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Original Research Article

Neuroprotective effect of *Ginkgobiloba* and ascorbic acid in brain against high fat diet and stress induced neurotoxicity

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ABSTRACT

Objective: Chronic exposure to stress and diet rich in saturated fat is one of the major reasons for the development of dementia and neurodegenerative disorders. The present study aims to examine the neuroprotective potential of *Ginkgobiloba* and Ascorbic acid against high fat diet and stress induced neurotoxicity in brain.

Materials and Methods: Animals were randomly divided into five groups. Group I received normal diet, Group II received high fat diet along with stress, Group III were treated with *Ginkgobiloba* 100mg/kg body weight, and Group IV were treated with Ascorbic acid 100mg/kg body weight, Group V were treated with *Ginkgobiloba* 100mg/kg body weight and Ascorbic acid 100mg/kg body weight. After the treatment all rats were sacrificed and brains were removed. Golgi staining was done and dendritic branching points and dendritic intersections were quantified with the help of cameralucida.

Results: There was a significant increase in dendritic length and branching points was observed in brain in rats treated with *Ginkgobiloba* and Ascorbic acid.

Conclusion: Present study concludes that *Ginkgobiloba* and Ascorbic acid have neuroprotective role against high fat diet and stress induced Wistar rats.

1. Introduction

Stress and western diets are one of the main causative factors for neurodegenerative diseases and dementia.[1,2] The no of people suffering with dementia in the world have more than doubled from 1990 to 2016.[3] Alzhemiers disease is the major cause of dementia accounting to 60 to 80 percent of total cases.[4] Studies have shown that stress and diets rich in saturated fat have led to cognitive decline and dementia.[5,6]

Ginkgobiloba is a Chinese medicinal plant, which has been used in treating neurological disorders and for memory impairment.[7] It has antioxidant, anti-apoptopic, and free radical scavenging and neuroregeneration properties.[8] The benefit of *Ginkgobiloba* is attributed to its two active chemical components, flavonoids and terpenoids.[9] Apart from that *Ginkgobiloba* has been proved to augment cholinergic system and increase the synaptic plasticity in central nervous system.[10,11]

Ascorbic acid is anantioxidant found in citrus fruits and vegetables and strawberries.[12] Studies have shown that Vitamin C enhanced the expression of genes involved in neurotransmission,[13] neurogenesis,[14] and maturation of embryonic stem cells of neurons.[15] Recent studies proved that the free radical scavenging properties of ascorbic acid enhanced learning and memory in rats.[16]

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Very few studies have addressed the changes in dendritic morphology of brain with exposure to stress along with intake of high saturated diet and the neuroprotective role of both *Ginkgobiloba* and Ascorbic acid against the toxicity caused on dendrites by stress and high saturated diet. With this back ground the present study was undertaken to explore the dendritic changes in various regions of brain.

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 ± 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 five groups with six animals in each group. The total duration of the study for 3 months.

1. **Group I**: Control animals received ad libitum diet and water (NC)
2. **Group II**: Fed with diet rich in high fat diet for three months and induced stress for 21 days (69^{th} -90^{th} day) (H+S).
3. **Group III:** Treated with *Ginkgobiloba* 100mg/kg.b.w for 15 days (75^{th}- 90^{th} day) (H+S+G).
4. **Group IV:** Treated with Ascorbic acid 100mg/kg.b.wfor 15 days (75^{th}- 90^{th} day) (H+S+C).
5. **Group V:** Treated with *Ginkgobiloba* 100mg/kg.b.w for 15 days and Ascorbic acid 100mg/kg.b.wt for 15 days (75^{th}- 90^{th} day) (H+S+G+C 100mg/kg).

3.1. Administration of high fat diet

Hyperlipidemia was produced by feeding with cholesterol-rich high-fat diet (H) 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.[17]

3.2. Induction of stress

Male wistar rats were placed in a wire mesh restrainer for 21 days, daily 6 hours. The restrainer was made up of a wooden base to which stainless steel wire mesh was hinged. A pad lock and latch were used to secure the rat. The dimensions were 8 cm (Length) x 4cm (Breadth) x 4 cm (Height). This type of wire mesh restrainer can only restrict the animal movement without any uneasiness, pain or suffocation.[18]

3.3. Administration of Ginkgobiloba

100 mg of *Ginkgobiloba* extract was dissolved in 1% gum acacia solution. This solution was administered to the animals at 100 mg/kg body weight with the help of oral gavage needle attached to a syringe for 15 days.[19]

3.4. Administration of ascorbic acid

200mg of Ascorbic acid was dissolved in the 20 ml of ordinary tap water, administered to the animals at the dose of 100mg/kg body weight with oral feeding needle.[19]

3.5. Golgi staining procedure

After 90 days of completion of experimental period, 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.[18]

3.6. 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.

