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Research article

ROLE OF *GLYCYRRHIZA GLABRA* ROOT EXTRACT IN DENDRITIC ARBORIZATION AND DENDRITIC INTERSECTIONS OF HIPPOCAMPAL CA1 NEURONS IN 3-MONTHS OLD RATS

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ABSTRACT: The hippocampus is located bilaterally in the medial temporal lobe; within the hippocampus the flow of information is unidirectional. Repeated psychological stress, aging and dementia may leads to the dendritic atrophy in CA1 pyramidal neurons of hippocampus. Accordingly, the present study was designed to investigate the role of aqueous root extract of *Glycyrrhiza glabra (Gg)* treatment on the dendritic arborization and dendritic intersections of hippocampal CA1 neurons in 3 months old male Wistar albino rats. The aqueous root extract of *Glycyrrhiza glabra* was administered orally in four different doses (75, 150, 225 and 300 mg/kg) for 2, 4 and 6 weeks duration, respectively. At the end of the spatial memory tests, the rats were sacrificed by deeply anesthetized Pentobarbitone, their brains were removed rapidly and Hippocampal CA1 region studied through Rapid Golgi staining. Hippocampal CA1 neurons were traced using camera lucida, and Quantification of dendritic branching points and dendritic intersections were quantified by using concentric circle method of Sholl. All the doses of aqueous root extract of Gg for 6 weeks showed significantly enhanced dendritic arborization 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. However, the rats treated for 2 and 4 weeks did not show any significant change in hippocampal CA1 neuronal dendritic arborization. Thus the constituents present in 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 may be useful in management of impaired learning, dementia, Alzheimer's disease and other neurodegenerative disorders.

Key words: Alzheimer's disease, dendritic arborization, dendritic intersections, *Glycyrrhiza glabra*, CA1 neurons.

INTRUDUCTION

The hippocampus is one of the important portions of the brain that is associated primarily with the memory and spatial navigation, within the hippocampus the flow of information is unidirectional, which proceeds from the dentate gyrus to CA3 layer to CA1 layer to the subiculum, then out of the hippocampus to the entorhinal cortex.

Hippocampal lesions like Alzheimer's disease and dementia will leads to memory dysfunction. Some degree of memory loss also occurs with stress, ageing, and changes in the blood flow or reduced oxygen supply to the hippocampus.

Ayurvedic system of medicine has been mentioned several natural medicinal plants to induce mental upliftment by enhancing learning and memory such plants are called 'medhya'. *Glycyrrhiza glabra* (family: Leguminosae), popularly known as licorice includes in such 'medhya' group of medicinal plants. The roots and rhizomes of Gg have anti-inflammatory (Yokota T et al, 1998), antioxidant (Ju HS et al, 1989), expectorant, diuretic and laxative properties. Previous studies have shown that *Glycyrrhiza glabra* (*Gg)* aqueous root extract treatment in Wistar albino rats enhances both spatial learning ability and retention of learned tasks (Kosuri Kalyan Chakravarthi et al, 2012), hence the present study was designed to evaluate the role of Gg root extract on 3 months old rat's hippocampal neurons particularly the Cornu Ammonis area 1 (CA1) subregion of the hippocampus.

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MATERIALS AND METHODS

Plant material

The roots of *Gg* were purchased from a local ayuredic store in Udupi, Karnataka, India during 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 3-months 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 º C ±2º 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 Groups*:* Rats were randomly divided into eight groups.

Group I- Control (n=6): Animals receive a known volume of distilled water.

Group II- Diazepam control (n=6): Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

Group III (n=6): animals receive aqueous root extract of Gg -75mg/kg/day.

Group IV (n=6): animals receive aqueous root extract of Gg -150mg/kg/day.

Group V (n=6): animals receive aqueous root extract of Gg -225mg/kg/day.

Group VI (n=6): animals receive aqueous root extract of Gg -300mg/kg/day.

Group VII (n=6): animals receive aqueous root extract of Gg -150mg/kg/day. Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

Group VIII (n=6): animals receive aqueous root extract of Gg -225mg/kg/day Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

n = number of animals.

Animals **(Group III to Group VIII)** are treated with the aqueous root extract of Gg for 2, 4 and 6 weeks durations (Total number of animals = 144).

