



## Effects of Atorvastatin and Simvastatin on Learning and Memory in Aged Swiss Albino Mice

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### ABSTRACT

Age induced dementia as a result of progressive neurodegenerative disorders like Alzheimer's Disease (AD) has several causes and increased cholesterol turnover, amyloid deposition and oxygen free radical mediated cell injuries are mainly implicated ones. Statins along with cholesterol lowering property show many pleiotropic effects and may help in this condition. So the current study was conducted to evaluate the effect of atorvastatin and simvastatin (5mg/kg/day for 14 days) on learning and memory in aged swiss albino mice. Both the statins lowered the serum cholesterol level significantly in comparison to the disease control groups. When tested for learning as well as memory retaining ability, the aged animals treated with statins and standard drug piracetam (400mg/kg/day for 7 days) outperformed relative to the untreated group and are at par with the healthy young mice. Following this, the animals were sacrificed and brain tissue homogenate was tested for malondialdehyde (MDA) and was found to be lower in statin treated groups indicating decreased oxidative stress. Statins showed significant improvement in learning and memory in aged animals which may be attributed to their cholesterol lowering and antioxidant property.

**Keywords:** Memory, dementia, statin.

### INTRODUCTION

Dementia is an acquired deterioration in cognitive abilities. It is a syndrome of many causes, the most common being Alzheimer's disease.<sup>1</sup> Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory deficits, cognitive impairment and personality changes. It usually begins after 50 years of age and gradually progress over 5-10 years.<sup>2</sup> The most important pathology in AD is neuritic plaque containing A $\beta$  amyloid which is a protein of 39-42 amino acids derived from larger transmembrane protein called amyloid precursor protein (APP).<sup>1</sup>

Cholesterol turnover appears to play a crucial role in the deposition and clearance of amyloid peptide in brain.<sup>3,4,5</sup> Oxygen free radicals produced as a by-product of mitochondrial respiration can cause cellular damage.<sup>6</sup> They are also implicated in aging and neurodegenerative diseases.<sup>7</sup>

The most widely used treatment for Alzheimer's disease at present are acetylcholinesterase inhibitors, which aim to prolong cognitive function through increased synaptic activity without neuroprotection. The treatment is only symptomatic and provides only modest outcome for the patients.<sup>8</sup>

On the other hand increased intake of vitamin E is reported to have reduced the risk of AD through its antioxidant property.<sup>9</sup> Vitamin C, a known antioxidant has been shown to have memory enhancing property.<sup>10</sup>

Statins, which act by inhibiting HMG Co-enzyme A reductase, the rate limiting enzyme in cholesterol biosynthesis, are widely prescribed for treatment of dyslipidemia. Other than lipid lowering effect, there are many other effects like anti-inflammatory, antioxidant activities and improvement of endothelial functions which may be attributed to the therapeutic effects of statins.<sup>11</sup>

On this background the present study is being undertaken to evaluate the role of statins on learning and memory in some animal behavioral models using swiss albino mice.

### MATERIALS AND METHODS

#### Drugs

Atorvastatin (Systopic Lab. Ltd), Simvastatin (USV Ltd) and Piracetam (Pegasus Pharmaco India(P) Ltd) were procured from local market. All other chemicals and reagents used were of analytical grade.

#### Animals

Apparently healthy inbred strains swiss albino mice of either sex were randomly selected from the animal house of the institute. Young mice (aged 3 months) were weighing around 20-25 gm and aged mice (7 months old) were weighing 25-30 gm. Before the experiment all animals were acclimatized in the laboratory for 5 days in standard laboratory condition and were allowed free access to food and water throughout the period of experiment. They were used only once in the experiment. All experiments were carried out in the day time from

10:00hr and 16:00hr. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA.

### Experimental Design

12 young mice were divided into 2 groups (Group I and Group VI) and 48 aged mice were divided into 8 groups (Group II to Group V and Group VII to Group X) with 6 animals in each group. Passive avoidance paradigm and Morris water maze were the two exteroceptive behavioral models used in this experiment. Group I to Group V were subjected to passive avoidance paradigm and Group VI to Group X were subjected to Morris water maze.

