Reversal of Memory Deficits by Atorvastatin and Simvastatin in Rats

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The present study was undertaken to investigate the beneficial effects of Atorvastatin and Simvastatin in cognitive dysfunctions of rats. Alprazolam, Scopolamine and high fat diet (HFD) induced amnesia served as interoceptive models where as, Water-maze and Elevated plus-maze served as exteroceptive models. A total of 38 groups of rats were used in this investigation. Escape latency time (ELT) recorded during acquisition trials conducted from day 1 to day 4, in water maze was taken as an index of acquisition, where as mean time spent in target quadrant during retrieval trial on day 5, was taken as the index of retrieval (memory). On elevated plus-maze, transfer latency (TL) measured on 1st d served as the index of acquisition and TL recorded on 2nd d was taken as the index of retrieval (memory). Alprazolam (0.5 mg kg⁻¹ intraperitoneally), Scopolamine (0.4 mg kg⁻¹ intraperitoneally) and HFD treated (for 90 days) rats exhibited amnesia as reflected by impairment in learning ability as well as memory, when tested on both, water maze and elevated plus maze. Atorvastatin (5 mg kg⁻¹ orally) as well as Simvastatin (5 mg kg⁻¹ orally) significantly attenuated Alprazolam, Scopolamine and HFD induced amnesia. These results highlight the ameliorative role of statins in experimental amnesia with possible involvement of their cholesterol dependent as well as cholesterol independent actions.

Key words—amnesia; statins; anterograde; cholesterol; memory; water maze

INTRODUCTION

Dementia is a syndrome of progressive nature marked by gross behavioral and personality disturbances. This syndrome occurs in Alzheimer’s disease (AD), cerebrovascular disease (i.e., multi-infarct dementia), and other conditions primarily or secondarily affecting the brain. Accumulation of peptide β-amyloid, in brains of AD patients leads to neurotoxicity and neurodegeneration. Elevated β-amyloid levels and apolipoprotein E4 (ApoE4) are risk factors for Alzheimer’s disease. Observational studies have revealed elevated serum cholesterol level as an important risk factor for Alzheimer’s disease, suggesting a pathophysiologic relation between β-amyloid and cholesterol levels. Experimental studies have shown that cholesterol fed wild type rabbits develop brain pathology similar to that of Alzheimer’s disease. Transgenic mouse model of AD exhibited increased β-amyloid plaques, when mice were fed with cholesterol rich diet. Cell culture and in vivo animal studies have shown that reducing cholesterol can inhibit β-amyloid synthesis. Statins are widely prescribed as cholesterol lowering drugs, which act by inhibiting Hydroxy-methyl-glutaryl Co-enzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis for the treatment of dyslipemias. Statin therapy is associated with distinct advantages as well as disadvantages in human beings. The major advantages observed with statin therapy include anti-inflammatory action, anti-thrombotic effect, antioxidant activity, improvement of endothelial dysfunction etc. in addition they have also been shown to possess some benefits in cognitive impairment. A recent study on 63 AD patients suggested that statin use improved the depressive symptoms associated with AD and slowed Alzheimer progression. Statins have been shown to reduce the risk of ischemic stroke and related memory impairment by a variety of mechanisms. Epidemiological studies have suggested that individuals above 50 years of age, who were receiving statins had a substantially lowered risk of developing dementia, independent of the presence or absence of untreated hyperlipidemia, or exposure to non-statin lipid-lowering drugs. On the other hand, the major disadvantages associated with statin therapy include rhabdomyolysis, immuno-suppression as well as cholesterol inhibition. Cholesterol synthesis is essential for neurons to function normally, so theoretically it appears that
excessive inhibition of cholesterol synthesis may result in neuro-cognitive decline.\textsuperscript{13} There are conflicting observations regarding the effect of statins on cognitive function. Although, there are a few studies showing cognitive decline,\textsuperscript{13} some studies showing no effect on memory,\textsuperscript{14,15} but several studies suggest improvement of cognitive functions with statin therapy. Therefore, the present study was designed to investigate the role of statins (Atorvastatin and Simvastatin) in Alprazolam; Scopolamine and high fat diet (HFD) induced amnesia in rats employing various memory models.

**MATERIALS AND METHODS**

**Animals** Wistar rats of either sex, weighing around 200 g were employed in the present study. They were exposed to alternate light and dark cycle of 12 h and had free access to food and water. They were procured from the disease free animal house of Chaudary Charan Singh, Haryana Agriculture University Hisar (India). The animals were acclimatized to the laboratory conditions for at least five days prior to the behavioral test. Experimental, protocol was approved by the institutional animal ethics committee (IAEC). Care of the animals was taken as per guidelines of committee for the purpose and control supervision of experiments on animals (CPCSEA), Ministry of Forests and Environment, Government of India.

**Drugs** Atorvastatin (Zydus research center, Ahmedabad, India), Simvastatin (Morgan Ltd., Baddi, HP, India), Alprazolam (Kim Laboratory, Ambala Cantt, India) and Scopolamine hydrobromide (Sigma-Aldrich, USA) were used in the present study. Atorvastatin and Simvastatin were suspended in 1% w/v carboxy methyl cellulose (CMC) sodium for oral administration. Alprazolam was suspended in 1% w/v CMC sodium and administered intraperitoneally (i.p.), where as Scopolamine HBr was dissolved in distilled water for intraperitoneal administration. Volume of administration was 10 ml/kg body weight of the rat.

