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The Neuroprotective Effect of Iloperidone on Scopolamine Induced Learning and Memory Impairment in Rats

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ABSTRACT

Background: Alzheimer's disease (AD) is the most common neurodegenerative disease in the world. One of the most commonly prescribed oral antipsychotic drugs, iloperidone, has been shown to have beneficial effects on restoring impaired cognitive function. In the present study, we investigated the effects of iloperidone on spatial memory in terms of alleviating scopolamine-induced learning and memory impairments in rats by using the Actophotometer, Morris water maze (MWM) test, elevated plus-maze (EPM) test and the pole climbing test. Furthermore, we investigated the possible mechanisms of action of iloperidone in preventing cognitive dysfunction. **Methods:** Thirty Wistar rats were randomly divided into five groups of six animals each. Group- I animals were administered with distilled water throughout the study. Group II received Scopolamine (1.4mg/kg i.p), Group III a positive control group received Donepezil (5mg/kg p.o), Group IV recieved Iloperidone at low dose (0.15mg/kg i.p), Group V received Iloperidone at high dose (0.30mg/kg i.p). The study lasted for 7 days. Cognitive impairment was assessed by the Actophotometer, Morris water maze (MWM) test, elevated plus-maze (EPM) test and the pole climbing test. Activities of cholinesterases as well as reduced malondialdehyde were evaluated in the brain tissue. **Results:** The results showed that scopolamine caused a decrease in locomotor activity, and an increase in escape latency time and transfer latency time. However, learning and memory impairment induced by scopolamine was reversed by the iloperidone. Moreover, Iloperidone inhibited the acetylcholinesterase enzyme, thereby elevating the acetylcholine concentration in the brain and also inhibiting lipid peroxidation by decreasing the MDA level in the brain. It is concluded that Iloperidone may be used as a neuroprotective and memory enhancer.

INTRODUCTION:

Alzheimer's disease (AD) also referred to simply as Alzheimer's, is a neurodegenerative disease that usually starts slowly and gradually worsens over time. It is the cause of 60–70% of cases of dementia. The most common early symptom is difficulty in remembering recent event As the disease advances, symptoms can include problems with language ,disorientation (including easily getting lost), mood swings loss of motivation, self-neglect, and behavioral issues.[1] AD is characterized by the presence of excessive amounts of neurotic plaques containing amyloid protein loss of cholinergic markers in the brain. Loss of cholinergic cells particularly in the basal forebrain is accompanied by loss of the neurotransmitter acetylcholine. A decrease in acetylcholine in the brain of patients with AD appears to be a critical element in producing dementia. [2]

The cause of Alzheimer's disease is poorly understood. About 70% of the risk is believed to be inherited from a person's parents, with many genes usually involved. [3] Other risk factors include a history of head injuries depression, and hypertension. The disease process is associated with amyloid beta (Aβ) plaques and neurofibrillary in the brain. [4] There are no medications or supplements that have been shown to decrease risk. [5] Loss of cognitive ability with age is considered to be a normal process whose rate and extent are very variable AD was originally defined as presenile dementia, but it now appears that the same pathology underlies the dementia irrespective of the age of onset. [6]

Neurodegeneration of the cholinergic neurons is accompanied by an alteration in the synthesis of acetylcholine or its presynaptic recapture which results in the progressive impairment of memory capacity. This information has led to the establishment of a cholinergic hypothesis of the disease as a research platform in finding lasting treatments for cholinergic deficits hence restoring the memory performance in AD patients. The cholinergic hypothesis can be applied by an injection of Scopolamine which induces cognitive deficits mimicking those observed in AD, and treatment will be aimed at restoring the activity of the cholinergic system by inhibiting acetylcholinesterase enzyme.[7]

Scopolamine, a muscarinic cholinergic receptor antagonist, has been widely adopted to study cognitive deficits in experimental animals. After intraperitoneal (i.p.) injection of scopolamine, the cholinergic neurotransmission was blockaded, leading to cholinergic dysfunction and impaired cognition in rats. Recently, it has been reported that memory

impairment induced by scopolamine in rats is associated with altered brain oxidative stress status. Therefore, rats with scopolamine-induced memory deficits were used as an animal model for screening antidementia drugs [8].