Atorvastatin, simvastatin and piracetam were suspended in 1% carboxy methyl cellulose (CMC) and administered per orally to respective groups. Group I, II, VI and VII were administered 1%w/v CMC (10ml/kg body wt. p.o.) for 14 days, Group III and VIII were administered piracetam (400 mg/kg b.w, p.o.) for 7 days, Group IV and IX were administered simvastatin (5 mg/kg b.w, p.o.) and Group V and X were administered atorvastatin (5 mg/kg b.w, p.o.) for 14 days before subjecting the animals to any exteroceptive behavioral model. During the acquisition trials (1day in Passive avoidance paradigm and 4 days in Morris water maze) and memory retention test, 1% CMC, piracetam, simvastatin and atorvastatin were administered each day to the respective groups 60 min before subjecting the animals to any exteroceptive model.

### Passive Avoidance Test

This is a sensitive model for testing the learning and memory. The apparatus used for this test consist of a transparent acrylic cage 27x27x27cm with a grid floor (3mm steel rods set 8 mm apart) inserted in the cage with a wooden platform 10x7x1.7cm in the center of the grid. The box was illuminated with a 15W bulb. Training was carried out in 2 sessions on 1st day followed by testing of memory retention on the second day. Step down latency (SDL) was noted in all sessions. SDL is the time taken by the mouse to step down from wooden platform on to the grid floor with all its 4 paws.

Session 1: Mouse was placed gently on wooden platform. When the mouse step down and place all the 4 legs on the grid floor, shock (20V, AC current) was delivered for 15seconds.<sup>18</sup>

Session 2: It was started 90 min after the 1st session. If the mouse steps down onto the grid within 60 sec then another electric shock was given to it. If it did not step down then it was be taken out of the platform and returned to its home cage.

Day 2: Retention of the experience of day 1 was tested in a similar manner. This time no shock was applied. Cut off time of 300 sec was taken as end of session.<sup>10,12</sup>

### Morris Water Maze

This maze represent more specific test of spatial memory. The technique of using escape from water to motivate learning is the basis of this model. The maze consist of circular tank (150 cm diameter and 45 cm height) filled with water at 25-28 °C. Water was made opaque by addition of small quantity of titanium dioxide.<sup>12</sup> Tank was divided into 4 quadrants (Q1,Q2,Q3,Q4) with the help of 2 threads. There was a hidden platform (white in color with a diameter of 10cm ) kept at the center of the 4th quadrant 1 cm below the water level throughout the training period. Animals were subjected to 4 consecutive trials on each day at 5 min interval for 4 days. The animal was released in the water facing towards the wall of the tank allowed to escape to the hidden platform and further allowed to remain there for 20 sec and the escape latency (ELT) was recorded. ELT is the time taken by the animal from getting dropped in to the tank to escape on to the platform. If the animal did not locate the platform within 120sec (cut off) then it was guided to the platform and further allowed to remain there for 20 sec. The sequence of starting quadrants of the trial was as follows:-Day1: Q1 →Q2 →Q3 →Q4. Day2: Q2 →Q3 →Q4 →Q1. Day3: Q3 →Q4 →Q1 →Q2. Day4: Q4 →Q1 →Q2 →Q3.

Mean escape latency (ELT) was derived from each trial. ELT of Day 4 was compared with that of Day 1. On the 5th day retention of memory was tested by doing a probe test in which platform was removed. The quadrant where the platform was originally kept during trial sessions was considered as target quadrant. The time spent in the target quadrant (Q4T) was noted and compared.<sup>10</sup>

### Locomotor Activity

Locomotor activity was measured in the control as well as in the drug treated groups using Rotarod before subjecting them to any behavioral model. The speed of Rotarod was set at 20 revolutions per minutes (RPM). Then the mice were placed on the rod and motor was started. As the rod rotated anticlockwise the animal walked forward to keep itself steady on it. In the initial phase of 180seconds the animals were placed as many times on the rod as they fell from it. In the second phase started 1hour apart, the animals were placed on the rod only once and the duration for which the animal remains on the rod was noted for comparison.

Immediately after the completion of exteroceptive behavioral experiment the animals were subjected to ether anaesthesia. Blood was collected by direct cardiac puncture for estimation of serum cholesterol by colorimetric method using commercially available kit from Crest Biosystems.

