A Comparative Study of Effect of Atorvastatin and Donepezil on Learning and Memory in Scopolamine Induced Amnesia in Albino Rats

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Abstract: *Learning and memory are some of the most intensively studied topic in neuroscience. Dementia is a syndrome of gradual onset and continuous decline of higher cognitive functioning. It is a common disorder in older persons. The common causes accounting for dementia are Alzheimer's disease (AD), vascular dementia, and other neurodegenerative diseases. Alzheimer's disease is progressive neurodegenerative disorder associated with loss of cholinergic neurons in distinct brain areas. Dementia and dyslipidemia may be co-morbid condition because both conditions predominantly affect elderly people. Drugs, currently available for dementia have several potential side effects. Further, with many drugs patients develop tolerance. Anti-dyslipidemic drug is one of the most commonly prescribed drugs today. Amongst anti-dislipidaemic drugs, statins have been found to have neuroprotective effects. The aim of the present study was to assess the cognitive effect of atorvastatin and to compare the same with donepezil and also to see blood glucose level in rats. Sixteen albino rats, divided into four groups and each group consisting of four animals, were recruited in the study and given respective drugs or agents. Morris Water Maze(MWM) and Elevated Plus Maze(EPM) were the animal models used. Mean escape latency(in MWM) and mean transfer latency(in EPM) were the parameters seen in all groups. Besides these, blood glucose level was also assessed in all animals. Results showed a decrease in mean escape latency and mean transfer latency suggesting neuroprotective effect of atorvastatin. No significant alteration in blood glucose level in different groups was seen.*

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I. Introduction

Learning and memory are the most intensively studied subjects in the field of neuroscience. Learning is the act of acquiring new or modifying and reinforcing existing knowledge, behaviors, skills, values or preferences which may lead to a potential change in synthesizing information, depth of the knowledge, attitude or behavior relative to the type and range of experience. Memory refers to the storage, retention and recall of information including past experiences, knowledge and thoughts.[1] Cognition is a general term that refers to all mental processes, such as perception, thinking, memory, movement, attention, emotions, ability to understand the intentions and thoughts of other people, decision making, and self-awareness. Dementia is a syndrome of gradual onset and continuous decline of higher cognitive functioning. It is a common disorder in older persons and becomes more prevalent with each decade of life. Approximately 10% of adults 65 years and older and 50% of adults older than 90 years have dementia.[2] The prevalence of dementia rapidly increases from about 2-3% among those aged 70–75 years to 20–25% among those aged 85 years or more.[3] Dementia rates are growing at alarming proportion in all regions of the world and are related to aging population.[4] The number of people living with dementia worldwide in 2015 was estimated at 47.47 million, reaching 75.63 million in 2030 and 135.46 million in 2050. Neurologic conditions, including dementia, were estimated by the Global Burden of Disease 2010 Study as the third leading cause of years lived with disability at global level.[6] The common causes accounting for dementia are Alzheimer's disease (AD), vascular dementia, and other neurodegenerative diseases. Other important causes are dementia associated with depression, Parkinsonism and Schizophrenia. Dementia of AD accounts for 50–70% of total cases of dementia. Alzheimer's disease is progressive neurodegenerative disorder associated with loss of cholinergic neurons in distinct brain areas. Selective loss of

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cholinergic neurons and decrease in cholineacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer type.[6] Dementia and dyslipidemia may be co-morbid condition because both conditions predominantly affect elderly people. Anti-dyslipidemic drug is one of the most commonly prescribed drug today. Amongst anti-dislipidaemic drugs, statins have been found to have neuroprotective effects. Clinical studies have demonstrated that cognitive decline is slowed in dementia patients that have been receiving statins.[7,8]

Extensive evidence indicates that relatively modest increases in circulating glucose concentrations enhance learning and memory processes in rodents and humans.[9] Glucose administration regulates many neural and behavioral processes in rodents, including learning and memory.[10]

