Original Research Article

Neuroprotective role of Ginkgobiloba and Rosuvastatin in CA1 region of Hippocampus against high fat diet induced neurotoxicity

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ABSTRACT

Objective: Diet rich in fat is one of the main risk factor for the development of Alzheimer's disease. Studies have shown that diet rich in fat disrupts memory and learning. The present study evaluates the ameliorative role of Ginkgobiloba and Rosuvastatin against high fat diet induced neurotoxicity in CA1 (Corona Ammonis) region of hippocampus.

Materials and Methods: Animals were randomly divided into six groups. Group I received normal diet, Group II received high fat diet, Group III & IV were treated with Ginkgobiloba 50mg/kg and 100mg/kg body weight, and Group V & VI were treated with Rosuvastatin 10mg/kg and 20 mg/kg body weight. All the rats were subjected to spatial learning (Morris water maze). Subsequently, rats were sacrificed and brains were removed. Golgi staining was done and CA1 neurons of hippocampus were traced using camera lucida. Dendritic branching points and dendritic intersections were quantified. Lipid profile and Super oxide (SOD) was also estimated.

Results: There was enhancement of spatial learning in treatment group rats. Furthermore, a significant increase in dendritic length and branching points was observed in CA1 region along with significant decrease in the Superoxide dismutase in rats treated with higher dose of Ginkgobiloba and Rosuvastatin.

Conclusion: Present study concludes that Ginkgobiloba and Rosuvastatin in higher dose have protective role against high fat diet induced neurotoxicity in CA1 region.

1. Introduction

Hippocampus is a part of limbic system. It is essential for the formation of stable declarative memory in humans[1] and spatial memory in rodents.[2] The hippocampus is divided into two major 'U' shaped interlocking sectors, the dentate gyrus and the hippocampus proper (cornu ammonis). Hippocampus proper is demarcated into four sub regions CA1, CA2, CA3 and CA4.[3]

CA1 is considered as the main output area of the hippocampal network and contributes to incremental learning and responsible for autobiographical memory. Lesions in CA1 region results in non-learning of contextual fear conditioning.[4,5]

Western diet rich in cholesterol and fat resulted in neuroinflammation of brain and led to memory impairment in rats.[6–8] Previous studies have shown that diet rich in calories and fat caused disruption of blood brain barrier in rats and affected long term potentiation in rats.[9,10] Ginkgobiloba is a Chinese plant, which has been used in traditional Chinese medicine for treating wide range of health disorders.[11] The benefits of Ginkgo biloba is attributed to two active chemical components, flavonoids and terpenoids.[12] These active constituents have been

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extensively studied and found to exhibit free radical scavenging properties and antagonize the platelet activating factor.[13,14] Apart from that Ginkgobiloba has a protective role in neurodegenerative diseases[15] and possesses a neurotrophic effect on the hippocampus.[16,17] It is proved to augment cholinergic system in central nervous system and enhances memory in rodents and human.[18,19]

Rosuvastatin is a second generation hydrophilic synthetic statin. It has been reported that Rosuvastatin reduced oxidative stress in rat's hippocampus and enhanced memory.[20] Cross sectional studies have indicated that Rosuvastatin reduces incidence of AD risk.[21] In the present study, the efficacy of Ginkgobiloba extract and Rosuvastatin has been evaluated against the neurotoxicity produced by high fat diet in CA1 region of hippocampus.

2. Materials and Methods

2.1. Animal care and maintenance

Adult in-bred male Wistar rats weighing about 120-150g were obtained from the Central Animal House, Mamata Medical College, Khammam. Prior approval of institutional animal ethical committee was obtained for the study (IAEC/DP-05/C16 2012-2013). All the experimental procedures were carried out according to the guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Animals were housed in polypropylene cages with paddy husk as the bedding material. Cages were maintained at standard temperature $25 \pm 2°C$, humidity (50-55%) under 12-hour light and dark cycle. Rats were fed ad libitum with a balanced diet containing 21.96% crude oil, 3.10% crude fibre, 7.37% ash, 1.38% sand silica.

3. Experimental design

Adult male Wistar rats were divided six groups with six animals in each group

Group I: control animals received ad libitum diet and water (NC).

Group II: Fed with diet rich in high fat diet for three months 90 days (HFD).

Group III: Fed with high fat diet for three months and treated with Ginkgobiloba 50mg/kg.b.w for 15 days (75th-90). (HFD+GINK 50mg/kg).

Group IV: Fed with high fat diet for three months and treated with Ginkgobiloba 100mg/kg.b.w for 15 days (75th-90). (HFD+GINK 100mg/kg).

Group V: Fed with high fat diet for three months and treated with Rosuvastatin 10mg/kg.b.w for 14 days (76th-90). (HFD+ROS 10mg/kg).

Group VI: Fed with high fat diet for three months and treated with Rosuvastatin 20mg/kg.b.w for 14 days (76th-90). (HFD+ROS 20mg/kg).

3.1. Estimation of the body weight

Weight of the animals was measured at the beginning of the study and at the end (after 3 months).

