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Original Article

Evaluation of Armodafinil's Anti-amnestic Activity in Scopolamine-induced Amnesia in Wistar rats

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ABSTRACT

Background: Amnesia is the loss of memory that majorly affects middle-aged to older people with a prevalence (1.0 - 2.6%) of the overall population. Studies reported that scopolamine induction results in memory dysfunctions observed in demented patients. The current study evaluated the therapeutic potentials of armodafinil and its beneficial role in dementia, learning, and memory impairment. Armodafinil is the (-)-R enantiomer of modafinil. It is a nootropic used for the treatment of narcolepsy and the reversal of anesthetic effects. It binds to and thereafter inhibits the dopamine-reuptake pump, enhancing the concentration of dopamine in the synaptic gaps. **Materials and Methods:** 30 albino Wistar rats (250-300g) were included in the study, and they were randomly divided into five groups of six rats each (n = 6), two of which worked as controls: a control negative group and a control positive group. Scopolamine (3 mg/kg) was administered intraperitoneal to the control positive group on the 16th day after receiving 200mg/kg of Brahmi extract orally daily for 15 days. Armodafinil (15&30mg/kg) was given orally to the test groups for 15 days, and scopolamine (3mg/kg) was administered on the 16th day. The behavioral measurements were performed at the end of the 16th day, after which the animals were euthanized and brains were retrieved for biochemical assessments. **Results:** In the present study, armodafinil groups showed a significant increase in the % alterations in Y-maze, time spent in Morris water maze, motor activity on the spinning rod, and the response of steps climbed in the staircase test, and also substantial variation in levels of antioxidants and neurotransmitters in drug-treated groups were found. Both behavioral and biochemical analyses indicated the neuroprotective effects of armodafinil in memory dysfunction and are appreciated as a potential therapeutic approach for Parkinson's disease (PD). **Conclusion:** From the study armodafinil-treated groups showed retrieval of memory when compared to control negative (scopolamine-treated) group which showed heavy alteration in memory. The current study explored the neurobehavioral and memory-enhancing effects of armodafinil in scopolamine-induced amnesia which can be an essential tool to current clinical approaches toward neuroprotection.

Keywords: Amnesia, Armodafinil, Brahmi, Memory, Oxidative stress, Scopolamine.

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INTRODUCTION

Amnesia is a complex condition with memory deficiency that emerges with an array of neurological and neurodegenerative diseases impacting the brain. Amnesia is associated with diseases like Alzheimer's disease, Parkinson's disease, traumatic brain injury, cerebrovascular accident, Huntington's disease (HD), and Lewy body disease where its progression leads to dementia or memory loss. Current pharmacological treatment includes cholinesterase inhibitors such as Donepezil, Rivastigmine, and Galantamine; and glutamate regulators such as Memantine, which are used in the treatment of amnesia in Alzheimer's disease.[1] New targets therapies for Parkinson's disease have been discovered, including Prasinezumab, an IgG_1 monoclonal antibody that targets aggregated-Synuclein.[2] Aducanumab is another medication, eradicating beta-amyloid in the brain, and has been reported of slowing cognitive and functional deterioration in people with early Alzheimer's.[3] Non-pharmacological treatments, especially cognitive behavioral therapy, are particularly beneficial because they strongly emphasize how one's perceptions, feelings, and mood affect their emotions and behavior, which in turn helps them develop their behavioral and cognitive skills.[4] The cranial stimulation techniques, such as transcranial stimulation and deep brain stimulation, are also effective in treating memory problems.[5]

Neurological disorders are the second leading cause of death, with a prevalence of over 30 million people. These account for 10% of the total disease burden in India.[6] Nearly 50 million individuals worldwide suffer from neurodegenerative disorders; by 2050, that number is anticipated to rise to 115 million.[7] To

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Table 1: Experimental groups.

Groups (*n*=6)	Treatment
I	Vehicle group
II	Negative control – treated with scopolamine (3mg/kg)
III	Positive control – Brahmi extract (200mg/kg) for 15 days followed by scopolamine (3mg/kg) on 16th day
IV	Test – low dose (15mg/kg) Armodafinil for 15 days followed by scopolamine (3mg/kg) on 16th day
V	Test – high dose (30mg/kg) Armodafinil for 15 days followed by scopolamine (3mg/kg) on the 16th day

deal with the rising burden of neurological illnesses in the nation, it is essential to strengthening clinical recognition and affordable medical care for these neurological disorders.