3.7. 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 cameralucida-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.[18]

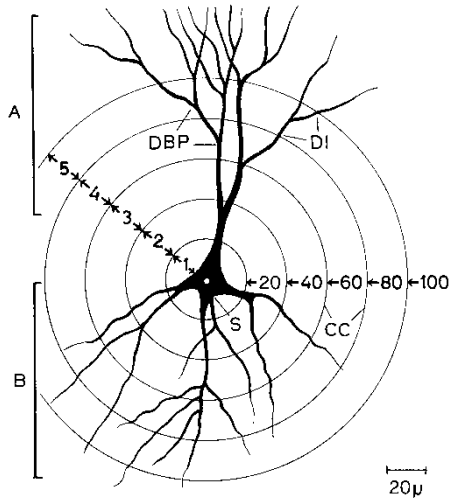

Fig. 1: Diagram showing a hippocampal CA3 neuron and the scheme of dendritic quantification. A transparent sheet with concentric circles of 20μ increment in radius (20μ-100μ) was placed on the camera lucida traced neuron so that the center of concentric circle coincides with the center of the cell body. Dendritic intersections at each concentric circle and dendritic branching points in each concentric zone werecounted. A- Apical dendrites, B- Basal dendrites, DBP- Dendritic branching points, DI- Dendritic intersections, S- Soma, CC- Concentric circles and 1 to 5- Concentric zones

3.8. 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. Apical dendritic intersections of CA3 neurons: Figures 2 and 6

Rats in H+S group showed significantly less number of apical intersections compared to NC 40, 60, 80 and 100 ($p<0.001$). Rats treated with only *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed significant increase in number of apical dendritic intersections compared to H+S. (H+S versus H+S+G): 60, 80 ($p<0.01$) and 100($p<0.05$); (H+S versus H+S+C): 40, 80, 100 ($p<0.01$) and 60 ($p<0.001$). Rats treated with both *Ginkgobiloba* and Ascorbic acid showed higher significance in increase in number of apical dendritic intersections compared to H+S group.(H+S versus H+S+G+C):40, 60, 80 and 100($p<0.001$).

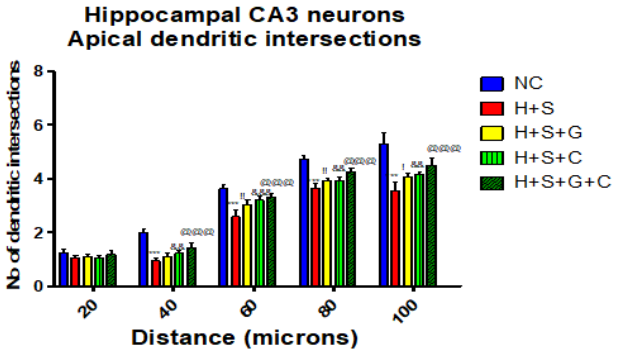

Fig. 2: Apical dendritic intersections of CA3 neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: $^{***}p<0.001$; H+S versus. H+S+ G:$^{!}p<0.05$,$^{!!}p<0.01$; H+S versus H+S+ C: $^{\&\&\&}p<0.001$,$^{\&\&}p<0.01$; H+S versus H+S+G+C $^{@@@}$ $p<0.001$ (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

4.2. Basal dendritic intersections of CA3 neurons Figures 3 and 6

Rats in H+S group showed significantly less number of dendritic intersections compared to NC 40, 60, 80 ($p<0.001$)and 100($p<0.01$). Rats treated with only *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed significant increase in number of dendritic intersections (H+S versus H+S+G): 60, 80 ($p<0.05$); (H+S versus H+S+C): 60,100 ($p<0.05$) and 80 ($p<0.001$). Rats treated with both Ginkgobiloba and Ascorbic acid showed higher significance in increase in number of dendritic intersections compared to H+S group.(H+S versus H+S+G+C):40, 80, ($p<0.001$), 60 ($p<0.01$and 100($p<0.05$).

4.3. Apical dendritic branching points of CA3 neurons Figures 4 and 6

A significantly less number of apical dendritic branching points were observed in H+S group compared to NC. 20-40, 80-100 ($p<0.001$) and 60-80 ($p<0.01$). A significant increase in number of apical dendritic branching points zones was observed in the rats treated with both *Ginkgobiloba* 100 mg/kg and Ascorbic acid 100mg/kg compared to H+S groups. H+S versus H+S+G+C):20-40, 60-80, 80-100 ($p<0.01$).

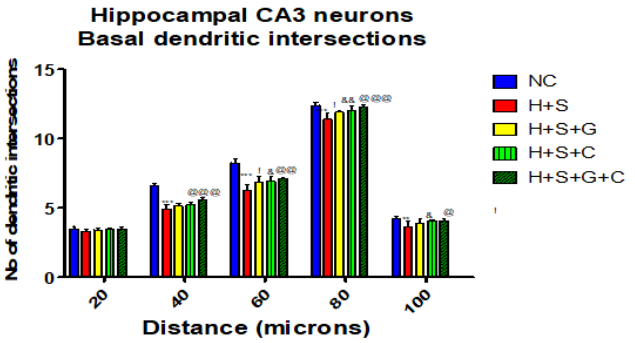

Fig. 3: Basal dendritic intersections of CA3 neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: ***p<0.001, ** p<0.01; H+S versus. H+S+ G:$^{!}$p<0.05; H+S versus H+S+ C: &&p<0.01, & p<0.05; H+S versus H+S+G+C $^{@@@}$ p<0.001,$^{@@}$ p<0.01$^{@}$ p<0.05 (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