Lipid profile: Blood samples were obtained by retro-orbital puncture. Serum cholesterol and triglycerides were estimated (after 3 months).[22]

3.2. Administration of high fat diet

Hyperlipidemia was produced by feeding with cholesterol-rich high-fat diet (HFD) for 3 months. Deoxycholic acid (5g) will be mixed thoroughly with 700g of powdered rat chow diet. Simultaneously cholesterol (5g) will be dissolved in 300g warm coconut oil. This oil solution of cholesterol will be added slowly into the powdered mixture to obtain a soft homogenous cake. This cholesterol-rich HFD will be molded into pellets of about 3g each and will be used to feed the animals.[23]

3.3. Administration of Ginkgobiloba:

100 mg and 50 mg of Ginkgobiloba extract was dissolved in 1% gum acacia solution. This solution was administered to the animals at 50mg/kg body weight and 100 mg/kg body weight with the help of oral gavage needle attached to a syringe for 15 days.[24]

3.4. Administration of Rosuvastatin

Rosuvastatin was obtained from Pfizer Pharmaceuticals private limited, Mumbai. It was given at doses10mg/kg body weight and 20mg/kg body weight orally with the help of an oral gavage needle attached to a syringe for 14 days.[25]

3.5. Morris water maze

Morris water maze spatial memory of the rats was tested on 90th day by using Morris water maze. The water maze consists of a circular tank of 1.80 m diameter and 75 cm depth. The pool was filled with water and maintained at a temperature of 24-26ºC to a depth of about 50 cm. It was divided into four quadrants and an escape platform of size 4"×4" was hidden approximately 2cm below the water surface in the target quadrant. Water in the pool was made opaque by adding milk just before the experiment. Permanently positioned distinctive objects were placed for facilitating spatial orientation of the animal. Positions of the cues were kept unchanged throughout the period of experiment. The rats were trained in the water maze in 10 sessions on 5 consecutive days, two sessions on each day. Each session consists of four trails. In each trial time taken to reach hidden platform was recorded. If the rat was unable to find the platform within two minutes, the training session was terminated and a maximum score of two minutes was assigned.

Twenty four hours after the last session, rats were subjected to a probe trial. This session was for 30 seconds in which the hidden platform was removed. Here time taken to reach the target quadrant (latency) was measured.[26]

3.6. Golgi staining procedure

Rats were deeply anesthetized with ether and sacrificed by cervical dislocation. Brains were removed quickly and placed in a petri dish containing freshly prepared Golgi-cox fixative. The hippocampus was dissected from both hemispheres of the brain. Tissue was processed for Golgi staining.[27]

3.7. Dendritic quantification

The dendritic quantification of hippocampal CA1 neurons was done by using the camera lucida technique.8 -10 well stained pyramidal neurons of CA1 region in hippocampus were selected from each rat and traced using camera lucida device (Dutta scientific works, Bangalore, India). Neurons that were darkly-stained throughout their arborization and with minimal overlap were selected. Neurons with truncated dendritic branches within a 100 μm radius from the cell body were excluded.

3.8. Quantification of dendritic branching points and dendritic intersections

The concentric circle method of Sholl was used for dendritic quantification. Concentric circles with radial distance of 20 μm were drawn on a transparent sheet and used for dendritic quantification. This sheet was placed on the camera lucida-traced neuron in order that centre of the cell body of the neuron coincided with the center of the concentric circles.

The number of branch points between two successive concentric circles i.e., within each successive 20 μm radial spheres were counted. The dendritic intersection was defined as the point where a dendrite touches or intersects the concentric circle. Both branch points and intersections were counted to a maximum radial distance of 100 μm from the center of the soma.[27]

3.9. Estimation of superoxide dismutase

The animals were euthanized by decapitation, and the brain was quickly removed and kept in a petridish located on ice. Hippocampus was dissected and homogenised in 1.5% potassium chloride, using a glass type Potter-Elvejhem homogeniser. The homogenate were centrifuged at 800×g for 10 minutes at 4 degree centrigrade.the pellet was discarded and the supernatant was stored at -70 degrees. Superoxide dismutase (SOD) activity, expressed as USOD/mg protein, was based on the decrease in the rate of autocatalytic adrenochrome formation at 480nm.[28]

3.10. Statistical analysis

The data were analysed with one way Anova followed by Bonferroni's post-test using Graph Pad Prism, version 5 (Graph Pad Prism Software inc., USA). The results were expressed as Mean ± SD, p value less than 0.05 was considered statistically significant.

4. Results

4.1. Weight of the animals & Blood tests Table 1

The weight of the animals fed with only high fat diet rich in cholesterol showed a significant increase in weight ($p<0.001$) when compared to NC group. The animals treated with Ginkgobiloba 100mg/kg & Ginkgobiloba 50mg/kg did not show any significant effect on the body weight compared to HFD group. The weight of the animals treated with Rosuvastatin10mg/kg and 20mg/kg has shown significant decrease in weight compared to HFD group ($p<0.01$) and ($p<0.001$).

4.2. Serum cholesterol & serum triglycerides

HFD group showed significant increase ($p<0.001$) of serum cholesterol and triglycerides compared to NC group. Rats treated with Ginkgobiloba 100mg/kg showed significant decrease ($p<0.05$), in serum cholesterol and triglycerides compared to HFD group. Rats treated with Rosuvastatin 10mg/kg and 20 mg/kg showed significant decrease in serum cholesterol and triglycerides compared to HFD group. ($p<0.01$) and ($p<0.001$)

Mean ± SD of Weight, Cholesterol and Triglycerides is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD vs. HFD +GINK 100: ^$p<0.05$; HFD Vs HFD+ROS10: && $p<0.01$; HFD Vs HFD+ROS20: ### $p<0.001$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt.