Scopolamine exhibits muscarinic antagonistic properties, which interfering with the learning process and causing serious deficits in memory and behavior. Scopolamine-induced amnesia is used to test anti-amnestic medications for central nervous system (CNS) impairment after amnestic dosages were administered since it caused a large rise in Acetylcholinesterase (AchE) activity. It is further supported by the increase in brain oxidative state following the administration of an amnestic dosage of scopolamine.[8]

Studies have reported that armodafinil (a racemic form of modafinil) exhibits stimulant action in the CNS and shows wakefulness-promoting activities.[9] It modulates the levels of chemical messengers in the brain and reduces extreme somnolence.[10] It is used in the treatment of narcolepsy and other brain disorders. It was reported that it increases dopamine levels in the synapses between brain cells and improves alertness by strengthening neural connections.[11] Armodafinil, a eugeroic medication that encourages improved thinking and perception and exhibits anti-amnestic activity by lowering AchE levels, was found to be neuroprotective in the current investigation. The (-)-R enantiomer of armodafinil, modafinil has been shown in earlier studies to have positive effects on increasing neuronal density and improving the survival of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -intoxicated dopaminergic neurons in the substantia nigra region of the brain, highlighting the drug's neuroprotective potential in the treatment of Parkinson's disease.[12]

Thus, the current study identified the neuroprotective action of armodafinil for examining the neurobehavioral and memory-improving effects in scopolamine-induced amnesia, which can be a potent asset for current clinical approaches to neuroprotection.

The predisposing factors include depressive thoughts, stress, and anxiety, which hinder cognitive performance and are one of the factors that impact an amnestic condition.[13] Memory and consciousness loss was generated by both minor head injuries and serious traumatic disorders.[14] Encephalitis or meningitis, or myelitis, are certain acute or chronic infections caused by pathogens that directly affect the brain cells that enter the CNS, which results in neurological deficits.[15] Changes in the stimulation of thyroid hormones are recognized as a risk factor for impaired cognition. The fluctuating levels of thyroid stimulating hormone affect the brain tissues, leading to deficits in memory and learning.[16] Chronic ethanol intake causes an oxidative stress response, and neuroinflammation, and alters neurotransmitter levels, leading to cognitive disruption. For instance, Wernicke-Korsakoff syndrome, in which there is a permanent alteration in the formation of new memories, results in serious amnesia.[17] As Vitamin B_{12} (cobalamin) is important for the synthesis of myelin, which is responsible for the fast conduction of nerve impulses, its deficiency results in a reduction of brain function.[18]

MATERIALS AND METHODS

Chemicals

For Brahmi treatment, tablet containing 250mg of *Bacopa monnieri* pack of 60 tablets from the Himalayan Store was purchased in Tirupati.

Experimental Animals

For the present study, albino Wistar rats (250 - 300g) were procured from Sri Raghavendra Enterprises, Bangalore, India. The rats were housed in polypropylene cages with free access to food and water, 12-hr light/dark cycle, under standardized conditions (temperature 23 ± 10°C). The animals were acclimatized for seven days and the study was conducted in accordance with the protocols approved by the Institutional Animal Ethical Committee (IAEC/XIII/04/RIPER/2019).

Study Protocol

A total of 30 albino Wistar rats (250-300g) were divided into 5 groups with 6 rats per group (Table 1). The Brahmi group was given extract 200mg/kg dose for 15 days of oral administration followed by scopolamine (3mg/kg) on the 16th day. The armodafinil (15 and 30mg/kg) groups were administered orally for 15 days and scopolamine (3mg/kg) was injected on the 16th day.

Behavioral studies

Y-Maze test

The Y-maze was performed for measurement of spontaneous alteration (SAP) which helps in the determination of spatial working memory/short-term memory. The Y-maze was cleaned

with an anti-bacterial agent entirely before starting the test. The sections of the maze were marked as A, B, and C in which each animal was placed into one arm of the Y-maze facing towards the center. During the first trial, one of the arms (C) was closed for the entry whereas the animals explore the other two arms (A&B). Later, the animals were liable to free access in all the arms during the trial phase. The animals were given a 5mins time period in each trial. The animal entry was considered only when it entered with complete four paws from the center and into the arm. The score was calculated based on five alterations ACB (1), CBA (2), BAC (3), ACB (4), and CBA (5). The maze was completely cleaned in between each trial to avoid pheromones produced by animals which affect the actions of next subjects.[19]

% Alteration = {No. of alterations/ (Total arm entries -2)} *100

Morris Water Maze (MWM)

Morris water maze or MWM test is extensively used to evaluate spatial memory of the rodents. Animals were trained for five successive days in a circular pool filled to a depth of 20 cm with water maintained at 28 ± 2°C. The test was executed to observe escape latency in which time was taken by the animal to discover the hidden platform placed in a certain quadrant. The water was made opaque with any harmless substance to keep the platform hidden from the animal. The animal will be guided to the platform if it couldn't discover the platform within 120 sec and compelled on staying on the platform for 30 sec. On the 15th day, each animal will be treated with the respective drug allotted to the group: positive control- Brahmi extract, test groups -armodafinil low dose and high dose subsequently with scopolamine through intraperitoneal route.[20]

Staircase test

Simiand *et al.* method was performed for the staircase test where the instrument consists of five steps (2.5 x 10 x 7.5 cm). The behavioral activity of a single animal was recorded after each mouse was mounted on the box's floor with its back to the staircase. When the animal laid complete four paws upon step it was regarded as climbing and when the animal rose on its hind legs to smell the air on the step was marked as rearing. The number of steps climbed, and rearing was counted for 3mins and noted.[21]