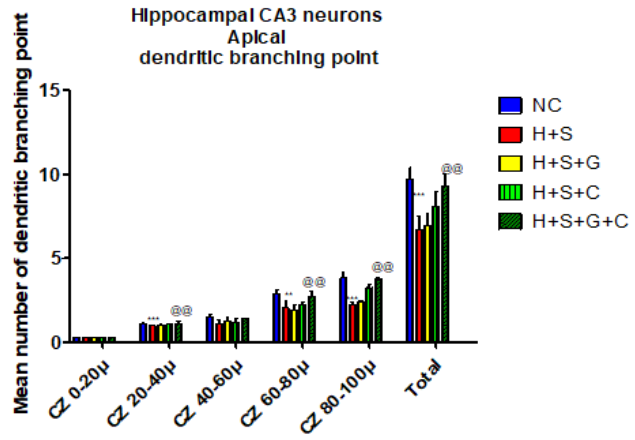

Fig. 4: Apical dendritic branching points of CA3 neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: *** p<0.001,** p<0.01; H+S versus H+S+G+C $^{@@}$ p<0.001 (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, Ascorbic acid 100 mg/kg body wt

4.4. Basal dendritic branching points of CA3 neurons Figures 5 and 6

A significantly less number of basal dendritic branching points were observed in the H+S group of rats compared to NC group. 20-40, 40-60, 60-80 and 80-100 (p<0.001). A significant increase in number of basal dendritic branching points was observed in the rats treated with only *Ginkgobiloba* 100 mg/kg (H+S versus H+S+G) 20-40, 60-80 (p<0.01) and 80-100 (p<0.001) and Ascorbic acid 100mg/kg compared to H+S group. (H+S versus H+S+C):20-40, 40-60, 60-80, 80-100 (p<0.01).

Rats treated with combination of both *Ginkgobiloba* and Ascorbic acid had higher significant increase in dendritic branching points when compard to H+S group. (H+S versus H+S+G+C):20-40, 40-60, 60-80, 80-100 (p<0.001).

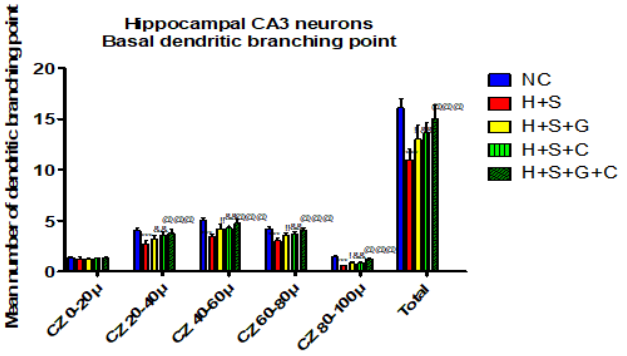

Fig. 5: Basal dendritic branching points of CA3 neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: *** p<0.001,** p<0.01; H+S versus H+S+G+C $^{@@}$ p<0.001 (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt

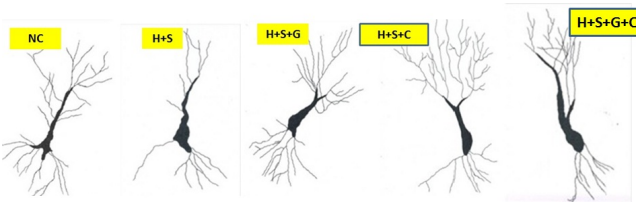

Fig. 6: Camera lucida tracings of CA3 neurons of Hippocampus showing the dendritic arborization in different groups (Golgi Cox staining). NC: normal control; H+S: High fat diet + Stress; G: *Ginkgobiloba* 100mg/kg; C: Ascorbic acid 100 mg/kg Note (i) increase in dendritic arborization in H+G & H+C compared to H+S group (ii) Significant increase in dendritic arborization in H+G+C compared to H+S group

4.5. Apical dendritic intrsections of Basolateral Amygdaloid neurons: Figures 7 and 9

Rats in H+S group showed significantly less number of apical dendritic intersections compared to NC 20, 40, 60, 80 (p<0.001) and 100(p<0.01). Rats treated with only Ginkgo biloba 100mg/kg showed significant increase in number of apical intersections compared to H+S. (H+S versus H+S+G): 20, 40, 60, (p<0.05), and 80(p<0.01). Rats treated with Ascorbic acid 100mg/kg also showed significant increase in number of apical intersections compared to H+S group. (H+S versus H+S+C): 20, 60, (p<0.05), 40, and 80(p<0.01). Rats treated with both *Ginkgobiloba* and Ascorbic acid showed higher significance in increase in

number of intersections compared to H+S group. (H+S Vs H+S+G+C):20. 40, 80 ($p<0.001$) 60 ($p<0.01$) and ($p<0.05$) 100.