After collection of blood samples animals were sacrificed by cervical dislocation and brains were carefully removed by opening the skull and their weight measured. Brain tissue was homogenized and fresh tissue homogenate was used for estimation of malondialdehyde (MDA) in the

brain tissue as indices of thiobarbituric acid reacting substances (TBARS) and lipid peroxidation.

**Statistical Analysis**

Results are given as mean ± SEM. Data was analyzed using one-way ANOVA followed by post-hoc Tukey's test. Values with P<0.05 were considered statistically significant.

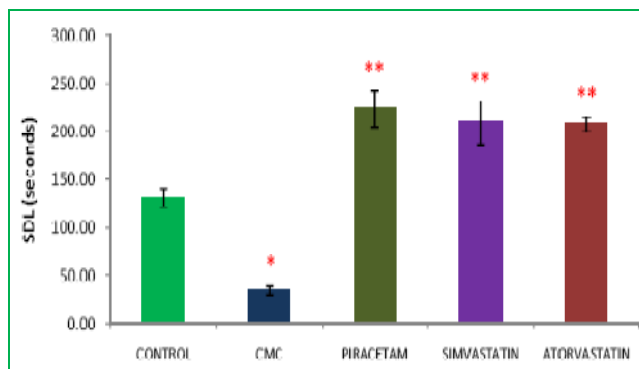
**RESULTS**

**Effect of drugs on locomotor activity**

When tested for locomotor activity on rotarod, there was no significant difference in the fall off time among different groups.

**Effect of drugs on step down latency (SDL)**

Effect of drugs on SDL in passive avoidance paradigm is shown in figure no. 1. It shows that SDL in aged mice treated with CMC (Group II) was significantly lower than that of young mice treated with CMC (Group I). But the SDL in aged mice treated with piracetam (Group III), simvastatin (Group IV) and atorvastatin (Group V) were significantly higher than that of aged mice treated with CMC (Group II).



n=6. Values expressed in mean ± SEM. \*\* denotes p<0.001 compared to control; \*\*\* denotes p<0.001 compared to CMC treated group

**Figure 1:** Effect of drugs on SDL in passive avoidance paradigm in age induced amnesia

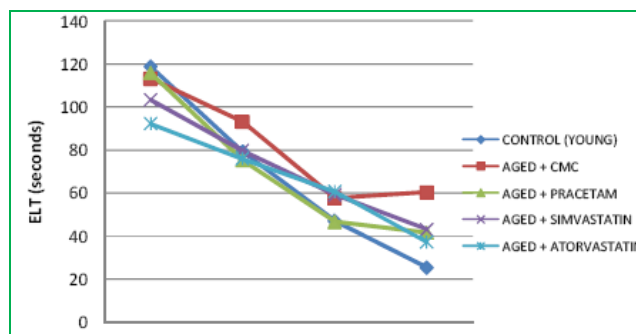
**Effect of drugs on escape latency (ELT)**

ELT as a measure of effective learning was measured during the acquisition trial in Morris water maze is shown in figure 2. It was found that, the day 4 ELTs in all groups, i.e. young (Group VI), CMC treated (Group VII), piracetam treated (Group VIII), simvastatin treated (Group IX) and atorvastatin treated (Group X) were significantly lower than their respective day 1 ELT. There is gradual decrease in ELT from day 1 to day 4 in all groups except in CMC treated group where there was slight increase in day 4 ELT in comparison to day 3 ELT which was found to be statistically insignificant.

**Effect of drugs on day 5 Q4T**

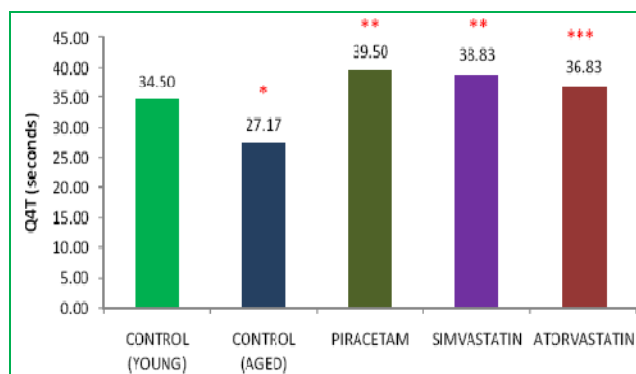
The effect of drugs on Q4T on 5<sup>th</sup> day (figure 3) served as a measure of memory shows that, the day 5 Q4T in aged mice treated with CMC was significantly lower than that of young mice treated with CMC. But the day 5 Q4Ts in

aged mice treated with piracetam, atorvastatin and simvastatin were significantly higher than that of disease control group, i.e. aged mice treated with CMC.



