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Effect of Atorvastatin on Memory in Albino Mice

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ABSTRACT

Objective: The aim and objective of the present study was to evaluate the effect of atorvastatin on learning and memory in albino mice.

Materials and Methods: Thirty Swiss albino mice were divided into 5groups (n=6). In group2, group4 and group5 hyperlipidemia was induced by high fat diet (HFD) orally for 28days. Atrorvastatin was given to group3, group4 and group5 orally for 14 d. Learning and memory was evaluated with Hebb Williams's maze, Elevated plus maze, Y maze and Step through latency. Continuous data were analyzed by one way ANOVA followed by Scheffe multiple range test, discrete data were analyzed by Kruskal - Wallis test. The level of significance was 5% ($p \leq 0.05$).

Result and Conclusion: HFD treatment had shown significant increase in body weight, significant impairment in learning and memory ($p < 0.05$). Only atorvastatin treated group had shown better learning and memory in comparison to HFD group. Atorvastatin 10mg/kg and 20 mg/kg had reversed the HFD induced impairment of learning and memory but there was no significant difference between the doses ($p > 0.05$).

Keywords: Atorvastatin, Elevated plus maze, High fat diet, Hebb williams's maze, Learning and memory, Step through latency, Y maze

INTRODUCTION

Memory is one of the complex functioning of brain comprising of perception, registration, consolidation, storage, recollection and decay. Any impairment in memory affects the quality of life. With increase in life expectancy, more cases of memory loss or dementia are being reported. In India there were 3.7 million people with dementia in 2010 while the numbers are expected to be doubled by 2030 [1] and to be over 100 million by 2050 [2]. Dementia is a syndrome of failing memory and other intellectual functions. Alzheimer's disease and increasing age are the most common causes of dementia. Most of these elderly age group people are associated with various co-morbid conditions like diabetes mellitus, hypertension, dyslipidemia, cardiovascular diseases etc.

Hyperlipidemia itself is one of the major risk factor for coronary artery disease (CAD).Various treatment modalities are available for treating hyperlipidemia. Life style modifications like dietary, physical activity modification and drug therapy includes HMG-CoA reductase inhibitors (Statins), bile acid sequestrant resins (cholestyramine, colestipol, colesevelam), fibrates (gemfibrozil, fenofibrate), nicotinic acid and its derivatives. Since the introduction of statins in 1987, it has become the most commonly prescribed agents for the treatment of dyslipidemia. Statins (3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors) are the most effective and widely used medicines to reduce low-density lipoprotein cholesterol and reduce cardiovascular events. Lovastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, rosuvastatin are the statins available in market. Among these most commonly used statin is atorvastatin

Statins are well tolerated and have minimal adverse effects, but several case reports and case series have suggested a potential association between statins and cognitive impairment. In 1998 Australian adverse drug reactions bulletin has quoted statin is one of the drug which causing memory impairment [3]. Recently FDA announced to include memory loss and confusion in labeling of statin [4]. Paradoxically some studies have also shown evidences of decreased risk of stroke, dementia, Alzheimer's disease [5,6] as statins interfere by a complex mechanism which is by antioxidant, anti-inflammatory, anti-thrombotic and improved endothelial function and thus prevents plaque formation and improved blood circulation. This pleiotropic statin effect appears to be independent of cholesterol lowering effects [7]. Controversy still exists to answer the question: "whether statins impair or improve memory?" The supportive literatures are meager and more controversial.

MATERIALS AND METHODS

Experimental animals: Thirty (30) Swiss albino mice (22-28gms body weight) of either sex were randomly selected and grouped into five groups (n=6). They were acclimatized and housed in animal house with 12h: 12h light-dark cycle at 27±2ºc temperature and 45-55% relative humidity. Food and water supplied ad libitum. This study was approved by the Institutional Animal Ethical Committee (IAEC) Lt No 14- 798/03/C/ CPCSEA-2003.

High Fat Diet (HFD): It was prepared by mixing Cholesterol 2%, Cholic acid 1%, Coconut oil 5%, Standard pellet diet powder 92% [8-12]. This mixture was made into small rounds and was baked. Hyperlipidemia was induced by feeding the albino mice with high fat diet (HFD) around 15 grams / animal /day was given instead of normal pellet diet to animals of groups 2, 4 and 5 for 28 days and drug was administered orally for 14 days i.e. from 29th day to 42nd day of the study [Table/Fig-1].