**Laboratory Models**

**Exteroceptive Behavioral Models** (a) Elevated plus-maze apparatus and (b) Morris water maze apparatus.

**Elevated Plus-maze** Elevated plus-maze was originally introduced as a model for studying anxiolytic agents. Later on, it was found that acquisition and retention processes of memory could also be studied using elevated plus-maze.\textsuperscript{16,17} However, the parameters used for testing these two categories of agents were distinctly different. The total number of explorations (arm entries) and time spent by the animal in open arm Vs enclosed arm are parameters of anxiety whereas; transfer latency (TL) is a parameter of memory.\textsuperscript{16,18} The TL was defined as the time in seconds taken by the animal to move into one of the enclosed arms with all its four legs. A single dose of Alprazolam (0.5 mg/kg, i.p.) has been demonstrated to induce amnesia in mice.\textsuperscript{19,20} The procedure, technique and end point for testing learning and memory was followed as per the parameters described by investigators working in the area of psychopharmacology.\textsuperscript{21–23} Briefly, the elevated plus maze apparatus for rats consisted of a central platform (10 cm×10 cm) connected to two open arms (50 cm×10 cm) and two covered (enclosed) arms (50 cm×40 cm×10 cm) the maze was elevated to a height of 50 cm from the floor. In order to record transfer latency (TL), each rat was placed at the end of an open arm facing away from the central platform. TL was recorded on first day for each animal. The rat was allowed to explore the maze for 20 s and then returned to its home cage. Retention of this learned task was examined 24 h after the first day trial.

**Morris Water Maze**\textsuperscript{24} It consisted of a circular water tank (diameter 150 cm and height 45 cm), filled with water maintained at 250°C. The water was made opaque with a white colored non toxic dye. The tank divided into four quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm^2) of 29 cm height was located in the center of one of these four quadrants. The position of platform was kept unaltered throughout the training sessions. In the present the target quadrant was Q4. Each animal was subjected to four consecutive trials on each day with gap of 5 min for four consecutive days, during which they were allowed to escape on to the hidden platform and to remain there for 20 s. In case the animal was unable to locate the hidden platform within 120 s, it was gently guided to the platform and allowed to remain on the platform for 20 s. Escape latency time to locate the hidden platform in water maze was taken as an index of acquisition or learning. Starting position on each day to conduct four acquisition trials was changed as described below and Q4 was maintained
as target quadrant in all the acquisition trials. The starting point for dropping the rat into water-maze on day one for four consecutive acquisition trials was in the sequence Q1, Q2, Q3, Q4 and so on.

Sequence of change of starting point:
- Day 1: Q1, Q2, Q3, Q4
- Day 2: Q2, Q3, Q4, Q1
- Day 3: Q3, Q4, Q1, Q2
- Day 4: Q4, Q1, Q2, Q3.

Mean escape latency time (ELT) was calculated for each day trial. On fifth day, the platform was removed; each rat was placed in water for 120 s. The animal was subjected to four such trials and each trial had a different starting point covering all the four quadrants. The mean time spent by the animal in all four quadrants i.e., Q1, Q2, Q3, Q4 was recorded. The time spent in the target quadrant Q4 as compared to time spent in other quadrants in search of missing platform was taken as an index of retrieval. Care was taken that relative location of water maze with respect to other objects in the laboratory serving as visual clues was not disturbed during the total duration of the study.

**Interceptive Behavioral Models**

(a) Alprazolam-induced amnesia. (b) Scopolamine amnesia. (c) High fat diet (HFD) induced amnesia.

Alprazolam (0.5 mg kg\(^{-1}\) i.p.) and Scopolamine hydrobromide (0.4 mg kg\(^{-1}\) i.p.) were administered before training for induction of anterograde amnesia and before retrieval trial for inducing retrograde amnesia. Scopolamine amnesia.

**Estimation of Serum Cholesterol Level**

Blood sample was withdrawn from retro orbit sinus. The sample was placed for half an hour. Then centrifuged for 15 min at 4,000 rpm to separate the serum from clot debris. Total serum cholesterol was estimated spectrophotometrically at 540 nm by Allain’s method using a commercially available kit (Monzyme India Limited, Secunderabad, India).

**Experimental Protocol**

The animals were divided into 38 groups. Each group comprised of 6 animals.

Group I (Control group): Rats were administered vehicle (1% w/v CMC sodium, 10 ml kg\(^{-1}\) i.p.). TL was noted after 30 min and then after 24 h (2nd d) using elevated plus-maze.

Group II (Alprazolam, before training): Rats were injected with Alprazolam (0.5 mg kg\(^{-1}\) i.p.) 30 min before exposure to elevated plus maze on first d. TL was noted 30 min after the injection and again after 24 h i.e., on 2nd d.

Group III (Alprazolam, before retrieval trial): Rats were administered Alprazolam immediately after exposure to elevated plus maze on first day. TL was recorded on 1st d and 24 h later.

Group IV and V (Atorvastatin/Simvastatin per se.): Rats were treated with Atorvastatin/Simvastatin 5 mg kg\(^{-1}\) each orally, 60 min prior to elevated plus maze exposure on 1st d. TL was measured on 1st and 2nd d.

Group VI and VIII (Atorvastatin/Simvastatin + Alprazolam, before training): Animals were administered Atorvastatin/Simvastatin and Alprazolam 60 and 30 min respectively, before first day exposure on elevated plus maze. TL was recorded on 1st d and 2nd d.

Group VII and IX (Atorvastatin/Simvastatin + Alprazolam, before retrieval trial): Rats were administered Atorvastatin/Simvastatin and Alprazolam immediately after first day exposure to elevated plus maze on first day. TL was recorded on 1st d and 2nd d.

On similar lines, Scopolamine (0.4 mg kg\(^{-1}\) i.p.) was used to induce amnesia in place of Alprazolam for studies involving reversal of memory deficits using elevated plus maze (Groups X-XV)/Morris water maze (Groups XXIX to XXXIV).

Group XVI and XVII (normal diet/high fat diet): Rats were subjected to normal standard laboratory diet/high fat diet (HFD) for a period of 90 days then their TL was recorded on elevated plus-maze on 91st d and 92nd d i.e., 24 h later.