Rotarod test

The Rotarod (by INCO Pvt Ltd.) test was performed to determine the tendency of rats to balance on a spinning rod, which provides an insight into an animal's motor coordination. Each animal was placed in a separate chamber and a start button was pressed which records automatically the time latency with the help of a time counter. The rota rod was programmed with different accelerations from 5 rpm to 40 rpm which is the maximum velocity. The rats were trained for five days, 3 trials per day from 5 rpm for 20 sec to 20 rpm for 300 sec to maintain coordination control. On the test day, the time taken by the animal to drop off from the rotating rod to the platform was recorded. The average time latency to the first drop of the animal from the rod was recorded.[22]

Biochemical Estimations

Preparation of Brain tissue homogenate

Animals were sacrificed by cervical dislocation and the brains were removed and placed in an ice-cold saline solution. All the brain tissues were homogenized in ice-cold phosphate buffer (pH 7.4) and the collected homogenate was centrifuged at 7000 rpm for 15 min. Then the clear supernatant fluid was used for different biochemical estimations.

Estimation of Superoxide Dismutase

The superoxide activity (SOD) was measured by the method of Misra and Fridovich. Briefly, 0.8ml of carbonate buffer (100mM, pH 10.2) and 100 µl of epinephrine (3mM) were added to the brain homogenate and the change in absorbance of each sample at 480 nm in spectrophotometer for 2 mins at a 15 sec interval were recorded. Simultaneously blank and standard solutions were run for SOD activity.[23]

Estimation of Catalase

The catalase activity (CAT) was measured by the method of Aebi. The supernatant (0.1 ml) was added to the cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Then 1.0ml of freshly prepared 30 mM hydrogen peroxide was added that the reaction was observed. The rate of decomposition of hydrogen peroxide will be measured spectrophotometrically from changes in absorbance at 240 nm. The activity of catalase will be expressed as units/mg protein.[24]

Estimation of Acetylcholinesterase

The AchE activity was evaluated by using Ellman's method. In 0.1M Phosphate buffer, the tissues are weighed and homogenized (pH 8). A 0.4ml aliquot of the homogenate is placed in a cuvette with 2.6 ml phosphate buffer (0.1M, pH 8) and 100l 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB). The components of the cuvette were thoroughly mixed with bubbling air, and the absorbance was measured in a spectrophotometer at 412 nm. The basal reading was taken when absorbance exceeds a stable value. A total of 20µl of the substrate (acetylthiocholine) was added, and the change in absorbance was measured. As a result, the change in absorbance per minute was calculated.[25]

Estimation of Glutamate

A portion of supernatant (1ml) will be taken from brain homogenate and dried at 70°C in an oven then the residue was regenerated in distilled water (100ml). Standard solutions of

glutamate at 2mM concentration including with the sample were spotted on chromatography paper with the help of a micropipette. The paper will be positioned in a chamber containing butanol: acetic acid: water (12: 3: 5 v/v) as solvent. The solvent front will extend to the top of the paper then it will be 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 min. 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 a spectrophotometer.[26]

Estimation of Lipid peroxidase (LPO)

The amount of lipid peroxidation was measured by the malondialdehyde (MDA) reactive products present in the homogenate samples of the brain using Ultraviolent (UV)-visible spectroscopy. This estimation was done mainly by the thiobarbituric acid reactive substances (TBARS) assay. To the 0.2ml of supernatant, sodium dodecyl sulfate (SDS) of 0.2ml, acetic acid 1.5ml, and thiobarbituric acid of 1.5ml were added. The components were mixed well and made up to 4ml with water and then heated (95°C for 60 min) in the water bath. After that, cooled and shaken well with the addition of 1 ml of water and 5 ml of n-butanol/pyridine mixture and centrifuged at 4000 rpm for 10 min. The separated organic layer was collected, and absorbance was measured at 532nm. The LPO levels were expressed as nmoles of MDA released/g wet tissue.[27]

Statistical Analysis

All the data were expressed as the means ± Standard Deviation (SD) for 6 animals in each group. Statistical analysis was performed using Graph pad Prism V 8.0 by the Analysis of Variance (ANOVA) and subsequently Tukey's test for comparison among experimental groups. *P*-values less than 0.05 was defined as the statistical significance.

RESULTS

In Scopolamine-induced models, there will be various causes which include neurochemical and behavioral alterations in the brain which lead to severe cognitive, and behavioral deficits. In the present study, the effects of armodafinil (15&30 mg/kg) with scopolamine (3mg/kg) were administered in albino Wistar rats. The animals were trained for five consecutive days in behavioral parameters like Y-maze, Morris water maze, staircase, and rotarod. After induction of scopolamine (3mg/kg) on the 16th day of the trial, each animal was monitored in behavioral parameters and biochemical estimations to know the effects of treatment in amnestic conditions and the above-mentioned parameter results were mentioned below.