Drug and Dose preparation: Atorvastatin dosage was prepared by suspending atorvastatin in 0.5% carboxymethyl cellulose. It was given orally daily for 14 d. Atorvastatin 10mg/kg to group 3, group 4 and 20mg/kg to group 5. Atorvastatin, vehicle and HFD were administered by oral route.

Elevated Plus Maze

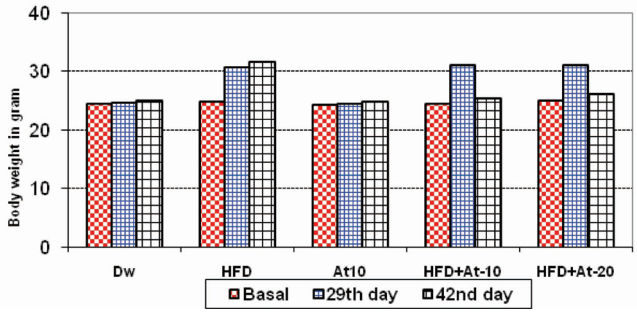
Animals were placed at the end of open arm. The time for each animal to move to the closed arm was taken as transfer latency (TL) and was noted for 90 sec. The 1st day (41st day of the study) the mice was allowed to move freely to explore the apparatus for at least 10 min. TL recorded on 1st day was acquisition (learning) and after 24 h (42nd day of the study) reflects the retention / consolidation (memory) for learning [5,13,14].

Y Maze

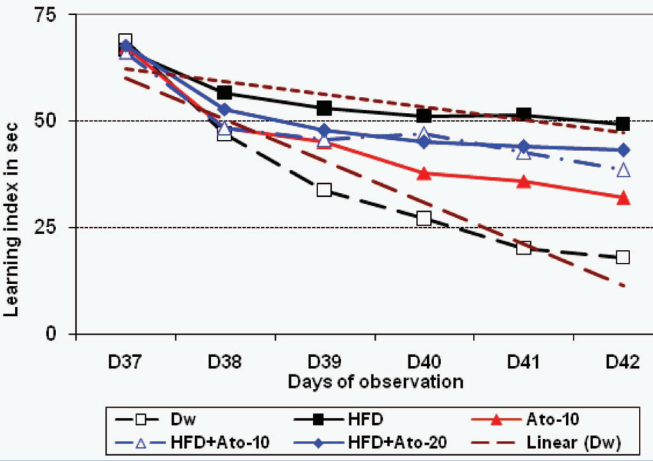
Each mouse was placed at each end and allowed to explore. The mice explored each arm. The ability to alternate was the ability of the mice to know that they already visited the arm. Alteration is

Group. No	Diet	Drug treatment [29-42day(14days)]
1.	Normal diet	Distilled water
2.	HFD diet	Distilled water
3.	Normal diet	Atorvastatin (10mg/kg)
4.	HFD diet	Atorvastatin (10mg/kg)
5.	HFD diet	Atorvastatin(20mg/kg)

[Table/Fig-1]: Study Groups, ** HFD is for 28 days (1-28day)*



[Table/Fig-2]: Effect of drugs on body weight in albino mice, *Dw= Distilled Water; HFD= High Fat Diet; Ato10= Atorvastatin 10mg/kg; HFD+Ato 10= High Fat Diet + Atorvastatin 10mg/kg; HFD+Ato= Atorvastatin*



[Table/Fig-3]: Effect of drugs on learning and memory in Hebb William maze, *Dw= Distilled Water; HFD= High Fat Diet; Ato10= Atorvastatin 10mg/kg;HFD+Ato 10= High Fat Diet + Atorvastatin 10mg/kg; HFD+Ato= Atorvastatin*

defined as the number of successive entries into the three arms. The percentage of alteration was calculated by following formula [15,16].