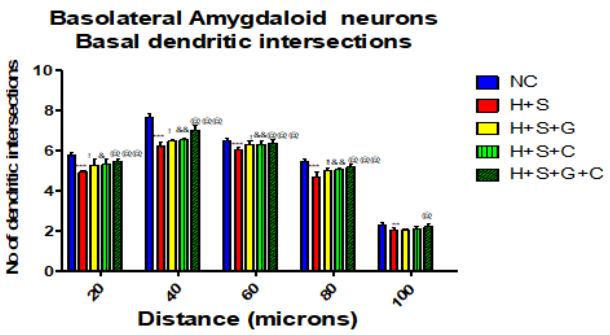

Fig. 7: Apical dendritic intersections of Basolateral Amygdaloid neurons; Mean± SD is shown, n = 6 in each group. NC versus H+S: *** $p<0.001$, **$p<0.01$; H+S versus. H+S+ G:$^{!}p<0.05$,$^{!!}p<0.01$; H+S versus H+S+ C: $^{\&}$ $p<0.05$,$^{\&\&}p<0.01$; H+S versus H+S+G+C $^{@}$ $p<0.05$,$^{@@}$ $p<0.01$,$^{@@@}p<0.001$ (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat dietplus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100mg/kg body wt

4.6. Basal dendritic points of Basolateral Amygdaloid neurons: Figures 8 and 9

H+S group of rats showed significantly less number of basal dendritic branching points at 20-40, 40-60, 80-100 ($p<0.01$) and 60-80 ($p<0.001$) zones compared to NC group. Rats that were administered only Ascorbic acid 100mg/kg showed significant increase in number of basal dendritic branching points compared to H+S group (H+S versus H+S+C): 80-100 ($p<0.05$). Rats treated with combination of *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg also showed significant increase in dendritic branching points. (H+S versus H+S+G+C): 20-40, 40-60, 80-100 ($p<0.05$).

4.7. Apical dendritic intersections of Motor cortex neurons: Figures 10 and 14

Rats in H+S group showed significantly less number of apical intersections compared to NC 40, 60, 80 and 100 ($p<0.001$). Rats treated with only *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed significant increase in number of dendritic intersections compared to H+S. (H+S versus H+S+G): 40, 60, 80 ($p<0.05$) and 100($p<0.01$); (H+S versus H+S+C): 40, ($p<0.05$) 60, 80 and 100 ($p<0.01$). Rats treated with combination of *Ginkgobiloba* and Ascorbic acid showed higher significance in increase in number of apical dendritic intersections compared to H+S group.(H+S versus H+S+G+C):40, ($p<0.01$) 60, 80 and 100 ($p<0.001$).

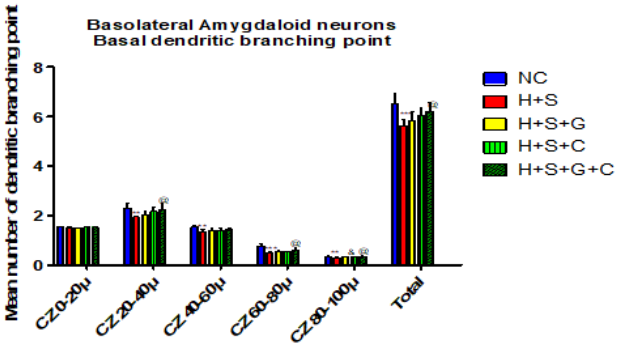

Fig. 8: Basal dendritic branching points of Basolateral Amygdaloid neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: *** $p<0.001$,** $p<0.01$; H+S versus H+S+C $^{\&}$ $p<0.05$; H+S versus H+S+G+C $^{@}$ $p<0.05$. (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba*100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt

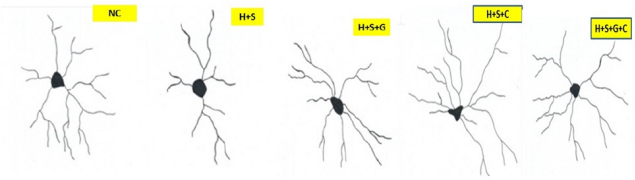

Fig. 9: Camera lucida tracings of Basolateral Amygdaloid neurons showing the dendritic arborization in different groups (Golgi Cox staining). NC: normal control; H+S: High fat diet + Stress; G: *Ginkgobiloba* 100mg/kg; C: Ascorbic acid 100 mg/kg Note (i) increase in dendritic arborization in H+G & H+C compared to H+S group (ii) Significant increase in dendritic arborization in H+G+C compared to H+S group

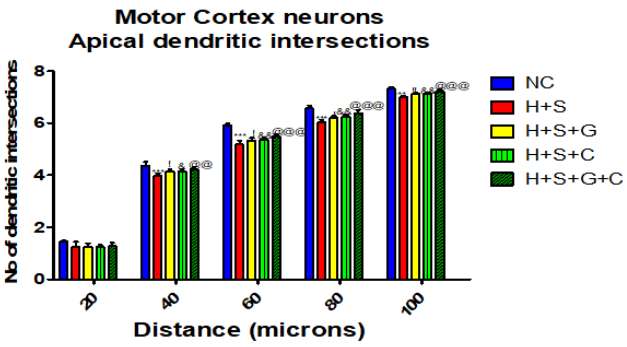

Fig. 10: Apical dendritic intersections of Motor Cortex neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S: *** $p<0.001$; H+S versus. H+S+ G:$^{!}p<0.05$,$^{!!}p<0.01$; H+S versus H+S+ C: $^{\&\&}$ $p<0.001$,$^{\&}p<0.05$; H+S versus H+S+G+C $^{@@}$ $p<0.01$ $^{@@@}$ $p<0.001$ (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

4.8. Basal dendritic intersections of Motor cortex neurons: Figures 11 and 14

Rats in H+S group showed significantly less number of basal dendritic intersections compared to NC20, 80 ($p<0.01$)40, 60 and 100 ($p<0.001$). Rats treated with only *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed significant increase in number of dendritic intersections compared to H+S. (H+S versus H+S+G): 40, and 60 ($p<0.05$); (H+S versus H+S+C): 40 and 60 ($p<0.01$). Rats treated with combination of *Ginkgobiloba* and Ascorbic acid showed higher significance in increase in number of apical dendritic intersections compared to H+S group.(H+S versus H+S+G+C):40, 100 ($p<0.01$) and 60 ($p<0.001$).

