

# NOOTROPIC ACTION OF GLYCYRRHIZA GLABRA ROOT EXTRACT ON THE DENDRITIC MORPHOLOGY OF HIPPOCAMPAL CA1 NEURONS IN ONE MONTH OLD RATS

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Abstract Our earlier studies have shown that Glycyrrhiza glabra (family: Leguminosae) aqueous root extract treatment in Wistar albino rats enhances both spatial learning ability and retention of learned tasks accordingly, the present study was designed to investigated the nootropic action of aqueous root extract of Glycyrrhiza glabra treatment on the dendritic morphology (dendritic arborization and dendritic intersections) of hippocampal CA1 neurons in one month old male Wistar albino rats. Methods: The aqueous root extract of Glycyrrhiza glabra was administered orally in four doses (75, 150, 225 and 300 mg/kg) for 6 weeks. After the treatment period, all experimental animals were subjected to spatial learning (Morris water maze, Hebb-William's maze and elevated plus maze) tests. At the end of the spatial memory tests, the rats were deeply anesthetized with Pentobarbitone and killed their brains were removed rapidly and fixed in rapid Golgi fixative. Hippocampal CA1 neurons were traced using Camera lucida, and dendritic arborization and intersections were quantified. These data were compared with those of age-matched control rats. Results: Results showed that all the doses of aqueous root extract of Gg significantly enhanced dendritic arborization (dendritic branching points) and dendritic intersections however in the dose of 150 and 225 mg/kg/p.o showed a significant (p<0.01) enhancement of dendritic arborization and dendritic intersections along the length of both apical and basal dendrites in hippocampal CA1 pyramidal neurons is comparable to control. Conclusion: Based on our results obtained, we conclude that constituents present in aqueous root extract of Glycyrrhiza glabra have neuronal dendritic growth stimulating properties.

Keywords: Camera lucida, dendritic arborization, Glycyrrhiza glabra, Hippocampal CA1 neurons.

#### INTRODUCTION

The hippocampus is a major component of the brain of humans and other mammals located bilaterally in the medial temporal lobe, underneath the cortical surface. It belongs to the limbic system and plays important roles in long-term memory and spatial navigation.

Traditional herbal extracts have been used to enhancing learning and memory.<sup>[14]</sup> In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been in clinical use for centuries. Though there are studies revealing the effect of *Glycyrrhiza glabra* [Gg] in improving learning and memory <sup>[5, 6]</sup> but the nature of neurons in the brain particularly hipp ocampal neuron is not studied. Accordingly, the present study was designed to study the nootropic action of Gg root extract on rat hipp ocampal neurons particularly the CA1subregion of the hipp ocampus.

#### MATERIALS AND METHODS

#### Plant material:

The roots of Gg were purchased from a local ayuredic store in Udupi, Karnataka, India during

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Kosuri Kalyan Chakravarthi, Department of Anatomy, Santhiram Medical College, NH-18, Nandyal-518501, Kurnool District, Andhra Pradesh, India 2/4/2012. The material was authentified by the Dr. Krishna Kumar, Chairman, Department of applied Botany, Mangalore University.

#### Preparation of Aqueous root extract:

The crude aqueous extract of Gg was prepared by macerating dried powdered root with respective solvent for 24 h. The macerated powdered roots were then extracted by using soxhlet extractor for 36 h, 1-2 cycles per hour. The extract was dried and weighed. A brownish black waxy residue with 16% yield was obtained. This aqueous extract of Gg was administrated orally to separate groups of 1-month old male Wistar albino rats in four different doses 75, 150, 225 and 300 mg/kg respectively.