Current anti-AD drugs show symptomatic relief and come with several side effects. [9] AchE inhibitors include rivastigmine, tacrine, donepezil, and galantamine while memantine has been also prescribed recently. The drugs approved for the therapy act by counteracting the acetylcholine deficit, they try to enhance the acetylcholine level in the brain. AchE presents some limitations, such as their short half-lives and excessive side effects caused by the activation of peripheral cholinergic systems, which is the most frequent and important side effect of these drug therapies. [10]

Iloperidone, a second-generation anti-psychotic agent demonstrates binding affinity to the receptors in central nervous system. The mainly iloperidone is used as the antischizophrenic agent. Iloperidone increases the acetylcholine level in the hippocampus. Iloperidone increase the acetylcholine release in the cortex through the complex mechanism which is enhanced by prefrontal 5-HT1A receptor activation. Iloperidone used in the symptoms of psychotic (mental) disorders, such as schizophrenia.

Materials and Methods

Animals

Wistar albino rats of either sex (150-200gm) were taken from the Animal House of Yashoda Technical Campus, faculty of Pharmacy Satara. All animals were kept under standard environmental conditions like 25 ± 3°C temperature, 45-50% relative humidity and 12 hr light and dark cycle before the initiation of the experiment. Free food and water were accessed *ad libitum.* All the protocols used in the experiment were approved by the Institutional Animal Ethical Committee of Yashoda Technical Campus, Faculty of Pharmacy, Satara. (1915/PO/ReBi/S/16/CPCSEA 04/11/2016) and ethical guidelines were strictly followed during the experiments. These animals were acclimatized for 48 hours to adapt the new environment before the experimental work.

Drugs and chemicals

Scopolamine (Manus Aktteva Biopharma LLP), Donepezil (Niksan Pharmaceuticals)

Iloperidone (Manus Aktteva Biopharma LLP) was purchased.

Experimental Design

The present study deals with the neuroprotective effect of Iloperidone on Scopolamine (1.4 mg/kg i.p) induced Alzheimer's Disease in Wistar rats. Neural degeneration was induced in groups II, III, IV, V by i.p. administration of Scopolamine at 1.4 mg/kg. Positive control (Group III) treated by Donepezil at 5 mg/kg p.o was ingested before 30 min of scopolamine challenge for 7 days. Group IV (Test 1) animals were treated with Iloperidone at (0.15mg/kg i.p.) and Group-V (Test 2) animals were treated by Iloperidone (0.30mg/kg i.p) before 30 min of scopolamine challenge for 7 days. A behavioural parameter was recorded on day 7 for actophotometer test, morris water maze test, elevated plus maze test, pole climbing test oxidative stress (MDA level) and neurochemical estimation (Acetylcholine level) was recorded.

Behavioral Tests: All the animals were trained for 2 days before drug administration.

Locomotor Activity

Locomotor activity is influenced by most of the CNS drugs in both humans and animals. The locomotor activity of the drug can be studied using actophotometer which operates on photoelectric cells that are connected in circuit with a counter when the beam of light falling on a photocell is cut off by the animal, a count is recorded. Animals were placed individually in the activity cage for 10 min and the activity was monitored. The test is done before 30 min and after the drug administration. The photo cell count is noted and a decrease or increase in locomotor activity is calculated.[11]

Elevated Plus Maze

Memory was evaluated by the elevated plus maze (EPM) apparatus. The maze consists of two closed arms (50 × 10 cm) with two opposite open arms (50 × 10 cm) which is plus-shaped and remain elevated above the floor level. Before experiment, each rat was trained by placing them at the end of an open arm and by using a stopwatch. Transfer latency time (sec), can be defined as the time taken by a rat to enter (with all four paws) into either of the closed arms. The transfer latency was observed and noted. The maze was cleaned with 70% ethanol between runs. The time taken by each animal to enter into the closed arm with all its four

limbs when positioned at the edge of one open arm facing away from the central platform was recorded as the initial transfer latency. A 60 sec time period cut-off was set. The rat was then allowed to move freely inside the maze regardless of open and closed arms for another 10 sec. After 24 hours the retention transfer latency test was performed in the same way as in the acquisition trial. If the rat did not enter the enclosed arm within 60 sec of 2nd trial, the transfer latency (day 0) was assigned 60 sec. The rats were again put into the elevated plus maze to evaluate the transfer latency. [12][13]