Rapid Golgi staining procedure

After the treatment period, all experimental animals were subjected to spatial learning (Morris water maze, and elevated plus maze) tests. At the end of the spatial memory tests, the rats were deeply anesthetized with Pentobarbitone and sacrificed; 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-µm thickness were excised, dehydrated, cleared and mounted with Distrin plasticizer xylene mounting media. (Rao BS et al, 1993)

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 (Sholl DA, 1956) was used for dendritic quantification. Concentric circles with a distance of 20 µm 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 and 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 µm and apical dendritic branching points and intersections up to a radial distance of 160 µm were counted 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.

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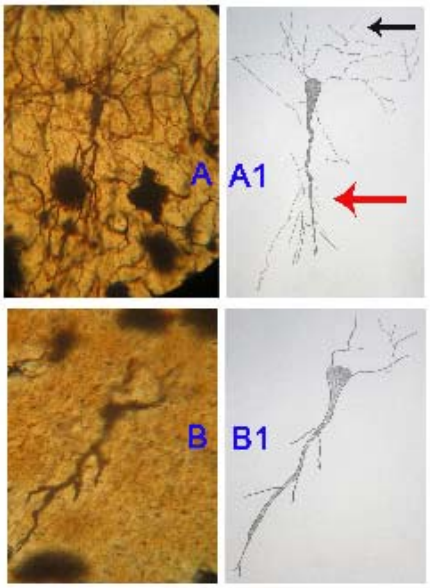
Statistical Analysis

Data was analyzed using ANOVA followed by Dunnett's multiple comparison test. p value < 0.05 were considered as statistically significant. The values were expressed as Mean ± Standard Error of Mean (SEM).

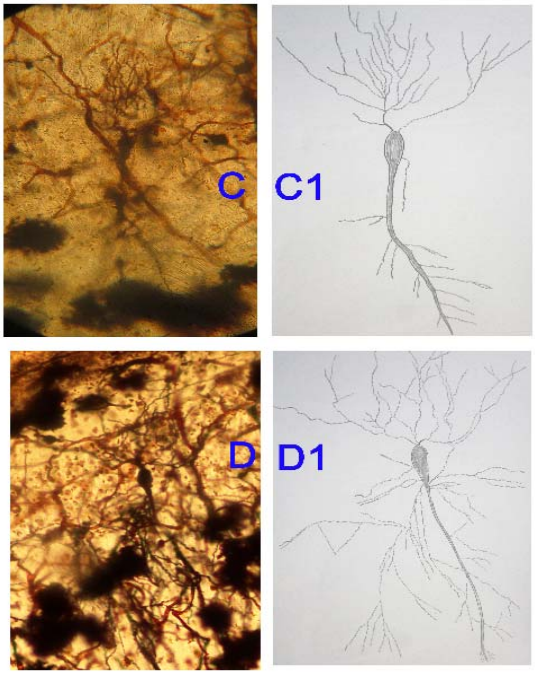
RESULTS

The aqueous extract of root of *Glycyrrhiza glabra* (6 weeks of treatment) showed significant enhancement in the dendritic arborization and dendritic intersections in a dose dependent manner [Figure -1, Figure -2, Figure -3 and Figure -4]



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

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



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 CA1neurons of rats treated with 150 mg/kg aqueous extract of *Glycyrrhiza glabra* orally every day for 6weeks (Group IV);

Figure- 2: Representative photomicrographs (C and D) and camera lucida tracings (C1and D1) of Golgi-stained hippocampal CA1 neurons.