#### Animals:

The experimental protocol was approved during September 2011 and September 2012 by the Institutional Animals Ethics Committee (IAEC), Yenepoya University and care of laboratory animals was taken as per CPCSEA guidelines. Rats were housed individually (Animal house, Yenepoya University, Reg.no 347/CPCSEA) in polypropylene cages of standard dimensions (22.5 × 35.5 × 15 cm) and maintained at temperature (25°C ±2° C) and light (light



period, 08.00–20.00) in a controlled room with relative humidity of 50-55%. Food and water were provided *ad libitum*. Experiments were carried out between 09:00 and 14:00 h.

**Experimental Design:** Rats were randomly divided into eight groups.

- **Group I- Control (n=6):** A known volume of distilled water was administrated orally each day for 6 weeks.
- Group II- Diazepam control (n=6): Diazepam 7 mg/kg was injected i.p. 20 min before the test session.
- **Group III (n=6):** Received 75 mg/kg aqueous extract of Gg orally every day for 6weeks.
- Group IV (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 6weeks.
- Group V (n=6): Received 225 mg/kg aqueous extract of Gg orally every day for 6 weeks.
- **Group VI (n=6):** Received 300 mg/kg aqueous extract of Gg orally every day for 6 weeks.
- Group VII- Gg 150mg + Diazepam (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 6 weeks. Diazepam 7 mg/kg was injected i.p. 20 min before the test session.
- Group VIII- Gg 225mg + Diazepam (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 6 weeks. Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

n = number of animals.

#### Rapid Golgi staining procedure:

After the treatment period, all experimental animals were subjected to spatial learning (Morris water maze, Hebb-William's maze and elevated plus maze) tests. At the end of the spatial memory tests, the rats were deeply anesthetized with Pentobarbitome and killed; their brains were removed rapidly and fixed in rapid Golgi fixative. Tissues were processed for rapid Golgi staining.

Briefly, tissues were fixed for 5 days in Golgi fixative and impregnated with a 1.5% aqueous silver nitrate solution for 48 hours. Sledge microtome sections of  $120-\mu$ m thickness were excised, dehydrated, cleared and mounted with Distrin plasticizer xylene mounting media. <sup>[7]</sup>

#### Camera lucida tracing:

From each rat, 8-10 hippocampal CA1 pyramidal neurons were traced using *Camera lucida* and their dendritic branching points (a measure of dendritic arborization) and dendritic intersections (a measure dendritic length) were quantified.

### Quantification of dendritic branching points and dendritic intersections:

The concentric circle method of Sholl <sup>[8]</sup> was used for dendritic quantification. Concentric circles with a distance of 20  $\mu$ m between 2 adjacent concentric circles were drawn on a transparent sheet for quantification of dendritic branching points and dendritic intersections.

The number of branching points between the two concentric circles, i.e., within each successive 20-mm concentric zone (circle), was counted. The dendritic intersections point (a dendrite intersected a given concentric circle) at each concentric circle were counted. Basal dendritic branching points and intersections up to a radial distance of 120  $\mu$ m and apical dendritic branching points and intersections up to a radial distance of from the center of the cell body of the CA1 neuron. Mean number of apical and basal dendritic quantification (dendritic branching points and dendritic intersections) in each concentric zone were calculated.

#### Statistical Analysis:

Data was analyzed using ANOVA followed by Dunnett's multiple comparison test. p value < 0.05 were considered as statistically significant.

#### RESULTS

Figure-1, Figure-2, Figure-3 and Figure-4 illustrates *Camera lucida* tracings (A1, B1, C1, D1, E1, F1, G1 and H1) of Golgi-stained (silver nitrate impregnated) hippocampal CA1 pyramidal neurons (A, B, C, D, E, F, G and H) of control and different doses of the aqueous root extract of *Glycyrrhiza glabra* treated rats for 6 weeks.

**Figure.1:** Representative photomicrographs (A and B) and *Camera lucida* tracings (A1and B1) of Golgi-stained hippocampal CA1neurons.



A and A1-Control (Group I); B and B1- Diazepam control (Group II); Black arrow- Basal dendrites of hippocam pal CA1 neurons; Red arrow-Apical dendrites of hippocam pal CA1 neurons.