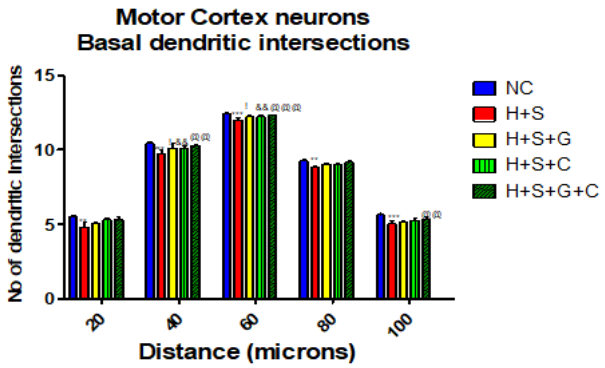

Fig. 11: Basal dendritic intersections of Motor Cortex neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S:** $p<0.01$ *** $p<0.001$; H+S versus. H+S+ G:! $p<0.05$; H+S versus H+S+ C: && $p<0.001$; H+S versus H+S+G+C @@ $p<0.01$ @@@ $p<0.001$ (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

4.9. Apical dendritic branching points of Motor Cortex neurons: Figures 12 and 14

A significantly less number of apical dendritic branching points were observed in H+S group compared to NC.20-40, 60-80, ($p<0.001$) and 80-100 ($p<0.01$). A significant increase in number of apical dendritic branching points zones was observed in the rats treated with only *Ginkgobiloba* 100 mg/kg and Ascorbic acid 100mg/kg compared to H+S groups. (H+S versus H+S+G): 60-80, 80-100 ($p<0.05$); (H+S versus H+S+C): 20-40, 60-80, ($p<0.01$) and 80-100 ($p<0.05$). Rats treated with combination of *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed higher significance in no of dendritic branching points.(H+S versus H+S+G+C):20-40, 60-80 and 80-100 ($p<0.01$).

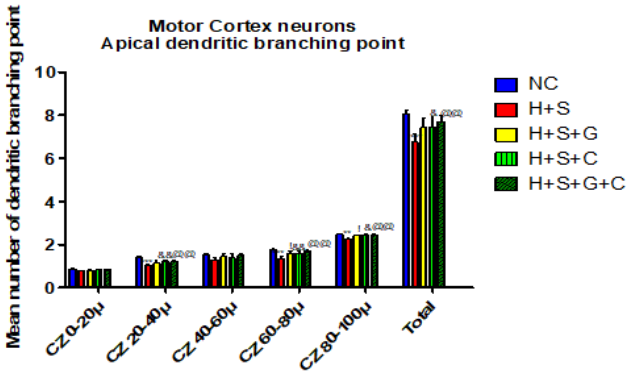

Fig. 12: Apical dendritic branching points of Motor Cortex neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S:** $p<0.01$ *** $p<0.001$; H+S versus. H+S+ G:! $p<0.05$; H+S versus H+S+ C: & $p<0.05$, && $p<0.001$; H+S versus H+S+G+C @@ $p<0.01$ (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

4.10. Basal dendritic branching points of Motor Cortex neurons: Figures 13 and 14

A significantly less number of apical dendritic branching points were observed in H+S group compared to NC.20-40, 60-80, ($p<0.001$) and 80-100 ($p<0.01$). A significant increase in number of apical dendritic branching points zones was observed in the rats treated with only *Ginkgobiloba* 100 mg/kg and Ascorbic acid 100mg/kg compared to H+S groups. (H+S versus H+S+G): 20-40, 40-60 and 60-80 ($p<0.05$); (H+S versus H+S+C): 20-40, 60-80, ($p<0.01$) and 40-60 ($p<0.05$). Rats treated with combination of *Ginkgobiloba* 100mg/kg and Ascorbic acid 100mg/kg showed higher significance in no of dendritic branching points.(H+S versus H+S+G+C):20-40, 60-80 ($p<0.001$), 40-60 and 80-100 ($p<0.01$).

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5. Discussion

Our study explored the neuroprotective role of *Ginkgobiloba* and Ascorbic acid against the high fat diet and stress induced neurotoxicity. The rats treated with *Ginkgobiloba* 100mg and Ascorbic acid 100mg showed significant increase in dendritic length and branching points but when given in combination there was higher significance in increase in dendritic length. The beneficial effects of *Ginkgobiloba* can be attributed to it pleitrophic effects.[20]

Ginkgobiloba have ameliorated the impairment of learning and memory abilities of rats induced by repeated high sustained +Gz (Positive acceleration) exposure.[21] There is a clear line of evidence that *Ginkgobiloba* have improved the memory and learning deficits in rats induced