Rationale behind this study is that the drugs currently available for dementia have no disease-modifying potential (i.e., do not slow down the progression of the disease), they just give symptomatic relief. Further, they have several side effects and, with many drugs patient develop tolerance. So, there is a need of exploring new drugs for the same. Rationale of comparing neuroprotective effect of atorvastatin with donepezil is that in many cases, dementia and dyslipidemia are seen as co-morbid conditions and atorvastatin is one of the most commonly prescribed anti-dyslipidemic drug. So, if the neuroprotective effects of atorvastatin would be found to be comparable with that of donepezil, we would be able to treat both dementia and dyslipidemia with a single drug (Atorvastatin) and further adverse effects of currently available drugs for dementia would be avoided. If the efficacy would not be comparable to that donepezil it would at least be used as an add-on drug with the standard drug of dementia.

Aim of the study
1. To study the effect of Atorvastatin in learning and memory in Scopolamine induced amnesia in albino rats.
2. To compare the effect of Atorvastatin with Donepezil in learning and memory in scopolamine induced amnesia in albino rats.
3. To see biochemical changes such as blood glucose level in rats with scopolamine-induced dementia.

## II.	Materials And Methods
This was an animal experimental study carried out on albino rats in the Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi. Approval of the Institutional Animal Ethics Committee (IAEC), RIMS, Ranchi was taken prior to the study.

ANIMALS:
Healthy Male Wister albino rats weighing between 150-250 grams were taken for the present study. The animals were kept in clean and dry cages, with 12 h: 12 h light-dark cycle at normal room temperature and humidity and were fed with standard laboratory diet consisting of soaked black gram and water was given ad libitum. Arrangements were made to ensure regular cleaning of cages and disposal of excreta and urine. The animals were acclimatized for 7 days to the laboratory conditions before behavioral experiments.

Animals were randomly divided into 4 groups with each group consisting of 4 animals. Each group had separate cages for them. All the cages were appropriately labeled. Animals in each cage were also labeled separately and color coded with the help of permanent marker. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) Guidelines and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

DRUGS USED :
1. Tab.Atorvastatin (Tab. Atora 20mg, Zydus Healthcare Ltd.)
2. Tab. Donepezil (Tab. Donep 5mg. Alkem Laboratories Ltd)
3. Inj. Scopolamine (Sigma Aldrich, U.S.A)

GROUPS:
The details of groups were as follow:

Group	No. of rats	Drugs	Dose
Group I	4	1% gum acacia	0.5ml of 1% gum acacia
Group II	4	Donepezil + Scopolamine	0.5mg/kg body wt. + 1mg/kg body wt.
Group III	4	Atorvastatin + Scopolamine	10mg/kg body wt. + 1mg/kg body wt.
Group IV	4	Scopolamine	1mg/kg body wt.

The dose of drugs was determined on the basis of conversion factor using surface area of rats and man (0.018). Then each animal was weighed and absolute dose was calculated as per body weight of the rats.

PREPARATION OF DRUGS:

1. Gum acacia: 1% suspension of gum acacia was prepared by mixing 1 gm of gum acacia and small amount of water in a mortar pestle and then making a final suspension of 100ml in distilled water.

2. Donepezil: Donepezil tablet was powdered and a uniform suspension was made using 1% gum acacia. The daily dose of donepezil for dementia in Alzheimer's disease in human is 5 mg /70 kg. Accordingly the daily dose for a 200 gm rat was calculated to be 0.1 mg (0.5mg/kg body weight of the rat).

3. Atorvastatin: Tablet was powdered and a uniform suspension was made using 1% gum acacia. Dose used for the study was 10mg/kg body weight of the rat. Therefore daily dose for a rat of 200 gm rat was calculated to be 2mg.

4. Scopolamine : Scopolamine was dissolved in normal saline and injected intraperitoneally in rats with the help of 1ml syringe and 26 gauge hypodermic needle at a dose of 1 mg/kg body weight. Therefore daily dose for a rat of 200 gm rat was calculated to be 0.2mg.

Administration: Atorvastatin and donepezil were freshly prepared daily and given orally using animal feeding tube (gavage tube) daily. Scopolamine was given intraperitoneally 30 min before the experiment.