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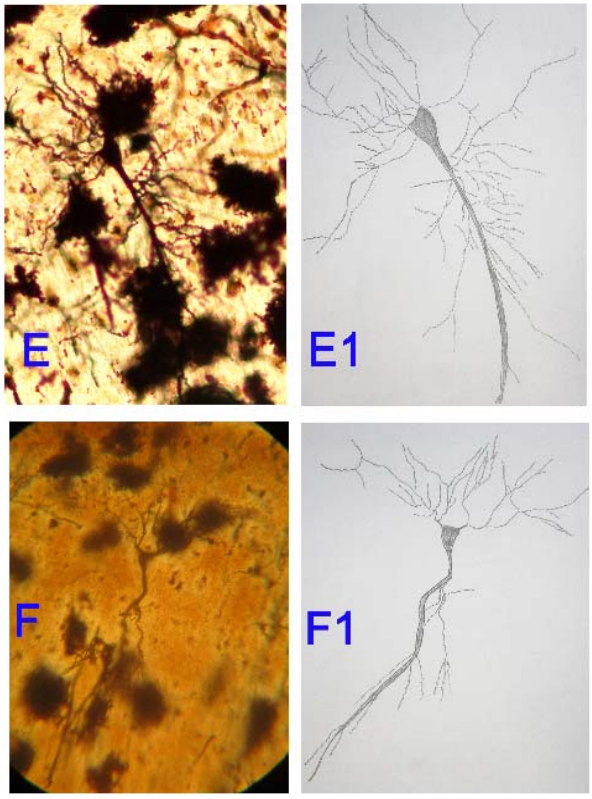
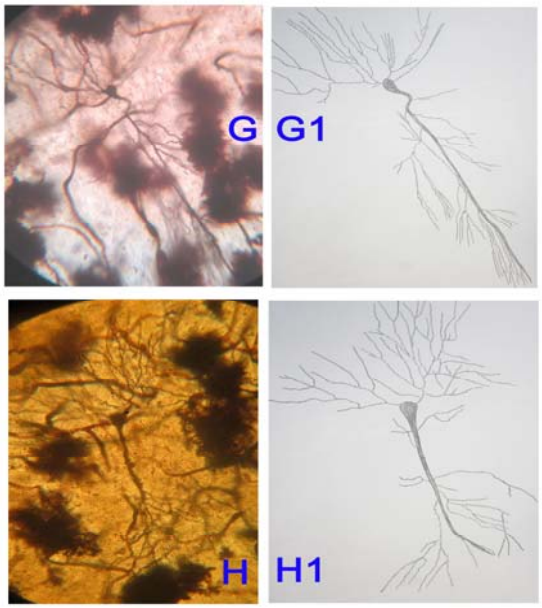


E and E1- hippocampal CA1neurons of rats treated with 225 mg/kg aqueous extract of *Glycyrrhiza glabra* orally every day for 6weeks (Group V); F and F1- hippocampal CA1 neurons of rats treated with 300 mg/kg aqueous extract of *Glycyrrhiza glabra* orally every day for 6 weeks (Group VI);

Figure- 3: Representative photomicrographs (E and F) and camera lucida tracings (E1and F1) of Golgi-stained hippocampal CA1 neurons.



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 VIII);

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.**

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Dendritic Quantification of Hippocampal CA1 pyramidal neurons (6 weeks of treatment)

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(0-20 µm, 20-40 µm, 40-60 µm, 60-80 µm, 80-100 µm and 100-120 µm) and apical (0-20 µm, 20-40 µm, 40-60 µm, 60-80 µm, 80-100 µm, 100-120 µm, 120-140 µm, 140-160 µm and 160-180 µm) dendrites in all the concentric zones is comparable to control rats [Table-1, Table- 2, Table-3 and Table-4].

Table-1: Basal dendritic branching points of hippocampal CA1 neurons at different concentric zones in 3-months old male Wistar albino rats (6 weeks of treatment)

Groups	Distance from soma, µm					
	0-20	20-40	40-60	60-80	80 -100	100-120
I. Control	0.50± 0.22	2.50± 0.22	3.33± 0.51	2.50± 0.22	2.66± 0.21	0.00± 0.00
II. Diazepam 7mg/kg/i.p	0.00± 0.00	1.00± 0.00	1.33± 0.21	0.00± 0.00	0.00± 0.00	0.00± 0.00
III. Gg 75mg/kg/p.o	0.50± 0.22	6.66± 0.21**	7.66± 0.21**	8.50± 0.22**	2.50± 0.22	0.00± 0.00
IV. Gg 150mg/kg/p.o	3.50± 0.22**	12.50± 0.22**	10.83± 0.16**	7.50± 0.22**	6.50± 0.22**	3.66± 0.21**
V. Gg 225mg/kg/p.o	6.50± 0.22**	11.50± 0.22**	10.50± 0.22**	7.66± 0.21**	6.33± 0.21**	3.33± 0.21**
VI. Gg 300mg/kg/p.o	0.50± 0.22	10.33± 0.21**	10.33± 0.21**	9.50± 0.22**	2.50± 0.22	0.00± 0.00
VII. Gg150mg+ Diazepam7mg/kg/i.p	3.33± 0.21**	8.50± 0.22**	13.33± 0.21**	6.50± 0.22**	5.66± 0.21**	2.83± 0.16**
VIII. Gg225mg+ Diazepam7mg/kg/i.p	4.50± 0.22**	9.66± 0.21**	13.50± 0.22**	8.50± 0.22**	7.33± 0.21**	3.50± 0.22**

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

Table-2: Basal dendritic intersections of hippocampal CA1 neurons at different concentric zones in 3-months old male Wistar albino rats (6 weeks of treatment).