Effect of Armodafinil on the Y-Maze test

In Y-maze, the % alterations or SAP was calculated as mentioned in the methods section. The graph shows (Figure 1) a significant difference ($p<0.05$) from the control to scopolamine treated group in percentage of spontaneous alternation. Treatment with Brahmi and armodafinil has moderately increased the

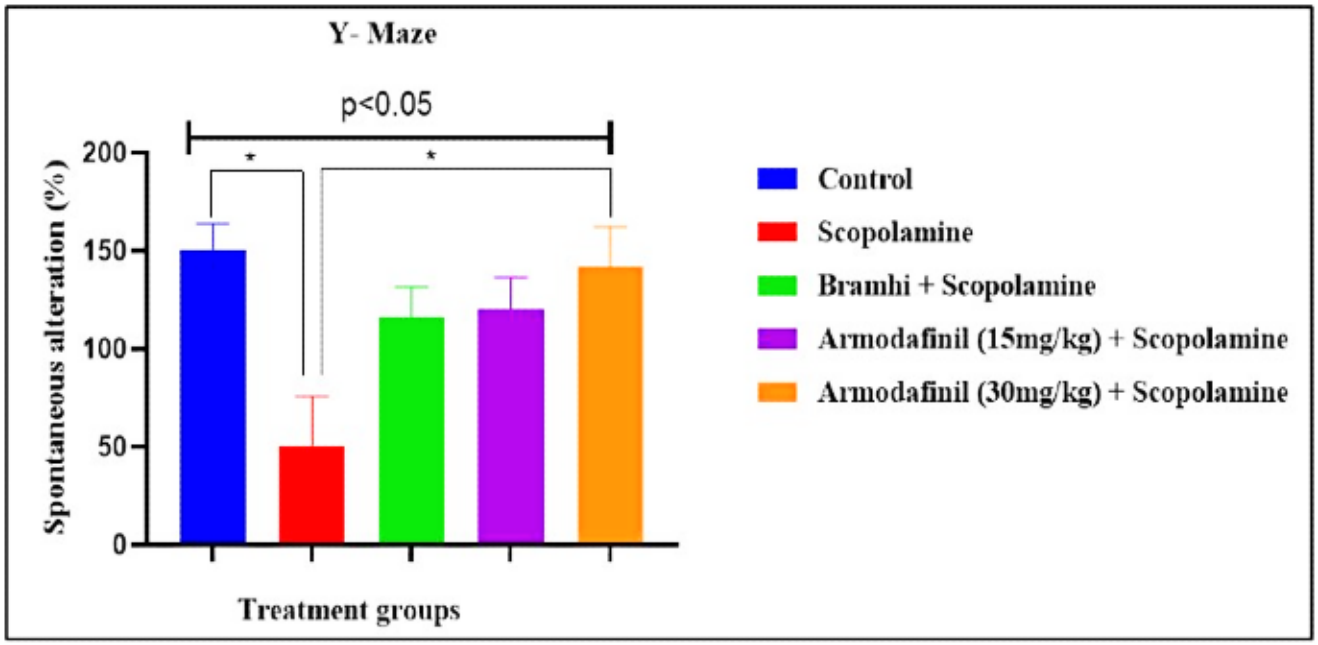

Figure 1: Effect of Armodafinil observed in the Y-Maze test. Graph representing % spontaneous alterations observed in all 5 experimental groups, control, scopolamine, Brahmi + scopolamine, Armodafinil (15mg/kg) + scopolamine and Armodafinil (30mg/kg) + scopolamine; mean ± SD (*n*=6). Calculations were done using ANOVA and Tukey's test for comparison among experimental groups for $p< 0.05$ was defined as statistical significance.

spontaneous alteration. However, the high dose (30 mg/kg) armodafinil treated group has shown significantly improved ($p<0.05$) response in improving the spontaneous alternation and completely reversed the pathological changes incurred by the treatment of scopolamine. Thus, the Y-maze study demonstrates improved cognition in Wistar rats.

Effect of Armodafinil on the Morris Water Maze test

In the MWM test each animal was trained for five consecutive days and allowed to swim in the pool for 120 sec for finding the hidden platform in the targeted quadrant. Figure 2 shows a significant difference ($p<0.05$) from the control to scopolamine treated group in the time spent in the targeted quadrant. The treated groups Brahmi and armodafinil (15mg/kg) have shown alleviative response in the escape latency and the time spent in the targeted quadrant with a significant increase ($p<0.05$) and also ameliorated the effect of scopolamine. Thus, the Morris water maze test demonstrates improved spatial memory in Wistar rats.

Effect of Armodafinil on the Staircase test

The animals were trained for five successive days, on the test day each animal was mounted on the box's floor and then allowed to climb for 3 min. The behavioral activity was observed when the animal placed the four paws on the step then the steps climbed were monitored. As shown in Figure 3, the staircase test results which have a significant increase ($p<0.05$) in the treated (Brahmi and armodafinil- 30mg/kg) groups and a significant difference among control, scopolamine alone treated group and armodafinil group (15mg/kg) which indicates the high dose of armodafinil (30mg/kg) reversed the scopolamine effects in the rat brain. These findings show that armodafinil (15mg/kg) has less response in scopolamine-induced mice.