DURATION OF THE EXPERIMENT:
All the rats were given respective drugs according to the group allocated for a period of 20 days.

Morris Water Maze test

The Morris Water Maze test was carried out from 15th to 20th day. The training days were designated as ―day 1, 2, 3, 4. A gap of 48hrs was given before performing the memory retention test. This time gap simulates normal forgetting, which occurs due to lack of rehearsal of learned task. Then on 20th day, rats were tested for retention of the learned task using Morris Water Maze. The memory test day was designated as 'day 6'.

Procedure:

* The Morris Water Maze is a large circular pool of diameter 1.8- 2.0 m and height of 0.4- 0.6m. The pool is filled with water and rendered opaque by addition of non-toxic color. The water temperature is maintained at 25ºC to 30ºC.
* The tank is marked off into four quadrants, i.e., N, S, E & W.
* An escape platform of 13 cm size with heavy base is placed in the middle of any fixed quadrant.
* For the hidden platform task, water is added till a level 2 cm above the platform.
* Extra maze cues are put around the maze that helps the rats to navigate the tank.
* Rats were placed in the water at a designated starting location and the time taken by the rats to find the hidden platform was defined as ―Escape Latency(EL).
* The rats were trained to navigate through the submerged platform. They were given a maximum time of 120 seconds (cut-off time) to find the hidden platform and were allowed to stay on it for 30 seconds to get oriented to the external cues.

Elevated Plus Maze test

The Elevated Plus Maze test was carried out on 18th day and 19th day.

Procedure :

* The plus maze consist of two open (50 ×10 cm) and two closed arms (50×10×40 cm) facing each other with an open roof. The entire maze is elevated from the ground to a height of 50 cm for rats.
* The animals are placed individually at the end of either of the open arms and the time animal takes to move from open to enclosed arm **(transfer latency)** is noted and transferred to Master chart.
* Transfer latency is the time elapsed between the time the animal is placed in the open arm and the time at which all its legs have crossed the white line into the enclosed arm.

Estimation of blood sugar level:

* Blood sugar level was estimated on 1st, 15th and 20th day.
* Blood samples were collected from the tail of rat, since it is the most venous part of body of rat.
* The tail of rat was cleaned with spirit cotton and then with the help of sterilized blade, it was cut 0.5mm just enough to allow one drop of blood to ooze out.
* The Glucometer was made ready before hand with the test strip attached to it. One drop of blood was allowed on the appropriate reaction zone of the strip.
* Within a few seconds, the level of fasting blood sugar appeared on the display that was noted down in the Master chart.
* After taking the blood sample betadine ointment was applied to tail of each rat to prevent the infection.

Inclusion Criteria:
* 1. All the animals used for the study were healthy and active in their cage.
* 2. Animals were male wistar rats.
* 3. Weight of the animals used was 150-250grams.

Exclusion criteria :
* 1. Diseased and inactive rats were excluded from the study.
* 2. Rats with weight less than 150 grams and above 250 grams were excluded from the study.

STATISTICAL ANALYSIS:
Statistical analysis of data was carried out by employing analysis of variance(ANOVA). One way ANOVA test was used to compare the effect of drugs on different groups. Tukey's HSD test was used for post-hoc analysis of significant overall differences.