4.3. Morris water maze test

Water maze performance during the training sessions:

Analysis of the spatial memory test on the water maze showed that during the first session all the rats failed to reach the escape platform. During the second and third day the NC group animal reached the platform in less time when compared to HFD which suggests memory impairment. In the fourth and fifth day HFD group took longer time to escape on to the platform when compared to NC group. This can be attributed to the deleterious effects of high fat diet on spatial memory.

Table 1: Weight, cholesterol, triglycerides

Groups	No	Weight (grams)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
NC	6	147.3 ± 8.16	90.71 ± 2.06	92.80 ± 5.96
HFD	6	$260.8 \pm 11.80{***}$	$163.12 \pm 4.91{***}$	$149.42 \pm 4.56{***}$
HFD+GINK50	6	253.3 ± 10.74	156.24 ± 3.16	142.08 ± 7.38
HFD+GINK100	6	243.5 ± 9.92	$148.83 \pm 2.20^{\wedge}$	$138.92 \pm 6.72^{\wedge}$
HFD+ROS10	6	$235.7 \pm 10.44^{\&\&}$	$140.08 \pm 2.15^{\&\&}$	$133.58 \pm 5.82^{\&\&}$
HFD+ROS20	6	$198.7 \pm 7.36^{\#\#\#}$	$120.08 \pm 4.12^{\#\#\#}$	$113.58 \pm 4.68^{\#\#\#}$

4.4. Latency to enter target quadrant Table 2

This was done on 96th day, twenty four hours after the 10th session HFD group took significantly ($p<0.001$) more time to reach the target quadrant when compared to NC (probe). The above results imply that high fat diet causes substantial memory impairment.

Rats treated with Ginkgobiloba 100mg/kg took significantly less time ($p<0.01$) to reach the target quadrant when compared to HFD group. The results of Ginkgobiloba 50 mg/kg treatment were not significant.

Rats treated with Rosuvastatin 20mg/kg als0 took significantly less time ($p<0.01$) to reach the target quadrant when compared to HFD group, whereas rats treated with Rosuvastatin 10 mg/kg didn't show significance compared to high fat diet group. The above results imply that Ginkgobiloba and Rosuvastatin were effective in higher dosage.

Mean ± SD of Latency to enter the target quadrant is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD vs. HFD +GINK 100: ^^$p<0.01$; HFD Vs HFD+ROS20: #$p<0.5$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt.

4.5. Dendritic Morphology of CA1 region Figures 1, 2, 3 and 4 Apical dendritic intersections Table 3

Rats in HFD group showed significantly less number of apical dendritic intersections compared to NC 40, 60, 80 and 100 ($p<0.001$). Rats treated with Ginkgobiloba 100mg/kg showed significant increase in number of apical dendritic intersections compared to HFD. (HFD versus HFD +GINK100: 40, (($p<0.001$)60, 80 and 100($p<0.01$). Rats treated with 50 mg/kg group showed significant increase in number of apical dendritic intersections compared to HFD. (HFD versus HFD+GINK50: 40 ($p<0.001$).

Rats treated with Rosuvastatin 20mg/kg showed significant increase in number of apical dendritic intersections compared to HFD (HFD versus HFD +ROS 20): 40, ($p<0.01$)60, 80 and 100($p<0.01$).

4.6. Basal dendritic intersections Table 4

HFD group rats showed significantly less number of basal dendritic intersections at 20, 40, 60, 80,100 ($p<0.001$).compared to NC group. Rats which were administered with Ginkgobiloba 100mg/kg rats showed significant increase in number of basal dendritic intersections compared to HFD groups (HFD versus HFD+GINK 100: 20($p<0.05$), 40, 60,80,100 ($p<0.01$). Rats which were administered with Rosuvastatin 20mg/kg showed significant increase in number of basal dendritic intersections compared to HFD group (HFD versus HFD+ROS 20: 20 ($p<0.05$), 40, 60, 80 and 100 ($p<0.01$).

4.7. Apical dendritic branching points Table 5

A significantly less number of apical dendritic branching points at 20-40, 40-60, 60-80 and 80-100 ($p<0.001$) zones were observed in the HFD group of rats when compared to NC group. A significant increase in number of apical dendritic branching points zones was observed in the rats treated with Ginkgobiloba 100 mg/kg compared to HFD groups (HFD versus HFD+GINK100): 20-40, ($p<0.05$) 40-60, 60-80, 80-100 ($p<0.01$). Rats treated with Ginkgobiloba 50mg/kg did not show any significance.

A significant increase in number of apical dendritic branching points zones was observed in the rats treated with ROS 20 mg/kg compared to HFD group (HFD versus HFD+ROS 20: 40-60($p<0.05$), 60-80, 80-100 ($p<0.01$). Rats treated with Rosuvastatin 10mg/kg showed no significance compared to high fat diet.