Morris Water Maze Test

Morris water maze was used to assess learning and memory in experimental mice. There are several advantages of Morris water maze over other models of learning and memory including the absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds [20, 21]. Briefly, it consists of a circular water tank, filled with opaque water, and one centimeter submerged platform. First, animals were trained to locate the platform. During acquisition, trial escape latency time (ELT), a time measure to locate the hidden platform, was noted as an index of acquisition. Each animal was subjected to the acquisition trial. The time spent by the animal, searching for the missing platform in target quadrant Q2 with respect to another quadrant (Q1, Q3, and Q4) on 5th day, was noted as an index of retrieval. For studying the effect of drug on acquisition, the drug solution was administered before the acquisition trial. [14][15]

Pole Climbing Test

When an electrical stimulus is given to the animal, it tries to escape from it and move to the near safe place. This equipment is designed in such a way to climb the pole when the stimulus is generated. Prior to the experiment, animals were trained. Training and testing is conducted in a 25x25x40 cm chamber that is enclosed in a dimly light, sound-attenuated box. Scrambled shock is delivered to the grid floor of the chamber. A smooth stainless steel pole, 2.5 cm in diameter, is suspended by a counter balance weight through a hole in the upper centre of the chamber. A micro switch is activated when the pole is pulled down by 3 mm. With weight greater than 200 gm. A response is recorded when a mice jumps on the pole and activates the micro switch. The activation of light and speaker together is used as conditioned stimulus. Each animal was placed six times per day. [16]

Histopathological Studies

After 7th day of treatment, the brains of different groups were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and postfixed in the same fixative overnight at 48°C. The brains were then routinely embedded in paraffin and stained with hematoxylin-Eosin. The hippocampal lesions were assessed microscopically at 40 magnification.

Dissection and Homogenization

On day 7, after behavioral assessments, animals were sacrificed by cervical dislocation. The brains were removed. Each brain was separately put on ice and rinsed with ice-cold isotonic saline. A (10% w/v) homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 minutes and aliquots of supernatant were separated and used for biochemical estimation. [17]

Biochemical Tests

AchE Estimation

Rat brain samples (0.03M sodium phosphate buffer, pH-7.4) a 10% (w/v) were prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 10,000 rpm. The brain homogenate was mixed with an equal volume of 1% Triton X-100 (1% w/v in 0.03M sodium phosphate buffer, pH -7.2) and centrifuged at 100,000 g at 40C in a Remi Ultracentrifuge using a fixed angle rotor for 60 min. The supernatant was collected and used for acetylcholinesterase activity. Acetylcholinesterase kinetic study was done by Ellman‘s method (1961) at 412 nm wavelength by using double beam spectrophotometer. Processed brain samples were mix with 0.1mM sodium phosphate buffer (pH 8.0) and after 5 min incubation acetylthiocholine iodide (154.38 mM) and Ellman’s reagent is 5, 5’-dithiobis (2-nitrobenzoate) also abbreviated as DTNB (10 mM) added just prior to reading. The specific activity of acetylcholinesterase is expressed in µmole/min/g of protein.

Estimation of malondialdehyde level

MDA levels in brain structures and peripheral tissues were estimated by the thiobarbituric acid (TBA) method. Samples were homogenized in 10 volumes of cold water. A MDA standard was prepared by hydrolysis of 16.4 µl of 1,1,3,3-tetraethoxypropane stock solution

