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Original article

Effect of *Glycyrrhiza glabra* Root Extract on Learning and Memory in Wistar Albino Rats.

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ABSTRACT

Aims: Memory is an organism's mental ability to store, retain and recall information. The hippocampus plays an important role in learning and memory. The present study was undertaken to investigate the effect of Glycyrrhiza glabra root extract on learning and memory in one month old male Wistar albino rats. Materials and Methods: The aqueous extract of root of Glycyrrhiza glabra was administered orally in four doses (75, 150, 225 and 300 mg/kg) for 4 weeks. Elevated plus-maze, Hebb-William maze and Morris water maze tests were conducted to evaluate the learning and memory parameters and served as the exteroceptive behavioral model. Diazepam induced amnesia served as the interoceptive behavioral model. Results: The aqueous extract of root of Glycyrrhiza glabra showed improvement in learning and memory in a dose dependent manner. However, 150 and 225 mg/kg doses have shown a significant ($p<0.01$) enhancement in learning and memory which is comparable to control. Aqueous extract of Gg (150 and 225 mg/kg, p.o) administrated for 4 weeks reversed amnesia induced by Diazepam. Conclusion: Hence Glycyrrhiza glabra appears to be a promising drug for improving memory and it would be worthwhile to explore the potential of this plant in the management of impaired learning, dementia, Alzheimer's disease and other neurodegenerative disorders. However, further studies are necessitated to identify the exact mechanism of action.

1.Introduction:

The brain is the center of the nervous system in all vertebrates. The cerebral cortex of the human brain contains roughly 15-33 billion neurons depending on gender and age.[1], linked with up to 10,000 synaptic connections each. Each cubic millimeter of cerebral cortex contains roughly one billion synapses.[2].These neurons communicate with one another by means of long protoplasmic fibers called axons, which carry trains of signal pulses called action potentials to distant parts of the brain or body and target them to specific recipient cells. From a biological perspective, the function of a brain is to generate behaviors that promote the genetic fitness of an animal.[3] The hippocampus is a major component of the brain of humans and other mammals. It belongs to the limbic system and plays important roles in long-term memory and spatial navigation. The central cholinergic pathways play a prominent role in learning and memory processes.[4]

Memory is the ability of an individual to record sensory stimuli, events, information and etc., retain them over a short or long period of time and recall the same at a later date when needed.[5].Learning is the process of acquiring knowledge about the world and memory could be considered as the retention of the acquired knowledge, which can be retrieved as and when, required.[6] Dementia is a mental disorder characterized by loss of intellectual ability which invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. Stressful conditions are often associated with loss of memory and cognitive functions which may lead to threats of schizophrenia and Alzheimer's disease.

Traditionally herbal drugs have been used to enhance cognitive functions and to alleviate other functions associate with the Alzheimer's disease. In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been in clinical use for centuries. Glycyrrhiza glabra consists of polysaccharides, flavonoids, triterpene, saponins, pectins, simple

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sugars, mineral salts, amino acids, and various other substances. Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of licorice root. Glycyrrhiza glabra (Gg) Roots have anti-ulcer[7], anti-inflammatory[8], antioxidant[9], expectorant, diuretic, laxative, and sedative[10] properties. They also possess antipyretic[11], antiherpes[12], antiviral[13], antimicrobial and anxiolytic[14] activities.

2.Materials and Methods

2.1.Preparation of Aqueous root extract of Glycyrrhiza glabra (Gg):

The roots of *Glycyrrhiza glabra* (Gg) was purchased from local ayuredic store, Udupi and its authenticity confirmed by the Dr. Krishna Kumar, Chairman, Department of applied Botany, Mangalore University.

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 in a soxhlet extractor for 36 h, 1-2 cycles per hour. The crude extract was evaporated to dryness using a rotary evaporator. The yield of the extract was 16%. The extract was administrated orally to separate groups of one month old male Wistar albino rats in four different doses 75, 150, 225 and 300 mg/kg respectively. Fresh solutions of root extract of Gg were prepared every day before the start of experiment by reconstituting the weighed quantity of the crude extract in a minimum amount of distilled water for oral administration.