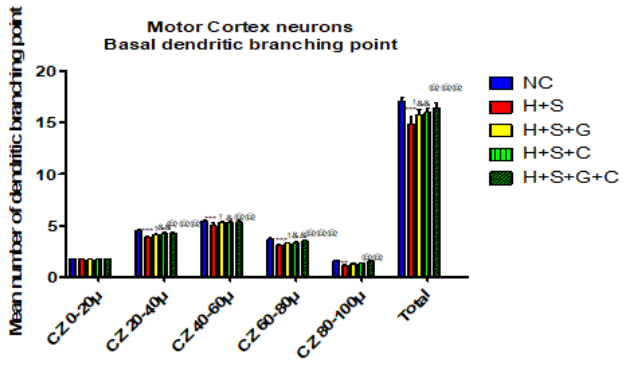

Fig. 13: Basal dendritic branching points of Motor Cortex neurons; Mean ± SD is shown, n = 6 in each group. NC versus H+S:** p<0.01 *** p<0.001; H+S versus. H+S+ G:$^{!}$p<0.05; H+S versus H+S+ C: $^{\&}$ p<0.05,$^{\&\&}$ p<0.001; H+S versus H+S+G+C $^{@@}$ p<0.01,$^{@@@}$ p<0.001 (One way ANOVA, Bonferroni's test). NC: Control, H+S: High fat diet plus Stress, G: *Ginkgobiloba* 100 mg/kg body wt, C: Ascorbic acid 100 mg/kg body wt.

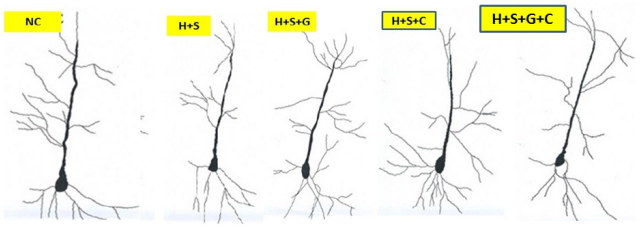

Fig. 14: Camera lucida tracings of Motor Cortex neurons showing the dendritic arborization in different groups (Golgi Cox staining). NC: normal control; H+S: High fat diet + Stress; G: *Ginkgobiloba* 100mg/kg; C: Ascorbic acid 100 mg/kg Note (i) increase in dendritic arborization in H+G & H+C compared to H+S group (ii) Significant increase in dendritic arborization in H+G+C compared to H+S group

by various stimuli such as chronic stress,[22] fluoride,[23] amyloid,[24] aluminum,[25] scopolamine,[26] ischemia[24] and aging.[25]

The cognitive enhancing properties of *Ginkgobiloba* can be ascribed to its potential effect on cholinergic system and neurotransmitters in brain.[26] Few studies have shown that it has improved the microcirculation in brain by modulating the nitric oxide levels[27] and by antagonizing the overproduction of endothelin-1 in the blood vessels.[28] The beneficial action is mainly due to its antioxidant properties of the flavonoids present in it, for instance rats treated with *Ginkgobiloba* for fourteen days was found to enhance the antioxidant levels in hippocampus against sodium nitrite induced neurotoxicity.[29] In another experiment it has reversed the mitochondrial dysfunction of hippocampi and platelets in middle aged ovariectomized rats.[30]

While the neuroprotective role of Gingkobiloba on dendrites of brain have been documented in very few studies for instance, aged rats treated with treated with *Ginkgobiloba* for eight weeks have shown increase in dendritic length and segments in CA1and CA3 regions of hippocampi.[31] Barkats et al,[32] reported that treatment with EGb 761 (50 mg/kg/day) for 7 months led to a significant increase in the projection field of intra- and infrapyramidal mossy fibers in the CA3 region of hippocampus. Dae Young Yoo[33] et al, observed a significant increase in tertiary dendrites in hippocampus along with neuroblast differentiation in Dentate Gyrus after administration of *Ginkgobiloba* 40 mg/kg and 100mg/kg for 28 days. However Tchantchou et al[34] did not find any significant change in the dendritic pattern in Alzheimer's mice model when treated with *Ginkgobiloba* for six months. This discrepancy in the result may be related to the transgenic mouse model used in the experiment.

The neuroprotective effect of Ascorbic acid is due to its free radical scavenging and antioxidant properties.[35] Many studies have revealed that Ascorbic acid have reduced the neurodegeneration caused by ischemia and exitotoxicity.[36–38] Vitamin C protected the lead induced apoptosis in hippocampus[39] and also attenuated the toxic effects of ethanol on neuroglial cells in brain.[40,41]

In guinea pigs, Ascorbic acid deficiency have reduced the hippocampal volume and decreased the neuronal migration in dentate gyrus[38,42–44] Furthermore invitro studies have shown that on Ascorbic acid recycling have regulated the growth in neurite of brain[43] and enhanced the neural stem cells in the Midbrain by enhancing the generation of Midbrain type dopamine (mDA). However in another experiment treatment with Ascorbic acid did not alter hippocampal neuronal morphology or synaptic plasticity in CA1 region of young guinea pigs.[44] The difference in the result can be attributed to the area of hippocampus selected. In that experiment the author have only observed the changes of the dendritic morphology in CA1 region only.