in 50 ml of 0.2 mM hydrochloric acid and incubated at 100 °C for 1 h. The MDA standard (10 mM) was further diluted to yield final concentrations of 1, 2, 3, 5, 7, and 10 μM to obtain the standard curve for estimation of total MDA. MDA levels in examined tissues were measured as follows: (1) 1 ml sample of homogenate were incubated with 1 ml of 0.37 % TBA in 50 mM NaOH and 1 ml of 2.8 % trichloroacetic acid in a boiling water bath for 20 min to develop a colored MDA–TBA adduct (TBA acid reactive species), and were then clarified by centrifugation at 1,500 rpm for 10 min, and 2); the resulting supernatants were aspirated, and the pink chromogen was measured at 532 nm using a Varian Cary 50 UV–Visible Spectrophotometer against a blank by comparison with the standard curve. The results were evaluated from the standard curve and calculated as μM MDA/g of tissue. All the analyses were performed in triplicate, and the average values were taken.

Statistical analysis: -

All analytical measures like behavior (using elevated plus maze), Escape latency time (using morris water maze test), and locomotor activity (using actophotometer) represented in the table, were denoted as mean ±S.E.M. (n=6). A one-way ANOVA statistical method followed by Tukey's multiple comparisons was adopted. A significant difference was considered b/w group when $p<0.05$. $p<0.001$. ##$p<0.0001$ Test compared with negative control. All analyses were done with GraphPad Prism 8.0 software.

Results

Locomotor Activity

Group-II (negative Control) treated with Scopolamine (1.4mg/kg i.p.) administration showed a significant decrease in locomotor activity in comparison to Group I (normal control). Group-III treated with Donepezil (5mg/kg p.o.) showed significant increase in locomotor activity at 7^{th} day as compared to Group-II (negative control), Group IV (Test 1) treated with low dose of Iloperidone (0.15mg/kg i.p.) showed significant increase in locomotor activity at 7^{th} day as compared to Group-II (negative control). Group V (Test 2) treated with a high dose of Iloperidone (0.30mg/kg i.p.) showed significant increase in locomotor activity at 7^{th} day as compared to Group-II (negative control). The result was found dose dependent.

Table 1. Effect of Iloperidone on Scopolamine learning and memory impairment in rats using actophotometer

Groups	Treatment/ Dose (mg/kg)	No. of Locomotor Activity in 10 min
Group-I Normal Control	Vehicle	694 ± 62.46
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	330 ± 19.8###
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	607 ± 42.49***
Group-IV (Test drug with low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	584 ± 51.52**
Group -V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	598 ± 55.7*

Each value represents the mean ± SEM (n = 6). The number of locomotor activity decreases for the Scopolamine group (###$p < 0.001$ vs. control), and the administration of iloperidone increases this number of locomotor activity (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine

Morris Water Maze Test

Group-II (negative Control) treated with Scopolamine (1.4mg/kg i.p.) administration showed a significant increase in Escape latency time in comparison to Group-I (normal control). Group-III treated with Donepezil (5mg/kg p.o.) showed a significant decrease in Escape latency time at 7th day as compared to Group-II (negative control). Group-IV (Test 1) treated with low dose of Iloperidone (0.15mg/kg i.p.) showed significant decrease in Escape latency time at 7th day as compared to negative control (Group-II). Group-IV (Test 2) treated with a

high dose of Iloperidone (0.30mg/kg i.p.) showed significant decrease in Escape latency time at 7th day as compared to Group-II (negative control). The result was found dose dependent.

Table 2. Effect of Iloperidone on Scopolamine induced learning and memory impairment in rats using morris water maze test.

Groups	Treatment/ Dose (mg/kg)	Escape Latency Time (sec)
Group-I Normal Control	Vehicle	19.5 ± 1.56
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	24.5 ± 2.20###
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	16 ± 1.28***
Group-IV (Test drug with low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	18 ± 1.36**
Group –V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	16 ± 1.23*

Each value represents the mean ± SEM (n = 6). The escape latency time increases for the Scopolamine group (###$p < 0.001$ vs. control), and the administration of iloperidone decreases the escape latency time (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine.