Effect of Armodafinil on the Rotarod test

In the present study, the tendency of rats was observed when were given 5 days of training on the rotarod apparatus. In the graph (Figure 4), the time spent was decreased significantly by $p<0.05$ in the scopolamine-treated group in comparison with the control group. The treated groups Brahmi and armodafinil showed a significant increase $p<0.05$ of time spent on rotarod which indicated that recovery of pathological changes was done by scopolamine alone treated group.

Effect of Armodafinil on antioxidant assays

As depicted in Table 2, SOD levels were increased in treatment groups- Brahmi and armodafinil (30 mg/kg). The scopolamine alone treated group shows a significant difference ($p<0.05$) compared to the control group. There is a significant difference of $p<0.05$ from the control to scopolamine alone treated group which indicated that more production of hydrogen peroxide released into the cytosol. This leads to reduced levels of catalase in brain tissue. The Brahmi and armodafinil treated groups showed increased levels of CAT compared to the scopolamine treated group significantly ($p<0.05$) which demonstrated that both SOD and CAT levels were ameliorated by the effect of armodafinil. Scopolamine alone treated group noticed an elevated level of MDA in brain tissues and showed a more significant difference of $p<0.05$ in comparison with the control group. The increased levels of MDA were significantly $p<0.05$ reversed by the treatment

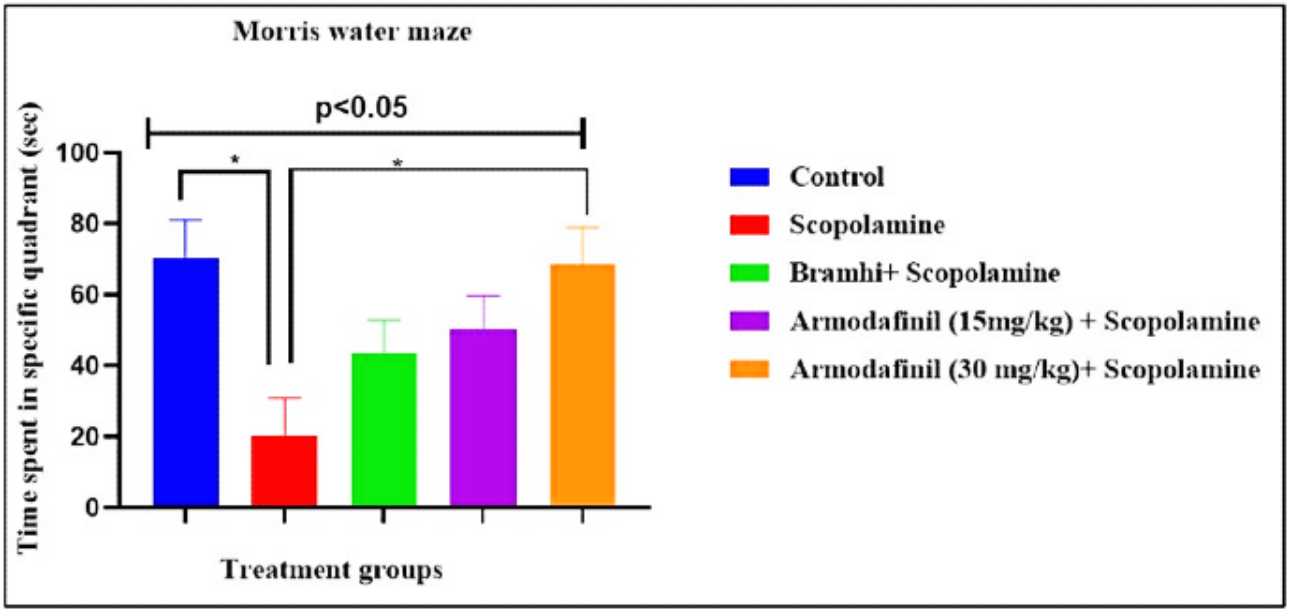

Figure 2: Effect of Armodafinil in Morris water maze. Graph representing time spent by animals in the specific quadrant in seconds in all 5 experimental groups, control, scopolamine, Brahmi + scopolamine, Armodafinil (15mg/kg) + scopolamine and Armodafinil (30mg/kg) + scopolamine. All the data were expressed as the mean ± SD (n=6). Calculations were done using ANOVA and Tukey's test for comparison among experimental groups for $p< 0.05$ was defined as statistical significance.