Group XVIII and XIX (HFD + Atorvastatin/Simvastatin): HFD animals were administered Atorvastatin/Simvastatin daily, orally for 15 days just before subjecting them to elevated plus-maze. TL was noted on 91st d and after 24 h.

Group XX (Control group): Rats were administered vehicle, daily 30 min prior to acquisition (from d 1 to d 4) and before retrieval trial (on d 5) using water maze.

Group XXI (Alprazolam, before training): Rats were administered Alprazolam daily (from d 1 to d 4) 30 min before training on water maze and vehicle on day 5, 30 min before retrieval trial.

Group XXII (Alprazolam, before retrieval trial): Animals were injected vehicle daily (from d 1 to d 4),
30 min before training on water maze and Alprazolam on d 5, 30 min before retrieval trial.

Group XXIII and XXIV (Atorvastatin/Simvastatin per se): Rats were treated with Atorvastatin/Simvastatin daily, orally (from d 1 to d 4) 60 min before training on water maze and vehicle on d 5, 30 min before retrieval trial.

Group XXV and XXVII (Atorvastatin/Simvastatin + Alprazolam before training): Animals were administered Atorvastatin/Simvastatin and Alprazolam daily (from d 1 to d 4), 60 min and 30 min respectively, before training on water maze and vehicle on d 5, 30 min before retrieval trial.

Group XXVI and XXVIII (Atorvastatin/Simvastatin + Alprazolam, before retrieval trial): Animals were administered vehicle daily (from d 1 to d 4) 30 min before training on water maze and vehicle on d 5, 30 min before retrieval trial.

Group XXXV and XXXVI (normal diet/high fat diet): Rats were subjected to normal diet/high fat diet (HFD) for 90 days before training (91st d to 94th d) and retention test (95th d) on water maze.

Group XXXVII and XXXVIII (HFD + Atorvastatin/Simvastatin): HFD animals were administered Atorvastatin/Simvastatin daily for 15 days and again for four consecutive days (d 1 to d 4) 30 min before training. On 5th d, the rats were administered vehicle 30 min before retrieval trial.

Statistical Analysis All the results were expressed as mean±S.E.M (Standard error of mean). The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple range test. p<0.05 was considered as statistically significant.

RESULTS

Effect of High Fat Diet on Body Weight of Rats

There was a significant increase (p<0.05) in the body weight of animals over the period of 90 days in rats receiving normal diet or high fat diet (HFD), when compared to the body weights of rats on day 1. Furthermore, HFD treatment for 90 days produced a significant increase (p<0.05) in body weight of rats as compared to those receiving normal diet for 90 days (Table 1).

Effect of Alprazolam, Scopolamine and High Fat Diet on Transfer Latency of Rats Using Elevated Plus-maze Transfer Latency (TL) of first day reflected learning behavior of animals. Whereas, TL of next day reflected retention of information or memory. TL of control (vehicle treated) group animals decreased significantly on 2nd d i.e., after 24 h of training on elevated plus-maze. Alprazolam (0.5 mg kg⁻¹ i.p.) administered either before (group II) or after (group III) elevated plus-maze exposure on 1st d, significantly increased (p<0.01) 2nd d TL, when compared to 2nd d TL in control group on elevated plus-maze. These observations suggested that Alprazolam had produced anterograde and retrograde amnesia, respectively (Table 2).

Similarly, Scopolamine (0.4 mg kg⁻¹ i.p.) administered either before (group X) or after (group XI) elevated plus-maze exposure on 1st d, significantly increased (p<0.01) 2nd d TL, when compared to 2nd d TL in control rats on elevated plus-maze, indicating induction of anterograde as well as retrograde amnesia, respectively. On the other hand, both Alprazolam and Scopolamine treatment did not produce any significant effect on first day TL of control rats. Animals receiving the high fat diet (HFD) for 90 days (group XVII) showed a significant increase (p<0.05) in first day as well as 2nd d TL (p<0.01), when compared to TL of rats receiving normal diet (group XVI), reflecting a strong amnesic action (Table 3).

Effect of Atorvastatin and Simvastatin on Alprazolam, Scopolamine and HFD Induced Amnesia in Rats Using Elevated Plus-maze Atorvastatin (group IV) and Simvastatin (group V) per se did not produce any significant effect on TL of rats. Atorvastatin (group VI, XII) and Simvastatin (group

### Table 1. Effect of High Fat Diet (HFD) on Body Weight of Rats

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>184±2.4 g</td>
<td>200 ± 2.3 g</td>
<td>218.4±2.7 g</td>
<td>238.4±2.7 g</td>
</tr>
<tr>
<td>High fat diet</td>
<td>185±3.3 g</td>
<td>205.8±3.1 g</td>
<td>228.2±3.2 g</td>
<td>256 ± 3.2 g</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean±S.E.M. a) Denotes p<0.05 compared to day 1 body weight (Student's t-test). b) Denotes p<0.05 compared to day 90 body weight of normal diet (control group).
Table 2: Effect of Atorvastatin and Simvastatin on Alprazolam Induced Changes in the Transfer Latency (TL) of Rats Using Elevated Plus-maze