Elevated plus maze

Group-II (negative Control) treated with Scopolamine (1.4mg/kg i.p.) administration showed a significant increase in Transfer latency time in comparison to Group I (normal control). Group-III treated with Donepezil (5mg/kg p.o.) showed a significant decrease in Transfer latency time at 7th day as compared to Group-II (negative control). Group IV (Test 1) treated with low dose of Iloperidone (0.15mg/kg i.p.) showed a significant decrease in Transfer latency time at 7th day as compared to Group II (negative control). Group V (Test 2) treated

with high dose of Iloperidone (0.30mg/kg i.p.) showed significant decrease in Transfer latency time at 7th day as compared to Group-II (negative control). The result was found dose-dependent.

Table 3. Effect of Iloperidone on Transfer Latency Time in scopolamine induced learning and memory impairment in rats using elevated plus maze test

Groups	**Treatment/ Dose (mg/kg)**	**Transfer Latency Time (sec)**
Group-I Normal Control	Vehicle	14.8 ± 1.18
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	29.1 ± 2.54###
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	16.5 ± 1.48***
Group-IV (Test drug with low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	18.8 ± 1.31**
Group –V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	17.4 ±1.21*

Each value represents the mean ± SEM (n = 6). The transfer latency time increases for the Scopolamine group ($^{###}p < 0.001$ vs. control), and the administration of iloperidone decreases the transfer latency time ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine.

Pole climbing test

Group-II (negative control) treated with Scopolamine (1.4mg/kg i.p.) administration showed significant increase in Escape latency time in comparison to Group I (normal control). Group III treated with Donepezil (5mg/kg p.o.) showed a significant decrease in Escape latency time at 7th day as compared to Group-II (negative control). Group IV (Test 1) treated with low dose of Iloperidone (0.15mg/kg i.p.) showed a significant decrease in Escape latency time at

7th day as compared to Group II (negative control). Group V (Test 2) treated with high dose of Iloperidone (0.30mg/kg i.p.) showed significant decrease in Escape latency time at 7th day as compared to Group-II (negative control). The result was found to be dependent.

Table 4. Effect of Iloperidone on Scopolamine learning and memory impairment in rats using pole climbing test

Groups	**Treatment/ Dose (mg/kg)**	**Escape Latency Time (sec)**
Group-I Normal Control	Vehicle	67 ± 3.48
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	94 ± 7.52###
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	54 ± 4.10***
Group-IV (Test drug with low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	66 ± 3.89**
Group -V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	64 ± 4.86*

Each value represents the mean ± SEM (n = 6). The escape latency time increases for the Scopolamine group (###$p < 0.001$ vs. control), and the escape latency time administration of iloperidone decreases the (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine.

Acetylcholinesterase (AchE) level

Group-II (negative Control) treated with Scopolamine (1.4 mg/kg i.p.) showed an increase in hippocampal Acetylcholinesterase level as compared with Group-I (normal control). Group-III treated with Donepezil (5 mg/kg p.o.) showed a more significant decrease in Acetylcholinesterase level compared to Group-II (negative control). Group-IV (Test 1) treated by low dose of Iloperidone (0.15 mg/kg i.p.) showed a significant decrease in

Acetylcholinesterase level compared to negative control Group (Group-II). Group-V (Test 2) treated by high dose of Iloperidone (0.30mg/kg i.p.) showed a significant decrease in Acetylcholinesterase level compared to negative control (Group II). The result was found to be dependent.

Table 5. Estimation of the acetylcholinesterase activity in rat brain homogenate in scopolamine induced learning and memory impairment in rats.

Groups	**Treatment/ Dose (mg/kg)**	**Acetylcholine esterase AchE activity in brain tissue (μmol/min/g)**
Group-I Normal Control	Vehicle	1.69 ± 0.14
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	2.43 ± 0.20$^{###}$
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	1.55 ± 0.10***
Group-IV (Test drug with low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	1.68 ± 0.14**
Group -V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	1.62 ± 0.09*

Each value represents the mean ± SEM (n = 6). The level of acetylcholinesterase level increases for the Scopolamine group ($^{##}p < 0.01$, $^{###}p < 0.001$ vs. control), and the administration of iloperidone decreases the acetylcholinesterase level ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine.

