Research Article

EVALUATION OF SLEEP DEPRIVATION (A NOVEL ALZHEIMER'S DISEASE MODEL) BY COMPARATIVE STUDY WITH SCOPOLAMINE AND DIAZEPAM INDUCED AMNESIA IN MICE

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ABSTRACT

Sleep disturbances and dementia increases with advancing age and about 45% of Alzheimer patients have disruptions in their sleep and sundowning agitation. Clinical evidence showed that people who suffer from chronic insomnia are about 11 times more likely to develop Alzheimer in latter life. The present study was undertaken to study the effect of Sleep deprivation in learning and memory and correlates with established method like Scopolamine and Diazepam induced Alzheimer models in mice. In this method, mice were deprived from sleep for 5 days by using multiple platform method and Behavioral changes were evaluated using passive avoidance, Y-maze, Elevated plus maze and Morris water-maze tests. Sleep deprivation in mice showed significant decrease in learning and memory as behavioral changes studied from passive avoidance, Y-maze, Elevated plus maze and Morris water-maze tests. The behavioral changes are comparable with the established methods like Scopolamine and Diazepam induced amnesia in mice. This finding suggests that Sleep Deprivation can be used as Alzheimer's model in experimental animal to study effect of drugs on learning and memory.

Keywords: Sleep deprivation, Morris water-maze test, Y-maze, Scopolamine, Alzheimer's model.

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive neurodegenerative disease of the brain that is characterized by impairment of memory and eventually by 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, 2]

Sleep disorders affect a large part of the general population, with up to 56% of individuals reporting sleeping problems in the USA.[3] Impairment of the ability to 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[4]. About 45% of Alzheimer's disease (AD) patients have disruptions in their sleep and sun-downing agitation[5]. Young and middle-aged adults who *suffer* from *insomnia* are 11 times more *likely to develop Alzheimer's* and depression in their later life [6]. *Chronic* lack of sleep may promote the development of *Alzheimer's* disease and for *people* suffering from *insomnia* and other sleep disorders increases the risk of *Alzheimer's in later life*[7].

The literature evidences which reveals 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[8]. 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 on learning and memory, whereas a sleep deficit results in performance impairment both in rodents and humans[9]. Numerous studies have demonstrated that sleep deprivation in laboratory animals produces memory deficits in several behavioral models, such as avoidance tasks[10, 11], Morris water maze task[12], and radial maze task[13] and in object recognition test[14].

MATERIALS AND METHODS

Drugs and Chemicals

The drugs used were Diazepam (Ranbaxy, India), Scopolamine (German Remedies) and Normal Saline. All drug solutions were prepared in normal saline before use.

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

Experimental Design

On the 1st day of the experiment, the animals were divided randomly into four groups of six animals in each. Group I: Normal control which received the vehicle (normal saline, i.p),

Group II: Subjected for 5 days sleep deprivation and they receive normal food and water.

Group III Scopolamine (0.4mg/kg) was injected i.p

Group IV: Diazepam (1mg/kg) was injected i.p route

Scopolamine and Diazepam injection were dissolved separately in normal saline and injected i.p., volume of i.p. injection was 1 ml/100 g of mouse.

Assessment of Memory and Retention

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[16, 17].

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. TL was noted after 45 min of injection of Diazepam, 1 mg/kg, i. p and after 24 h and for Scopolamine (0.4 mg/kg, i.p.)[18] administered to mice and TL was noted after 45 min of injection and after 24 h.[16] and immediately after sleep deprivation first trial was given and after 24 hr TL was noted for second time[19].

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[20, 21, 16]. 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 sec during the first trail were used for the second trail and subsequently for the retention test after 24 hr of fist trial. Memory retention was examined 24 h after the first day trial on the second day. SDL was noted after 45 min of injection of Diazepam, 1 mg/kg, i. p and after 24 h; Scopolamine (0.4 mg/kg, i.p.) administered to mice and SDL was noted after 45 min of injection and after 24h.[16] and immediately after sleep deprivation first trial was given and after 24 hr SDL was noted for second time[22, 17].

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[23, 19]. 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.

Morris Water Maze Test

The water maze test was performed according to the method of Morris (1984) with some modifications. The apparatus is a circular water tank filled to a depth of 20 cm with 25°C water. Four points equally distributed along the perimeter of the tank serve as starting locations. The tank is divided in four equal quadrants and a platform (19 cm height) is located in the centre of one of the quadrants. The platform remains in the same position during the training session. Animals are given 2 – 4 trails per day for 4 – 5 days. The animals are released into the water and allowed to find the platform, cut-off time being 90 seconds. Well trained animals escape in less than 10 seconds. One day after the last training session, each animal was subjected to a probe trail (120 Seconds) without platform. During the retention test the latency to reach the escape platform is measured in seconds[21, 24].