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (kg⁻¹)</th>
<th>TL (training) in s</th>
<th>TL after 24h in s</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Vehicle 10 ml i.p.</td>
<td>46.8 ± 3.2</td>
<td>26.6 ± 2.8</td>
</tr>
<tr>
<td>II</td>
<td>Alprazolam (before training)</td>
<td>0.5 mg i.p.</td>
<td>49.2 ± 2.6</td>
<td>62.2 ± 3.4</td>
</tr>
<tr>
<td>III</td>
<td>Alprazolam (before retrieval)</td>
<td>0.5 mg i.p.</td>
<td>47.8 ± 3.3</td>
<td>60.4 ± 3.7</td>
</tr>
<tr>
<td>IV</td>
<td>Atorvastatin</td>
<td>5 mg p.o.</td>
<td>39.8 ± 1.9</td>
<td>24.4 ± 2.5</td>
</tr>
<tr>
<td>V</td>
<td>Simvastatin</td>
<td>5 mg p.o.</td>
<td>41.4 ± 2.6</td>
<td>26 ± 2.2</td>
</tr>
<tr>
<td>VI</td>
<td>Atorvastatin + Alprazolam (before training)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>47.8 ± 3.2</td>
<td>28.6 ± 2.6</td>
</tr>
<tr>
<td>VII</td>
<td>Atorvastatin + Alprazolam (before retrieval)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>44.8 ± 2.9</td>
<td>28.2 ± 2.1</td>
</tr>
<tr>
<td>VIII</td>
<td>Simvastatin + Alprazolam (before training)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>48.4 ± 3.3</td>
<td>38.2 ± 2.2</td>
</tr>
<tr>
<td>IX</td>
<td>Simvastatin + Alprazolam (before retrieval)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>46.4 ± 3.2</td>
<td>29.4 ± 3</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean ± S.E.M.  
(a) Denotes, p<0.05 as compared to first day TL in vehicle control.  
(b) Denotes, p<0.05 as compared to 2nd day TL in control group.  
(c) Denotes, p<0.05 as compared to 2nd day TL in Alprazolam (before training) group.  
(d) Denotes, p<0.05 as compared to 2nd day TL in Alprazolam (before retrieval) group. ANOVA followed by Tukey’s multiple range test.

Table 3: Effect of Atorvastatin and Simvastatin on Scopolamine and High Fat Diet (HFD) Induced Amnesia in Rats Using Elevated Plus-maze

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (kg⁻¹)</th>
<th>TL (training) in s</th>
<th>TL after 24h in s</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (vehicle)</td>
<td>10 ml i.p.</td>
<td>46.8 ± 3.2</td>
<td>26.6 ± 2.8</td>
</tr>
<tr>
<td>X</td>
<td>Scopolamine (before training)</td>
<td>0.4 mg i.p.</td>
<td>48.4 ± 3.1</td>
<td>64.2 ± 3.7</td>
</tr>
<tr>
<td>XI</td>
<td>Scopolamine (before retrieval)</td>
<td>0.4 mg i.p.</td>
<td>47.2 ± 2.4</td>
<td>61.8 ± 3.2</td>
</tr>
<tr>
<td>XII</td>
<td>Atorvastatin + Scopolamine (before training)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>47.8 ± 3.3</td>
<td>33.8 ± 2.1</td>
</tr>
<tr>
<td>XIII</td>
<td>Atorvastatin + Scopolamine (before retrieval)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>46.4 ± 3.2</td>
<td>34.2 ± 2.6</td>
</tr>
<tr>
<td>XIV</td>
<td>Simvastatin + Scopolamine (before training)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>47.2 ± 2.6</td>
<td>35.2 ± 2.2</td>
</tr>
<tr>
<td>XV</td>
<td>Simvastatin + Scopolamine (before retrieval)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>47.4 ± 3.8</td>
<td>35.8 ± 2.6</td>
</tr>
<tr>
<td>XVI</td>
<td>Normal diet (control)</td>
<td>10 ml i.p.</td>
<td>46.8 ± 2.9</td>
<td>26.6 ± 2.1</td>
</tr>
<tr>
<td>XVII</td>
<td>HFD for 90 days</td>
<td>46.8 ± 2.9</td>
<td>63.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>XVIII</td>
<td>HFD + Atorvastatin</td>
<td>HFD + 5 mg p.o.</td>
<td>44.8 ± 3.6</td>
<td>33.2 ± 3</td>
</tr>
<tr>
<td>XIX</td>
<td>HFD + Simvastatin</td>
<td>HFD + 5 mg p.o.</td>
<td>45.8 ± 3.1</td>
<td>34.8 ± 2.5</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean ± S.E.M.  
(a) Denotes p<0.05 as compared to first day TL in vehicle control/normal diet group.  
(b) Denotes p<0.05 as compared to 2nd day TL in control group/normal diet group.  
(c) Denotes p<0.05 as compared to 2nd day TL in Scopolamine (before training) group.  
(d) Denotes p<0.05 as compared to 2nd day TL in Scopolamine (before retrieval) group.  
(e) Denotes p<0.01 as compared to 2nd day TL in HFD treated group.  
(f) Denotes p<0.05 as compared to 2nd day TL in HFD treated group. ANOVA followed by Tukey’s multiple range test.

VIII, XIV administered before elevated plus-maze trial, significantly (p<0.05) attenuated Alprazolam and Scopolamine (administered prior to training on elevated plus-maze) induced increase in TL measured on 2nd d. These observations suggested that Atorvastatin and Simvastatin had reversed Alprazolam and Scopolamine induced anterograde amnesia (Tables 2 and 3).

Furthermore, Atorvastatin (group VII, XIII) and Simvastatin (group IX, XV), when administered after plus-maze trial on first day, significantly (p<0.05) decreased Alprazolam and Scopolamine (injected after elevated plus-maze training on 1st d) induced increase in TL recorded on 2nd d also. These results suggested that Atorvastatin and Simvastatin had reversed Alprazolam and Scopolamine induced retrograde amnesia as well (Table 1 and 2). HFD rats, (rats receiving high fat diet for 90 days successively),
when, treated with Atorvastatin (group XVIII)/Simvastatin (XIX) for 15 days successively produced a significant decrease in 1st d (p<0.05) and 2nd d TL (p<0.01 and p<0.05) when, compared to 1st and 2nd d TL of HFD rats respectively. These observations suggested that Statins had attenuated HFD induced amnesia (Table 3).

