



## AMELIORATIVE ROLE OF HYPOCHOLESTEROLEMIC DRUGS IN HIGH FAT DIET INDUCED AMNESIA IN RATS

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### ABSTRACT

Investigating the effects of various classes of hypocholesterolemic drugs on memory deficits associated with Alzheimer's type dementia in rats can give important clues in development of drugs in this therapeutic area. In this work learning and memory potential of dementia induced animals was assessed using exteroceptive and interoceptive models, brain acetylcholinesterase (AChE) activity; lipid profile and histopathological studies. High fat diet (HFD) produced a significant impairment of learning and memory indicated by increased transfer latency, decreased locomotor activity, higher levels of AChE activity and lipid profile. The tested hypocholesterolemic drugs significantly attenuated HFD-induced memory deficits and biochemical changes. Activity of simvastatin and rosuvastatin was found to be better than that of fenofibrate and nicotinic acid. Histopathology study further corroborated the results. This study demonstrates the potential of hypocholesterolemic drugs in memory dysfunctions associated with dementia and provides evidence of their cholesterol-dependent actions.

**Key words:** Simvastatin, Rosuvastatin, Fenofibrate, Nicotinic acid, High fat diet, Dementia.

### INTRODUCTION

Recently, the type of dementia associated with Alzheimer's disease (AD) has gained much concern. The key pathological features in the AD brain are deposition of insoluble  $\beta$ -amyloid peptides ( $\beta$ A), formation of neurofibrillary tangles and neuroinflammation that ultimately leads to neuronal cell death (Bales KR *et al.*, 1997). Brain cholesterol is an essential component of neuronal cell membranes and is involved in several biological functions, such as membrane trafficking, signal transduction, myelin formation and synaptogenesis (Valenza M & Cattaneo E, 2006). Given these widespread activities, it is not surprising that dysfunctions in cholesterol synthesis, storage, transport and removal can lead to human brain diseases. In AD, there is a link between cholesterol metabolism and formation and

deposition of  $\beta$ A (Korade Z & Kenworthy AK, 2008).

Dementia places an enormous burden on individuals, families and society. Consequently, a tremendous effort is being devoted to development of drugs that prevent or delay neurodegeneration. Clinical options to contain dementia are limited, and, so far, acetylcholinesterase (AChE) inhibitors, such as donepezil, rivastigmine and galantamine, are considered the gold standard therapy for AD (Yoshida M, 2003).

Very recently, focus has been directed towards 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors, which are better known as statins. Statins not only lower cholesterol level but also possess actions that are independent of their cholesterol lowering property. For example, statins have been demonstrated to exert potential anti-inflammatory, antioxidant and neuroprotective actions (Suribhatla S *et al.*, 2005). Although few studies have reported negative effects of statins on cognitive functions (Bayten SH *et al.*, 2006), a

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number of recent experimental and clinical reports have documented positive effects of statins in memory dysfunctions associated with dementias (Miida T *et al.*, 2007). In previous studies, ameliorative effects of simvastatin, atorvastatin and pitavastatin in experimental amnesia was reported (Sharma B *et al.*, 2008). However, differential effects of various hypocholesterolemic drugs on modulation of experimental amnesia remain to be elucidated. Therefore, the present study was designed to investigate the differential effects of various classes of hypocholesterolemic drugs (simvastatin, rosuvastatin, fenofibrate, and nicotinic acid) on high fat diet induced memory deficits in rats.

## MATERIALS AND METHODS

**Animals:** Male wistar rats aged about 6 weeks were employed in the present study. The animals were exposed to alternate light and dark cycle of 12 h and had free access to food and water. The animals were acclimatized to the laboratory conditions for at least seven 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:** Simvastatin (Artemis Biotech, Hyderabad), Rosuvastatin (MSN Laboratories Ltd., Patanceru), Fenofibrate (Alembic Limited, Vadodara), Nicotinic acid (Dr. Reddy's, Hyderabad) and Donepezil (Dr. Reddy's, Hyderabad) were procured. Simvastatin, Rosuvastatin, Fenofibrate, Nicotinic acid and Donepezil were suspended in 1% w/v sodium carboxy methyl cellulose (NaCMC) for oral administration.

**Composition and preparation of high fat diet:** High fat diet contains cholesterol (2%), cholic acid (1%), dalda (20%) and coconut oil (6%) as the major constituents. Rat feed was finely powdered and mixed properly with weighed ingredients. The mixture was spread on to a tray and baked in oven at 100°C for 1 hour. It was then cut into small pieces and provided to animals.

**Induction of hyperlipidemia:** Animals were fed with prepared high fat diet 100 gm/kg of body weight for 12 weeks. Initial lipid profile of animals was compared with lipid profile measured after 12 weeks of induction of hyperlipidemia. Animals that showed elevated lipid profile were selected for the study.

## EXPERIMENTAL DESIGN

The animals were divided into 11 groups. Each group comprised of 6 animals.

Group	Treatment	Dose (mg/kg)
1	Control (only vehicle)	-
2	Only High Fat Diet	-
3	Donepezil	5
4	Simvastatin	5
5	Rosuvastatin	5
6	Fenofibrate	65
7	Nicotinic acid	85
8	Donepezil + Simvastatin	5+5
9	Donepezil + Rosuvastatin	5+5
10	Donepezil + Fenofibrate	5+65
11	Donepezil + Nicotinic acid	5+85

## LABORATORY MODELS:

**Exteroceptive Behavioral Models:** a) Elevated plus-maze, b) Rectangular maze and c) Actophotometer

**Elevated Plus-maze (EPM):** The EPM serves as an exteroceptive behavioural model to evaluate learning and memory in rats (Itoh J *et al.*, 1991; Reddy DS & Kulkarni SK, 1998). Transfer latency (TL) is a parameter of memory which was defined as the time in seconds taken by the animal to move into one of the closed arms with all its four legs (Dhingra D *et al.*, 2003; Parle M & Singh N, 2007). The training trials were carried out for three days before initiation of behavioural study and treatment with test drugs, the average was taken as basal score. During behavioural study the animal was placed at the end of an open arm facing away from the central platform, the time taken to place all the four paws in the closed arm has noted as TL. A maximum of 120 seconds was given to the animal to explore closed arm, the animal which failed to explore the closed arm within 120 seconds was given a score of 120. The animal was allowed to explore the maze for 15s and then returned to its home cage. Behavioural study was carried for 14 days, where the animals were administered with vehicle or test drugs (doses in mg/kg, p.o.). TL was recorded for all animals one hour after the administration of test compounds, animals were allowed to explore the maze.