Estimation of MDA level

Group-II (negative Control) treated with Scopolamine (1.4 mg/kg i.p.) caused a marked increase in lipid peroxidation compared with Group I (normal control). Group-III treated with Donepezil (5 mg/kg p.o.) showed significant decrease in MDA level as compared to Group-II (negative control). Group-IV (Test 1) treated with a low dose of Iloperidone (0.15 mg/kg i.p.)

showed more significant decrease in MDA level as compared to Group-II (negative control). Group-V (Test 2) treated with high dose of Iloperidone (0.30 mg/kg i.p.) showed a more significant decrease in MDA level as compared to Group-II (negative control). The result was found dose-dependent.

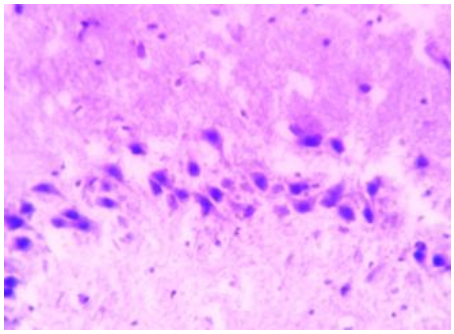
Table 6. Estimation of the MDA level in rat brain homogenate in scopolamine induced learning and memory impairment in rats.

Groups	**Treatment/ Dose (mg/kg)**	**MDA - nM of MDA/mg suspension of brain**
Group-I Normal Control	Vehicle	18.36 ± 1.28
Group-II Negative Control	Scopolamine(1.4mg/kg i.p)	53.25 ± 4.26$^{###}$
Group-III (Positive control)	Scopolamine(1.4mg/kg i.p) + Donepezil (5mg/kg p.o)	44.23 ± 3.09***
Group-IV (Test drug with a low dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.15mg/kg i.p)	48.69 ± 3.89**
Group -V (Test drug with high dose)	Scopolamine(1.4mg/kg i.p) + Iloperidone (0.30mg/kg i.p)	36.26 ± 2.90*

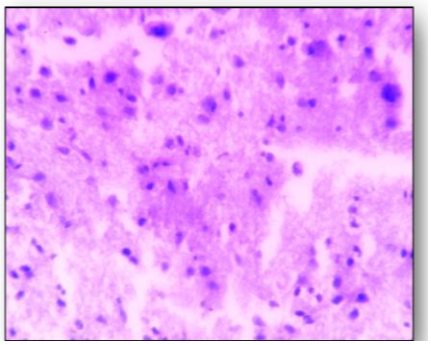
Each value represents the mean ± SEM (n = 6). The level of MDA increases and the antioxidant enzyme level decreases for the Scopolamine group ($^{##}p < 0.01$, $^{###}p < 0.001$ vs. control), and the administration of iloperidone decreases the level of MDA by increasing the level of antioxidant enzymes ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. Scopo). One-way ANOVA followed by the Tukey multiple comparison test. Scopo: Scopolamine.

Histopathological studies

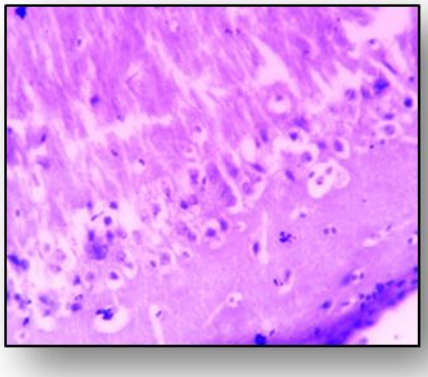
Group I-Normal control (a)



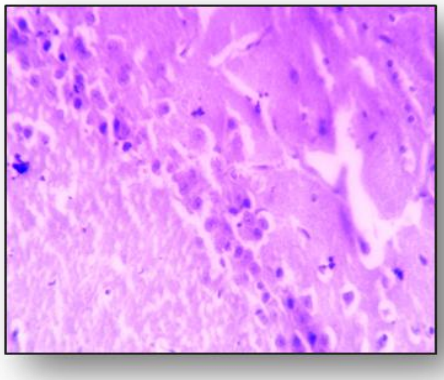
Group II- Toxicant (b)



Group III- Standard (c)