III. Result

WATER MORRIS MAZE TEST
Learning and memory was assessed in terms of mean Escape Latency which is the average of time(in seconds) taken by the rats to reach the platform in Morris Water Maze. The mean EL on different days for different groups was as follows-
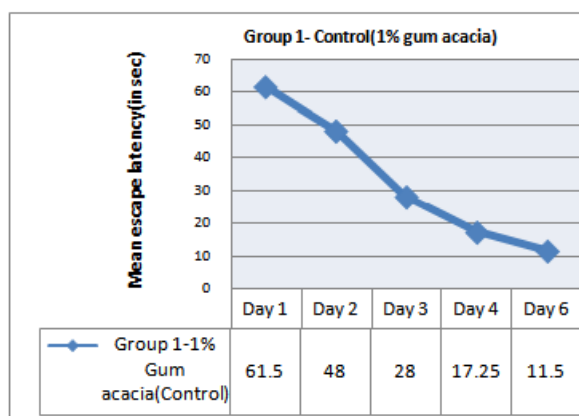
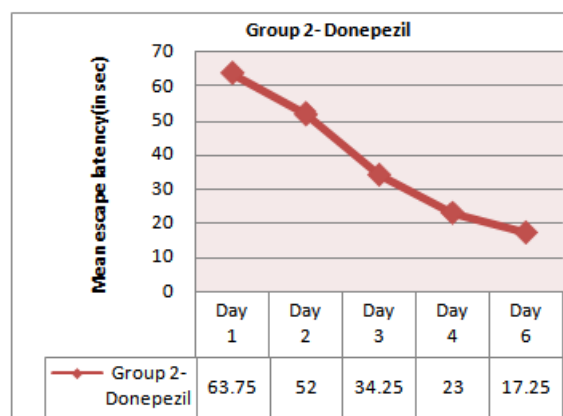**Figure 1: Mean EL time (in sec) of rats in Group 1 (1% gum acacia)   Figure 2: Mean EL time (in sec) of rats in Group 2 (Donepezil)**
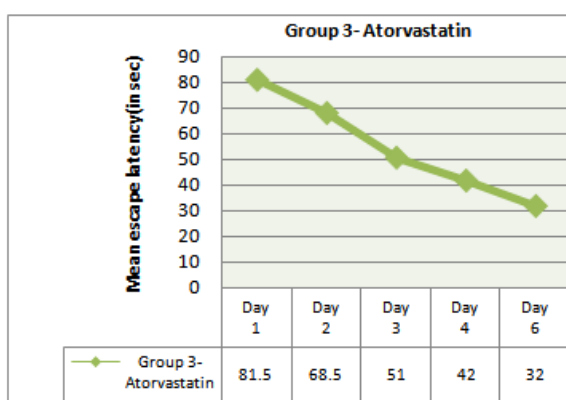

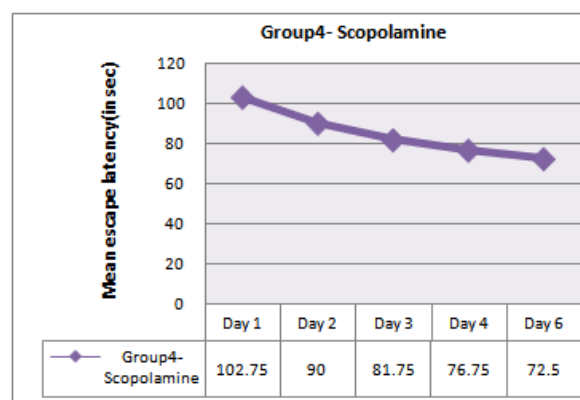**Figure. 3: Mean EL time (in sec) of rats in Group-3(Atorvastatin)   Figure. 4: Mean EL time (in sec) of rats in Group 4(Scopolamine)**
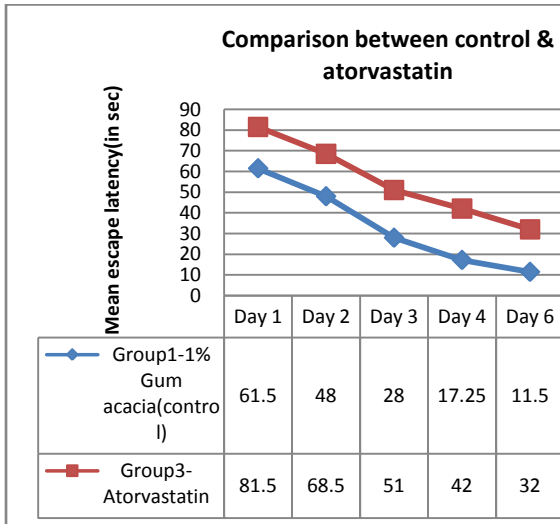
Fig 5: Comparison of mean EL(in sec) between Control time (in sec) between
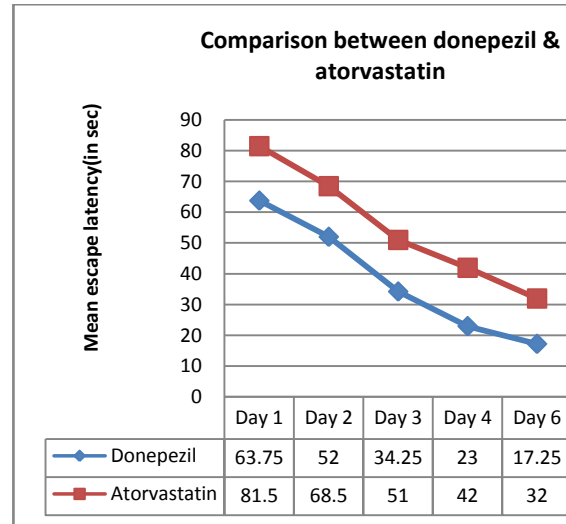**(Group 1) and Atorvastatin (Group-3)-**