n=6. Values expressed in mean ± SEM

**Figure 2:** Effect of drugs on ELT during acquisition trial in age induced amnesia

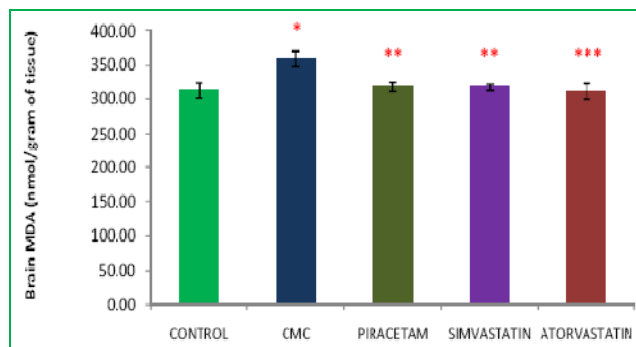


n=6. Values expressed in mean ± SEM; \*\* denotes p<0.05 vs control (young), \*\*\* denotes p<0.001 and \*\*\*\* denotes p<0.01 vs control (aged)

**Figure 3:** Effect of drugs on Q4T on 5<sup>th</sup> day

**Effect of drugs on brain MDA level**

In the present experiment the MDA level in brain tissue of mice were measured irrespective of the behavioral models they were subjected to and were expressed as nmol/gram of tissue. MDA level among aged mice treated with CMC was significantly higher than that among young mice treated with CMC. But the MDA level in groups pre-treated with piracetam, atorvastatin or simvastatin was significantly lower than the MDA level in CMC treated group (figure 4).



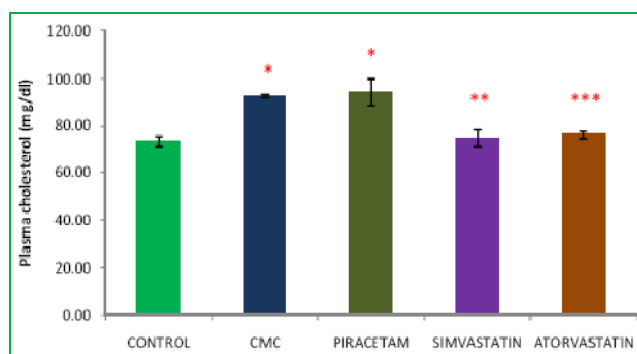
n=6. Values expressed in mean ± SEM; \*\* denotes p<0.05 compared to control, \*\*\* denotes p<0.05 compared to CMC treated group, \*\*\*\* denotes p<0.01 compared to CMC treated group

**Figure 4:** Effect of drugs on MDA in age induced amnesia



### Effect of drugs on plasma cholesterol level

The effect of drugs on plasma cholesterol level in both the behavioral models was also combinedly studied as shown in figure 5. It was found that, the plasma cholesterol level in aged mice treated with CMC was significantly higher than that of young mice treated with CMC. But the plasma cholesterol levels in aged mice treated with atorvastatin and simvastatin were significantly lower than that of disease control group. The standard drug piracetam did not produce significant decrease in plasma cholesterol level in comparison to disease control group.



n=6. Values expressed in mean  $\pm$  SEM; '\*' denotes  $p < 0.01$  compared to control, '\*\*' denotes  $p < 0.01$  compared to CMC treated group, '\*\*\*' denotes  $p < 0.05$  compared to CMC treated group

**Figure 5:** Effect of drugs on plasma cholesterol in age induced amnesia

### DISCUSSION

The present study has been undertaken to explore the memory enhancing effect of commonly used statins like simvastatin and atorvastatin on age induced amnesia model using different exteroceptive behavioral models like passive avoidance paradigm (PAP) and Morris water maze (MWM).

Swiss albino mice have been selected as the rodents (rats / mice) are standardized experimental animal for behavioral study.<sup>13</sup> These animals are small in size, so feeding, handling and drug administration are relatively easy.