Effect of Atorvastatin and Simvastatin on Alprazolam, Scopolamine and HFD Induced Enhancement in Escape Latency Time (ELT) of Rats Using Water Maze

Control group rats (group XX), produced a significant (p<0.05) decrease in their day 4, ELT, when compared to their 1st d ELT. Rats receiving normal diet for 90 days (group XXXV) also produced a significant (p<0.05) decrease in their day 4, ELT as compared to their 1st d, ELT. Alprazolam and Scopolamine, when administered 30 min before training trials conducted on d 1 to d 4, as well as HFD treatment for 90 days (group XXXVI) significantly attenuated (p<0.05 and p<0.01) the decrease in d 4, ELT of respective control groups. These observations suggested that Alprazolam, Scopolamine and HFD had produced impairment of acquisition. Atorvastatin (group XXXII) and Simvastatin (group XXIV) per se group animals did not produce any significant change in ELT pattern of control (vehicle treated) rats. Further, Alprazolam and Scopolamine induced increase in ELT during successive training trials was reversed by pretreatment with Atorvastatin (group XXV and XXXI) and Simvastatin (group XXVI and XXXIII), as reflected by a significant decrease (p<0.05) in ELT of these animals. 15 days treatment with Atorvastatin (group XXXVI) or Simvastatin (XXXVII) produced a significant decrease (p<0.01 and p<0.05) in HFD induced increase in ELT (Table 4).

Effect of Atorvastatin and Simvastatin on Alprazolam/Scopolamine/HFD Induced Alterations in the Time Spent in Target Quadrant (TSTQ) during Retrieval Trials on Water Maze

Rats, receiving normal diet spent significantly (p<0.05) more time in target quadrant as compared to time spent in other quadrants during retrieval trial on day 5. Alprazolam and Scopolamine injected either before training trials (group XXI and XXIX) or before retrieval trial (group XXII and XXX) produced a significant decrease (p<0.05) in the mean time spent in target quadrant in search of missing platform as compared to time spent in target quadrant by control group rats. These observations suggested that Alprazolam as well

### Table 4. Effect of Atorvastatin and Simvastatin on Alprazolam, Scopolamine and High Fat Diet (HFD) Induced Changes in the Escape Latency Time (ELT) of Rats Using Morris Water-maze

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (kg⁻¹)</th>
<th>ELT (d 1) in s</th>
<th>ELT (d 4) in s</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>Control (vehicle)</td>
<td>10 ml i.p.</td>
<td>63.6±3.9</td>
<td>16.8±3.5ᵃ</td>
</tr>
<tr>
<td>X XI</td>
<td>Alprazolam (before training)</td>
<td>0.5 mg i.p.</td>
<td>66.2±4.8</td>
<td>58.6±4.2ᵇ</td>
</tr>
<tr>
<td>XXIII</td>
<td>Atorvastatin</td>
<td>5 mg p.o.</td>
<td>58.6±2.5</td>
<td>14.2±2.7</td>
</tr>
<tr>
<td>XXIV</td>
<td>Simvastatin</td>
<td>5 mg p.o.</td>
<td>61.9±4.2</td>
<td>16.8±3.9</td>
</tr>
<tr>
<td>XXV</td>
<td>Atorvastatin + Alprazolam (before training)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>64.8±4</td>
<td>25.8±3.2ᶜ</td>
</tr>
<tr>
<td>XXVII</td>
<td>Simvastatin + Alprazolam (before training)</td>
<td>5 mg p.o. + 0.5 mg i.p.</td>
<td>60.2±2.1</td>
<td>27.2±1.9ᶜ</td>
</tr>
<tr>
<td>XXIX</td>
<td>Scopolamine (before training)</td>
<td>0.4 mg i.p.</td>
<td>63.8±3.8</td>
<td>55.7±4.4ᶜ</td>
</tr>
<tr>
<td>XXXI</td>
<td>Atorvastatin + Scopolamine (before training)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>58.6±2.8</td>
<td>14.2±2.4ᶜ</td>
</tr>
<tr>
<td>XXXIII</td>
<td>Simvastatin + Scopolamine (before training)</td>
<td>5 mg p.o. + 0.4 mg i.p.</td>
<td>61.9±3.7</td>
<td>16.8±4.3ᶜ</td>
</tr>
<tr>
<td>XXXV</td>
<td>Normal diet (90 days)</td>
<td></td>
<td>55.6±3.2</td>
<td>14.4±3.6ᶜ</td>
</tr>
<tr>
<td>XXXVI</td>
<td>HFD for 90 days</td>
<td></td>
<td>68.6±4.7</td>
<td>62.8±5.5ᶜ</td>
</tr>
<tr>
<td>XXXVII</td>
<td>HFD + Atorvastatin</td>
<td>HFD + 5 mg p.o.</td>
<td>62.4±2.6</td>
<td>16.2±3.1ᶜ</td>
</tr>
<tr>
<td>XXXVIII</td>
<td>HFD + Simvastatin</td>
<td>HFD + 5 mg p.o.</td>
<td>61.8±2.7</td>
<td>18.4±3.1ᶜ</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean±S.E.M.

ᵃ Denotes p<0.05 as compared to 1st day ELT in vehicle control/normal diet control.
ᵇ Denotes p<0.05 as compared to day 4, ELT in vehicle control.
ᶜ Denotes p<0.05 as compared to day 4, ELT in Alprazolam alone group.
ᵈ Denotes p<0.05 as compared to day 4, ELT in Scopolamine alone group.
ᵉ Denotes p<0.01 as compared to day 4, ELT in HFD normal diet group.
ᶠ Denotes p<0.01 as compared to day 4, ELT in HFD rats. ANOVA followed by Tukey’s multiple range test.
as Scopolamine had produced anterograde and retrograde amnesia. Rats treated with Atorvastatin (group XXIII)/Simvastatin (group XXIV) alone did not produce any significant change in the time spent in target quadrant in search of missing platform, when compared to control group rats (Table 5 and 6).