6. Conclusion

In conclusion we observed that there was a higher significance in increase in dendritic brancing points and intersections in the brain in groups which were treated with both *Ginkgobiloba* and Ascorbic acid. Our findings are consistent with the findings of previous research. However the precise mechanisms responsible for this efficacy of *Ginkgobiloba* and Ascorbic acid have to be investigated in detail. Findings of our study provide a neuroanatomical basis and support therapeutic potential of *Ginkgobiloba* and Ascorbic acid in neurodegenerative diseases.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare no conflict of interest.

References

1. Qizilbash N, Gregson J, Johnson ME, Pearce N, Douglas I, Wing K, et al. BMI and risk of dementia in two million people over two decades: a retrospective cohort study. *Lancet Diabetes Endocrinol*. 2015;3(6):431–6.
2. Song H, Sieurin J, Wirdefelt K, Pedersen NL, Almqvist C, Larsson H. Association of stress-related disorders with subsequent neurodegenerative diseases. *JAMA Neurol*. 2020;77(6):700–9. doi:10.1001/jamaneurol.2020.0117.
3. Regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study. *Lancet Neurol*. 2016;18(1):88–106.
4. 2020 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2020;16(3):391–460.
5. Justice NJ. The relationship between stress and Alzheimer's disease. *Neurobiol Stress*. 2018;8:127–133.
6. Mielke MM, Zandi PP, Shao H. The 32-year relationship between cholesterol and dementia from midlife to late life. *Neurology*. 2010;75(21):187–98.
7. Singh SK, Srivastav S, Castellani RJ, Plascencia-Villa G, Perry G. Neuroprotective and Antioxidant Effect of Ginkgo biloba Extract Against AD and Other Neurological Disorders. *Neurotherapeutics*. 2019;16(3):666–74.
8. Feng Z, Sun Q, Chen W. The neuroprotective mechanisms of ginkgolides and bilobalide in cerebral ischemic injury: a literature review. *Mol Med*. 2019;25:57–60.
9. Oken BS, Storzbach DM, Kaye J. A - The efficacy of Ginkgo biloba on cognitive function in Alzheimer disease. *Arch Neurol*. 1998;55(11):1409–15.
10. Winkler J, Thal LJ, Gage FH, Fisher LJ. Cholinergic strategies for Alzheimer's disease. *J Mol Med (Berl)*. 1998;76(8):555–67.
11. Freo U, Pizzolato G, Dam M, Ori C, Battistin L. A short review of cognitive and functional neuroimaging studies of cholinergic drugs: implications for therapeutic potentials. *J Neural Transm*. 2002;109:857–870.
12. Kumar A, Saini RV, Saini AK. Neuroprotective role of ascorbic acid: antioxidant and non-antioxidant functions. *Asian J Pharmaceutical Clin Res*. 2018;11:30–3.
13. Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med*. 2009;46(6):719–30.
14. Kratzing CC, Kelly JD. Tissue levels of ascorbic acid during rat gestation. *Int J Vitam Nutr Res*. 1982;52:326–32.
15. Shin DM, Ahn JI, Lee KH, Lee YS, Lee YS. Ascorbic acid responsive genes during neuronal differentiation of embryonic stem cells. *Neuroreport*. 2004;15:1959–63.
16. Salehi I, Soleimani MS, Pourjafar M, Moravej FG, Soleimani S. Vitamin C Can Alter Lead-Induced Passive Avoidance Learning Impairment in Rats. *Anat Sci*. 2015;12(3):137–40.
17. Kumar V, Singh S, Khanna AK, Khan MM, Chander R, Mahdi F, et al. Hypoliedimic activity of anthocephalus indicus (kadam) in hyperlidemic rats. *Med Chem Res*. 2008;17(2-7):152–8.
18. Rao BSS, Madhavi R, Sunanda, Raju TR. Complete reversal of dendritic atrophy in CA3 neurons of hippocampusby rehabilitation in restraint stressed rats. *Curr Sci*. 2001;80(5):653–9.
19. Jetti R, Raghuveer CV, Rao MC, Somayaji SN, Babu BP. Neuroprotective effect of Ascorbic acid and Ginkgo biloba against Fluoride caused Neurotoxicity. *J Environ Sci Toxicol Food Technol*. 2014;8(1):30–6.
20. Siegel G, Jumar A, Mey C, Ermilov E. Pleiotropic effects of Ginkgo biloba: implication in blood pressure regulation. *Hypertension*. 2012;60:A301. doi:10.1161/hyp.60.suppl_1.A301.
21. Chen LE, Wu F, Zhao A, Ge H, Zhan H. Protection Efficacy of the Extract of Ginkgo biloba against the Learning and Memory Damage of Rats under Repeated High Sustained +Gz Exposure. *Evid Based Complement Alternat Med*. 2016;2016:6320586. doi:10.1155/2016/6320586.
22. Walesiuk A, Trofimiuk E, Braszko JJ. Ginkgo biloba normalizes stress- and corticosterone-induced impairment of recall in rats. *Pharmacol Res*. 2006;53(2):123–8.
23. Raju S, Sivanesan SK, Gudemalla K. Cognitive enhancement effect of Ginkgo biloba extract on memory and learning impairments induced by fluoride neurotoxicity. *Int J Res Pharm Sci*. 2019;10(1):129–34.