4.8. Basal dendritic branching points Table 6

HFD group of rats showed significantly less number of basal dendritic branching points at 0-20, 20-40, 40-60, 60-80, and 80-100 ($p<0.001$) zones compared to NC group. Rats that were administered Ginkgobiloba 100mg/kg showed significant increase in number of basal dendritic branching points compared to HFD group (HFD versus HFD+GINK100: 20-40 ($p<0.001$), 40-60, 60-80, and 80-100 ($p<0.01$)

Rats that were administered Rosuvastatin 20mg/kg showed significant increase in number of basal dendritic branching points compared to HFD group HFD versus HFD+ROS 20: 20-40, 40-60, 60-80, and 80-100 ($p<0.01$).

Table 2: Morris water maze test

Groups	no	Latency to enter the target quadrant (sec)
NC	6	3.02 ± 0.69
HFD	6	4.88 ± 0.62***
HFD+GINK50	6	4.66 ± 0.78
HFD+GINK100	6	3.29 ± 0.76^^
HFD+ROS10	6	4.75 ± 0.31
HFD+ROS20	6	3.65 ± 0.66#

Table 3: Apical dendritic intersections

Groups	n	Distance from soma (μ)				
		20	40	60	80	100
NC	6	0.923 ± 0.11	2.522 ± 0.14	3.220 ± 0.12	5.116 ± 0.51	5.207 ± 0.34
HFD	6	0.772 ± 0.19	1.031 ± 0.19***	1.820 ± 0.37***	2.657 ± 0.45***	3.320 ± 0.52***
HFD+ GINK50	6	0.825 ± 0.10	1.390 ± 0.32$$$	2.038 ± 0.44	3.029 ± 0.12	3.387 ± 0.79
HFD+ GINK 100	6	0.868 ± 0.11	1.798 ± 0.29^^^	2.578 ± 0.46^^	4.024 ± 0.55^^	4.044 ± 0.58^^
HFD+ROS10	6	0.786 ± 0.07	0.906 ± 0.04	2..09 ± 0.1	3.193 ± 0.12	3.443 ± 0.13
HFD+ROS20	6	0.842 ± 0.18	1.387± 0.11###	2.530 ± 0.48##	3.990 ± 43##	3.199 ± 0.88##

Apical dendritic intersections of hippocampal CA1 neurons; Mean ± SD is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD Vs. HFD+ GINK50: $$$ $p<0.001$; HFD Vs. HFD +GINK 100: ^^^ $p<0.001$, ^^$p<0.01$; HFD Vs HFD+ROS20 ### $p<0.001$, ## $p<0.01$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt.

Table 4: Basal dendritic intersections

Groups	n	Distance from soma (μ)				
		20	40	60	80	100
NC	6	3.533 ± 0.35	7.556 ± 0.26	12.76 ± 0.48	7.819 ± 0.19	3.639 ± 0.38
HFD	6	2.544 ± 0.25***	4.611 ± 0.96***	5.223 ± 0.80***	4.224 ± 0.25***	2.003 ± 0.49***
HFD+GINK50	6	2.639 ± 0.25	4.962 ± 0.35	5.832 ± 0.92	4.297 ± 0.39	2.476 ± 0.10
HFD+GINK100	6	3.192 ± 0.58^	6.238 ± 0.69^^	7.984 ± 0.16^^	5.838 ± 0.36^^	3.161 ± 0.16^^
HFD+ROS10	6	2.402 ± 0.61	4.869 ± 0.77	5.618 ± 0.26	4.520 ± 0.54	2.371 ± 0.37
HFD+ROS20	6	3.10 ± 0.35#	6.142 ± 0.59##	7.334 ± 0.77##	5.749 ± 0.91##	2.882 ± 61##

Basal dendritic intersections of hippocampal CA1 neurons; Mean ± SD is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD vs. HFD +GINK 100: ^^^ $p<0.001$, ^^$p<0.01$,^$p<0.1$; HFD Vs HFD+ROS20 ### $p<0.001$, ## $p<0.01$, # $p<0.05$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt.

Table 5: Apical dendritic branching points

Groups	n	Concentric zones (μ)					Total number of branching points
		0-20	20-40	40-60	60-80	80-100	
NC	6	0.289 ± 0.02	0.764 ± 0.08	0.955 ± 0.03	1.220 ± 0.16	1.275 ± 0.20	4.503 ± 0.49
HFD	6	0.256 ± 0.02	0.504 ± 0.07***	0.672 ± 0.08***	0.837 ± 0.13***	0.829 ± 0.22***	3.098 ± 0.52 ***
HFD+GINK50	6	0.260 ± 0.01	0.600 ± 0.02	0.767 ± 0.07	0.901 ± 0.06	0.906 ± 0.05	3.434 ± 0.21
HFD+GINK 100	6	0.345 ± 0.08	0.697 ± 0.04^	0.859 ± 0.08^^	1.086 ± 0.09^^	1.083 ± 0.05^^	4.070 ± 0.34^^
HFD+ROS 10	6	0.270 ± 0.20	0.615 ± 0.06	0.675 ± 0.19	0.850 ± 0.07	0.871 ± 0.09	3.280 ± 0.52
HFD+ROS 20	6	0.294 ± 0.92	0.620 ± 0.08	0.824 ±0.07#	1.050 ± 0.07##	1.091± 0.09##	3.879 ±1.23##