**Figure.2:** Representative photomicrographs (C and D) and *Camera lucida* tracings (C1and D1) of Golgi-stained hipp ocampal CA1 neurons.



C and C1- hippocampal CA1 neurons of rats treated with 75 mg/kg aqueous extract of *Glycyrrhiza glabra* orally every day for 6 weeks (Group III); D and D1- hippocampal CA1 neurons of rats treated with 150 mg/kg aqueous extract of *Glycyrrhiza glabra* orally every day for 6weeks (Group IV);

**Figure.3:** Representative photomicrographs (E and F) and *Camera lucida* tracings (E1and F1) of Golgi-stained hipp ocampal CA1 neurons.



E and E1- hippocampal CA1 neurons of rats treated with 225 mg/kg aqueous extract of Glycyrrhiza glabra orally every day for 6weeks (Group V); F and F1- hippocampal CA1 neurons of rats treated with 300 mg/kg aqueous extract of Glycyrrhiza glabra orally every day for 6 weeks (Group VI);

## Dendritic Quantification of Hippocampal CA1 pyramidal neurons:

The aqueous root extract of Gg in the dose of 150 and 225 mg/kg/p.o showed significantly (p<0.01) increased numbers of dendritic branching points and dendritic length along the length of both basal(o- $20\mu$ m, 20-40 $\mu$ m, 40-60 $\mu$ m, 60-80 $\mu$ m, 80-100 $\mu$ m and 100-120 $\mu$ m) and apical (0-20 $\mu$ m, 20-40 $\mu$ m, 40-60 $\mu$ m, 60-80 $\mu$ m, 80-100 $\mu$ m, 100-120 $\mu$ m, 120-140 $\mu$ m, 140-160 $\mu$ m and 160-180 $\mu$ m) dendrites in all the concentric zones is comparable to control rats [Table-1, Table- 2, Table-3 and Table-4].

**Figure.4:** Representative photomicrographs (G and H) and *Camera lucida* tracings (G1and H1) of Golgi-stained hippocampal CA1 neurons of rats treated with aqueous extract of *Glycyrrhiza glabra* (Gg) for 6 weeks.



G and G1- hippocampal CA1 neurons of rats treated with Gg150mg/kg/p.o+ Diazepam 7mg/kg/i.p (Group VII); H and H1- hippocampal CA1 neurons of rats treated with Gg225mg/kg/p.o+ Diazepam 7mg/kg/i.p (Group VII);

**Table.1:** Basal dendritic branching points of hippocampal CA1 neurons at different concentric zones in one month old male Wistar albino rats (Six weeks duration)

Groups	Distance from soma, µm						
dioups	0-20	20-40	40-60	60-80	80 -100	100-120	
I. Control	2.17±	3.67±	1.33±	1.33±	2.50±	0.00±	
	0.17	0.21	0.21	0.21	0.22	0.00	
II. Di azep am 7mg/kg/i.p	0.83±	1.66±	0.00±	0.00±	0.00±	0.00±	
	0.16	0.21	0.00	0.00	0.00	0.00	
III. Gg 75mg/kg/p.o	3.50±	13.33±	7.16±	4.50±	2.33±	0.17±	
	0.22**	0.21**	0.30**	0.22**	0.21	0.17±	
IV. Gg	6.67±	15.83±	19.50±	7.67±	5.83±	5.83±	
150mg/kg/p.o	0.33**	0.30**	0.22**	0.21**	0.30**	0.30**	
V. Gg	5.50±	16.33±	14.50±	13,50±	9.50±	6.33±	
225mg/kg/p.o	0.22**	0.33**	0.22**	0.22**	0.22**	0.22**	
VI. Gg	4.50±	12.17±	9.83±	5.50±	2.17±	0.17±	
300mg/kg/p.o	0.22**	0.30 **	0.22**	0.22**	0.17	0.17±	
VII. Gg150mg+	5.67±	8.00 ±	15.50±	11.00±	13.50±	8.17±	
Diazepam7mg/kg/i.p	0.21**	0.36**	0.22**	0.36**	0.22**	0.30**	
VIII. Gg225mg+	4.33±	11.83±	13.50±	9.50±	7.50±	7.67±	
Diazenam7mg/kg/i.n	0.21**	0.30**	0.22**	0.21**	0.22**	0.21**	