Group IV- Test I (d)



Group V-Test II (e)

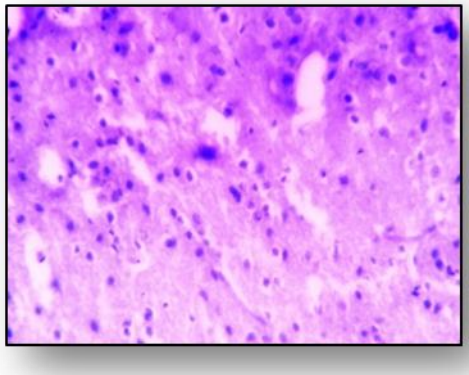


Figure: Histopathological studies. These figures (a), (b), (c), (d) and (e) are normal control, scopolamine (disease control), donepezil (standard), iloperidone (low dose), iloperidone (high dose) respectively, representing the histological sections of the brain tissue showing neurological lesions.

Effect of Iloperidone on histopathology of hippocampus in Alzheimer's rats:

The slides of the brain tissue of rats were observed under 40x magnification for histopathological changes in the brain.

Group-I (normal control) showed intact cells. Brains of Group-II (negative Control) Scopolamine (1.4mg/kg i.p.) rat showed decreased numbers of memory cell neurons, Degeneration of neurons and neuronophagia. Group-III (positive control) Donepezil (5mg/kg p.o.) showed mild necrosis with more intact cells. Group-IV (Test 1) Iloperidone (0.15mg/kg i.p.) showed little effect on neurodegeneration and recovered the neuron shrinkage. Group-V (Test 2) Iloperidone (0.30mg/kg i.p.) showed a decrease in neurodegeneration and neurological lesions with intact cell and recovered the neuron shrinkage and diminished vacuoles around the neuron.

DISCUSSION

The findings of this current investigation revealed the neuroprotective actions of Iloperidone against Scopolamine-induced Alzheimer's disease in the investigational rats. The cognitive impairments were regarded as a foremost clinical sign and severe health related risk by scopolamine in AD.[18] Cognition is the neurological mechanism of understanding, which involves knowledge, interpretation, thinking, and decision-making. Alzheimer's disease is considered a protein misfolding disease due to the aggregation of misfolded β-amyloid protein in the brain of Alzheimer's patients. Dementia is described as the gradual deterioration of memory and intellectual skills, and Alzheimer's disease has gained much attention in the last decade as a major cause of memory deterioration.[19] Brain aging is known to be related to a decrease in acetylcholine levels, neuronal loss, increased inflammation, and oxidative stress. In AD patients acetylcholine levels are depleted in the brain. One method for this is blocking the activity of AchE, the enzyme-degrading acetylcholine.[20] The cognitive-enhancing activity of Iloperidone on the scopolamine-induced memory impairments in rats was investigated using Actophotometer, Morris water maze test, Elevated plus maze test, Pole climbing test and biochemical assessments.[21]

In Actophotometer, the number of locomotor activity was decreased in Scopolamine (1.4mg/kg) administered rats as compared to normal control Group. Group-III treated by Donepezil (5mg/kg p.o.) showed significant increase in locomotor activity at 1st day and more significant increase in locomotor activity at 3rd day and 7th day. Group-IV (Test 1) treated by Iloperidone (0.15mg/kg i.p.) did not show significant increase in locomotor

activity at 1st day and 3rd day but it could show significant increase in locomotor activity at 7th day. Group-V (Test 2) treated by Iloperidone (0.30mg/kg i.p.) did not show significant increase in locomotor activity at 1st day but it could show significant increase in locomotor activity at 3rd day and 7th day.

Scopolamine-treated rats showed cognitive impairment and memory deficit as indicated by increased escape latency time in MWM test. The Escape latency time was increased in Scopolamine (1.4mg/kg) administered rats as compared to the normal control Group. Group-III treated by Donepezil (5mg/kg p.o.) showed significant decrease in Escape latency time at 1st day and more significant decrease at 3rd day and 7th day. Group-IV (Test 1) treated by Iloperidone (0.15mg/kg i.p.) did not show significant decrease in Escape latency time at 1st day and 3rd day but it could show significant decrease in at 7th day. Group-V (Test 2) treated by Iloperidone (0.30mg/kg i.p.) did not show significant decrease in Escape latency time at 1st day but it could show significant decrease in Escape latency time at 3rd day and 7th day. Both doses of Iloperidone in scopolamine pretreated rats significantly showed improvement in acquisition trials as evidenced by reduction in the escape latency time as compared with scopolamine group.