Groups	Distance from soma, µm					
	0-20	20-40	40-60	60-80	80 -100	100-120
I. Control	0.33± 0.21	1.66± 0.21	3.33± 0.21	3.50± 0.22	3.50± 0.22	0.00± 0.00
II. Diazepam 7mg/kg/i.p	0.00± 0.00	0.00± 0.00	1.16± 0.16	1.33± 0.21	0.00± 0.00	0.00± 0.00
III. Gg 75mg/kg/p.o	0.50± 0.22	3.50± 0.22**	3.50± 0.22	3.66± 0.21	8.50± 0.22**	0.00± 0.00
IV. Gg 150mg/kg/p.o	2.50± 0.22**	5.50± 0.22**	7.66± 0.21**	8.50± 0.22**	13.50± 0.22**	9.66± 0.21**
V. Gg 225mg/kg/p.o	3.33± 0.21**	5.50± 0.22**	10.50± 0.22**	6.66± 0.21**	10.50± 0.22**	6.66± 0.21**
VI. Gg 300mg/kg/p.o	0.50± 0.22	3.66 ± 0.21**	3.50± 0.22	3.66± 0.21	12.16± 0.16**	0.00± 0.00
VII. Gg150mg+ Diazepam7mg/kg/i.p	2.33± 0.21**	4.50 ± 0.22**	8.66± 0.21**	7.50± 0.22**	7.83± 0.16**	5.50± 0.22**
VIII. Gg225mg+ Diazepam7mg/kg/i.p	2.66± 0.21**	5.50± 0.22**	10.33± 0.21**	10.66± 0.21**	12.16± 0.16**	10.50± 0.22**

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

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Table-3: Apical dendritic branching points of hippocampal CA1 neurons at different concentric zones in 3-months old male Wistar albino rats (6 weeks of treatment).

Distance from soma, µm	GROUPS (control and Gg treated rats)							
	I	**II**	III	IV	V	VI	VII	VIII
0-20	0.50± 0.22	0.00± 0.00	0.66± 0.21	4.50± 0.22**	3.66± 0.21**	0.66± 0.21	3.50± 0.22**	4.33± 0.21**
20-40	1.00± 0.00	0.00± 0.00	1.33± 0.21	3.66± 0.21**	13.50± 0.22**	1.16± 0.16	3.16± 0.16**	3.33± 0.21**
40-60	1.50± 0.22	0.00± 0.00	1.16± 0.16	8.50± 0.22**	11.50± 0.22**	1.16± 0.16	6.50± 0.22**	3.67± 0.21**
60-80	0.16± 0.21	0.00± 0.00	1.33 0.21	5.50± 0.22**	9.50± 0.22**	1.83± 0.16	5.50± 0.22**	4.50± 0.22**
80-100	1.83± 0.16	0.00± 0.00	1.50± 0.22	6.66± 0.21**	3.66± 0.21**	1.83± 0.16	5.66± 0.21**	3.33± 0.21**
100-120	2.00± 0.00	0.00± 0.00	1.33± 0.21	6.50± 0.22**	6.50± 0.22**	1.50± 0.22	6.83± 0.16**	13.50± 0.22**
120-140	0.16± 0.16	0.00± 0.00	0.00± 0.00	4.50± 0.22**	4.50± 0.22**	0.00± 0.00	4.33± 0.21**	4.50± 0.22**
140-160	0.00± 0.00	0.00± 0.00	0.00± 0.00	5.16± 0.16**	2.50± 0.22**	0.00± 0.00	3.50± 0.22**	2.00± 0.00**

n=6; values (number of apical dendritic branching points) 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*.

Table-4: Apical dendritic intersections of hippocampal CA1 neurons at different concentric zones in 3-months old male Wistar albino rats (6 weeks of treatment).