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 T2and cleaning them carefully [25]. After 45 min of administration drugs Diazepam and scopolamine first trial is given and same in case of sleep deprived groups followed by 24 hr acquisition period and then final reading are taken[14].

Sleep Deprivation (SD) Method

This method of sleep deprivation used was an adaptation of the multiple platform method, originally developed for rats [26]. The animals which were subjected for 5 days sleep deprivation by multiple platform method[27]. 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[28]. A 100-W light illuminates the chamber during the period of sleep deprivation[29]. 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 was considered to be statistically significant.

RESULTS

Elevated Plus Maze

The results are given in Table 1 and plotted graph is shown in Fig 1. The Transfer Latency (TL) of the Group II (sleep deprived) animals were significantly increased in comparison with the Group I (normal control) animals (p< 0.01). Increase in Transfer Latency (TL) time for group III (scopolamine treated) and group IV(diazepam treated) in comparison with group I are significant (p<0.001 and p<0.05 respectively). The increase in TL indicates decrease in cognition due to memory impairment.

Table 1: Transfer Latency (TL) time on Elevated plus Maze

Groups	Transfer Latency(TL) in Sec; (Mean ± SEM)
Group I	23.50 ± 2.52
Group II	45.33 ± 4.52**
Group III	54.67 ± 4.84***
Group IV	41.50 ± 3.25*

Values represented in (Mean ± S.E.M, n=6), *p<0.05, **p<0.01, ***p<0.001; p compared vs. Group I.

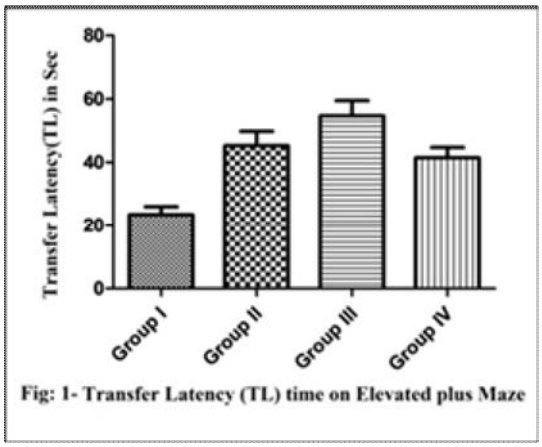

Fig: 1- Transfer Latency (TL) time on Elevated plus Maze

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(sleep deprived) was found to be reduced in comparison with Group I animals in terms of Step down Latency (SDL) significantly (p<0.01). Decrease in Step down Latency (SDL) time for group III (scopolamine treated) and group IV(diazepam treated) in comparison with group I are significant (p<0.001 and p<0.01 respectively). The decrease in SDL indicates decrease in memory.

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

Groups	Step down Latency in Sec; (Mean ± SEM)
Group I	52.17 ± 4.84
Group II	29.33 ± 4.15**
Group III	24.83 ± 2.65***
Group IV	32.83 ± 3.71**

Values represented in (Mean ± S.E.M, n=6), *p<0.05, **p<0.01, ***p<0.001; p compared vs. Group I.

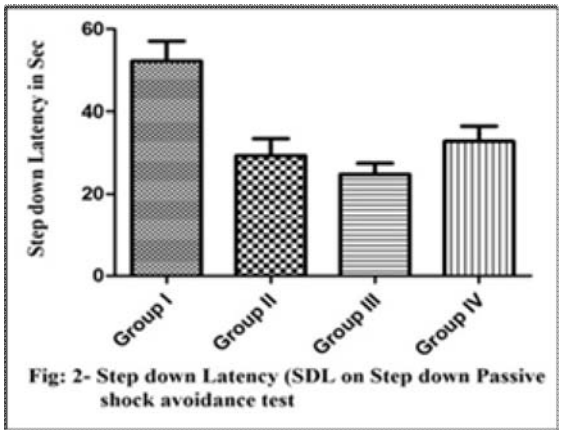

Fig: 2- Step down Latency (SDL on Step down Passive shock avoidance test

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<0.05). Decrease in percentage of alteration for group III (scopolamine treated) and group IV (diazepam treated) in comparison with group I are significant (p<0.01 and p<0.05 respectively). The decrease in percentage alteration indicates decrease of spatial working memory.