2.2.Experimental animals:

The experimental protocol was approved 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 (22.5× 35.5× 15 cm) and maintained at temperature (25 ±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.

2.3.Drugs:

Diazepam (Vishal enterprises, Mangalore) was used in the present study.

2.4.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 4 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 4 weeks.

Group IV (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 4 weeks.

Group V (n=6): Received 225 mg/kg aqueous extract of Gg orally every day for 4 weeks.

Group VI (n=6): Received 300 mg/kg aqueous extract of Gg orally every day for 4 weeks.

Group VII- Gg 150mg + Diazepam (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 4 weeks. Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

n = number of animals.

Group VIII- Gg 225mg + Diazepam (n=6): Received 150 mg/kg aqueous extract of Gg orally every day for 4 weeks. Diazepam 7 mg/kg was injected i.p. 20 min before the test session.

n = number of animals.

3.Experimental procedures

All experimental animals were tested for spatial memory by Elevated plus maze, Hebb-William maze and Morris water maze tests 90 minutes after the last dose.

3.1.Elevated plus maze:

The elevated plus-maze served as the exteroceptive behavioral model to evaluate learning and memory in rats. It was made of wood-block and consisted of four arms (50 cm long×10 breadth× 40 cm wide) fixed to a central platform (10 cm × 10 cm): two had 12 cm high walls (closed-arms) and two had no border in place of the walls (open-arms). The maze was elevated to a height of 50 cm. On the first day (i.e., after the last dose of 4 weeks duration), each rat was placed at the end of the open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the closed arm with its four legs. TL was recorded on the first day (training session) for each animal. Retention of this learned-task (memory) was examined 24 hr after the first day trail (i.e., 29th day, 24 hr after the last dose of 4 weeks duration). Significant reduction in TL value of retention indicated improvement in memory.

3.2.Hebb-William maze

The Hebb-William consists of completely enclosed rectangular box with an entry and a reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just one twisting corridor leading from the entry to the reward chamber.

The learning assessment for control and drug treated animals was conducted at end of drug treatment under zero watt red coloured bulb so as to minimize the nocturnal cycle disturbances. Before the training all the animals were familiarized with Hebb-William maze for a period of 10 min. From 1st -3rd day (i.e., 28th - 30th day, after the last dose of 4 weeks duration), the rats received four consecutive trials of training per day in the maze. In each trial the rat was placed in the entry chamber and the timer was activated as soon as the rat leaves the chamber. The time taken for the mice to reach the reward chamber (T L in minutes) was taken as the learning score of the trial. The average of four trials was taken as the learning score for the day. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. Retention of this learned-task (memory) was examined 96 hr after the last day trail (i.e., 34th day). Significant reduction in TL value of retention indicated improvement in memory.

3.3.Morris water maze:

It is a well-validated test for spatial learning and memory. The technique of using escape from water to motivate learning has been used for many years. [15,16,17] There are several advantages of Morris water maze over other models of learning and memory including absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds.

Morris water maze was used to assess learning and memory in experimental one month old male albino Wistar rats. Each animal was subjected to the four acquisition trials per day for 4 consecutive days (i.e., after the last dose of 4 weeks duration) and their memory was tested in the 5th day, during which the platform was removed (Probe trial). A plastic circular swimming pool (117 cm in diameter, 60 cm high) was filled to a depth of 25 cm with water. Two hundred milliliters of evaporated milk was added to make the water opaque and prevent visualization of the platform. Four points on the rim of the pool were designated north (N), south (S), east (E), and west (W), thus dividing the pool into four quadrants (NW, NE, SE, SW). An 8 × 8 cm Plexiglas platform, onto which the rat could escape, was positioned in the center of one of the quadrants, 1 cm below the water surface. One day before the test, each rat was placed in the pool for 60 seconds without the platform present; this free swim enabled the rat to become habituated to the training environment. The latency (TL) from immersion into the pool to escape onto the platform was recorded for each trial. If the rat did not find the platform in 120 seconds it was manually placed on the platform for a 30-second rest. On day 5 (i.e., 32nd day, 96 hr after the last dose of 4 weeks duration), the platform was removed. The rat was allowed 60 seconds of free swimming. The time spent in the quadrant where the platform was previously located was measured (probe trial), which was considered to assess memory for platform location.