Apical dendritic branching points of hippocampal CA1 neurons; Mean ± SD is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD vs. HFD +GINK 100: ^^^ $p<0.001$, ^^$p<0.01$,^$p<0.05$; HFD Vs HFD+ROS20 ### $p<0.001$, ## $p<0.01$, # $p<0.05$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt

Table 6: Basal dendritic branching points

Groups	n	Concentric zones (μ)					Total number of branching points
		0-20	20-40	40-60	60-80	80-100	
NC	6	1.530 ± 0.16	2.975 ± 0.06	3.955 ± 0.06	2.976 ± 0.12	0.961 ± 0.09	12.397 ± 0.49
HFD	6	1.070 ± 0.46***	1.556 ± 0.16***	2.372 ± 0.15***	1.422 ± 0.10***	0.567 ± 0.15***	6.987 ± 1.02***
HFD+GINK 50	6	1.040 ± 0.28	1.670 ± 0.27	2.392 ± 0.17	1.488 ± 0.40	6.309 ± 0.12	12.899 ± 1.24
HFD+GINK 100	6	1.112 ± 0.09	2.248 ± 0.28^^^	3.321 ± 0.16^^	2.189 ± 0.12^^	0.884 ± 0.06^^	9.754 ± 0.71^^
HFD+ROS 10	6	0.991± 0.06	1.692 ± 0.16	2.454 ± 0.33	1.416 ± 0.19	0.651 ± 0.25	7.204 ± 0.99
HFD+ROS 20	6	1.101 ± 0.06	2.112 ± 0.18##	3.324 ± 0.33##	2.206 ± 0.09##	0.871 ± 0.15##	9.614 ± 0.81##