**Rectangular maze:** The maze consists of completely enclosed rectangular box with an entry (A) and reward chamber (B) appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just twisting corridor (C) leading from the entry (A) to the reward chamber (B) (Prakash A *et al.*, 2007). The learning assessment for control and treated rats was conducted at end of treatment. On the first day, all the rats were familiarized with the rectangular maze for a period of ten minutes followed by the training trials were carried out for three days before initiation of behavioural study. In each trial animal was placed in the entry chamber and the timer was activated as soon as the rat leave the chamber (Agarwal A *et al.*, 2002), time taken by the rat to

reach the reward chamber (transfer latency (TL)) was taken as the learning score of the trial. The average of three trials was taken as the learning score. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. During learning assessment the animals were exposed to food and water ad libitum only for 1 hour after the maze exposure for the day was completed to ensure motivation towards reward area (B) (Saxena Vasundhara *et al.*, 2013; Dhingra D *et al.*, 2004).

During behavioural study the animal was placed at an entry chamber (A), the time taken by the animal to reach the reward chamber (B) (TL) was recorded. A maximum of 10 min was given for the animal to explore the reward chamber (B), the animal who failed to explore the B within 10 min was given a score of 600 and the animals were returned to its home cage. Behavioural study was carried for 14 days, where the animals were administered with vehicle or test drugs (doses in mg/kg, p.o.). TL was recorded for all animals one hour after the administration of test compounds, animals were allowed to explore the maze.

**Actophotometer:** This method aims to evaluate the locomotor activity of the control and treated animals. The locomotor activity will be measured using actophotometer (Joshi H & Parle M, 2006). Each animal will be placed individually in the actophotometer for 3 min and the basal activity score will be obtained. Subsequently, the animals were divided into 11 groups, each group consisting of six animals. The training trials were carried out for three days before initiation of behavioural study, the average was taken as basal activity score. It was followed by behavioural study which was conducted for 14 days and recording of activity score was noted as described earlier (Pragati Khare *et al.*, 2014). The animals were administered with vehicle or test drugs (doses in mg/kg, p.o.) on daily basis for 14 days. Basal activity score was recorded for all animals one hour after the administration of test compounds, animals were allowed to explore the maze.

**Estimation of cholinesterase enzyme:** The cholinergic marker, acetylcholinesterase was estimated in the whole brain. Briefly, the brains of the rats were removed over ice and the brains were separated using fine forceps. The tissue was then homogenized in 0.03 M sodium phosphate buffer, pH 7.4. 25  $\mu$ l of this homogenate was incubated for 5 min with 75  $\mu$ l of TrisHCl and 75  $\mu$ l of DTNB (Kirti Kulkarni S *et al.*, 2010; Ellman GL *et al.*, 1961). Then, 0.1ml of freshly prepared acetyl thiocholine iodide, pH 8 was added and the absorbance was read at 412 nm.

**Estimation of serum biochemical parameters:** Total cholesterol was estimated by CHOD-PAP method,

triglycerides by GPO method and HDL, LDL & VLDL were estimated by phosphotungstic acid precipitation method.

**Brain histopathology:** After the treatment and behavioral studies, two animals in each group were sacrificed by excessive ether anesthesia and the brains were isolated and were kept in 10% formaldehyde solution. The brain was stained with cresylviolet, cerebellum and basal ganglia were studied under light microscope (Pramodinee Kulkarni D *et al.*, 2011).

**Statistical Analysis:** All the results were expressed as mean  $\pm$  SEM. The data were analyzed using two way analysis of variance (ANOVA) followed by Dunnett's test. Significance was observed at \* $P < 0.05$ , \*\* $P < 0.01$  & \*\*\* $P < 0.001$ .

## RESULTS

### Effect of hypocholesterolemic drugs on Body weight:

There was a significant ( $P < 0.001$ ) increase in the body weight of animals over the period of 12 weeks in rats receiving high fat diet, when compared to the body weights of rats on day 1. The rats in all treatment groups except donepezil-treated group showed marked reduction in body weight. There was substantial decrease in body weight of the animals treated with simvastatin (Group 4), rosuvastatin (Group 5), fenofibrate (Group 6) and nicotinic acid (Group 7). The reduction in body weight obtained with rosuvastatin was found to be significant ( $P < 0.05$ ) than that of simvastatin, fenofibrate and nicotinic acid. The reduction in TL observed with the combination of above compounds with donepezil was 12% better than that of compounds alone. The reduction in body weight obtained with simvastatin (Group 8) and fenofibrate (Group 10) along with donepezil was found to be significant ( $P < 0.01$ ) than that of rosuvastatin (Group 9) and nicotinic acid (Group 11) with donepezil.

### Effect of hypocholesterolemic drugs on high fat diet induced amnesia in rats using Elevated plus-maze:

HFD rats (rats receiving high fat diet for 12 weeks successively), when, treated with simvastatin (Group 4), rosuvastatin (Group 5) and fenofibrate (Group 6) for 14 days successively produced a significant ( $P < 0.001$ ) decrease in TL when compared to TL of HFD rats respectively. The attenuating effect of simvastatin was relatively better than that of rosuvastatin and fenofibrate. The reduction in TL observed with the combination of above compounds with donepezil was 8% better than that of compounds alone. The administration of simvastatin (Group 8), rosuvastatin (Group 9) and nicotinic acid (Group 11) along with donepezil showed significant ( $P < 0.001$ ) reduction in TL when compared to that of fenofibrate (Group 10) with donepezil. These

observations suggested that hypocholesterolemic drugs had attenuated HFD induced amnesia.

**Effect of hypocholesterolemic drugs on high fat diet induced amnesia in rats using Rectangular maze:** The learning scores (Transfer latency) obtained by each group were suggestive of the fact that rats took lesser time on day 14 compared to high fat diet group. The transfer latency score obtained by Group 1 (Positive control) was higher than those afforded by Groups 4-7 indicating better and efficient learning in them, as compared to the positive control. Group 2 (Negative control group) showed an increase ( $P<0.001$ ) in transfer latency score due to the memory deficit induced by high fat diet. The transfer latency scores observed for simvastatin (Group 4), rosuvastatin (Group 5) and fenofibrate (Group 6) afforded a significant ( $P<0.001$ ) memory compared to high fat diet group. The reduction in TL observed with the combination of above compounds with donepezil was 36% better than that of compounds alone. Administration of simvastatin (Group 8) and nicotinic acid (Group 11) along with donepezil afforded better ( $P<0.01$ ) learning scores than that of fenofibrate (Group 10) and rosuvastatin (Group 9) with donepezil.