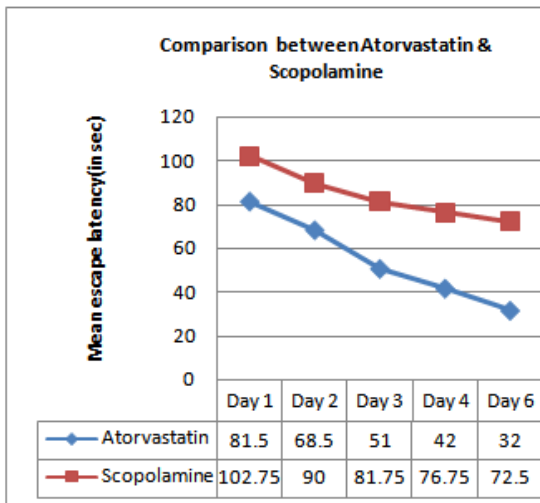**Fig 6: Comparison of mean escape latency**

Donepezil(Group 2) Atorvastatin (Group-3)-

Fig 7: Comparison of mean EL time (in sec) between Atorvastatin
latency time (in sec)
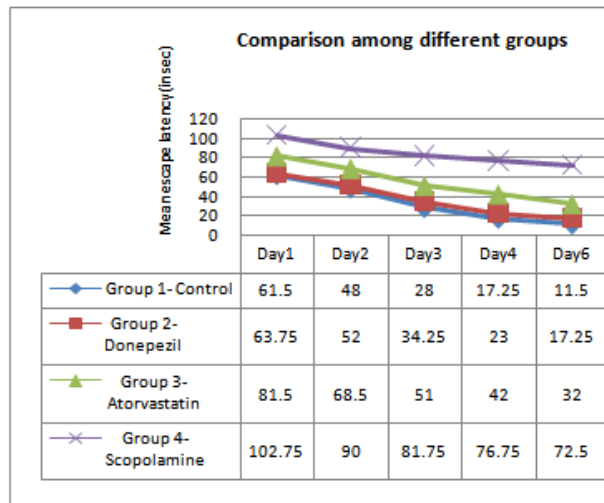**(Group-3) and Scopolamine (Group-4)-**

