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Ameliorative Effect of Apremilast on Scopolamine-Induced Alzheimer in Wistar rats

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Abstract:
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a gradual decline in memory associated with shrinkage of brain tissue, with localized loss of neurons mainly in the hippocampus and basal forebrain, with diminished level of central cholinergic neurotransmitter-acetylcholine and also reported to be associated with accumulation of in neuronal inclusions and also with signs of inflammation. The second messengers cAMP and cGMP mediate fundamental aspects of brain function relevant to memory, learning and cognitive functions. Consequently, cyclic nucleotide phosphodiesterases (PDEs), the enzymes that inactivate the cyclic nucleotides, are promising targets for the development of cognition-enhancing drugs. Therefore, the present study investigated the effects of Apremilast as a PDE-4 inhibitor on scopolamine-induced learning and memory impairments in rats. Passive avoidance test and Morris water maze test were conducted to evaluate the learning and memory parameters. Various biochemical parameters such as acetylcholinesterase (AChE), SOD, GSH and catalase activity were also assessed. The present study demonstrates that Apremilast had potential therapeutic effects on improving the antiamnesic activity in rats via inhibiting oxidative stress and acetylcholinesterase activity in brain.

Keywords:. Phosphodiestrase, Antioxidant, Alzheimer's disease, oxidative stress, cAMP

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is identified by loss of memory, diminished cognitive performance, reduced learning and intellectual abilities causing a common public health problem [1-2]. AD has a number of pathologies which mainly includes cholinergic transmission degeneration, neuro-inflammation, oxidative stress and decreased level of brain derived neurotrophic factor (BDNF) [3,4].

Evidences from the literature show that the key symptoms of AD are mainly caused by cholinergic system dysfunction which has a role in cognitive disorders [1,5]. It is known that acetylcholine (ACh) levels in synaptic cleft are regulated by Acetylcholinesterase (AChE) activity where AChE responds to many different insults including oxidative stress that plays important role in the progression of a variety of mental disorders. Therefore, this enzyme might be a target to treat AD [6]. Cholinergic pathways involved in learning and memory functions are projected from the basal forebrain to the cortex, the hippocampus and the amygdale. Muscarinic receptors (mAChRs) like M subtype also have a major role in learning, memory, cognitive functions and emotional state [6, 7]. It is known that mAChRs blockages cause to memory disorders. Scopolamine (SCOP) is a mAChRs antagonist that stimulates memory impairment and partially mimics AD and dementia [1].

The AChE inhibitors especially Galantamine and Donepezil are used in treatment of AD, nowadays, lost their effectiveness in time due to loss of efficacy.

Therefore, there is no available permanent treatment currently to cure AD or to change its progression. In addition to these, anti-inflammation and anti-oxidant agents are well-known agents used in the adjuvant therapy of AD [8].

Cyclic nucleotide (cAMP and cGMP) signaling is fundamentally involved in brain mechanisms that actively mediate cognitive processes, as well as in brain development and homeostasis thatprovides the milieu for cognition. As a consequence, there has been significant interest in tar-geting cyclic nucleotide phosphodiesterases (PDEs), the enzymes that hydrolyze and inactivatethese second messengers, as cognition-enhancing drugs [9-14]. A well-known intracellular messenger cyclic adenosine monophosphate (cAMP) is abundantly present in inflammatory cells and is responsible for regulating inflammatory responses. Among the super family of Phosphodiesterase (PDE) enzymes, the four-gene PDE4 family is claimed to be responsible for the degradation of cAMP in the inflammatory cells, vascular endothelial cell linings, and cells of the outermost layer of the skin. Currently, it is believed that Phosphodiesterase type-4 (PDE-4) enzymes is playing a central role in all kinds of cAMP mediated cell signaling [15] PDE-4 inhibitors are emerged as a new class of drugs having broad spectrum anti-inflammatory influences (in both *in-vitro* and *in-vivo*) against asthma, pulmonary granulocytosis, diseases of bones and joints (osteoporosis and arthritis), inflammatory diseases of large intestine, sclerosis, and other conditions [16] last is an orally available selective inhibitor of the PDE-4 enzyme and inhibits spontaneous production of pro-inflammatory cytokines [17]. Apremilast is a small-molecular novel compound available orally taken by mouth and approved by FDA for psoriatic arthritis (March 2014) and plaque psoriasis (September 2014)[18]. Apremilast inhibits PDE-4 which inturn elevates intracellular levels of cAMP and thus prevents generations of several pro-inflammatory mediators and nitric oxide synthase [19, 20]. Therefore, The research study was an attempt to determine the protective role of apremilast against scopolamine-induced Alzheimer's disease via modulation of oxidative stress and AchE in rats.