**Effect of hypocholesterolemic drugs on high fat diet induced amnesia in rats using actophotometer:** Rats subjected to high fat diet (Group 2) showed a prominent reduction in basal activity score when compared to normal (Group 1) rats. Hypocholesterolemic drugs, when administered after high fat diet, simvastatin (Group 4), rosuvastatin (Group 5), fenofibrate (Group 6) and nicotinic acid (Group 7) significantly ( $P<0.001$ ) augmented basal activity score compared to high fat diet. The enhancement in locomotor activity produced by simvastatin, rosuvastatin, and fenofibrate was far better than that of nicotinic acid and comparable to that of standard donepezil (Group 3). The enhancement of basal activity score when simvastatin (Group 8), rosuvastatin (Group 9), fenofibrate (Group 10) and nicotinic acid (Group 11) were administered along with donepezil was 16% better than that of drugs when administered alone.

**Effect of hypocholesterolemic drugs on acetylcholinesterase activity:** High fat diet treated animals (Group 2) showed elevated levels of acetylcholinesterase indicating its memory reducing potential. The animals of positive control group treated with standard drug donepezil (Group 3) produced significant ( $P<0.01$ ) reduction of acetylcholinesterase enzyme activity in comparison with normal control (Group 1). In the treatment group, the animals exposed to high fat diet and treated with simvastatin (Group 4) significantly ( $P<0.05$ ) decreased the acetylcholinesterase activity in comparison with negative control. The activity

of this compound was found to be better than that of rosuvastatin (Group 5), fenofibrate (Group 6) and nicotinic acid (Group 7). The reduction of acetylcholinesterase activity when simvastatin (Group 8), rosuvastatin (Group 9), fenofibrate (Group 10) and nicotinic acid (Group 11) were administered along with donepezil was 10% better than that of above drugs when administered alone. There was significant ( $P<0.01$ ) reduction in acetylcholinesterase activity when simvastatin (Group 8) were administered along with donepezil and it was better than that of rosuvastatin (Group 9), fenofibrate (Group 10) and nicotinic acid (Group 11) with donepezil.

**Effect of hypocholesterolemic drugs on lipid profile:** Rats subjected to high fat diet for 12 weeks (Group 2) showed significant ( $P<0.001$ ) increase in their total cholesterol, triglycerides, LDL, VLDL and decreased HDL, when compared to normal diet (Group 1) rats. Treatment with donepezil (Group 3) showed significant ( $P<0.001$ ) alteration in lipid profile compared to high fat diet rats (Group 2). Simvastatin (Group 4), rosuvastatin (Group 5), fenofibrate (Group 6) and nicotinic acid (Group 7) treatment produced a significant ( $P<0.001$ ) change in lipid profile. Similarly, administration of simvastatin (Group 8), rosuvastatin (Group 9), fenofibrate (Group 10) and nicotinic acid (Group 11) along with donepezil produced significant ( $P<0.001$ ) decrease in lipid profile. The change in serum lipid profile with hypocholesterolemic drugs administered along with donepezil was 36% better than that of above drugs administered alone.

**Brain Histopathology:** Microscopic examination of brain sections of negative control (Group-1) showed normal cerebellum. The molecular layer and purkinjiec cells were unremarkable. The basal ganglion showed normal morphology. There was no neuronal edema or degeneration or gliosis. Micrographs of brain section of amnesia-induced group showed severe neuronal edema with marked gliosis and neuronal degeneration (Group-2).

Micrograph of brain section of amnesia-induced rats treated with donepezil (Group-3) showed very mild histopathological alteration in the basal ganglion. Micrograph of brain sections of amnesia-induced rats treated with simvastatin (Group-4) and rosuvastatin (Group-5) showed mild gliosis of the basal ganglia. While amnesia-induced rats treated with fenofibrate (Group-6) and nicotinic acid (Group-7) showed moderate edema, gliosis and degeneration in basal ganglion.

Micrograph of brain sections of amnesia-induced rats treated with simvastatin (Group 8) and rosuvastatin (Group 9) along with donepezil showed mild gliosis otherwise normal in basal ganglia. Amnesia-induced rats treated with fenofibrate (Group 10) and nicotinic acid

(Group 11) moderate neuronal edema and gliosis in basal ganglia.

**Table 1. Effect of hypocholesterolemic drugs on Body Weights**

Group No.	Treatment & Dose	Initial (gms.)	Week-12 (gms.)	Week-14 (gms.)
1	Control (1% Na CMC p.o)	215.38± 9.33	ND	201.02± 12.69
2	Only High Fat Diet (HFD)	126.00±7.98	285.08±19.25 <sup>***</sup>	278.32±19.62
3	Donepezil 5 mg/kg	154.17±7.96	279.83±9.14 <sup>***</sup>	266.00±6.745
4	Simvastatin 5 mg/kg	116.67±10.98	280.48±11.41 <sup>***</sup>	276.00±11.52
5	Rosuvastatin 5 mg/kg	108.5±7.42	281.77±21.87 <sup>***</sup>	303.05±17.04 <sup>A</sup>
6	Fenofibrate 65 mg/kg	125.5±6.54	279.17±11.82 <sup>***</sup>	281.50±7.42
7	Nicotinic Acid 85 mg/kg	105.83±3.94	264.3±19.90 <sup>***</sup>	272.52±15.07
8	Donepezil 5 mg/kg + Simvastatin 5 mg/kg	95.5±5.20	228.68±6.66 <sup>***</sup>	239.33±14.21 <sup>B</sup>
9	Donepezil 5 mg/kg + Rosuvastatin 5 mg/kg	121.67±10.29	287.8±15.37 <sup>***</sup>	278.00±14.48
10	Donepezil 5 mg/kg + Fenofibrate 65 mg/kg	137.00±5.88	206.18±17.41 <sup>***</sup>	206.83±21.44 <sup>BB</sup>
11	Donepezil 5 mg/kg + Nicotinic Acid 85 mg/kg	127.00±5.53	264.05±10.71 <sup>***</sup>	270.67±11.63

Each group (n=6), each value represents Mean±SEM.

a) Denotes \* $P<0.05$ , \*\* $P<0.01$ , and \*\*\* $P<0.001$  compared to initial body weight.

b) Denotes <sup>A</sup> $p<0.05$  compared with HFD-group and <sup>B</sup> $p<0.05$  and <sup>BB</sup> $P<0.01$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by dunnett's test. NA - Not determined.