n=6; values (number of apical dendritic branching points) are expressed as Mean  $\pm$  SEM; \* p<0.05, \*\* P<0.01 (ANOVA followed by Dunnett's multiple comparison test); Gg -*Glycyrr hiza glabra*.

**Table.2:** Basal dendritic intersections of hippocampal CA1 neurons at different concentric zones in one month old male Wistar albino rats (Six weeks duration)

Croups	Distance from soma, µm						
dioups	0-20	20-40	40-60	60-80	80 -100	100-120	
I. Cont rol	1.17±	2.33±	3.33±	3.67±	2.16±	0.00±	
	0.17	0.21	0.21	0.21	0.16	0.00	
II. Di azep am 7mg/kg/i.p	0.00±	0.66±	1.00±	1.16±	0.00±	0.00±	
	0.00	0.21	0.36	0.30	0.00	0.00	
III. Gg 75mg/kg/p.o	1.33±	7.83±	3.67±	9.17±	6.33±	0.33±	
	0.21	0.30**	0.33	0.82**	0.21**	0.21±	
IV. Gg	3.67±	9.67±	10.50±	12.50±	13.00±	7.67±	
150mg/kg/p.o	0.21**	0.33**	0.22**	0.22**	0.36**	0.49**	
V. Gg	3.33±	8.50±	9.50±	11.50±	16.67±	11.17±	
225mg/kg/p.o	1.33**	0.22**	0.22**	0.22**	0.21**	0.54**	
VI. Gg	1.33±	4.50 ±	4.00±	12.83±	9.05±	0.33±	
300mg/kg/p.o	0.21	0.22**	0.25	0.17**	0.22**	0.21	
VII. Gg150mg+	3.33±	9.50 ±	6.33±	12.50±	16.50±	14.00±	
Di azep am7mg/kg/i.p	0.21**	0.22**	0.49**	0.22**	0.22**	0.25**	
VIII. Gg225mg+	3.16±	5.83±	6.83±	10.17±	12.33±	9.00±	
Diazepam7mg/kg/i.p	0.30**	0.30**	0.30**	0.30**	0.21**	0.25**	

n=6; values (number of basal dendritic intersections) are expressed as Mean  $\pm$  SEM; \* p<0.05, \*\* P<0.01 (ANOVA followed by Dunnett's multiple comparison test); Gg -Glycyrrhiza glabra

**Table.3:** Apical dendritic branching points of hippocampal CA1 neurons at different concentric zones in one month old male Wistar albino rats (Six weeks duration)