Table 3: Percentage alteration on Y maze task

Groups	Percentage alteration; (Mean ± SEM)
Group I	56.50 ± 5.04
Group II	39.17 ± 3.72*
Group III	36.50 ± 4.11**
Group IV	39.50 ± 3.47*

Values represented in (Mean ± S.E.M, n=6), *p<0.05, **p<0.01, ***p<0.001; p compared vs. Group I.

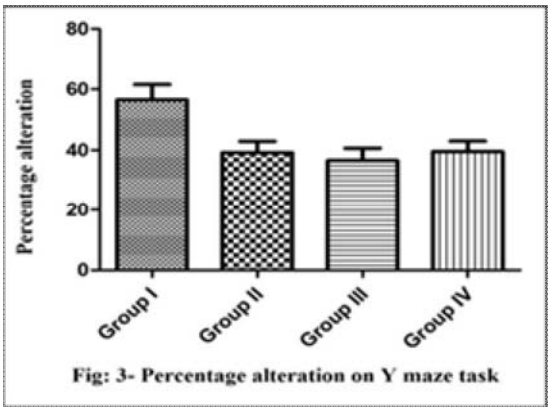

Fig: 3- Percentage alteration on Y maze task

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). The increase in escape latency for group III (scopolamine treated) and group IV (diazepam treated) in comparison with group I are significant (p<0.001 and p<0.001 respectively). The increase in latency to escape onto the hidden platform in comparison with the Group I animals (p<0.001) indicates loss of memory retention and non-spatial working memory.

Table 4: Escape Latency on Morris water maze task

Groups	Escape Latency in Sec; (Mean ± SEM)
Group I	14.33 ± 2.18
Group II	47.83 ± 4.39***
Group III	56.33 ± 4.47***
Group IV	44.33 ± 3.16***

Values represented in (Mean ± S.E.M, n=6), *p<0.05, **p<0.01, ***p<0.001; p compared vs. Group I.

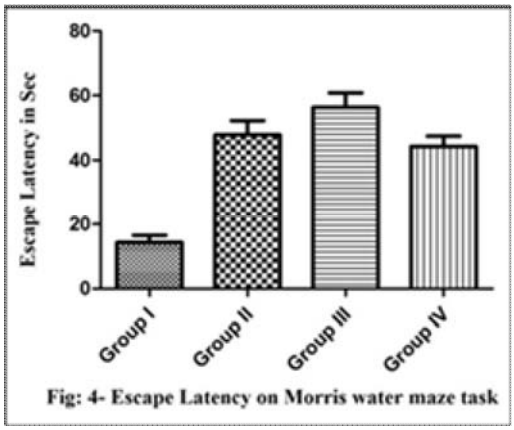

Fig: 4- Escape Latency on Morris water maze task

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<0.05). Time needed for exploring a novel as well as familiar object was increased in group III(scopolamine treated) and group IV (diazepam treated) in comparison with group I are significant (p<0.01 and p<0.05 respectively). The increase in exploration time indicates decreased learning and memory retention of objects.

Table 5: Exploration time on Object recognition task

Groups	Exploration time in sec (familiar object); (Mean ± SEM)	Exploration time in sec (new object); (Mean ± SEM)
Group I	4.66 ± 0.57	7.83 ± 0.70
Group II	7.83 ± 0.83*	12.17 ± 0.94*
Group III	8.33 ± 0.88**	12.67 ± 1.45**
Group IV	7.55 ± 0.76*	12.17 ± 0.61*

Values represented in (Mean ± S.E.M, n=6), *p<0.05, **p<0.01, ***p<0.001; p compared vs. Group I.

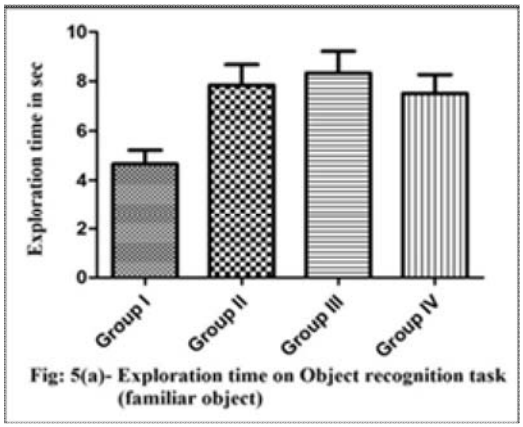

Fig: 5(a)- Exploration time on Object recognition task (familiar object)