**Table 2. Effect of hypocholesterolemic drugs on transfer latency using Elevated plus-maze**

Group No.	Treatment & Dose	Initial (Sec.)	Day-1 (Sec.)	Day-7 (Sec.)	Day-14 (Sec.)
1	Control (1% Na CMC p.o)	39.89± 7.76	23.5± 6.48	9.17± 2.33	15.33± 3.78
2	Only High Fat Diet (HFD)	35.28±6.03	46.33±5.1	66.17±5.28	105.00±2.77
3	Donepezil 5 mg/kg	46.06±8.33	35.50±4.13	55.00±6.63	43.00±5.44
4	Simvastatin 5 mg/kg	22.17±2.11	44.50±5.37	82.00±2.58 <sup>AAA</sup>	70.67±2.09 <sup>+++</sup> , <sup>BBB</sup>
5	Rosuvastatin 5 mg/kg	16.72±2.29	34.33±4.37	71.17±6.52	72.33±3.16 <sup>+++</sup> , <sup>BBB</sup>
6	Fenofibrate 65 mg/kg	21.72±5.62	69.67±7.86	80.83±5.69 <sup>AAA</sup>	84.5±4.59 <sup>+</sup> , <sup>BBB</sup>
7	Nicotinic Acid 85 mg/kg	37.22±5.26	54.50±7.22	79.17±5.67 <sup>AA</sup>	104.83±3.40 <sup>BBB</sup>
8	Donepezil 5 mg/kg + Simvastatin 5 mg/kg	23.61±2.42	26.00±3.96	39.50±4.28 <sup>***</sup>	33.00±3.67 <sup>+++</sup>
9	Donepezil 5 mg/kg + Rosuvastatin 5 mg/kg	32.67±5.66	39.83±3.08	78.83±3.12 <sup>AA</sup>	68.83±2.6 <sup>+++</sup> , <sup>BBB</sup>
10	Donepezil 5 mg/kg + Fenofibrate 65 mg/kg	32.67±7.75	59.17±5.02	84.33±6.94 <sup>+</sup> , <sup>AAA</sup>	97.00±4.59 <sup>00</sup> , <sup>BBB</sup>
11	Donepezil 5 mg/kg + Nicotinic Acid 85 mg/kg	28.72±2.51	47.67±6.21	77.67±3.57 <sup>AA</sup>	74.50±7.50 <sup>+++</sup> , <sup>BBB</sup>

Each group (n=6), each value represents Mean±SEM.

a) Denotes \* $P<0.05$ , \*\* $P<0.01$ , and \*\*\* $P<0.001$  (Day-7) & <sup>A</sup> $P<0.05$ , <sup>+</sup> $P<0.01$ , and <sup>+++</sup> $P<0.001$  compared to initial total latency (Day-14).

b) Denotes <sup>A</sup> $p<0.05$  compared with HFD-group and <sup>B</sup> $p<0.05$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by dunnett's test.

**Table 3. Effect of hypocholesterolemic drugs on transfer latency using Rectangular maze**

Group No.	Treatment & Dose	Initial (Sec.)	Day-1 (Sec.)	Day-7 (Sec.)	Day-14 (Sec.)
1	Control (1% Na CMC p.o)	53.56±22.79	117.00±52.39	145.67±21.84	63.33±11.95
2	Only High Fat Diet (HFD)	66.94±24.92	250.17±17.55	449.17±20.91	546.67±15.04
3	Donepezil 5 mg/kg	69.11±10.07	127.67±16.73	204.33±14.57 <sup>***</sup>	176.17±13.93 <sup>+++</sup>
4	Simvastatin 5 mg/kg	60.11±8.53	210.00±13.80	253.67±16.35 <sup>***</sup>	257.17±16.66 <sup>+++</sup> , <sup>BB</sup>
5	Rosuvastatin 5 mg/kg	63.39±16.98	316.67±21.74	288.67±15.26 <sup>***</sup> , <sup>AA</sup>	255.33±13.29 <sup>+++</sup> , <sup>BB</sup>
6	Fenofibrate 65 mg/kg	36.11±6.82	252.83±8.08	373.50±15.68 <sup>**</sup> , <sup>AAA</sup>	444.83±25.32 <sup>+++</sup> , <sup>BBB</sup>
7	Nicotinic Acid 85 mg/kg	44.56±6.41	280.00±7.94	405.67±21.04 <sup>AAA</sup>	487.83±15.5 <sup>BBB</sup>
8	Donepezil 5 mg/kg+Simvastatin 5 mg/kg	35.17±10.23	106.17±15.01	108.67±17.45 <sup>***</sup> , <sup>AAA</sup>	111.33±15.12 <sup>+++</sup> , <sup>B</sup>
9	Donepezil 5 mg/kg+Rosuvastatin 5mg/kg	75.39±12.45	173.67±14.36	154.00±14.44 <sup>***</sup>	160.00±10.66 <sup>+++</sup>
10	Donepezil 5 mg/kg+Fenofibrate 65 mg/kg	48.33±10.48	252.33±13.55	237.83±18.24 <sup>***</sup>	213.67±10.92 <sup>+++</sup>
11	Donepezil 5 mg/kg+Nicotinic Acid 85mg/kg	85.50±21.88	295.33±34.52	311.17±26.55 <sup>***</sup> , <sup>AAA</sup>	436.5±22.22 <sup>+++</sup> , <sup>BBB</sup>

Each group (n=6), each value represents Mean±SEM.

a) Denotes \* $P<0.05$ , \*\* $P<0.01$ , and \*\*\* $P<0.001$  (Day-7) & <sup>A</sup> $P<0.05$ , <sup>+</sup> $P<0.01$ , and <sup>+++</sup> $P<0.001$  compared to initial total latency (Day-14).

b) Denotes <sup>A</sup> $p<0.05$  compared with HFD-group and <sup>B</sup> $p<0.05$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by dunnett's test.