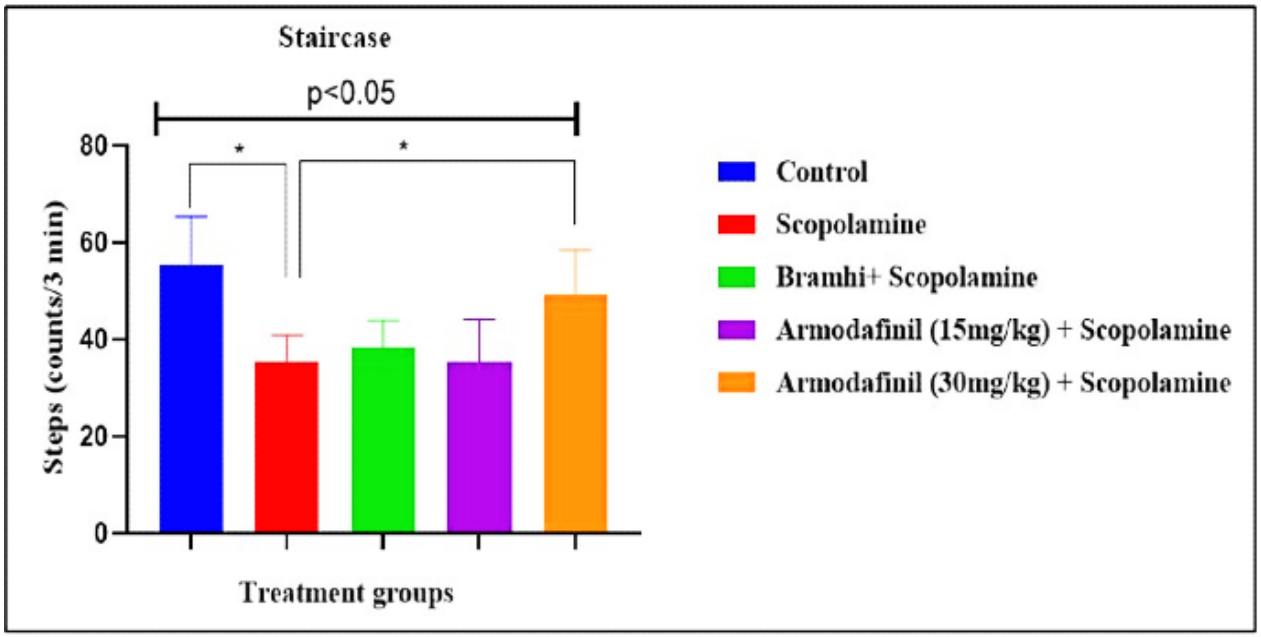

Figure 3: Effect of Armodafinil in Staircase test. Graph representing steps taken (counts/ 3min) by animals in all 5 experimental groups, control, scopolamine, Brahmi + scopolamine, Armodafinil (15mg/kg) + scopolamine and Armodafinil (30mg/kg) + scopolamine. All the data were expressed as the mean ± SD (*n*=6). Calculations were done using ANOVA and Tukey's test for comparison among experimental groups for $p < 0.05$ was defined as statistical significance.