Atorvastatin (group XXV and XXXI) and Simvastatin (groups XXVII and XXXIII) administered before training trial (from day 1 to 4), significantly (p<0.05) attenuated Alprazolam and Scopolamine induced decrease in time spent in target quadrant during retrieval test on day 5. The observation suggested reversal of Alprazolam/Scopolamine induced anterograde amnesia by Atorvastatin and Simvastatin. Furthermore, Atorvastatin (group XXVI and XXXII)/Simvastatin (group XXVIII and XXXIV) administered before retrieval trial (on day 5, on water maze), significantly (p<0.05) attenuated Alprazolam and Scopolamine induced decrease in time spent in target quadrant during retrieval test on day 5. These observations indicated reversal of Alprazolam/Scopolamine induced retrograde amnesia by Atorvastatin as well as Simvastatin (Tables 4 and 5).

HFD rats (group XXXVI) showed a significant decrease in time spent in target quadrant during retrieval trials on day 5, when compared to time spent in target quadrant of normal diet rats (group XXXV). HFD rats, when subjected to retrieval trials

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (g⁻¹)</th>
<th>Time spent in target quadrant (TSTQ) Q4 (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>Control (vehicle)</td>
<td>10 ml i.p.</td>
<td>69.2±4.2</td>
</tr>
<tr>
<td>XIX</td>
<td>Alprazolam (before training)</td>
<td>0.5 mg i.p.</td>
<td>31.8±3.9</td>
</tr>
<tr>
<td>XXII</td>
<td>Alprazolam (before retrieval)</td>
<td>0.5 mg i.p.</td>
<td>35.4±4.1</td>
</tr>
<tr>
<td>XXIII</td>
<td>Atorvastatin</td>
<td>5 mg p.o.</td>
<td>68.6±4.3</td>
</tr>
<tr>
<td>XXIV</td>
<td>Simvastatin</td>
<td>5 mg p.o.</td>
<td>66.8±3.8</td>
</tr>
<tr>
<td>XXV</td>
<td>Atorvastatin+Alprazolam (before training)</td>
<td>5 mg p.o.+0.5 mg i.p.</td>
<td>63.2±4.4</td>
</tr>
<tr>
<td>XXVI</td>
<td>Atorvastatin+Alprazolam (before retrieval)</td>
<td>5 mg p.o.+0.5 mg i.p.</td>
<td>62.6±3.8</td>
</tr>
<tr>
<td>XXVII</td>
<td>Simvastatin+Alprazolam (before training)</td>
<td>5 mg p.o.+0.5 mg i.p.</td>
<td>58.8±4.3</td>
</tr>
<tr>
<td>XXVIII</td>
<td>Simvastatin+Alprazolam (before retrieval)</td>
<td>5 mg p.o.+0.5 mg i.p.</td>
<td>61.2±4.1</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean±S.E.M. a) Denotes p<0.05 as compared to mean TSTQ of vehicle control. b) Denotes p<0.05 as compared to mean TSTQ of Alprazolam (before training) group. c) p<0.05 as compared to mean TSTQ of Alprazolam (before retrieval) group. ANOVA followed by Tukey’s multiple range test.

Table 6. Effect of Atorvastatin and Simvastatin on Scopolamine and HFD Induced Alterations in the Mean Time Spent in the Target Quadrant (TSTQ) Q4 Using Morris Water-maze

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (g⁻¹)</th>
<th>Time spent in target quadrant (TSTQ) Q4 (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>Control (vehicle)</td>
<td>10 ml i.p.</td>
<td>69.2±4.2</td>
</tr>
<tr>
<td>XXIX</td>
<td>Scopolamine (before training)</td>
<td>0.4 mg i.p.</td>
<td>33.6±3.8</td>
</tr>
<tr>
<td>XXX</td>
<td>Scopolamine (before retrieval)</td>
<td>0.4 mg i.p.</td>
<td>41.4±5.1</td>
</tr>
<tr>
<td>XXXX</td>
<td>Atorvastatin+Scopolamine (before training)</td>
<td>5 mg p.o.+0.4 mg i.p.</td>
<td>52±3.6</td>
</tr>
<tr>
<td>XXXXI</td>
<td>Atorvastatin+Scopolamine (before retrieval)</td>
<td>5 mg p.o.+0.4 mg i.p.</td>
<td>50±4.9</td>
</tr>
<tr>
<td>XXXXII</td>
<td>Simvastatin+Scopolamine (before training)</td>
<td>5 mg p.o.+0.4 mg i.p.</td>
<td>60.8±4.7</td>
</tr>
<tr>
<td>XXXXIII</td>
<td>Simvastatin+Scopolamine (before retrieval)</td>
<td>5 mg p.o.+0.4 mg i.p.</td>
<td>64.6±3.7</td>
</tr>
<tr>
<td>XXXV</td>
<td>Normal diet (90 days)</td>
<td>68.2±3.7</td>
<td></td>
</tr>
<tr>
<td>XXXVI</td>
<td>HFD for 90 days</td>
<td>22.4±4.1</td>
<td></td>
</tr>
<tr>
<td>XXXVII</td>
<td>HFD+Atorvastatin</td>
<td>HFD+5 mg p.o.</td>
<td>59.8±4.4</td>
</tr>
<tr>
<td>XXXVIII</td>
<td>HFD+Simvastatin</td>
<td>HFD+5 mg p.o.</td>
<td>38.2±3.6</td>
</tr>
</tbody>
</table>

Each group (n=6), each value represents mean±S.E.M. a) Denotes p<0.05 as compared to mean TSTQ of vehicle control/normal diet. b) Denotes p<0.05 as compared to mean TSTQ of Scopolamine (before training) group. c) Denotes p<0.05 as compared to mean TSTQ of Scopolamine (before retrieval) group. d) Denotes p<0.05 as compared to mean TSTQ of normal diet. e) Denotes p<0.05 as compared to mean TSTQ of HFD group. ANOVA followed by Tukey’s multiple range test.
on water maze after 15 days treatment with Atorvastatin (group XXXVII)/Simvastatin (XXXVIII) produced a significant increase ($p<0.01$ and $p<0.05$) in the time spent in target quadrant (Table 6). This observation indicated attenuation of HFD induced amnesia by Atorvastatin and Simvastatin.