**Table 4. Effect of hypocholesterolemic drugs on locomotor activity using Actophotometer**

Group No.	Treatment & Dose	Initial (Nos.)	Day-1 (Nos.)	Day-7 (Nos.)	Day-14 (Nos.)
1	Control (1% Na CMC p.o)	303.22±24.40	287.33±38.24	342.00±20.00	377.17±9.46
2	Only High Fat Diet (HFD)	295.67±31.29	230.00±10.28	238.00±19.49	76.00±7.20
3	Donepezil 5 mg/kg	325.67±28.03	298.00±15.07	223.00±20.07***	429.00±27.71 <sup>+++</sup>
4	Simvastatin 5 mg/kg	286.67±14.52	284.00±24.91	270.00±26.62***	328.00±22.89 <sup>+++</sup>
5	Rosuvastatin 5 mg/kg	421.00±28.69	380.00±34.87	380.00±25.09***	320.00±27.23 <sup>+++</sup> , B
6	Fenofibrate 65 mg/kg	330.67±12.24	260.00±18.34	261.00±13.28**	320.00±28.24 <sup>+++</sup> , B
7	Nicotinic Acid 85 mg/kg	314.67±9.96	242.00±6.44	232.00±9.93	218.00±7.51 <sup>+++</sup> , BBB
8	Donepezil 5 mg/kg + Simvastatin 5 mg/kg	357.33±18.92	298.00±19.55	218.00±41.58 <sup>***</sup> , A	310.00±28.80 <sup>+++</sup>
9	Donepezil 5 mg/kg + Rosuvastatin 5 mg/kg	400.00±54.91	300.00±3.73	310.00±17.39***	323.00±31.74 <sup>+++</sup>
10	Donepezil 5 mg/kg + Fenofibrate 65 mg/kg	368.67±24.61	321.00±16.35	225.00±32.9***	287.00±22.07 <sup>+++</sup>
11	Donepezil 5 mg/kg + Nicotinic Acid 85 mg/kg	350.33±11.72	291.00±14.31	320.00±22.63***	487.00±26.03 <sup>+++</sup>

Each group (n=6), each value represents Mean±SEM.

a) Denotes \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  (Day-7) & <sup>+</sup> $P < 0.05$ , <sup>++</sup> $P < 0.01$ , and <sup>+++</sup> $P < 0.001$  compared to initial basal activity (Day-14).

b) Denotes <sup>A</sup> $p < 0.05$  compared with HFD-group and <sup>B</sup> $p < 0.05$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by dunnett's test.

**Table 5. Effect of hypocholesterolemic drugs on Brain Acetylcholinesterase levels**

Group No.	Treatment & Dose	AChE (moles/min/gm)
1	Control (1% Na CMC)	0.028±0.00065
2	Only High Fat Diet (HFD)	0.032±0.0004
3	Donepezil 5 mg/kg	0.014±0.0009 <sup>**</sup> , AAA
4	Simvastatin 5 mg/kg	0.019±0.0003
5	Rosuvastatin 5 mg/kg	0.021±0.0004
6	Fenofibrate 65 mg/kg	0.025±0.0017
7	Nicotinic Acid 85 mg/kg	0.022±0.0005
8	Donepezil 5 mg/kg + Simvastatin 5 mg/kg	0.017±0.0004 <sup>*</sup> , AA
9	Donepezil 5 mg/kg + Rosuvastatin 5 mg/kg	0.019±0.0005
10	Donepezil 5 mg/kg + Fenofibrate 65 mg/kg	0.022±0.0008
11	Donepezil 5 mg/kg + Nicotinic Acid 85 mg/kg	0.020±0.0008

Each group (n=2), each value represents Mean±SEM.

a) Denotes \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  (Day-7) & <sup>+</sup> $P < 0.05$ , <sup>++</sup> $P < 0.01$ , and <sup>+++</sup> $P < 0.001$  compared to initial basal activity (Day-14).

b) Denotes <sup>A</sup> $p < 0.05$  compared with HFD-group and <sup>B</sup> $p < 0.05$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by dunnett's test.

**Table 6. Effect of hypocholesterolemic drugs on serum lipid profile**

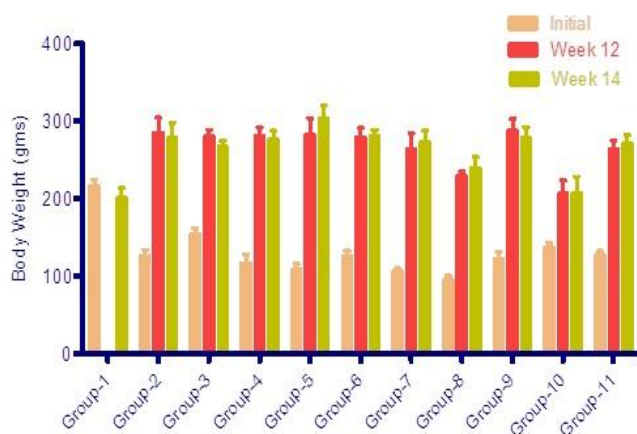
Group no.	Treatment & dose	Test	Initial	Post induction1	Final
1	Control (1% Na CMC)	HDL mg dl	98.60±2.24	-	103.15±0.99
		LDL mg/dl	73.37±7.45	-	131.33±7.82
		TC mg/dl	47.63±2.11	-	56.55±0.52
		TG mg/dl	36.29±0.8	-	20.33±1.33
		VLDL mg/dl	14.67±1.49	-	26.27±1.56
2	Only High Fat Diet (HFD)	HDL mg/dl	106.10±3.41	214.90±6.90 <sup>***</sup>	217.5±7.85
		LDL mg/dl	91.92±10.71	197.98±22.55 <sup>***</sup>	190.98±20.33
		TC mg/dl	56.88±3.88	27.55±1.33 <sup>***</sup>	31.48±1.36
		TG mg/dl	30.83±2.84	147.75±7.03 <sup>***</sup>	147.82±9.6
		VLDL mg/dl	18.38±2.14	39.60±4.51 <sup>***</sup>	38.20±4.07
3	Donepezil 5 mg/kg	HDL mg/dl	103.82±4.25	172.92±10.7 <sup>***</sup>	176.9±9.51 <sup>AAA</sup>
		LDL mg/dl	70.90±4.87	119.77±4.49 <sup>***</sup>	99.95±2.66 <sup>AAA</sup>
		TC mg/dl	55.23±2.78	41.28±2.70 <sup>***</sup>	46.42±2.98 <sup>AAA</sup>
		TG mg/dl	34.40±4.47	107.68±11.73 <sup>***</sup>	110.49±10.60 <sup>AAA, BBB</sup>
		VLDL mg/dl	14.18±0.97	23.95±0.90 <sup>***</sup>	19.99±0.53 <sup>AAA</sup>
4	Simvastatin 5 mg/kg	HDL mg/dl	99.30±2.52	204.97±2.29	128.73±2.54 <sup>A, BB</sup>
		LDL mg/dl	89.25±2.71	176.60±6.77	129.07±6.88 <sup>AAA, BBB</sup>
		TC mg/dl	46.23±1.16	30.43±1.04 <sup>***</sup>	38.05±1.39 <sup>AAA, BBB</sup>
		TG mg/dl	35.22±2.70	139.21±1.77	64.87±2.17 <sup>AAA, BBB</sup>