Distance	GROUPS (control and Gg treated rats)							
from soma, µm	I	II	Ш	IV	V	VI	VII	VII
0-20	0.16±	0.00±	0.33±	5.50±	3.66±	0.33±	4.50±	3.33±
	0.16	0.00	0.21	0.22**	0.21**	0.21	0.22**	0.21**
20-40	2.50±	0.00±	2.50±	4.67±	12.50±	2.00±	5.00±	6.67±
	0.22	0.00	0.22	0.21**	0.22**	0.25	0.25**	0.21**
40-60	1.67±	0.50±	1.33±	7.17±	6.67	1.50±	6.33±	6.67±
	0.21	0.22	0.21	0.17**	0.21**	0.22	0.21**	0.21**
60-80	0.16±	0.00±	6.67	6.50±	11.67±	4.50±	7•33±	8.33±
	0.16	0.00	0.21**	0.25**	0.21**	0.22**	0.33**	0.21**
80-100	1.00± 0.00	0.00± 0.00	2.33± 0.21**	6.50± 0.22**	6.17± 0.16**	2.33± 0.21**	8.00± 0.63**	6.50± 0.22**
100-120	1.67± 0.21	0.00± 0.00	1.83± 0.30	10.50± 0.22 <b>**</b>	5.33± 0.21**	1.83± 0.16	4.50± 0.22**	4.66± 0.22**
120-140	0.16± 0.16	0.00± 0.00	0.16± 0.16	7.50± 0.22**	4·33± 0.21**	0.16± 0.16	3.50± 022**	7.83± 0.47**
140-160	0.00± 0.00	0.00± 0.00	0.00± 0.00	8.67± 0.21**	4.67± 0.21**	0.00± 0.00	2.83± 0.17**	9.50± 0.22**
160-180	0.00± 0.00	0.00± 0.00	0.00± 0.00	4.67± 0.21**	5.50± 0.22**	0.00± 0.00	3.50± 0.22**	12.17± 0.66**

n=6; values (number of apical dendritic branching points) are expressed as Mean  $\pm$  SEM; \* p<0.05, \*\* P<0.01 (ANOVA followed by Dunnett's multiple comparison test); I- Control; II- Diazepam 7mg/kg/i.p; III- Gg 75mg/kg/p.o; IV- Gg 150mg/kg/p.o; V- Gg 225mg/kg/p.o; VI- Gg 300mg/kg/p.o; VI- Gg150mg+Diazepam7mg/kg/i.p; VII- Gg225mg+Diazepam7mg/kg/i.p; Gg-Glycyrrhiza glabra.

Table.4: Apical dendritic inter	rsections of hippocampal C	A1 neurons at different	concentric zones in	one month old
male Wistar albino rats (Six w	veeks duration)			

Distance	GROUPS (control and Gg treated rats)							
from soma, µm	I	Ш	Ш	IV	V	VI	VII	VII
0-20	0.83±	0.00±	0.16±	3.50±	2.66±	1.00±	2.50±	2.33±
	0.16	0.00	0.16	0.22**	0.21**	0.00	0.22**	0.21**
20-40	0.17±	0.00±	2.50±	4.50±	6.50±	2.66±	6.83±	6.83±
	0.17	0.00	0.22**	0.22**	0.22**	0.21**	0.30**	0.30**
40-60	1.83±	0.50±	2.16±	5.66±	9.50±	2.00	6.83±	8.50±
	0.16	0.22	0.16	0.21**	0.22**	0.36	0.30**	0.22**
60-80	1.16±	0.00±	1.33±	5.83±	9.67±	1.16±	6.33±	8.50±
	0.16	0.00	0.21	0.16**	0.21**	0.16	0.21**	0.22**
80-100	1.33±	0.00±	6.67±	8.50±	8.50±	6.83±	7.83±	7.50±
	0.21	0.00	0.21**	0.22**	0.22**	0.16**	0.16**	0.22**
100-120	2.00±	0.33±	2.16±	9.33±	6.66±	2.16±	8.66±	6.50±
	0.00	0.21	0.16	0.21**	0.21**	0.16	0.21**	0.22**
120-140	0.50±	0.00±	2.67±	6.50±	6.67±	2.67±	4.33±	4.83±
	0.22	0.00	0.21**	0.22**	0.21**	0.21**	021**	0.16**
140-160	0.00±	0.00±	0.00±	9.83±	4.33±	0.00±	3.50±	4.66±
	0.00	0.00	0.00	0.30**	0.21**	0.00	0.22**	0.21**
160-180	0.00±	0.00±	0.00±	11.50±	8.00±	0.00±	3.83±	16.67±
	0.00	0.00	0.00	0.22**	0.25**	0.00	0.16**	0.33**

n=6; values (number of apical dendritic intersections) are expressed as Mean ± SEM; \* p<0.05, \*\* P<0.01 (ANOVA followed by Dunnett's multiple comparison test); I- Control; II- Diazepam 7mg/kg/i.p; III- Gg 75mg/kg/p.o; IV- Gg 150mg/kg/p.o; V- Gg 225mg/kg/p.o; VI- Gg 300mg/kg/p.o; VII- Gg150mg+Diazepam7mg/kg/i.p; VIII- Gg225mg+Diazepam7mg/kg/i.p; Gg -Glycyrrhiza glabra