Percentage of alteration = (X-2)/n

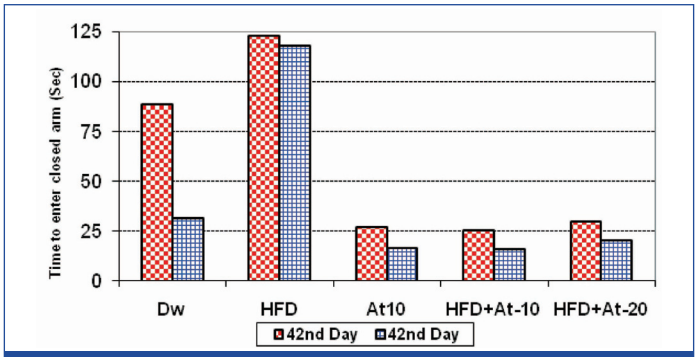
Where X = number of alterations, n= number of arms visited

Hebb-William's Maze

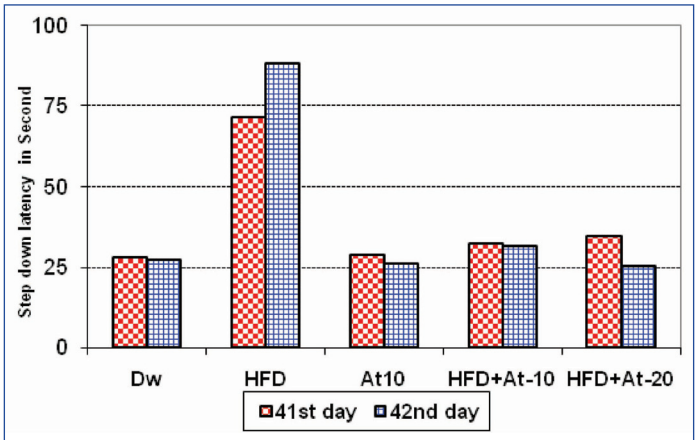
Hebb Williams Maze consists of three compartments. Animal placement chamber (A), leading to the middle exploratory chamber, the maze (B); which leads to reward chamber (C). Food was kept in the reward chamber. After 12 h of fast, the mouse was placed in the chamber A. Once it enters the chamber B, it was closed to prevent back entry into the chamber A. The total time taken by the animal to reach the reward chamber was noted. The animals were trained for 5 d i.e. from 37th day to 42nd day of the study, putting food in reward chamber on last 2 days (41st, 42nd day of the study) [17,18].

Step through Passive Avoidance

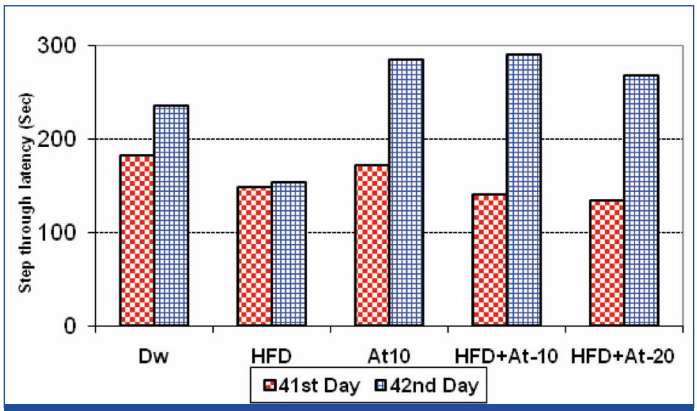
This model was used to observe the acquisition and retention of learned task. The apparatus consists of an outer illuminated box with an inner small dark box at the center. Electric shock through grid floor was applied to the animal entering the dark box. The mouse was placed on the stage (on the top of inner box). The animal gets down, tries to explore the area and enters into the inner box due to its tendency to move towards the dark area. The animal was thus



[Table/Fig-4]: Effect of drugs on learning and memory in Plus maze, *Dw= Distilled Water; HFD= High Fat Diet; At10 = Atorvastatin 10mg/kg;HFD+At10= High Fat Diet + Atorvastatin 10mg/kg; HFD+Ato= Atorvastatin 20mg/kg*



[Table/Fig-5]: Effect of drugs on learning and memory by Step down latency, *Dw= Distilled Water; HFD= High Fat Diet; At10 = Atorvastatin 10mg/kg;HFD+At10= High Fat Diet + Atorvastatin 10mg/kg; HFD+At= Atorvastatin 20mg/kg*