5	Rosuvastatin 5 mg/kg	VLDL mg/dl	17.85±0.54	35.32±1.35	25.81±1.38 <sup>AAA,BBB</sup>
		HDL mg/dl	102.87±0.75	207.08±1.03	129.88±1.95 <sup>AAA,BB</sup>
		LDL mg/dl	75.87±0.77	155.15±2.08	103.10±1.63 <sup>AAA,BBB</sup>
		TC mg/dl	57.68±1.11	42.77±0.9 <sup>***</sup>	54.13±1.41 <sup>AAA, BBB</sup>
		TG mg/dl	30.01±1.2	133.29±1.5 <sup>***</sup>	55.13±2.72 <sup>AAA</sup>
6	Fenofibrate 65 mg/kg	VLDL mg/dl	15.17±0.15	31.03±0.42	20.62±0.33 <sup>AAA</sup>
		HDL mg/dl	103.92±1.28	210.63±0.91	131.23±1.75 <sup>AAA,B</sup>
		LDL mg/dl	73.75±1.22	144.52±1.47	95.42±2.21 <sup>AAA,BBB</sup>
		TC mg/dl	55.67±1.24	39.33±0.91 <sup>***</sup>	52.48±1.20 <sup>AAA, BBB</sup>
		TG mg/dl	33.50±1.99	142.4±1.66 <sup>***</sup>	59.67±2.65 <sup>AAA</sup>
7	Nicotinic Acid 85 mg/kg	VLDL mg/dl	14.75±0.24	28.90±0.29	19.08±0.44 <sup>AAA</sup>
		HDL mg/dl	104.17±1.5	217.53±2.21	196.47±2.55 <sup>AAA</sup>
		LDL mg/dl	83.62±1.86	169.43±4.51	144.85±6.17 <sup>AAA</sup>
		TC mg/dl	55.08±1.89	41.37±2.83 <sup>***</sup>	50.92±3.76 <sup>AA, BBB</sup>
		TG mg/dl	32.36±2.98	142.28±3.97 <sup>***</sup>	116.58±3.97 <sup>AAA,BBB</sup>
8	Donepezil 5 mg/kg + Simvastatin 5 mg/kg	VLDL mg/dl	16.72±0.37	33.89±0.90	28.97±1.23 <sup>AAA,BBB</sup>
		HDL mg/dl	101.47±1.06	204.02±4.31	158.77±2.9 <sup>AAA</sup>
		LDL mg/dl	63.72±4.03	125.68±7.16	80.43±1.66 <sup>AAA,B</sup>
		TC mg/dl	59.95±1.37	37.13±1.44 <sup>***</sup>	47.00±1.36 <sup>AAA, BB</sup>
		TG mg/dl	28.77±1.32	141.75±5.97	95.68±3.97 <sup>AAA,B</sup>
9	Donepezil 5 mg/kg + Rosuvastatin 5 mg/kg	VLDL mg/dl	12.74±0.81	25.14±1.43	16.09±0.33 <sup>AAA,B</sup>
		HDL mg/dl	104.73±1.76	204.23±3.31	147.78±3.74 <sup>AAA</sup>
		LDL mg/dl	69.75±3.57	138.25±5.25	104.00±2.53 <sup>AAA,BBB</sup>
		TC mg/dl	56.78±1.34	41.4±1.28 <sup>***</sup>	50.78±1.57 <sup>AAA, BBB</sup>
		TG mg/dl	34.00±1.22	135.18±2.98	76.20±3.51 <sup>AAA</sup>
10	Donepezil 5 mg/kg + Fenofibrate 65 mg/kg	VLDL mg/dl	13.95±0.71	27.65±1.05	20.80±0.51 <sup>AAA</sup>
		HDL mg/dl	102.05±0.97	211.18±1.07	143.15±2.76 <sup>AAA</sup>
		LDL mg/dl	92.90±7.44	148.15±3.23	110.15±3.21 <sup>AAA,BBB</sup>
		TC mg/dl	54.73±0.71	39.38±0.97 <sup>***</sup>	47.08±0.90 <sup>AAA, BBB</sup>
		TG mg/dl	28.74±1.68	142.17±0.77	74.04±2.05 <sup>AAA</sup>
11	Donepezil 5 mg/kg + Nicotinic Acid 85 mg/kg	VLDL mg/dl	18.58±1.49	29.63±0.65	22.03±0.64 <sup>AAA</sup>
		HDL mg/dl	106.42±2.25	218.63±3.28	150.28±4.4 <sup>AAA</sup>
		LDL mg/dl	72.44±9.03	141.43±4.60	94.57±3.92 <sup>AAA,BBB</sup>
		TC mg/dl	55.83±1.54	40.92±1.53 <sup>***</sup>	47.22±1.21 <sup>AAA, BBB</sup>
		TG mg/dl	36.09±2.77	149.43±4.21	84.15±4.54 <sup>AAA</sup>
		VLDL mg/dl	14.49±1.81	28.29±0.92	18.91±0.78 <sup>AAA</sup>

Each group (n=6), each value represents Mean±SEM.

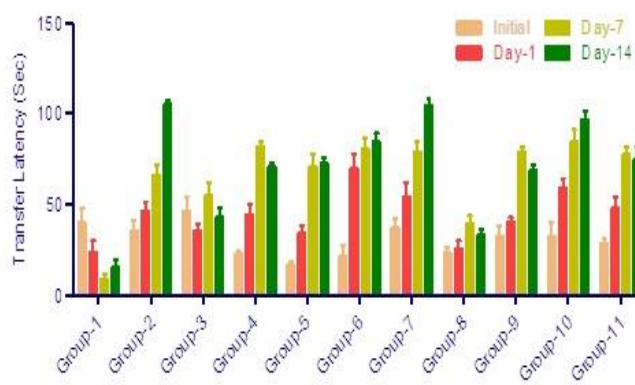
a) Denotes \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  compared to initial lipid profile. b) Denotes <sup>A</sup> $p < 0.05$  compared with HFD-group and <sup>B</sup> $p < 0.05$  and <sup>BB</sup> $p < 0.01$  compared with Donepezil 5 mg/kg at week-14, ANOVA followed by Dunnett's test.

**Figure 1. Effect of hypocholesterolemic drugs on Body Weights in animals receiving high fat diet (HFD).**



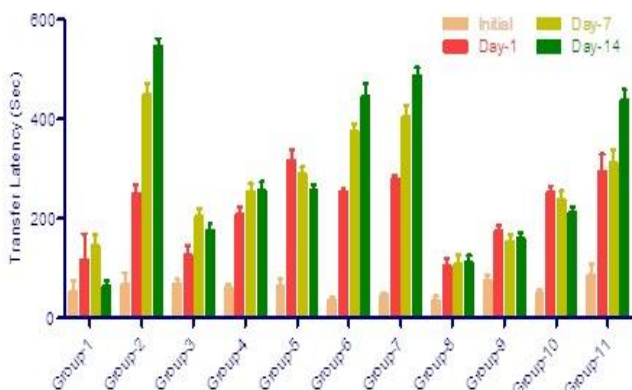
Each group (n=6), each value represents Mean±SEM.