Fig 8: Comparison of mean escape

among different groups

Elevated Plus Maze Test

In Elevated Plus Maze learning and memory are assessed in terms of mean Transfer Latency(TL) which is the average of time (in seconds) taken by rats to enter closed arm of the maze. The mean TL of rats on different days for different groups was as shown below-
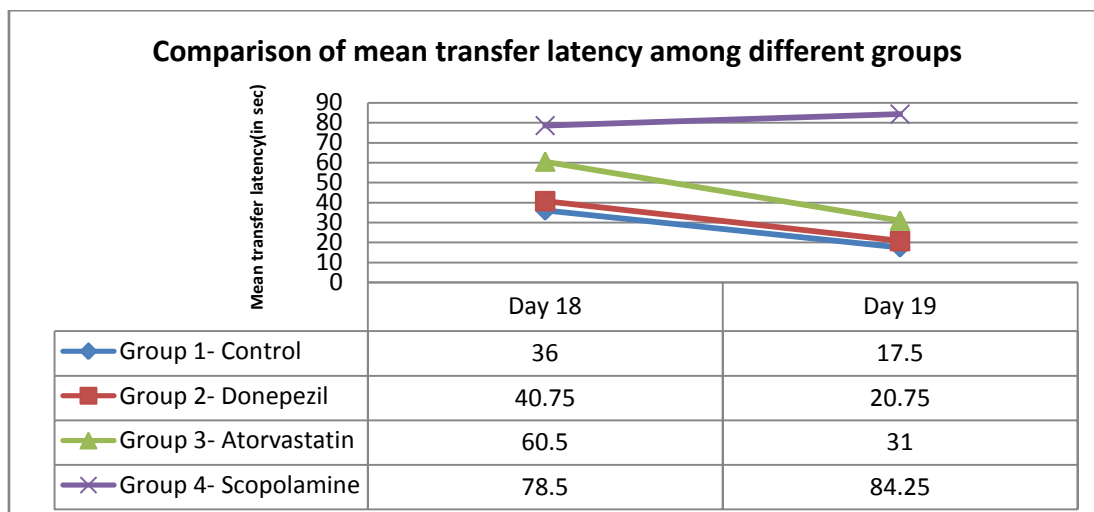
Comparison of mean transfer latency among different groups

	Day 18	Day 19
Group 1- Control	36	17.5
Group 2- Donepezil	40.75	20.75
Group 3- Atorvastatin	60.5	31
Group 4- Scopolamine	78.5	84.25

Fig 9: Comparison of mean transfer latency among different groups

BLOOD SUGAR LEVEL

Table 1: Comparison of mean blood glucose level (in mg/dL) among different groups-

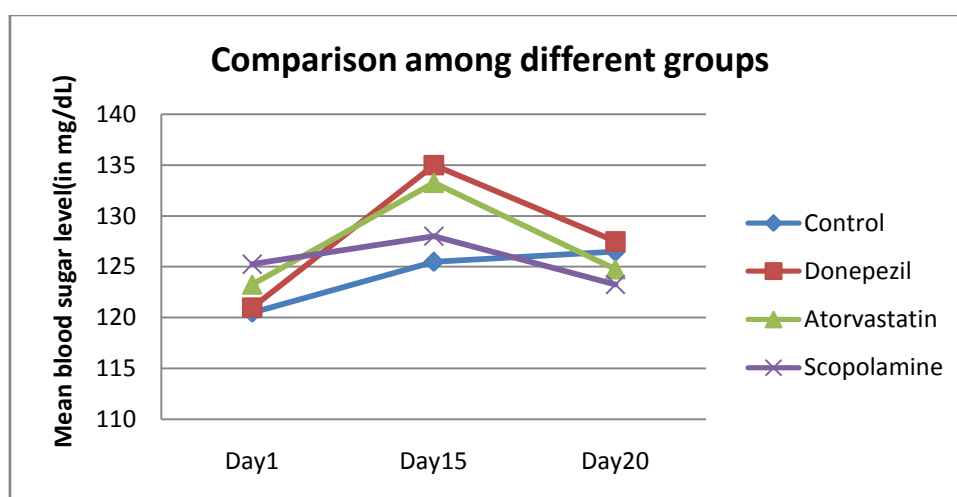| Group	Day 1	Day 15	Day 20
1	120.50	125.50	126.50
2	121.00	135.00	127.50
3	123.25	133.25	124.75
4	125.25	128.00	123.25

Fig 10: Comparison of mean blood sugar level among different groups

IV.   Discussion

Morris Water Maze

The data obtained from this research work indicates a decrease in mean Escape Latency (EL) in all groups. This is due to the learning and memory process due to repetition of the task in all groups. The rate of learning and the extent of memory retention were different in different groups.