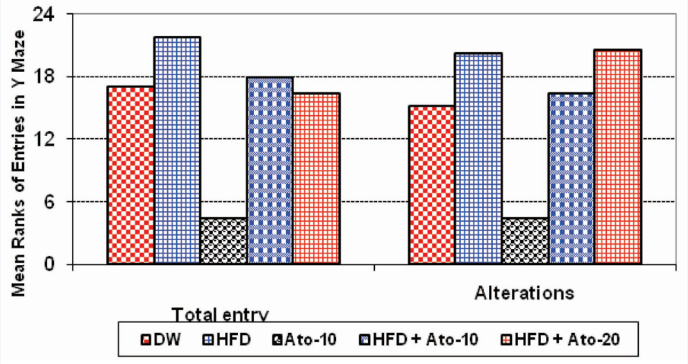
[Table/Fig-6]: Effect of drug on learning and memory by Step through latency, *Dw= Distilled Water; HFD= High Fat Diet; At10 = Atorvastatin 10mg/kg;HFD+At10= High Fat Diet + Atorvastatin 10mg/kg; HFD+At= Atorvastatin 20mg/kg*

allowed to explore on day 1 (41st day of the study) without electric shock. On day 2 (42nd day of the study) after subsequent entry into the inner box, electric shock of 0.2-0.3mA was applied for about 2 sec. The time taken for the mice to get down the box was the step down latency and enter the dark area was the step through latency. After this learning trial, retention of memory was assessed after 24 h. The maximum cut off time for entering into the dark area was taken as 5 min [13,19,20].

RESULTS

Effect of HFD and atorvastatin on body weight

Basal body weights of the mice in all groups were identical ($p > 0.05$). 28 d treatment with HFD significantly ($p < 0.05$) increases the body weight in group 2, group 4 and group 5. Atorvastatin 10mg/kg, 20mg/kg treatment significantly ($p < 0.05$) decrease the body weight in group 4 and group 5.



[Table/Fig-7]: Effect of drug on learning and memory in Y Maze,
Dw= Distilled Water; HFD= High Fat Diet; At10 = Atorvastatin 10mg/kg;HFD+At10= High Fat Diet + Atorvastatin 10mg/kg; HFD+At= Atorvastatin 20mg/kg

Effect of drugs on learning and memory in Hebb - Williams's maze

Only atorvastatin treated group had taken more time (32 ± 14.5 sec) cf the group 1(18 ± 8.6 sec) but difference was not significant ($p > 0.05$). Mice fed with HFD had taken significantly more time (51.5 ± 22.6 sec). It indicates learning and memory was impaired by HFD.

Effect of drugs on learning and memory in elevated plus maze

The experiment on elevated plus maze indicate that the learning was significantly different in different groups ($p < 0.001$). In group 1 the latency had decreased significantly from 88.5 ± 8.7 sec (on 41st day) to 31.5 ± 3 sec (on 42nd day). Where as in group 2 latency remained significantly high (122.83 ± 24.3). In group 3, 4 and 5 the latency decreased significantly (16.66, 15.66, 20.33 sec respectively) ($p < 0.05$). This indicates atorvastatin restrict the HFD induced impairment in learning and memory.

Effect of drugs on learning and memory in step down and step through latency

The observations on the 41st day were taken as an index of learning. Then the mice were exposed to shock and the observations on the 42nd day were taken as an index of memory. In group 2 step down latency had shown high (71.66 ± 13.2sec) cf distilled water (28.16 ± 2.5 sec).

On 42nd day, in group 2 step through latency remains unchanged. Group 4 and group 5 had shown no significant change in step through latency. It indicates atorvastatin restrict the HFD induced impairment in learning and memory.

Effect of drugs on learning and memory in Y- Maze

The Mean rank in both the number of entries and alterations of group 2 was higher than other groups. The group 3 had shown decrease in the mean rank compared to all other groups indicating better memory.

DISCUSSION

This study was conducted to evaluate the effect of atorvastatin on learning and memory in albino mice. Swiss albino mice were used in this study because they are the preferable animal for evaluation of learning and memory. Hyperlipidemia was induced by replacing the normal diet with high fat diet (HFD) consisting of cholesterol, cholic acid and coconut oil mixed in powdered normal pellet diet [8-12].

Effect of HFD and Atorvastatin on body weight

HFD had shown significant increase in body weight in group 2, 4 and 5. Atorvastatin 10mg/kg and 20mg/kg significantly decreased the body weight in group 4 and 5 by 18.61% and 16.18% respectively [Table/Fig-2].