4Statistical Analysis:

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

5.Results

5.1.Effect on transfer latency (By Elevated Plus-Maze):

Transfer Latency (TL) in seconds was defined as a time taken by the animal to move from the open arm into one of the covered arm with all its four legs. Significant reduction in TL value of retention indicated improvement of memory. Aqueous extract of Gg (75,150, 225 and 300mg/kg, P.o) administrated for 4 weeks showed reduction in TL of 29th day, in one month old male albino Wistar rats, when compared to respective control groups indicating significant improvement in memory. Aqueous extract of Gg (150 and 225 mg/kg, p.o) administrated for 4 weeks showed more significant improvement in memory, when compared to all groups. Diazepam injected before training significantly increased TL of 29th day, of 4 weeks duration indicating impairment of memory (amnesia). Aqueous extract of Gg (150 and 225 mg/kg, p.o) administrated for 4 weeks reversed amnesia induced by Diazepam (Table-1).

Table-1: Effects of Aqueous root extract of Gg on Transfer latency (TL) of one month old male Wistar albino rats by Elevated plus maze

Groups	Treatment	TL on 28th day (Learning)	TL after 24 h (Retention)
Group –I	Control	54.35±1.42	45.62±1.14
Group –II	Diazepam 7mg/kg/i.p	69.37±2.13	72.15±0.59
Group –III	Gg 75mg/kg/p.o	48.77±0.36*	42.11±0.51*
Group –IV	Gg150mg/kg/p.o	22.42±1.24**	9.10±0.71**
Group –V	Gg225mg/kg/p.o	26.20±1.15**	9.08±0.72**
Group –VI	Gg300mg/kg/p.o	48.67±0.44*	42.15±0.56*
Group –VII	Gg150mg+Diazepam7mg/kg/i.p	23.00±1.02**	9.08±0.72**
Group –VIII	Gg 225mg+Diazepam7mg/kg/i.p	23.86±1.05**	8.45±0.91**

n=6; values are expressed as Mean ± SEM in seconds; *p<0.05, ** P<0.01 (ANOVA followed by Dunnett's multiple comparison test; Gg – Glycyrrhiza glabra; TL– Transverse latency.

5.2.Effect on transfer latency (By Hebb-William maze):

Significant reduction in TL value of retention indicated improvement of memory. The time taken by the animal (Learning score) to reach the reward chamber from the entry chamber in all the doses of aqueous root extract of Gg (75, 150, 225 and 300 mg/kg, p.o) treated animals was reduced on day 1, 2, and 3 (i.e., 28th - 30th day), when compared to respective control groups indicating significant improvement in memory. Even there was significant reduction in TL value of retention (memory) on day 7th (i.e., 34th day) at all the doses of aqueous root extract of Gg (75, 150, 225 and 300mg/kg, p.o) but with a significant result ($p<0.01$) with 150 and 225mg/kg (Table-4 & Graph-1). Diazepam injected before training significantly increased TL on day 1, 2, and 3, of 4 weeks duration indicating impairment of memory (amnesia). Aqueous extract of Gg (150 and 225 mg/kg, p.o) administrated for 4 weeks reversed amnesia induced by Diazepam (Table-2).