Basal dendritic branching points of hippocampal CA1 neurons; Mean ± SD is shown, n = 6 in each group. NC Vs. HFD: *** $p<0.001$; HFD vs. HFD+GINK 100: ^^^ $p<0.001$, ^^$p<0.01$; HFD Vs HFD+ROS20: ## $p<0.01$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvatatin 20mg/kg. body.wt
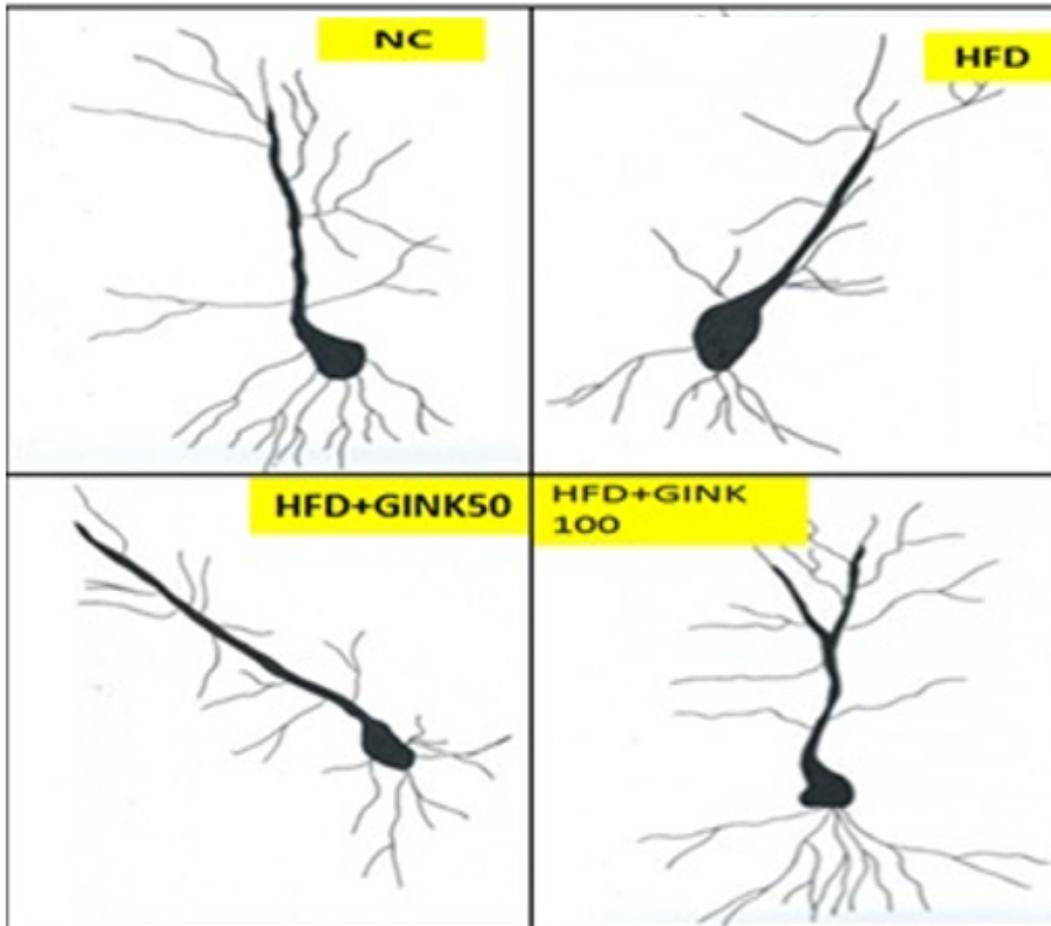
Fig. 1: Camera lucida tracings of CA1 neurons of Hippocampus showing the dendritic arborization indifferent groups (Golgi Coxstaining). NC: normal control; HFD: High fat diet; GINK50: Ginkgo biloba 50mg/kg; GINK 100: Ginkgo biloba 100mg/kg; Note (i) significant increase in dendritic arborization in HFD +GINK 100 compared to HFD group
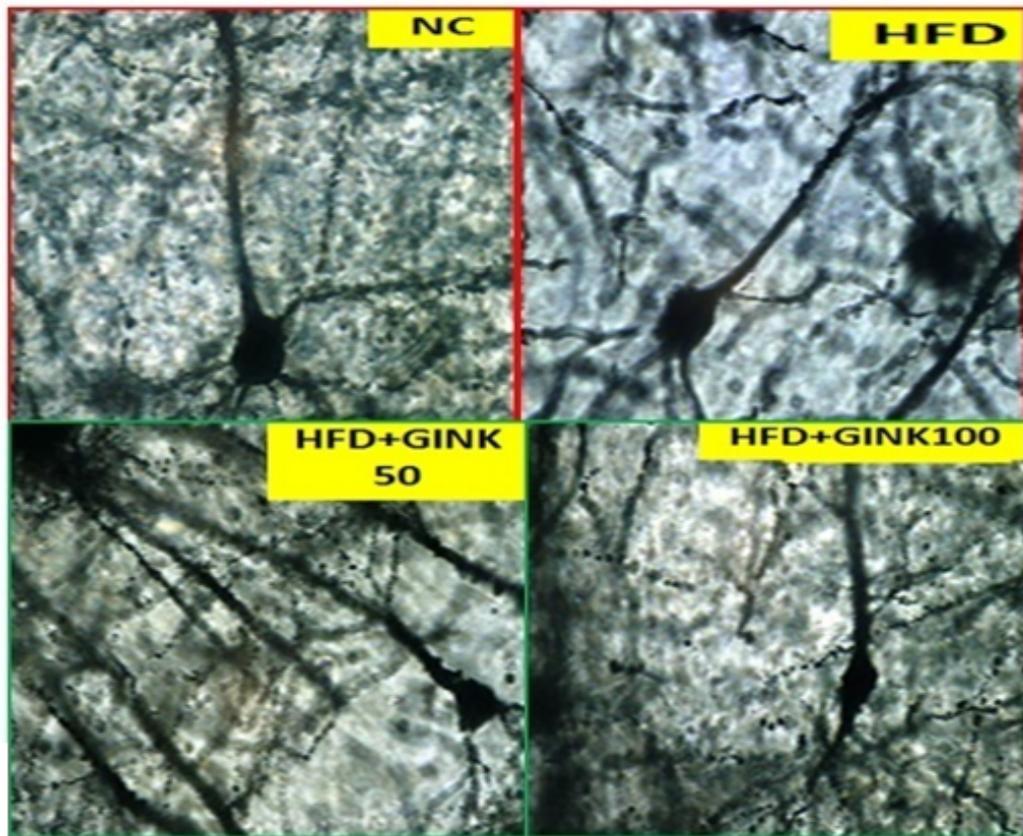
Fig. 2: Photomicrographs (40X) of CA1 neurons of Hippocampus showing the dendritic arborization indifferent groups (Golgi Cox staining). NC: normal control; HFD: High fat diet; GINK 50: Ginkgo biloba 50mg/kg; GINK 100: Ginkgo biloba 100mg/kg; Note (i) significant increase in dendritic arborization in HFD +GINK 100 compared to HFD group

4.9. Superoxide dismutase activity of rat brain Homogenate Table 7

Rats fed with HFD group showed significant decrease in super oxidase dismutase (p<0.01) levels of hippocampus compared to NC group. Rats treated with Ginkgobiloba 100mg/kg showed a significant increase in superoxide dismutase levels (p<0.01) compared to HFD group. Rats treated with Rosuvastatin 20mg/kg also showed a significant increase in superoxidase dismutase levels (p<0.05) compared to HFD group.

5. Discussion

The present study explored the neuroprotective role of Ginkgobiloba and Rosuvastatin in higher doses against the high fat diet induced neurotoxicity. The rats treated with Ginkgobiloba 100mg and Rosuvastatin 20mg showed significant reduction in time taken to enter the target quadrant (Morris water maze) when compared to rats fed with high fat diet which implies both were effective in improving the memory and learning in rats. Apart from that there was a significant increase in dendritic length and branching points in the CA1 region of hippocampus along with decreased superoxide dismutase levels that suggests the ameliorative effect of both the drugs in dose dependant manner.

Previous studies on Ginkgobiloba have shown that rats treated with Ginkgobiloba 100mg/kg body weight for three weeks showed significant improvement in short term memory on passive avoidance test and improved membrane fluidity in aged mice.[29] In another experiment administration of Ginkgobiloba at dose of 50 mg/kg, 200 mg/kg body weight showed significant improvement in spatial learning on Morris water maze in aluminum-treated rats[30] but in our study we could not find any significant improvement in spatial learning in rats treated with 50mg/kg.