Effect of drugs on learning and memory in Hebb - Williams's maze

Animals fed with HFD had shown significantly more time (51.5sec) to reach the reward chamber cf the group1(18sec) indicates learning and memory was impaired by HFD in group 2, group 4 and group 5. Atorvastatin in doses studied in group 3, 4 and 5 did not show any beneficial effect in Hebbwilliams maze. This indicates that atorvastatin has no effect on learning and memory in this model [Table/Fig-3].

Effect of drugs on learning and memory in elevated plus maze

The time taken by the animal to enter the closed arm from the open arm was recorded as transfer latency period. In group 2 HFD fed mice, had shown significant increase in latency (118.0 sec) compared to DW treated group (31.5sec). In groups 3, 4 and 5 atorvastatin treatment had significantly decrease the latency period to 16.66, 15.66 and 20.33 sec respectively from 31.5sec in distilled water treated group [Table/Fig-4]. This indicates only with HFD fed group had impaired learning and memory, whereas the atorvastatin treatment had significantly prevented HFD induced impairment in learning and memory. This result was observed to be similar to that of Millid et al., [5].

Effect of drugs on learning and memory in step down latency

Mouse placed on the high platform has a tendency to get down and explore. On 41st day i.e. before exposing them to shock, all the groups were identical except group 2 where step down latency period remained significantly high 71.66 sec [Table/Fig-5] indicating HFD impairs the learning. Group 3, 4, 5 values reveal that atorvastatin as such has no effect but normalized the memory deficit in HFD treated animal. The observations on 42nd day were almost similar.

Effect of drugs on learning and memory in step through latency

On the 41st day, the step through latency which was taken as the index of memory remained identical as the mice tends to enter the dark area and then shock was given. On the 42nd day, in group 2 there was no difference in latency (153 seconds) indicating impaired memory. In groups 3, 4 and 5 step through latency remained high (285, 290, 286 seconds respectively) indicating that the animal retained the memory of shock and avoided to step through, to the shock zone [Table/Fig-6].

Effect of drugs on learning and memory in Y- Maze

Aorvastatin treatment in group 3 significantly decreases both the number of entries and alterations. The total number of entries indicates the exploration behavior and the alterations indicate higher performance of retention of memory. If more is the alteration, less is the memory or discrimination power of the animal to remember which arm it has already explored. Group 3 animals had shown to decrease the alteration when compared to all other groups indicating better memory [Table/Fig-7]. It is very difficult to substantiate from this single observation that only atorvastatin increased the learning and memory in HFD induced learning and memory impairment.

[Table/Fig-3,5,6] clearly depict HFD caused deficit in learning and memory. These observations are similar to other literature that has demonstrated impaired memory with HFD [5]. This could be because the increased cholesterol could result in excess formation of β- amyloid and lowering the cholesterol levels have shown to inhibit its formation [21,22]. The groups 4, 5 which were given HFD and atorvastatin have shown significant improvement in learning and memory when compared to group 2. This indicates atorvastatin may have some role in improving memory.Some studies have shown similar observation [5,23-26]. Most probably this could be

because statins help in decreasing the cholesterol levels. Studies have suggested that net brain cholesterol concentration is regulated by serum cholesterol and has link between both the pools [5]. Apart from lowering cholesterol levels, statins have also shown antioxidant, anti-inflammatory, anti-thrombotic and improved endothelial function and thus prevents plaque formation and improved blood circulation [27]. Some studies and case reports contradict these findings and suggest deterioration in memory [28-31]. This could be probably due to reduction of neurite loss and death of neurons as shown in some studies [32,33]. Statins produce a dose dependent reduction in coenzyme Q10 concentrations which also plays a role in memory. Decrease in circulating levels of CoQ10 may affect the synthesis of polyunsaturated fatty acids that are integral to neuronal membranes [34]. All these lead to impaired neuropeptide synthesis which in turn causes impaired myelin formation and thus impaired memory. Exact role of statins in learning and memory has not been documented but various hypotheses are suggested.

LIMITATIONS

Appropriate co-relation with cholesterol and memory couldn't be exactly made as body weight is taken instead of cholesterol levels. Lipid profile couldn't be measured because of small size of animals and difficulty in blood collection.

CONCLUSION

In this study HFD had demonstrated significant impairment in learning and memory. Atorvastatin 10mg/kg and 20 mg/kg had reversed the HFD induced impairment of learning and memory in all the models except Hebb William's maze.

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