Material and methods:

Drugs: Scopolamine, Apremilast and Donepezil were purchased from Sigma Aldrich, India. Scopolamine was dissolved in saline (0.9% NaCl), Donepezil was freshly prepared in 1% tween 80 in water and Apremilast was dissolved in DMSO .

Animals:

Animals were obtained from animal house facility of R. V. Northland Institute Dadri, Greater Noida. The animal were acclimatizes for one week then was divided into the six groups (n=6), and maintained on rats fed with normal pellet diet and water *ad libitum.* All the animals had kept with good and standard laboratory condition like light (12 h light &12 h dark) at controlled room temperature 25±2 0 C and at maintained relative humidity (50±15).

Study design:

Animals were divided into four groups, each group consisting of six rats and their treatment schedule was as follows: (1) Group I: rats fed with normal saline orally for 17 days; (2) group II: rats received 1mg/kg scopolamine (i.p) from 9^{th} to 17^{th} day (toxic control); (3) group III: rats received1 mg/kg scopolamine (i.p)from 9^{th} to17^{th} day +20 mg/kg (p.o) for 8 days; (4) group IV: rats received 1mg/kg scopolamine (i.p) from

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9^{th} to 17^{th} day + 1 mg/kg Donepezil (p.o) for 8 days.

Behavioural Tests:

All the animals were trained for 2 days before drugs administration.

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 absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds. 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), time measure to locate the hidden platform, was noted as an index of acquisition. Each animal was subjected to the four acquisition trials per day for 4 consecutive days. The time spent by the animal, searching for the missing platform in target quadrant Q2 with respect to other quadrant (Q1, Q3, and Q4) on 5th day, was noted as an index of retrieval. For studying the effect of drug on acquisition, the drugs were administered before acquisition trial[21].

Passive Avoidance (PA) Test:

PA test apparatus consists 2-compartment slight illuminated (50W bulb) and dark separated by a guillotine door (5 x 5 cm)) with dimensions of 20 x 20 x 20 cm box each. Floor of dark compartment consists of 2 mm bronze bars arranged parallel each 1 cm apart. Experiment was conducted in two separate trials acquisition & retention trial on day7 & 8. & closed once animal enters dark compartment, immediately a low intense foot shock (0.5 mA for 3 sec) was subjected. Total duration 300 sec, first trial was considered as acquisition trial & second trial after 24 h trial was considered as retention trial. In case if the animal did not enter the dark compartment within 300sec, it can be concluded that animal remembers the acquisition trial. Successful training indicates increase in (TLT) in retention trial when compared to acquisition trial. One hour prior to the acquisition trial, rats were administered with drugs. Control group was administered with normal saline solution[22]

Biochemical test:

AchE Estimation. The cholinergic marker, acetylcholinesterase, was estimated in the whole brain according to the method of Ellman method. Ellman's reagent is 5, 5_-dithiobis (2-nitrobenzoate) and it is also abbreviated as DTNB. This homogenate was incubated for 5 min with 2.7mL of phosphate buffer and 0.1mL of DTNB. Then, 0.1mL of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412nm[23].

Oxidative stress parameters in brain tissue:

The levels of thiobarbituric acid reactive substances (TBARS), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated by the previous published methods respectively[23].

Histopathological examination:

The brain tissues were collected from different groups and fixed in 10% neutral buffered for-malin then processed for obtaining 4 µm paraffin-embedded sections. The sections were stained with hematoxylin, and eosin stain then examined under the microscope [24].