In addition studies on Ginkgobiloba have revealed that it has reduced the neurodegeneration in hippocampus against fluoride[24] and acrylamide[31] induced neurotoxicity in rats. Another experiment on old aged rats have shown that, chronic treatment with Ginkgobiloba have significantly
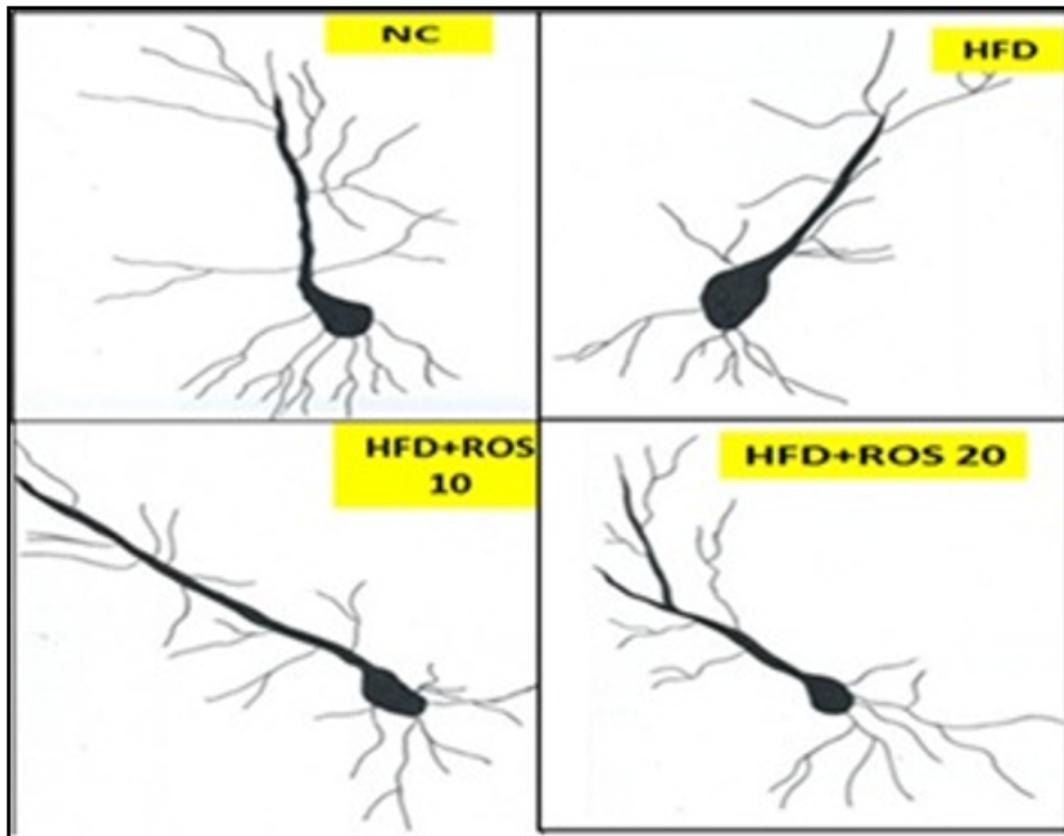
Fig. 3: Camera Lucida tracings of CA1 neurons of Hippocampus showing the dendritic arborization indifferent groups (Golgi Cox staining). NC: normal control; HFD: High fat diet;ROS 10:Rosuvastatin 10mg/kg; ROS 20: Rosuvastatin 20mg/kg Note (i) significant increase in dendritic arborization in HFD +ROS 20 compared to HFD group

Table 7:

Groups	No	Superoxide dismutase ((U/g protein).
NC	6	11.52 ± 0.69
HFD	6	$10.88 \pm 0.57**$
HFD+GINK50	6	10.66 ± 0.68
HFD+GINK100	6	$11.29 \pm 0.76^{\wedge\wedge}$
HFD+ROS10	6	10.05 ± 0.31
HFD+ROS20	6	$11.01 \pm 0.66^{\#}$

Mean $\pm$ SD of superoxide dismutase is shown, n = 6 in each group. NC Vs. HFD: ** $p<0.01$; HFD vs. HFD +GINK 100: ^^$p<0.01$; HFD Vs HFD+ROS20: $^{\#}p<0.05$ (One way ANOVA, Bonferroni's test). NC: Control, HFD: High fat diet, GINK 50: Ginkgobiloba 50 mg/kg body wt, GINK 100: Ginkgobiloba 100 mg/kg body wt, ROS 10: Rosuvatatin 10mg/kg. body.wt, ROS 20: Rosuvastatin 20mg/kg. body.wt.

increased the dendritic branching pattern in CA1 and CA3 regions of hippocampus.[32]

The positive effects of this extract in CA1 region of hippocampus can be attributed to antioxidant, free radical scavenging,[33] and antiapoptopic properties.[34] In our study also there was a decrease in superoxide dismutase levels. Treatment with leaf extract of Ginkgobiloba enhanced the memory in rats by improving the cerebral blood flow and[35] and increasing the brain derived neurotrophic factor which is necessary for long term potentiation.[36] Futhermore it also acts on neurotransmitters of brain and improves the cognition.[37,38] Recent studies on Ginkgibiloba have proved to inhibit the formation of beta amyloid in brain of transgenic rats.[39]

