induced cognitive impairment by lowering oxidative stress, increasing cholinergic activity, and increasing BDNF levels, and histopathological findings back up this claim. However, additional research is required to confirm the findings.

ETHICAL APPROVAL

The experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/62/2017 dated 23.09.2017).

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