Oxygen free radicals are implicated in the process of aging and are responsible for development of Alzheimer's disease. Some studies reported that in Alzheimer's disease there is increase in serum cholesterol level. So this study result showing the anti-amnesic effect of statins may be due to their hypocholesterolemic as well as antioxidant properties.<sup>11</sup>

Piracetam is a standard memory enhancer and is used as standard in different published research articles as standard.<sup>12</sup> The dose of piracetam, simvastatin and atorvastatin has been taken from the previous studies which were published in different journals. All the drugs are dissolved in 1 % w/v CMC administered through oral route.<sup>14</sup>

Passive avoidance paradigm is based on negative reinforcement and used to examine the long term

memory. It is a standard method to evaluate the long term memory by recording step down latency (SDL).<sup>13</sup> In this present study it is observed that atorvastatin and simvastatin in 5mg/kg dose each, SDL significantly increased in comparison to that of disease control group which received only CMC. The increase in SDL of piracetam group is significantly higher than that of disease control group. Simvastatin and atorvastatin showed the comparable effect with the standard drug piracetam on SDL. This result agrees with the previous study.<sup>14</sup>

Morris water maze is a standard method to assess the spatial learning and memory of rodents. Escape latency (ELT) on day 4 was taken as index of acquisition and Q4T on day 5, time spent in target quadrant, served as index for retrieval of memory.<sup>15</sup> The current study showed that there is marked decrease in ELT in subsequent trials from day 1 to day 4 in all aged mice served as standard and tests. The day 4 ELT in all the drug treated groups as well as in the vehicle treated groups decreased significantly in respect to corresponding day 1 values. This type of result denoted the acquisition of memory in all groups of animals.<sup>16</sup> The Q4T on 5<sup>th</sup> day in search of hidden platform during retrieval test decreased significantly in disease control group comparing with young control. But the same was significantly increased in all drug treated groups in comparison to disease control group. This result is consistent with earlier study reports.<sup>14, 17</sup>

There was also no significant difference in fall off time in rotarod performance in different disease models in comparison to normal control. Although there is decreased motor performance with increasing age due to various musculoskeletal causes, in our study there is no deterioration of motor coordination due to disease in animals. This indicates that differences in performances if any during the behavioral tests were not due to any musculoskeletal cause.

During the biochemical estimation it was observed in this study that brain tissue MDA level in disease control group was significantly higher than that of the normal control (young mice treated with CMC). Piracetam, simvastatin and atorvastatin produced significant decrease in brain MDA level in comparison to that of disease control group.<sup>11</sup> The effects produced by statins are not significantly different from piracetam treated group. This effect proved the anti-amnesic effect of statins may be due to their antioxidant properties.<sup>18</sup>

The aged mice treated with vehicle (CMC) only showed significant rise in plasma cholesterol level in comparison to that of normal control (young mice). This increase in plasma cholesterol level in age induced amnesia corroborates with other study results that elevated serum cholesterol level is an important risk factor for Alzheimer's disease. Atorvastatin and simvastatin in 5mg/kg dose significantly decreased the plasma cholesterol level whereas piracetam did not show any

significant change in plasma cholesterol level. Piracetam probably produces its anti-amnesic property by other mechanism such as stimulating cholinergic transmission which is not the case with statins. So in addition to the antioxidant property, hypocholesterolemic effect of statins can also be implicated for the anti-amnesic effect.<sup>19</sup>

### CONCLUSION

The study was conducted to evaluate the anti-amnesic property of statins (simvastatin and atorvastatin) vis-a-vis their hypocholesterolemic and antioxidant property. Advanced age significantly impaired the learning and memory of swiss albino mice. This impairment was successfully reversed by the standard drug Piracetam and by either of the statins when given prior to the experiment. It was also found in this study that memory enhancing property of statins was accompanied with decrease in plasma cholesterol and brain MDA level which points towards a definite relation of hypocholesterolemic and antioxidant property with the learning and memory. As piracetam also enhanced the learning and memory along with decrease in MDA without any visible effect on plasma cholesterol level, this indicates its cholesterol independent mechanisms like antioxidant and anticholinesterase activity.

The present study was conducted on laboratory animals. Further long term studies should be conducted on other species and finally on human subjects to firmly establish the result and for large scale clinical use for prevention and treatment of dementia.

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