Statistical analysis:

The data were analyzed using computer software, post-hoc LSD using SPSS version 16. The probability level less than 0.05 was considered as significant.

Results:

Behavoural studies:

Passive Avoidance Test:

Effect of Apremilast on the exchange latency time (TLT) fundamentally expanded in the first, second, third, and fourth maintenance

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preliminaries when contrasted with obtaining preliminary in control. In the scopolamine treated gathering there was no critical expansion in the TLT in the first, second, third, and fourth maintenance preliminaries when contrasted with obtaining preliminary (Fig-1).

Morris water maze test:

The activity of apremilast was evaluated using Morris water maze. The mice treatment groups except scopolamine-treated group showed significant transfer latency with platform and without platform which was given in Fig-2.

This indicates memory enhancing capacity of the apremilast. Donepezil (5 mg/kg) treatment for successive 8 days acts as standard drug, possessed significant (*P* <0.001) decrease in transfer latency when compared to toxic control (scopolamine) using software.

Effect on oxidative stress and AChE activity in brain tissue:

The status of antioxidant parameters are shown in Table 1. Scopolamine administered rats showed significant increase (p<0.001) in AchE activity and decrease (p<0.001) in GSH, SOD and CAT levels as compared with normal control rats. Administration of apremilast and donepezil (5 mg/kg/day, p.o.) showed significant decrease (p < 0.001) in AchE activity and similary increase (p < 0.001) in brain GSH, SOD, CAT levels when compared with scopolamine treated rats.

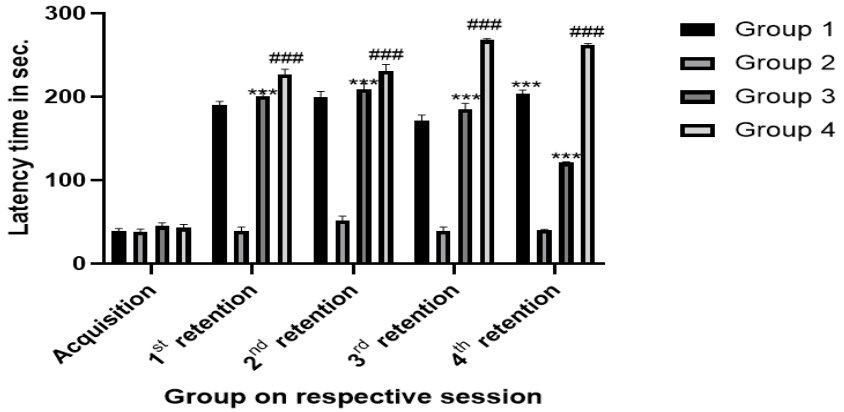

Figure 1 Showing effect of apremilast on scopolamine induced Alzheimer in rats in passive avoidance test. Graph showing mean ± SEM of latency time in seconds. ***P < 0.001, ###P < 0.001, compared with corresponding values of toxic control (Group II).

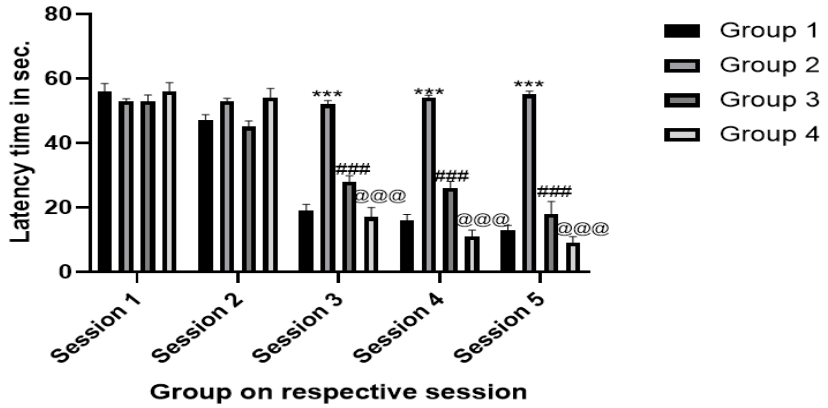

Figure 2 Morris water maze test. Showing effect of apremilast on latency time compared to the toxic control (Group II). (Mean ± SEM, n = 6). Graph showing mean ± SEM of latency time in seconds. ***P < 0.001 comparing toxic control (Group II) vs. normal control (