Control (group 1) rats showed a normal learning behaviour, which is evident from a continuous decrease in mean Escape latency (EL) time for the finding hidden platform in Morris Water Maze Test.**(Figure 1)**

Present study revealed that exposure to Scopolamine, which was used as an amnesic agent, impaired the learning and memory processing activities in rodents, i.e. there was delayed learning in the Scopolamine group (Group 4).**(Figure 4).** This was evident from an increased mean Escape Latency (EL) in Scopolamine group (Group 4) which was 102.75sec, 90.00sec, 81.75sec, 76.75sec and 72.50sec as compared to that in Control group (Group1) which was 61.50sec, 48.00sec, 28.00sec, 17.25sec and 11.50sec respectively on Day 1, Day 2, Day 3, Day 4, and Day 6. **(Figure 1).** The data showed that there was a significant impairment in learning as well as memory in Scopolamine treated group (Group 4) on all days (p value < 0.05, table 12).

Donepezil (Group 2) which was used as a reference drug showed improvement in learning of task which is evident from decrease in mean escape latency time from day 1 (63.75 sec) through day 2 (52.00 sec), day 3 (34.25 sec), day 4 (23.00 sec) and day 6 (17.25 sec). **(Figure 2).**

Atorvastatin treated rats (group 3) showed an improvement in learning of task in Morris Water Maze Test which is evident by decrease in mean escape latency time from day 1 to day 6 i.e., 81.25 sec, 64.75 sec, 46.75 sec, 38.50 sec and 25.00 sec. **(Figure 3).** Atorvastatin treated rats (Group 3) showed higher mean EL time, i.e., 81.25 sec(day 1), 64.75 sec(day 2), 46.75 sec(day 3), 38.50 sec(day 4) and 25.00 sec(day 6) than that of control, i.e. 61.50sec(day1),, 48.00sec(day2),, 28.00sec(day3), 17.25sec(day 4), and 11.50sec(day 6). The difference in mean EL between these two groups was statistically significant ($p < 0.05$) on day 1, day 2 and day 3 and highly significant ($p<0.01$) on day4 and day 6 suggesting that although atorvastatin improved learning and memory but the extent was lower than in control **(Figure 5)**. Further, mean EL of atorvastatin group (group 3), i.e. 81.25 sec(day 1), 64.75 sec(day 2), 46.75 sec(day 3), 38.50 sec(day 4) and 25.00 sec(day 6) was much lower than that of Scopolamine group (group 4) ,i.e. 102.75sec(day 1), 90.00sec(day 2), 81.75sec(day 3), 76.75sec(day 4), and 72.50sec(day 6). The difference in mean EL between these two groups was statistically significant ($p<0.05$) on day 1 and day 2 and highly significant on day 3, day 4 and day 6($p<0.01$) suggesting that atorvastatin does have nootropic effect**(Figure7).** Atorvastatin was found to be inferior to Donepezil in improving learning and memory which is evident when mean escape latency of Atorvastatin (Group 3) i.e., 81.25 sec(Day 1), 64.75 sec(Day 2), 46.75 sec(Day 3), 38.50 sec(Day 4) and 25.00 sec(Day 6) was compared with that of Donepezil (Group 2) i.e., 61.50 sec(day 1), 48.00 sec (day 2), 28.00 sec(day 3), 17.25 sec(day 4) and 11.50 sec(day 6) respectively. The difference was statistically significant on day 4 and day 6. **(Figure 6).**

This finding was similar to that of the study conducted **Georgieva - Kotetarova et al(2013)**[11] which stated that atorvastatin improved learning and memory in diazepam and induced amnesia in Wistar rats and also similar to that of the study conducted by **Chowta et al(2015)**[12] which stated that atorvastatin improved learning and memory in scopolamine induced amnesia in albino rats.