**Effect of Statins on Total Serum Cholesterol**

Rats subjected to high fat diet for 90 days showed a significant ($p<0.05$) increase in their total serum cholesterol levels, when compared to normal diet (control group) rats. Atorvastatin and Simvastatin treatment for 15 days produced a significant ($p<0.05$) fall in total serum cholesterol levels of HFD rats. On the other hand, administration of these statins for 1, 2 or 5 days, failed to produce any significant decrease in total serum cholesterol levels of control (vehicle), Alprazolam and Scopolamine treated rats (Figs. 1 and 2).

**DISCUSSION**

In water maze model, a marked decrease in escape latency time (ELT), during subsequent trials as compared to the first exposure, denotes normal learning ability. Whereas, enhancement in the time spent by the animal in the target quadrant (in search of missing platform) reflects successful retention of learned task (or memory). In the present investigation, control (vehicle treated) group rats showed a significant decrease in escape latency time (ELT) on day 4 as compared to their 1st day ELT. Furthermore, the rats receiving normal diet spent significantly longer time in the target quadrant as compared to time spent in other quadrants on day 5, thereby, indicating normal learning ability and memory. These observations are in line with the results of earlier reports. Similarly, a significant decrease in transfer latency time of rats noted on 2nd d as compared to their TL on 1st day indicated normal memory in elevated plus-maze test. A significant rise in body weight of rats was observed after 90 days of normal diet/HFD administration. Furthermore, rats subjected to HFD for 90 days produced a significant rise in body weight, when compared to those fed for 90 days with normal diet. The swimming ability or driving motivation to the platform was not altered despite of increased body weight of rats on 90th day, since there was no significant variation in the ELT of rats recorded on 91st day (whether receiving normal diet or HFD) as compared to 1st day ELT of control rats. In other words, changes in body weight did not interfere with the swimming ability of rats in any way. It is noteworthy here that the water maze model and elevated plus maze test are based on an entirely different kind of animal behavior. Since, both of these different memory models produced uniform results on memory score, the built in limitation if any present in an individual experiment model is automatically taken care of.

Amnesia is inability to remember past experiences or loss of memory. Anterograde amnesia is impairment of memory for events occurring after the accident/drug treatment. In such a case, new memories are not formed. Whereas, retrograde amnesia is impairment of memory of events, which have occurred before the accident/drug treatment. In such a case, new memories can be formed, but old memories are lost. In the present study, Alprazolam, when ad-
ministered before training showed anterograde amnesia as indicated by significant decrease in more time spent in target quadrant on 5th d, in water maze model. On parallel lines, there was a significant increase in the 2nd d transfer latency with Alprazolam, when administered before training in elevated plus-maze test. Alprazolam, when administered immediately after training on elevated plus-maze on 1st d or before retrieval trial on 5th d in water maze model produced significant increase in 2nd d TL and a significant decrease in mean time spent in target quadrant respectively, reflecting retrograde amnesia. These findings are in conformity with the earlier reports where in, some benzodiazepine (Bz) including Alprazolam have been shown to produce both, anterograde as well as retrograde amnesia.19,20,28,29 The anti-anxiety effect of Alprazolam could be dissociated from its amnesic effect on the basis of total number of entries of the animal into the two (open and enclosed) arms. Alprazolam has been shown to enhance the number of entries into the enclosed/open arm owing to its anti-anxiety effect. In the present study, since the TL time was increased by Alprazolam, it appears that this effect was due to the amnesic action rather than anti-anxiety effect of Alprazolam. The latter possibility is in line with previous studies.28,29 Several reports are there wherein, it has been indicated that benzodiazepine mediate some of their actions by modulating NO/cGMP pathway.30–32 In the present study, pretreatments with Atorvastatin or Simvastatin reversed Alprazolam induced anterograde as well as retrograde amnesia. Though, most of the statins are hydrophilic, Atorvastatin and Simvastatin employed in the present study are lipophilic in nature and have relatively less heparo-selectivity than hydrophilic statins.33,34 Therefore, both Atorvastatin and Simvastatin qualify as good candidates for crossing the blood-brain barrier. It has been shown that Simvastatin reduced cerebral ischemia and protected against stroke by a mechanism unrelated to serum cholesterol levels. These neuroprotective effects of Simvastatin reflect profound brain penetration and appear to be mediated via enhanced NO production.35 Thus, the target site for the central actions of these statins appears to be situated in the close vicinity of the brain. Recent reports revealed that statins evoke several actions independent of their cholesterol lowering properties.5,50 These effects of statins were probably mediated via nitric oxide (NO) or APoE.36,37 Statins have been reported to increase NO production by direct stimulation and up regulation of endothelial NO-synthase i.e., eNOS,38,39 Sim- vastatin (at doses of 0.2, 2 or 20 mg kg−1, s.c.) significantly increased the cerebral blood flow and prevented the occurrence of ischemic stroke in normocholesterolemic mice in the absence of significant changes in serum cholesterol level. This cholesterol independent effect of Simvastatin was completely reversed by L-mevalonate indicating that up-regulation of endothelial nitric-oxide synthase expression by Simvastatin.35 This study underlines the fact that the dose of 5 mg kg−1 of Simvastatin used in the present study was sufficient to elicit its effects on cognitive function through NO system. Further, statins have also been shown to enhance eNOS activity via posttranslational activation of the phosphatidylinositol-3-kinase/protein kinase Akt (PI3K/Akt) pathway.30 Many studies in the recent years have implicated a vital role for NO in neurophysiological process of learning and memory.40,41 Inhibition of NO system impaired memory in rats where as, stimulation of NO production improved cognitive functions in Alzheimer patients.44,45 NO, donors like molsidomine reversed Scopolamine induced amnesia in rats.45 NO probably acts as a retrograde messenger in the formation of long term potentiation (LTP) at the molecular level of learning and memory processes.46 PI3K activates phospholipase lipase C (PLC) which in turn hydrolyses inositol phosphatidylibiphsophate (PIP2) thereby yielding inositol triphosphate (IP3) and diacyl glycerol (DAG).47 DAG so formed activates protein kinase C (PKC) which brings about phosphorylation and activation of NMDA receptors,47 the prominent brain receptor participating in LTP. Activation of NMDA receptors increase influx of extracellular calcium in the neuronal cells, thereby increasing free intracellular calcium, which binds to calmodulin and subsequently stimulates relevant gene expression that eventually leads to enhancement of short term and long term memory.48–50 Therefore, it may be possible that the observed ameliorative effect of statins on Alprazolam induced amnesia may be mediated through enhanced brain level of NO and through activation of NMDA receptors.