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Table 1. Effect of Apremilast on SOD, GSH, CAT and AChE Activity In Scopolamine Induced Rats

Treatment	SOD (U/mg Protein)	GSH (µg/mg protein)	**CAT (nmoles of H2O2/min /mg protein)**	**AchE activity (µM/Min./mg protein)**
Group I	22.23±2.14	90.26±2.01	40.3±0.303	0.034±0.01
Group II	8.32±1.18***	30.19±2.36***	21.9±0.327***	0.150±0.02***
Group III	15.62±1.46###	85.95±0.88###	32.2±0.059###	0.085±0.023###
Group IV	20.50±1.60†††	92.18±2.60†††	34.6±0.209†††	0.033±0.01†††

Data are expressed as mean ± SEM (n = 6 animals per group). ***p < 0.001 HFD *vs.* normal control; ###p < 0.001 apremilast *vs.* scopolamine; ††† p < 0.001 donepezil *vs.* scopolamine,

Discussion:

The scopolamine amnesia test is widely used as primary screening test for various anti-Alzheimer drugs [25]. There recently has been an increased appreciation of the role that inflammation plays in the pathogenesis of Alzheimer's disease that has arisen principally from epidemiological studies showing a dramatic effect of long-term NSAID treatment on Alzheimer's disease risk. However, the molecuar mechanisms by which NSAIDs intervene in the pathological processes that underlie cognitive decline and neuronal loss remain unclear [26]. Recently, many studies reported that memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress within the brain [27]. Oxidative stress is the cytotoxic consequence of oxyradical and oxidant formation and the reaction with cellular constituents. Reactive oxidative species (ROS) are generated continuously in nervous system during normal metabolism and neuronal activity. The nervous system is particularly vulnerable to the deleterious effects of ROS. Because the brain has a high consumption of oxygen, large amount of polyunsaturated fatty acids (PUFAs), high contents of free ions, and low levels of antioxidants defense were compared to other organs [28]. Increased MDA level as one of the ROS has been shown to be an important marker for in vivo lipid peroxidation. From the behavioral test, that is, rectangular maze test and Morris water maze test, it is clearly seen that there was a general decrease in the transfer latency in all treated groups compared to the scopolamine-treated group. The memory loss effect of scopolamine is more prominent compared to the control group. In comparison with Donepezil, the drug-treated groups had almost equal performance which indicates protective effect of against

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memory loss. The major antioxidant and oxidative free radical scavenging enzymes like glutathione, SOD, and catalase play an important role to reduce oxidative stress in brain. These enzyme levels are decreased in the scopolamine-treated group compared to the control group. The enzyme levels are almost equal in combination group and the standard group. Individual groups are showing less than standard group. It supports the antioxidant action of drugs. In the present study rats after scopolamine treatment showed a significant decrease in the brain levels of AchE as compared with toxic group, which is the measure of lipid peroxidation and free radical generation. In this work, donepezil, which is a potent AChEI, ameliorated cholinergic deficits and decrease in oxidative stress in the brain of scopolamine-induced Alzheimer rats. These findings are in concordance with prior studies[29] and emphasize the effectiveness of donepezil as standard anti-amnestic agent for screening novel therapeutics for treating Alzheimer disease. In this study, Scopolamine-induced Alzheimer resulted in cholinergic system dysfunction as evidenced by elevation in AChE activity, an important enzyme which hydrolyses ACh, an essential neurotransmitter involved in learning and memory. This finding is in line with prior studies[29].

Conclusion:

The present study concluded that apremilast has therapeutic effects on improving the antiamnesic activity in rats through inhibiting oxidative, and decreasing acetylcholinesterase (AchE) activity in scopolamine induced Alzheimer disease in rats.

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Conflict of Interest: There is no conflict of interest.

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