**Figure 2. Effect of hypocholesterolemic drugs on transfer latency using Elevated plus-maze in animals receiving high fat diet (HFD).**



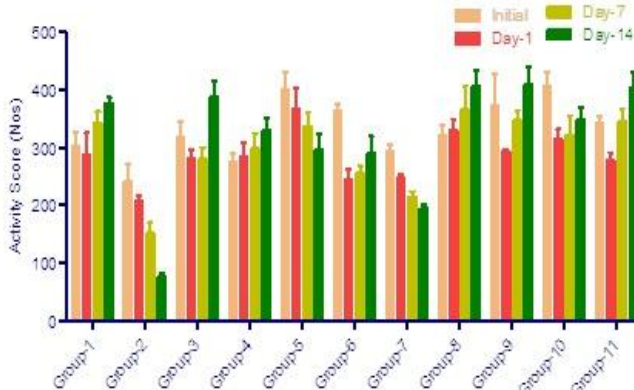
Each group (n=6), each value represents Mean±SEM.

**Figure 3. Effect of hypocholesterolemic drugs on transfer latency using Rectangular maze in animals receiving high fat diet (HFD).**



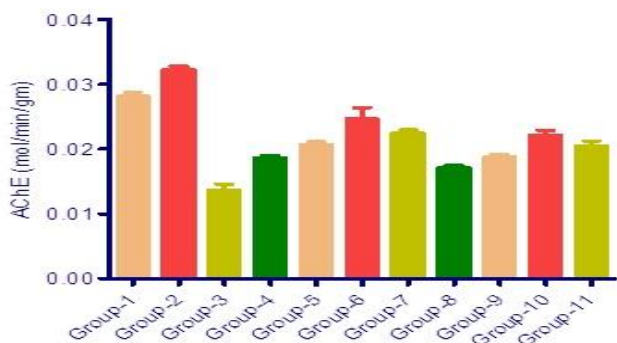
Each group (n=6), each value represents Mean±SEM.

**Figure 4. Effect of hypocholesterolemic drugs on locomotor activity using Actophotometer in animals receiving high fat diet (HFD).**



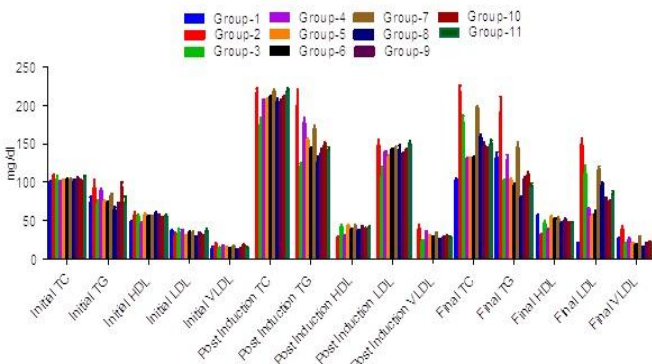
Each group (n=6), each value represents Mean±SEM.

**Figure 5. Effect of hypocholesterolemic drugs on Brain Acetylcholinesterase levels in animals receiving high fat diet (HFD).**



Each group (n=2), each value represents Mean±SEM.

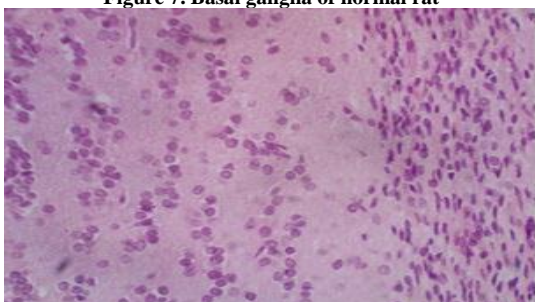
**Figure 6. Effect of hypocholesterolemic drugs on serum lipid profile in animals receiving high fat diet (HFD).**



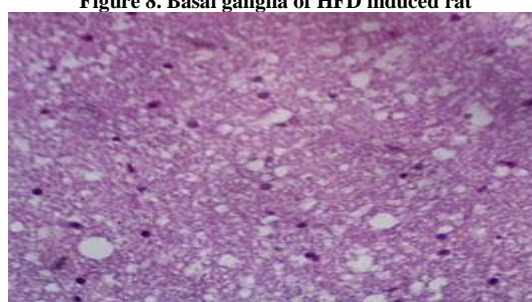
Each group (n=6), each value represents Mean±SEM.

**Brain Histopathology pictures of high fat diet (HFD) induced amnesia in Rats**

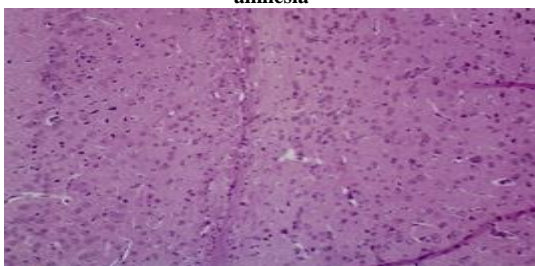
**Figure 7. Basal ganglia of normal rat**



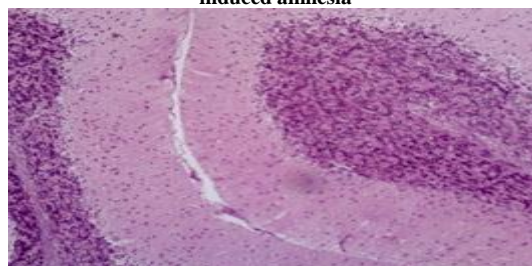
**Figure 8. Basal ganglia of HFD induced rat**