Distance from soma, µm	GROUPS (control and Gg treated rats)							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
0-20	0.50± 0.22	0.00± 0.00	0.50± 0.22	4.50± 0.22**	3.50± 0.22**	0.50± 0.22	3.50± 0.22**	3.33± 0.21**
20-40	0.66± 0.21	0.00± 0.00	0.50± 0.22	4.83± 0.16**	7.50± 0.22**	0.50± 0.22	3.66± 0.21**	2.50± 0.22**
40-60	1.33± 0.21	0.00± 0.00	3.66± 0.21**	6.66± 0.21**	13.50± 0.22**	3.50± 0.22**	4.50± 0.22**	4.50± 0.22**
60-80	1.16± 0.21	0.50± 0.22	1.50± 0.22	6.66± 0.21**	12.50± 0.22 **	1.33± 0.21	6.33± 0.21**	3.50± 0.22**
80-100	1.83± 0.16	0.66± 0.21	4.66± 0.21**	12.50± 0.22**	4.50± 0.22**	6.33± 0.21**	4.66± 0.21**	4.50± 0.22**
100-120	1.83± 0.16	0.00± 0.00	2.00± 0.00	4.50± 0.22**	6.50± 0.22**	1.66± 0.21	4.66± 0.21**	6.50± 0.22**
120-140	0.00± 0.00	0.00± 0.00	0.00± 0.00	8.50± 0.22**	3.50± 0.22**	0.00± 0.00	9.83± 0.16**	7.33± 0.21**
140-160	0.00± 0.00	0.00± 0.00	0.00± 0.00	7.50± 0.22**	4.50± 0.22**	0.00± 0.00	12.66± 0.21**	6.50± 0.22**

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*

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 number of both apical and basal dendritic branching points and dendritic intersections in all the concentric zones. In the dose of Gg 75mg/kg/p.o and 300mg/kg/p.o showed a significant ($p<0.01$) increase in the basal dendritic arborization and dendritic intersections in the 0-20 µm, 40-60 µm and 60-80 µm concentric zones. In the apical dendritic arborization and dendritic intersections the aqueous root extract of Gg in the dose of 75mg/kg/p.o and 300mg/kg/p.o showed a significant ($p<0.01$) increase in apical dendritic intersections (20-40 µm and 80-100 µm concentric zones).
However, the rats treated for 2 and 4 weeks of duration did not show any significant change in hippocampal CA1 neuronal dendritic arborization.

DISCUSSION

The CA1 Pyramidal neurons are found in the CA1 region of the hippocampus in the medial temporal lobe, each neuron consists of pyramidal shaped cell body or soma with two distinct dendrites (basal and apical dendrites). The basal dendrites emerge from the base and the apical dendrites from the apex of the pyramidal cell body or soma of the pyramidal neuron. The CA1 Pyramidal neurons receive input from the CA3 region of the hippocampus, entorhinal cortex, thalamus, in turn, CA1 region projects to the subiculum. Neurodegenerative, inflammatory processes and Oxygen free-radicals may leads to dendritic atrophy in CA1 pyramidal neurons of the hippocampus. Yamada M, et al, 1988 and Lolova I, 1989 has been reported that the number of dendritic branches in CA1 pyramidal neurons is decreased in aged rats and in Alzheimer's patients, such regions when exposed to enriched environments have been shown to significantly increase the density of spines and dendritic complexity (Moser MB, 1995).
Large number of dendritic spines is present on the pyramidal neurons of the hippocampus which consider as a major site for the excitatory synaptic transmission. Increase in the dendritic arborization will directly increase the number of dendritic spines and functions of pyramidal neurons of hippocampus. The present study showed that all the doses (75, 150, 225 and 300 mg/kg) of aqueous root extract of Gg (6 weeks of treatment) 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.
Such enhancement of dendritic arborization and dendritic intersections in hippocampal CA1 pyramidal neurons 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 may be useful in management of impaired learning, dementia, Alzheimer's disease and other neurodegenerative disorders.

CONCLUSION

Based on our results we concluded that all the doses of aqueous root extract of Gg (6 weeks of treatment) significantly enhanced dendritic arborization (dendritic branching points) and dendritic intersections, however in the dose of 150 mg/kg/p.o , 225 mg/kg/p.o and Diazepam induced amnesia group 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 which is comparable to control. However, the rats treated for 2 and 4 weeks of duration did not show any significant change in hippocampal CA1 neuronal dendritic arborization and dendritic intersections. Such significantly enhanced dendritic arborization (dendritic branching points) and dendritic intersections properties noted in this study may alter the activity of neurotransmitters that are involved in learning and memory, probably is one reason for the enhanced learning and memory in rats has been reported previously (Kosuri Kalyan Chakravarthi et al, 2013). However further studies regarding the role of constituents present in aqueous extract of root of Gg responsible for exact mechanism are necessary in order to develop and ideal agent for the treatment of various learning and memory related disorders.

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