Central cholinergic system plays a crucial role in the process of learning and memory. Cholinomimetic drugs have been shown to enhance memory, whereas
centrally acting cholinergic antagonists like Scopolamine are reported to impair memory and therefore have been widely used to study the antiamnestic potential of new drugs.\[51,52\] In the present study, Scopolamine has produced impairment of both, learning ability and retention capacity (memory), which is in agreement with previous reports.\[33,54\] Earlier studies have indicated that activation of muscarinic cholinergic receptors (mAChRs) led to the activation of protein kinase A\[55\] with subsequent activation of downstream protein kinases like MAPK/ERK\[56\] and PKC\[57\] which are involved in acquisition and retrieval of memory.\[58\] In the present study, pretreatment with Atorvastatin and Simvastatin have reversed Scopolamine induced amnesia. Statins are known to activate PI3K/Akt pathway\[59\] which ultimately activate PLC to hydrolyze PIP2 to produce IP3 and DAG.\[43\] DAG so formed activates PKC and IP3 increasing free intracellular Ca\[^{2+}\].\[48\] The increased free intracellular Ca\[^{2+}\] is known to increase the release of acetylcholine from nerve terminals.\[60\] Moreover increased intracellular Ca\[^{2+}\] increases cAMP levels, which subsequently activates PKA.\[61\] Therefore, reversal of Scopolamine induced amnesia by Atorvastatin and Simvastatin may be due to the activation of PI3K/Akt pathway.

In the present study, chronic administration (90 days) of high fat diet (HFD) not only produced significant increase in the total serum cholesterol levels, but also impaired memory. Clinical studies suggested that the net brain cholesterol concentration is regulated by serum cholesterol level and there is a cross talk between the CNS and peripheral cholesterol pools.\[62–65\] Therefore, it is plausible that peripheral cholesterol levels modulate CNS cholesterol levels and vice versa. Cholesterol turnover appears to play a crucial role in the deposition and clearance of amyloid peptide in brain. Furthermore, serum cholesterol, atherosclerosis, apolipoprotein-E and AD all appear to be interconnected.\[66–69\] ApoE is a cholesterol transporting protein that is associated with amyloid deposits.\[69–71\] Elevated serum cholesterol levels not only lead to atherosclerosis but also carry a high risk of developing AD.\[72\] Epidemiological studies revealed that individuals with high peripheral cholesterol levels show more susceptibility to Alzheimer’s disease.\[66–68,73\] and the incidence of AD is higher in countries with high-fat and high-calorie diets.\[74\] It has been reported that rats fed with a special diet having higher amount of fats showed memory deficits.,\[75–77\] In a double-blind study the administration of Simvastatin for six month period to AD patients was found to decrease CNS beta-amyloid levels.\[78\] Studies involving cultured rat cortical neurons have revealed neuroprotective action of Atorvastatin against glutamate induced excitotoxicity.\[79\] Statins exerted cognitive benefits in AD\[80–82\] and were reported to affect CNS cholesterol homeostasis.\[83\] This contention is further confirmed by the present study, wherein, Atorvastatin and Simvastatin significantly prevented HFD induced memory deficits. However, anti-inflammatory and anti-oxidant actions of statins to enhance memory can not totally be ignored at this point and might have contributed to the beneficial effect on memory. Thus Atorvastatin and Simvastatin successfully reversed the memory deficits induced by high fat diet/Alprazolam/Scopolamine injection.

CONCLUSION

Atorvastatin and Simvastatin successfully reversed the memory deficits induced by high fat diet, Scopolamine/Alprazolam injection probably through their cholesterol dependent as well as cholesterol independent effects.

Acknowledgements We are thankful to Dr. R. P. Bajpai, Hon’ble Vice Chancellor of Guru Jambheshwar University of Science and Technology, Hisar and Prof. Manjeet Singh, former Head, Department of Pharmaceutical Sciences and Drug Research Punjab University Patiala for their constant encouragement and keen interest in this project. We are also thankful to Zydis research center, Ahmedabad, Morpen Ltd., Baddi and Kim Labs Pvt Ltd., Ambala Cantt, India for supplying the free samples of Atorvastatin, Simvastatin and Alprazolam respectively.

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