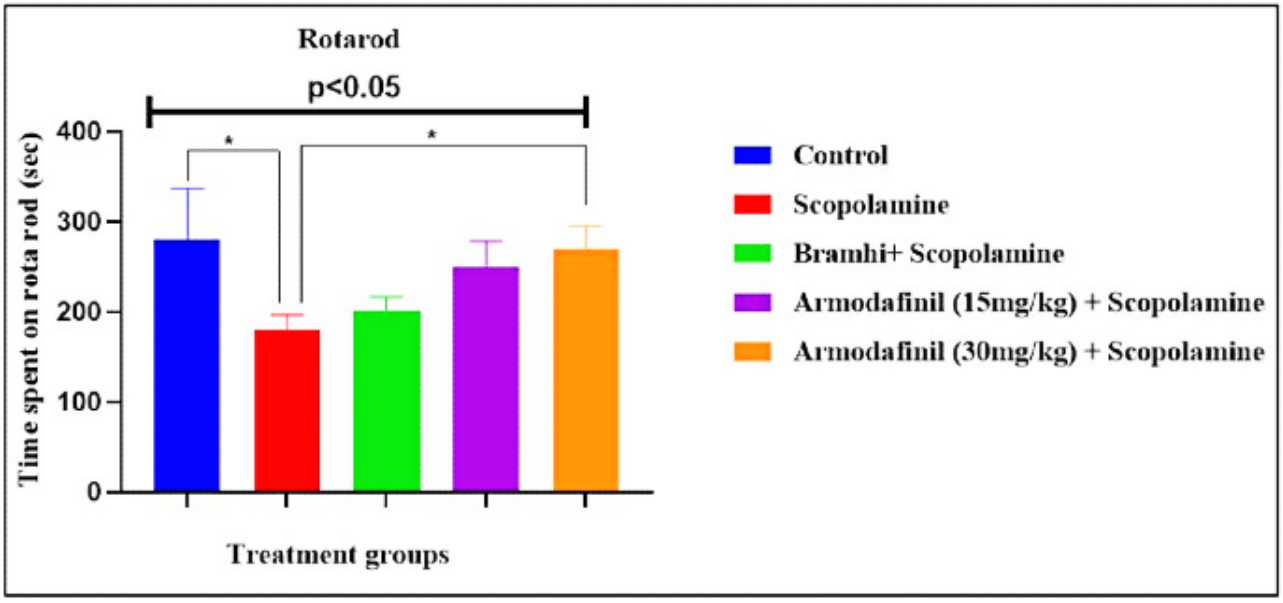

Figure 4: Effect of Armodafinil in Rotarod. Graph representing time spent on Rotarod in seconds by animals in all 5 experimental groups, control, scopolamine, Brahmi + scopolamine, Armodafinil (15mg/kg) + scopolamine and Armodafinil (30mg/kg) + scopolamine. All the data were expressed as the mean ± SD (*n*=6). Calculations were done using ANOVA and Tukey's test for comparison among experimental groups for $p < 0.05$ was defined as statistical significance.

groups Brahmi and armodafinil which indicates the protective role against lipid peroxidation.

Effect of Armodafinil on Acetylcholinesterase levels

As shown in Table 3, the scopolamine alone treated group resulted in upregulation of AchE activity with a less significant difference $p<0.05$ compared to the control group. The treatment groups Brahmi and armodafinil groups which are nootropic agents showed downregulation of AchE activity with a more significant improvement $p<0.05$ compared to the scopolamine treated group.

Table 2: Effect of Armodafinil in antioxidant assays.

Groups	SOD (U/mg of protein)3.3	Catalase (mmol H_2O_2/mg of protein)	Lipid peroxidase (µmol/mg of protein)
Control	150.23 ± 13.56	70.12 ± 10.96	55.34 ± 10.06
Scopolamine (3mg/kg)	50.23 ± 25.5	20.12 ± 10.77	35.45 ± 5.45
Brahmi (200mg/kg) + Scopolamine (3mg/kg)	116.12 ± 15.43	43.46 ± 9.38	38.34 ± 5.61
Armodafinil (15mg/kg) + Scopolamine (3mg/kg)	120.13 ± 16.29	50.24 ± 9.55	35.32 ± 8.90
Armodafinil (30mg/kg) + Scopolamine (3mg/kg)	142.33 ± 19.86	68.74 ± 10.23	49.53 ± 8.97

All the data were expressed as the means ± SD (n=6).

Table 3: Effect of Armodafinil on Acetylcholinesterase assay.

Groups	AChE levels (U/mg of protein)
Control	5.60 ± 0.51
Scopolamine (3mg/kg)	5.24 ± 0.52
Brahmi (200mg/kg) + Scopolamine (3mg/kg)	3.12 ± 0.56
Armodafinil (15mg/kg) + Scopolamine (3mg/kg)	3.53 ± 0.58
Armodafinil (30mg/kg) + Scopolamine (3mg/kg)	4.55 ± 1.23

All the data were expressed as the means ± SD (n=6).

Table 4: Effect of Armodafinil on Glutamate assay.

Groups	Glutamate (Conc µg/protein)
Control	0.62 ± 0.09
Scopolamine (3mg/kg)	0.28 ± 0.06
Brahmi (200mg/kg) + Scopolamine (3mg/kg)	0.42 ± 0.07
Armodafinil (15mg/kg) + Scopolamine (3mg/kg)	0.47 ± 0.09
Armodafinil (30mg/kg) + Scopolamine (3mg/kg)	0.53 ± 0.08

All the data were expressed as the means ± SD (n=6).

Effect of Armodafinil on Glutamate levels

The levels of glutamate in the scopolamine-treated group were significantly decreased and showed a difference ($p<0.05$) compared to the control group as shown in Table 4. Glutamate levels were ameliorated by the Brahmi and armodafinil treated groups with significant improvement $p<0.05$ compared to the scopolamine group indicating that neurotransmission of glutamate changes associated with cognition. Therefore, our study demonstrated that a significant increase in the armodafinil group triggered the changes that occurred with the scopolamine treatment.

DISCUSSION

Amnesia is a memory loss condition that impacts the neurons in the hippocampus, causing certain structural and functional abnormalities. Oxidative stress (OS) is one of the factors that contribute to neurodegenerative diseases. OS response in the brain is a change in the balance between the antioxidant defense system and free radical generation. Oxidative damage is susceptible to more oxygen demand, low antioxidants, and less regenerative capacity. When the neurons are protected from OS that led to cognitive impairment contributes to better treatment for neurodegenerative diseases.[28] This contributes to the evaluation of neurobehavioral and cognitive enhancement action of armodafinil in neurodegenerative diseases. Based on the treatment plan, the frequently used scopolamine induction model was selected using albino Wistar rats. The main principle of the study is to evaluate the cognitive enhancement effect of armodafinil on scopolamine-induced amnesia in Wistar rats. This was assessed by screening various behavioral and biochemical parameters.

There is a devoid of information on the improvement of cognition with armodafinil made us investigate the evaluation of armodonafil involved in the protection of neurons for the treatment of neurodegenerative diseases. Thus, we evaluated the behavioral changes, antioxidant activities, and neurotransmitters by estimating superoxide dismutase, catalase, malondialdehyde, and AchE and glutamate levels.