The EPM apparatus is used to examine anxiety in rodents, and it can be employed to test prospective anxiolytic or anxiogenic agents, as well as being applied as a basic testing tool for the analysis of neurophysiological anxiety. Animals are less anxious when they spend more time in the open arms of the apparatus. The Transfer latency time was increased in Scopolamine (1.4mg/kg) administered rats as compared to the normal control Group. Group-III treated by Donepezil (5mg/kg p.o.) showed significant decrease in Transfer latency time at 1st day and more significant decrease at 3rd day and 7th day. Group-IV (Test 1) treated by Iloperidone (0.15mg/kg i.p.) did not show significant decrease in Transfer latency time at 1st day and 3rd day but it could show significant decrease in at 7th day. Group-V (Test 2) treated by Iloperidone (0.30mg/kg i.p.) did not show a significant decrease in Transfer latency time on 1st day but it could show a significant decrease in Transfer latency time at 3rd day and 7th day. The administration of Iloperidone had a dose-dependent inhibitory effect on the transfer latency against scopolamine-induced amnesia in the EPM trial in this study. Furthermore, the decreased transfer latency time during the retention phase suggests that Iloperidone may help to resolve scopolamine induced memory and learning impairment.

In Cook's pole climbing test, The Escape latency time was increased in Scopolamine (1.4mg/kg) administered rats as compared to normal control Group. Group-III treated by Donepezil (5mg/kg p.o.) showed significant decrease in Escape latency time at 1st day and more significant decrease at 3rd day and 7th day. Group-IV (Test 1) treated by Iloperidone (0.15mg/kg i.p.) did not show significant decrease in Escape latency time at 1st day and 3rd day but it could show significant decrease in at 7th day. Group-V (Test 2) treated by Iloperidone (0.30mg/kg i.p.) did not show significant decrease in Escape latency time at 1st day but it could show significant decrease in Escape latency time at 3rd day and 7th day. Both doses of Iloperidone in scopolamine pretreated rats significantly showed improvement by reduction in the escape latency time as compared with scopolamine group. And it was significantly decreased in rats pre-treated with donepezil and Iloperidone. This indicated that Iloperidone pre-treatment significantly reduced the loss of memory in scopolamine treated rats.

The present study showed that scopolamine treatment in rats significantly increased the AchE activity in the hippocampus which was evident by the decreased concentration of acetylcholine because AchE breakdowns into acetyl and choline and it leads to decreased acetylcholine concentration in hippocampus and amnesia is induced in rats. However, rats pre-treated with donepezil and Iloperidone significantly decreased the AchE activity resulting in significantly increased acetylcholine concentration to improve the cognitive functions. [22]

In the present study rats after scopolamine treatment showed a significant increase in the brain levels of malondialdehyde, which is the measure of lipid peroxidation and free radical generation. In the drug-treated groups, there is a significant decrease in the levels of malondialdehyde which is nearly equal to the standard group. The antioxidant activity of Iloperidone is clear from the biochemical tests, which include the estimation of antioxidant enzymes.

CONCLUSION

In conclusion the present study demonstrate that the administration of test drug Iloperidone had therapeutic effect on improving the amnesic activity in rats at both lower and higher dose. The preventive response was indicated by the improved learning tendency according to the increase in locomotor activity, decrease in escape latency time and transfer latency time at

both doses of iloperidone as compared with disease control. In the present study Iloperidone inhibited acetylcholinesterase enzyme, thereby elevating the acetylcholine concentration in the brain and also inhibiting lipid peroxidation by decreasing the MDA level in the brain. Thus the present data indicates that the Iloperidone may be used as neuroprotective and memory enhancer.

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