24. Tang F, Nag S, Shiu SYW, Pang SF. The effects of melatonin and Ginkgo biloba extract on memory loss and choline acetyltransferase activities in the brain of rats infused intracerebroventricularly with β-amyloid 1-40. *Life Sciences vol*. 2002;71(22):2625–31.
25. Wang Y, Wang L, Wu J, Cai J. The in vivo synaptic plasticity mechanism of EGb 761-induced enhancement of spatial learning and memory in aged rats. *Br J Pharmacol*. 2006;148(2):147–53.
26. Naik SR, Pilgaonkar VW, Panda VS. Neuropharmacological evaluation of Ginkgo biloba phytosomes in rodents. *Phytother Res*. 2006;20(10):901–5.
27. Sun BL, Xia ZL, Yang MF, Qiu PM. Effects of Ginkgo biloba extract on somatosensory evoked potential, nitric oxide levels in serum and brain tissue in rats with cerebral vasospasm after subarachnoid hemorrhage. *Clin Hemorheol Microcirc*. 2000;23:139–44.
28. Sun BL, Zhang J, Xia WXC, Zhang SM, WJ Y, Yuan H. Effects of extract of Ginkgo biloba on spasms of the basilar artery and cerebral microcirculatory perfusion in rats with subarachnoid hemorrhage. *Clin Hemorheol Microcirc*. 2003;29:231–8.
29. Naik S, Pilgaonkar VW, Panda VS. Evaluation of antioxidant activity of Ginkgo biloba phytosomes in rat brain. *Phytother Res*. 2006;20(11):1013–6.
30. Shi C, David LF, Yew DT, Fang L, Yao Z, Xu J. Ginkgo biloba extract EGb761 protects against mitochondrial dysfunction in platelets and hippocampi in ovariectomized rats. *Platelets*. 2010;21(1):53–9.
31. Hosseini-Sharifabad M, Anvari M. Effects of Ginkgobiloba extract on the structure of Cornu Ammonis in aged rat: A morphometric study. *Iran J Basic Med Sci*. 2015;18(9):932–7.
32. Barkats M, Venault P, Christen Y, Cohen-Salmon C. Effect of long-term treatment with EGb 761 on age-dependent structural changes in the hippocampi of three inbred mouse strains. *Life Sci*. 1995;56:213–22.
33. Yoo DY, Nam Y, Kim W, Yoo KY, Park J, Lee CH, et al. Effects of Ginkgo biloba extract on promotion of neurogenesis in the hippocampal dentate gyrus in C57BL/6 mice. *J Vet Med Sci*. 2011;73(1):71–6.
34. Tchantchou F, Xu Y, Wu Y, Christen Y, Luo Y. EGb 761 enhances adult hippocampal neurogenesis and phosphorylationof CREB in transgenic mouse model of Alzheimer's disease. *FASEB J*. 2007;21:2400–8.
35. Njus D, Kelley PM, Tu YJ, Schlegel HB. Ascorbic acid: The chemistry underlying its antioxidant properties. *Free Radic Biol Med*. 2020;159:37–43.
36. MacGregor DG, Higgins MJ, Jones PA, Maxwell WL, Watson MW, Graham DI, et al. Ascorbate attenuates the systemic kainate-induced neurotoxicity in the rat hippocampus. *Brain Res*. 1996;727(1-2):133–44.
37. Majewska MD, Bell JA, London ED. Regulation of the NMDA receptor by redox phenomena: inhibitory role of ascorbate. *Brain Res*. 1990;537(1-2):328–32.
38. Lykkesfeldt J, Trueba GP, Poulsen HE, Christen S. Vitamin C deficiency in weanling guinea pigs: Differential expression of oxidative stress and DNA repair in liver and brain. *Br J Nutr*. 2007;98:1116–9.
39. Han JM, Chang BJ, Li TZ, Choe NH, Quan FS, Jang BJ, et al. Protective effects of ascorbic acid against lead-induced apoptotic neurodegeneration in the developing rat hippocampus in vivo. *Brain Res*. 2007;1185:68–74.
40. Naseer MI, Najeebullah, Ikramullah, Zubair H, Hassan M, Yang BC, et al. Vitamin-C protect ethanol induced apoptotic neurodegeneration in postnatal rat brain. *Pak J Med Sci*. 2009;25(9):718–22.

41. Sánchez-Moreno C, Paniagua M, Madrid A, Martín A. Protective effect of vitamin C against the ethanol mediated toxic effects on human brain glial cells. *J Nutr Biochem*. 2003;14(10):606–13.
42. Espinoza F, Magdalena R, Saldivia N, Jara N, Martínez F, Ferrada L, et al. Vitamin C Recycling Regulates Neurite Growth in Neurospheres Differentiated In Vitro. *Antioxidants*. 2020;9(12):1276.
43. Wulansari N, Kim EH, Sulistio YA, Rhee YH, Song JJ, Lee SH. Vitamin C-Induced Epigenetic Modifications in Donor NSCs Establish Midbrain Marker Expressions Critical for Cell-Based Therapy in Parkinson's Disease. *Stem Cell Reports*. 2017;9(4):1192–1206.
44. Hansen SN, Jørgensen JMB, Nyengaard JR, Lykkesfeldt J, Tveden-Nyborg P. Early Life Vitamin C Deficiency Does Not Alter Morphology of Hippocampal CA1 Pyramidal Neurons or Markers of Synaptic Plasticity in a Guinea Pig Model. *Nutrients*. 2018;10(6):749.

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