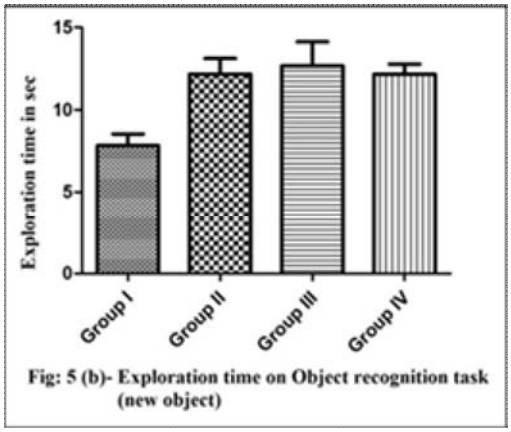

Fig: 5 (b)- Exploration time on Object recognition task (new object)

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. Our behavioral data show a significant learning deficit in Elevated plus maze, Y-maze, Morris water maze and in Object recognition task after sleep deprivation. The present study demonstrates that sleep deprivation for a longer period affect in cognition and memory. The decrease in cognition and memory impairment may for decrease in melatonin and for decline in neurotransmitters level in the brain. Currently Alzheimer's patients responding well

with melatonin treatment and shows lots of improvement in cognition and memory[30]. Melatonin was recently reported to be an effective free radical scavenger and antioxidant. Melatonin is believed to scavenge the highly toxic hydroxyl radical, the peroxy nitrite anion, and possibly the peroxyl radical. Also, secondarily, it reportedly scavenges the superoxide anion radical and it quenches singlet oxygen. Additionally, it stimulates mRNA levels for superoxide dismutase and the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (all of which are antioxidative enzymes), thereby increasing its antioxidative capacity. Also, melatonin, at least at some sites, inhibits nitric oxide synthase, a pro-oxidative enzyme. 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[31]. 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[31]. Melatonin also reduces the hyperphosphorylation of tau protein, which leads to the neurofibrillary tangles of Alzheimer's disease [32].

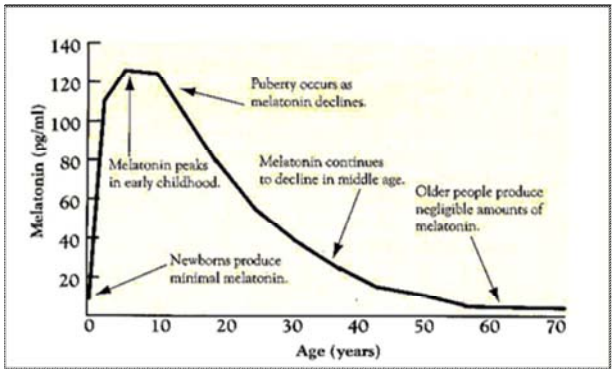

Figure 6: Decline of melatonin with age. [Journal of anti-aging medicine; Pierpaoli W; 2(4):343-348 (1999)

The synthesis and secretion of melatonin and other neurotransmitter occur mainly in sleep cycle. The paradoxical sleep is important for learning and acquisition of memory[14]. In our study due to sleep deprivation in mice for 5 days may induce a global reduction (down regulation) in the number of postsynaptic noradrenergic receptors in the brain[33].

The mechanisms underlying learning and memory deficits following sleep deprivation are not understood at present. Data from different studies have shown that 96 h of SD before training impairs acquisition and consolidation of aversive tasks[34], and that treatment with the cholinergic agonist pilocarpine during the deprivation period blocks SD effects on inhibitory avoidance tasks[35].

Different research studies suggest that sleep deprivation would reduce the antioxidant defenses[36]. Sleep might involve the elimination of toxic compounds (e.g. free radicals) and the replenishment of energy stores. The hippocampal increase in oxidative stress 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[37]. Increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases. Scopolamine produced amnesia by muscarinic receptor antagonism and decreases in Ach level in brain and induces deficits in learning and memory paradigms[38]. Diazepam induced amnesia in the experimental animals by on the benzodiazepine site of the GABA / benzodiazepine receptor complex and impaired both prospective and retrospective memory[39].

CONCLUSION

In conclusion, our data for sleep deprivation not only correlates with established Alzheimer's model of scopolamine and diazepam induced Alzheimer in mice. Sleep plays an important role in memory formation and sleep deprivation is a natural Alzheimer's disease animal model may be a better option than Scopolamine and Diazepam induced Alzheimer's disease because Sleep deprivation can be as one of the most important factor for Alzheimer's disease. However, further research and investigation are required to adapt sleep deprivation as Alzheimer's disease model in experimental animals.

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