All the animals were trained for five consecutive days in the Y-maze, MWM, staircase, and rotarod for assessment of behavioral changes. In the present study, the scopolamine alone treated group showed a significant decrease ($p<0.05$) in the % SAP which indicates a decrease in memory retrieval, and this was ameliorated by the treated groups Brahmi and armodafinil groups. In the MWM test, the time spent in the targeted quadrant

region declined in the scopolamine alone treated group owing to its impairment in the spatial memory and the treated groups recovered these scopolamine effects by improving the duration of time in the targeted quadrant. There is a connection between motor activities with cognition in neurodegenerative diseases[29] so to explore the motor activity changes in neurodegenerative disorders such as Parkinson's disease, multiple sclerosis (MS), and Huntington's disease. The response of steps climbed by the animals in the staircase test significantly differed ($p<0.05$) from scopolamine to treated groups which resulted in alteration in the memory. The scopolamine alone treated group significantly decreased ($p<0.05$) the tendency of animals to hold on the spinning rod which indicated the motor activity and the duration of time spent on the spinning rod improved significantly in the Brahmi and armodafinil groups. The reactive oxygen species (ROS) levels will be decreased through the antioxidant defense system in the body. The antioxidants SOD, CAT, and MDA levels were estimated in the present study in both disease and treatment groups. The superoxide transforms into hydrogen peroxide with the help of SOD which then generates the highly reactive hydroxyl radicals that participate in cellular activities. This highly reactive hydroxyl radical will further divide into water and oxygen in peroxisomes in presence of catalase enzyme.[30] The scopolamine treated group showed a significant decrease ($p<0.05$) in SOD and CAT levels which indicated the damage in the neurons and the treatment groups Brahmi and armodafinil restored the levels with a significant increase in the antioxidant levels.

When there is more oxygen demand in the brain the reactive oxygen species (ROS) will be catalysed. There will be high levels of phospholipids in the cell membrane of the brain which act as substrates for lipid peroxidation which is related to oxidative damage to the tissue. The increase in free radicals damages the cell membrane due to instability in the phospholipids which results in cellular toxicity and accumulation of MDA known as lipid peroxidation chain reaction.[31] The polyunsaturated fatty acids indicate MDA levels which were assayed and considered as markers for oxidative damage to the cell membrane. The increase in MDA levels indicated cellular toxicity and lipid peroxidation chain reaction in the scopolamine-treated group. The MDA levels were significantly decreased ($p<0.05$) in the armodafinil and Brahmi-treated groups demonstrating reduced damage to the brain tissue.

The cholinergic neurotransmitter acetylcholine plays an important role in the improvement of cognition. The levels of acetylcholine esterase activity will be diminished in a state of cognitive decline.[32] The scopolamine disrupts the cholinergic neurotransmission with increased levels of acetylcholine in the brain causing deficits in memory which indicated a significant increase ($p<0.05$) in the scopolamine alone treated group in comparison with the control group. The armodafinil and Brahmi treated groups reimposed the AchE levels with a significant increase ($p<0.05$) that designated the improvement in cognition.

Glutamate is a requisite neurotransmitter, and its receptors were involved in the acquisition of memory. In previous studies, modafinil increased the extracellular glutamate in the hippocampal region. [33] This suggested that modafinil treatment indicated increased glutamate levels in the brain tissue. The alterations of glutamate levels which were significantly lowered in scopolamine-treated group constitute a decline in memory and the levels were significantly ameliorated and restored in armodafinil (15 and 30 mg/kg) treated groups indicating its enhancing cognition.

CONCLUSION

Armodafinil treated groups, both low and high doses have shown impressive results in various behavioral and biochemical measurements performed in this present study appears to be a promising approach Furthermore, armodafinil-treated groups exhibited retrieval of memory when compared to disease control (scopolamine-treated) groups with potent antioxidant activity. Armodafinil also significantly enhanced levels of glutamate and ameliorated AchE levels which have important functions regarding learning and memory. The present study concludes that armodafinil is beneficial for the improvement of cognition in scopolamine-induced amnesia. Further study is needed to explore its exact mechanism on cognition.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

AchE: Acetylcholinesterase; **ANOVA:** Analysis of variance; **CAT:** Catalase; DTNB: 5,5'- Dithiobis (2-nitrobenzoic acid); **HD:** Huntington's Disease; **IAEC:** Institutional Animal Ethics Committee; **LPO:** Lipid Peroxidase; **MDA:** Malondialdehyde; **MS:** Multiple Sclerosis; **MWM:** Morris Water Maze; **OS:** Oxidative Stress; **PD:** Parkinson's Disease; **ROS:** Reactive Oxygen Species; **SOD:** Superoxide dismutase; **SOS:** Sodium dodecyl sulphate; **SD:** Standard Deviation; **SAP:** Spontaneous Alteration; **TBARS:** Thiobarbituric acid reactive substances; **UV:** Ultraviolent.

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