Furthermore Diazepam induced amnesia reversed by the aqueous root extract of Gg (150 and 225 mg/kg, p.o) has shown a significant (p<0.01) increased numbers of both apical and basal dendritic branching points and dendritic intersections in all the concentric zones.

In addition, the aqueous root extract of Gg in the dose of 75 mg/kg/p.o and 300mg/kg/p.o has shown a significant (p<0.001) increased basal dendritic arborization in the 0-20  $\mu$ m, 20-40  $\mu$ m, 40-60  $\mu$ m and 60-80  $\mu$ m concentric zones and increased basal dendritic intersections (p<0.01) in the 60–80  $\mu$ m, 80–100  $\mu$ m, 100-120  $\mu$  and 120-140  $\mu$ m concentric zones. The dose of 75 mg/kg/p.o and 300mg/kg/p.o has also shown a significant (p<0.01) increased apical dendritic arborization in the 60-80  $\mu$ m and 80-100  $\mu$ m concentric zones and increased apical dendritic intersections in the 60-80  $\mu$ m and 80-100  $\mu$ m concentric zones and increased (p<0.01) apical dendritic intersections in the 20-40  $\mu$ m, 80–100  $\mu$ m and 120-140  $\mu$ m concentric zones.

#### DISCUSSION

Dendrites undergo dynamic changes under physiological and pathological conditions. It is believed that some areas of the brain particularly the hippocampus vulnerable to glutamate, ischemia, inflammatory processes, repeated psychosocial or oxidative stress, <sup>[9, 10]</sup> may leads to dendritic atrophy in CA1 pyramidal neurons of the hippocampus, accompanied by specific cognitive deficits in spatial learning and memory. It has been reported that the number of dendritic branches in CA1 pyramidal neurons is decreased in aged rats and in Alzheimer's patients. <sup>[11, 12]</sup> Such areas of brain structures has been shown to significantly increase the density of spines and dendritic complexity due to repeated exposure to enriched environments.<sup>[9]</sup> Increase in the dendritic arborization and dendritic intersections in hippocampal CA1pyramidal neurons may result in alterations in synaptic connectivity. It may result in alteration in learning and memory.

The present study showed that all the doses (75, 150, 225 and 300 mg/kg) of aqueous root extract of Gg significantly enhanced dendritic arborization (dendritic branching points) and dendritic intersections however in the dose of 150 and 225 mg/kg/p.o showed a significant (p<0.01) enhancement of dendritic arborization and dendritic intersections in hippocampal CA1 pyramidal neurons is comparable to control. May result in alterations in synaptic connectivity, which probably is one reason for the enhanced learning and memory in rats has been reported previously. [14, 15] Thus the aqueous root extract of Gg may stimulate the release of neuromodulators or neuronal dendritic growth stimulating factors that alter the activity of neurotransmitters that are involved in learning and memory, which thereby contributes to enhanced learning and memory.

#### CONCLUSION

In conclusion, all the doses of aqueous root extract of Gg significantly enhanced dendritic arborization (dendritic branching points) and dendritic intersections, however in the dose of 150 and 225 mg/kg/p.o showed a significant (p<0.01) enhancement of dendritic arborization and dendritic intersections along the length of both apical and basal dendrites in hippocampal CA1 pyramidal neurons is comparable to control. Based on our results obtained, we conclude that constituents present in aqueous extract of root of Gg have neuronal dendritic growth stimulating properties.

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