**Figure 9. Basal ganglia of rat treated with Donepezil in HFD induced amnesia**

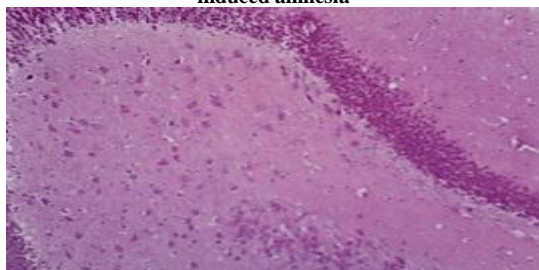


**Figure 10. Basal ganglia of rat treated with Simvastatin in HFD induced amnesia**

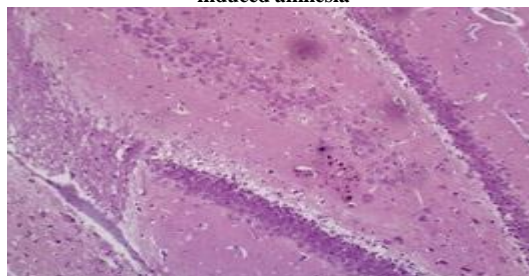




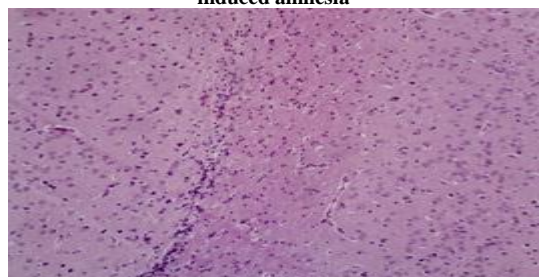
**Figure 11. Basal ganglia of rat treated with Rosuvastatin in HFD induced amnesia**



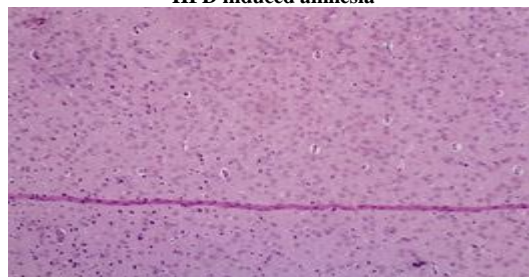
**Figure 12. Basal ganglia of rat treated with Fenofibrate in HFD induced amnesia**



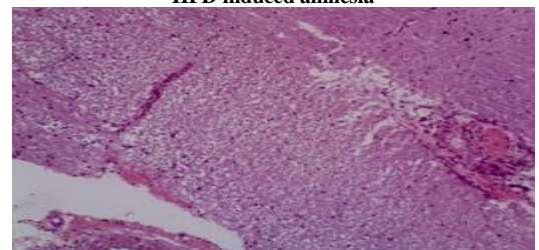
**Figure 13. Basal ganglia of rat treated with Nicotinic acid in HFD induced amnesia**



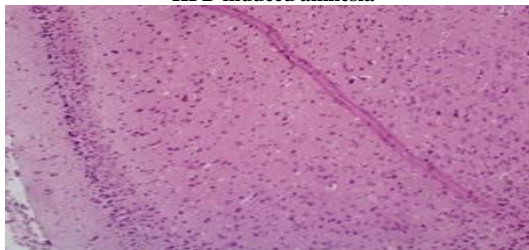
**Figure 14. Basal ganglia of rat treated with Donepezil+Simvastatin in HFD induced amnesia**



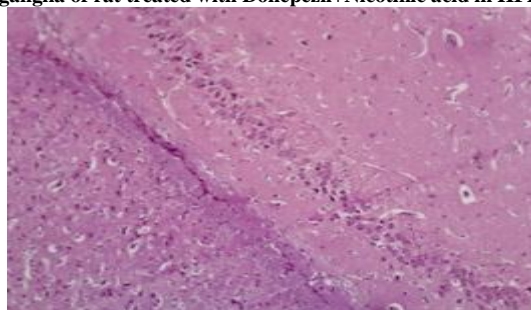
**Figure 15. Basal ganglia of rat treated with Donepezil+Rosuvastatin in HFD induced amnesia**



**Figure 16. Basal ganglia of rat treated with Donepezil+Fenofibrate in HFD induced amnesia**



**Figure 17. Basal ganglia of rat treated with Donepezil+Nicotinic acid in HFD induced amnesia**



## DISCUSSION

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, chronic administration (12 weeks) of high fat diet (HFD) not only produced significant increase in the total serum cholesterol levels,

but also impaired memory. This impairment of memory might be due to the increased CNS cholesterol pool because there is a cross talk between CNS and peripheral cholesterol levels. The increased CNS cholesterol might lead to deposition of amyloid peptide in brain (Ashutosh Agarwal *et al.*, 2002). Statins exerted cognitive benefits in AD and were reported to affect CNS cholesterol homeostasis (Haley RW & Dietsch JM, 2000). This contention is further confirmed by the present study, wherein, simvastatin and rosuvastatin significantly prevented HFD induced memory deficits indicated by augmentation of transfer latency and reduction in basal

activity score. In addition to statins, fenofibrate and nicotinic acid also ameliorated HFD induced memory deficits.

The current study revealed that HFD caused elevation of serum LDL, VLDL, total cholesterol, triglycerides and reduction of HDL levels. Simvastatin, rosuvastatin, fenofibrate and nicotinic acid reverted back the altered lipid profile. These drugs might have improved the memory by reducing the serum cholesterol level with subsequent reduction of CNS cholesterol and deposition of  $\beta$ -amyloid peptide. However, anti-inflammatory and anti-oxidant actions of statins to enhance memory cannot totally be ignored at this point and might have contributed to the beneficial effect on memory.

The tested drugs were also found to decrease the acetylcholinesterase levels. This activity of the above compounds might also contribute to their memory enhancing effect by decreasing the metabolism of acetylcholine.

Histopathological study further corroborated the result. Standard donepezil successfully reversed the marked gliosis and neurodegeneration of basal ganglia induced by scopolamine. The photomicrographs of brain sections treated with hypocholesterolemic drugs showed mild gliosis and lack of neurodegeneration of basal ganglia. It indicated neuroprotective role of hypocholesterolemic drugs.

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Hypocholesterolemic drugs from various classes were found to reverse memory deficits induced by high fat diet through their cholesterol dependent effects.

## CONCLUSIONS

Treatment of amnesia-induced rats with hypocholesterolemic drugs significantly ameliorates the cholinergic dysfunction and inflammation-induced neurodegeneration characterizing Alzheimer's disease. Noteworthy, statins revealed more pronounced modulatory effect on most of the measured physical and biochemical parameters as well as histological feature of the brain than fibrates and nicotinic acid. The successful reversal of memory deficits induced by high fat diet might be through their cholesterol dependent effects. However, anti-inflammatory and antioxidant actions of hypocholesterolemic drugs can not totally be ignored at this point and might have contributed to the beneficial effect on memory. The selected drugs may represent new therapeutic approaches for intervention of the progressive neurological damage associated with Alzheimer's disease.

**ACKNOWLEDGEMENT:** None

## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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