The neuroprotective activity of Rosuvastatin is due to its pleotrophic effects.[40] Previous experiments on Rosuvastatin have shown to improve the memory deficits caused iron loading and aging.[41] Georgieva-Kotetarova[42] reported that treatment with Rosuvastatin10 mg/kg has preserved long-term memory and enhanced cognitive functions in rats with diazepam-induced amnesia. Furthermore treatment with Rosuvastatin has reversed the changes in hippocampus
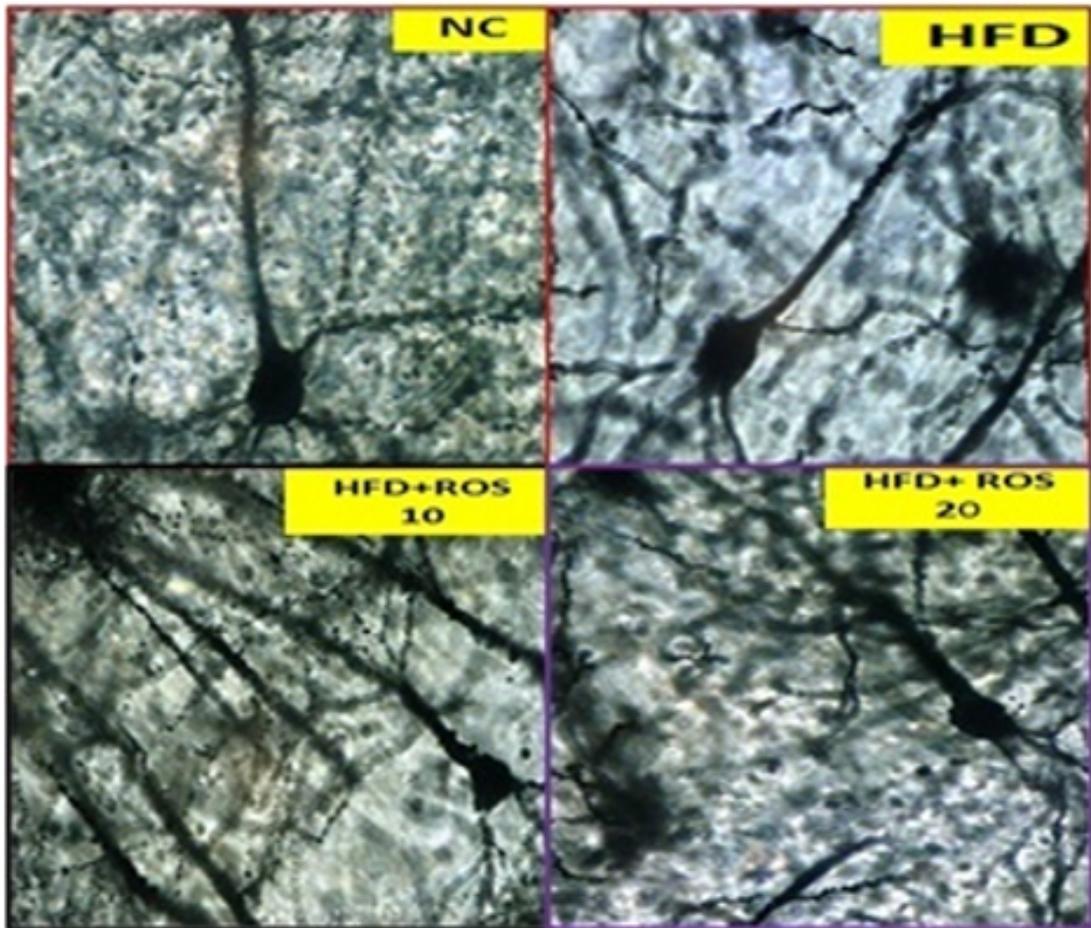
Fig. 4: Photomicrographs (40X) of CA1 neurons of Hippocampus showing the dendritic arborization indifferent groups (Golgi Cox staining). NC: normal control; HFD: High fat diet; ROS 10: Rosuvastatin 10mg/kg; ROS 20: Rosuvastatin 20mg/kg Note (i) significant increase in dendritic arborization in HFD +ROS 20 compared to HFD group.

caused by Olanzapine an antipsychotic drug.[43] The protective role of Rosuvastatin can be explained by its antioxidant,[44] anti-inflammatory and anti-hyperlipidemic properties.[45] Studies have shown that treatment with Rosuvastatin have prevented Parkinsons disease in an in vitro model[46] and modulated the nitric oxide synthase expressions.[47]

Many clinical and preclinical studies confirm the neuroprotective role of Rosuvastatin against Alzheimer's disease, Parkinson's, and Multiple Sclerosis.[48–50]

6. Conclusion

In conclusion, we observed dose dependent increase of dendritic brancing points and intersections in the region of CA1 region of hippocampus in groups treated with Ginkgobiloba and Rosuvastatin. The antioxidant properties, anti-inflammatory and pleiotrophic properties have decreased the neurotoxicity produced by diet rich in high fat. The present study is consistent with the findings of previous research. In our study we proved that by using golgi staining and counting the number of dendritic intersections and branching points.

7. Source of Funding

8. Conflict of Interest

Authors declares no conflict of interest.

Acknowledgements

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