Elevated plus maze

The data obtained from this research work showed a decrease in mean Transfer Latency (TL) on from Day 18 to Day 19 in all groups except Scopolamine group(Group 4) suggesting better memory and retention in all groups except Scopolamine group(Group 4)**. (Figure 9)**

Scopolamine was found to be much inferior to 1% Gum acacia(p-value<0.01) in improving cognitive behaviour which is evident when mean Transfer Latency(TL) of Scopolamine(Group 4) on Day 18(78.50 sec) and Day 19(84.25 sec) was compared with that of control(Group 1) i.e., Day 18(36.00 sec) and Day 19(17.50 sec)**. (Figure 9)**

Donepezil was found to improve learning and memory which can be appreciated when mean Transfer Latency of Donepezil group (Group 2) on Day 18(40.75 sec) and Day 19(20.75 sec) was compared with that of Scopolamine (Group 4) i.e., Day 18(78.50 sec) and Day 19(84.25 sec). The difference was highly significant suggesting that donepezil was highly effective in improving learning and memory in scopolamine induced amnesia($p<0.01$)**.** Mean TL of Donepezil group on Day 18(40.75sec) and Day 19(20.75 sec) was higher than that of Control (Group 1) i.e., Day 18(36.00 sec) and Day 19(17.50 sec). The difference was statistically insignificant ($p>0.05$) suggesting that Donepezil reversed scopolamine amnesia in the rats to an extent, although inferior but comparable to Control**. (Figure 9)**

Atorvastatin showed improvement in learning and memory in scopolamine-induced amnesia in albino rats which could be appreciated when mean TL of Atorvastatin group (Group 3) on Day 18(60.50 sec) and Day 19(31.00 sec) was compared with that of Scopolamine group (Group 4) i.e., Day 18(78.50 sec) and Day 16(84.25 sec) respectively. The difference was highly significant on both Day 18 and Day 19.($P<0.01$)**.** The mean TL of Atorvastatin group (Group 3) on Day 18(60.50 sec) and Day 19(31.00 sec) was higher than that of Donepezil group (Group 2) on Day 18(40.75 sec) and Day 19(20.75sec) respectively and the difference was highly significant($p<0.01$) suggesting that atorvastatin was much inferior to Donepezil in improving learning and memory in scopolamine-induced amnesia in albino rats in elevated plus maze model**.**

The finding of present study on elevated plus model was similar to that of study conducted by **Parle et al(2007)**[13]and **Biswas et al(2014)**[14]**.** Parle et al found that atorvastatin was able to improve learning and memory in scopolamine-induced amnesia in rats in elevated plus maze test. Biswas et al found that atorvastatin was able to improve learning and memory of tasks on elevated plus model in rodent.

Blood glucose level

Various evidences from literature supported a co-relation between cognitive behaviours and circulating blood sugar level. An increased blood sugar level has been found to prevent and reverse dementia in human as well as rodents, indirectly suggesting a co-relation between low blood glucose level and dementia.

The data obtained from this research work showed that, although extent of acquisition and retention of memory was different in different groups, there was no significant difference in mean blood sugar level on Day

1, Day 15 and Day 20 in rats of various groups. **(Table 1, Figure 10).** This suggests that dementia is not associated with alteration in blood glucose level.

Limitations of the study
- The first and foremost limitation of the present study is small sample size.
- Taking animal ethics into consideration, duration of study was taken short. A long term study could give even more precise and significant results.
- Our study also lacks biochemical analysis like Microdialysis of Acetylcholine release at synapses as well as histopathological examinations to see the changes in brain with cognitive deficit.
- Animal models prepared by Aβ amyloid infusion in rodents or transgenic rodents provide better simulation with the Alzheimer's disease and if used in the present study could have given even better results.

V. Conclusion
- Observation from this research work shows that learning and memory improve in all groups of rats suggesting that repeated tasks improve these cognitive behaviors.
- Donepezil had highest impact on improving learning and memory among all groups.
- Atorvastatin does improve learning and memory. But it was less effective in improving learning and memory as compared to donepezil in scopolamine induced dementia in rats both in Morris Water Maze Test and Elevated Plus Maze Test.
- From this study we also found that blood glucose level was not significantly altered in rats with scopolamine induced dementia which hence, appears unlikely to be the cause of dementia.

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