Table-2: Effects of Aqueous root extract of Gg on Transfer latency (TL) of one month old male Wistar albino rats by Hebb-William maze:

Groups	Treatment	TL on 1St Day (Learning)	TL on 2nd Day (Learning)	TL on 3rd Day (Learning)	TL on 7th Day (Retention)
Group –I	Control	5.48±0.03	5.07±0.12	4.50±0.02	4.54±0.18
Group –II	Diazepam7mg/kg/i.p	6.92±0.17	7.0±0.22	7.16±0.12	6.90±0.15
Group –III	Gg75mg/kg/p.o	5.02±0.15*	4.55±0.10*	4.02±0.12*	3.89±0.19*
Group –IV	Gg150mg/kg/p.o	3.49±0.03**	2.06±0.02**	0.96±0.07**	0.15±0.02**
Group –V	Gg225mg/kg/p.o	3.46±0.03**	2.16±0.06**	1.07±0.11**	0.17±0.01**
Group –VI	Gg300mg/kg/p.o	5.06±0.16*	4.55±0.14*	4.04±0.17*	3.95±0.23*
Group –VII	Gg150mg+Diazepam7mg/kg/i.p	3.43±0.03**	2.23±0.05**	1.04±0.02**	0.16±0.01**
Group –VIII	Gg 225mg+Diazepam7mg/kg/i.p	3.37±0.01**	2.13±0.06**	1.04±0.10**	0.15±0.02**

n=6; values are expressed as Mean ± SEM in minutes; *p<0.05, ** P<0.01 (ANOVA followed by Dunnett's multiple comparison test; Gg–Glycyrrhiza glabra; TL–Transverse latency.

5.3.Morris water maze:

In this test, the rats that were treated with aqueous root extract of Gg (75,150, 225 and 300mg/kg, p.o) for 4 weeks learned the platform location faster than the controls. Aqueous extract of Gg (150 and 225mg/kg, p.o) administrated for 4 weeks also reversed amnesia induced by Diazepam (Table-2 & 3). Even in the probe trial there was more preference for platform location in the target quadrant at all the doses of aqueous root extract of Gg (75, 150, 225 and 300mg/kg, p.o) but with a significant result ($p<0.01$) with 150 and 225mg/kg (Table - 4 & Graph-1).

Table-3: Effects of Aqueous root extract of Gg in one month old male Wistar albino rats by Morris water maze: (4 acquisition trials per day for 4 consecutive days)

Groups	Treatment	1St Day	2nd Day	3rd Day	4th Day
Group –I	Control	52.82±0.67	50.04±0.60	39.79±0.71	30.85±0.66
Group –II	Diazepam 7mg/kg/i.p	71.76±0.50	71.98±0.46	71.12±1.14	74.13±0.91
Group –III	Gg75mg/kg/p.o	49.99±0.42*	46.40±0.77*	36.40±0.58*	27.92±0.59*
Group –IV	Gg150mg/kg/p.o	21.30±0.81**	16.00±0.92**	11.59±0.53**	5.80±0.35**
Group –V	Gg225mg/kg/p.o	23.71±0.45**	15.87±1.12**	11.21±0.66**	6.62±0.87**
Group –VI	Gg300mg/kg/p.o	49.84±0.47*	46.36±0.64*	36.51±0.55*	28.02±0.47*
Group –VII	Gg150mg+Diazepam7mg/kg/i.p	22.30±0.83**	17.20±1.11**	12.22±0.66**	5.76±0.55**
Group –VIII	Gg 225mg+Diazepam7mg/kg/i.p	21.99±0.83**	16.74±0.76**	11.42±0.83**	6.81±0.74**

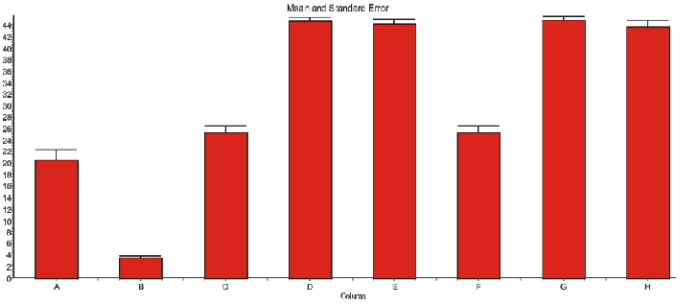
Mean latencies (Learning scores) across trails in the Morris water maze task; n=6; values are expressed as Mean ± SEM in seconds; * p<0.05, ** P<0.01 (ANOVA followed by Dunnett's multiple comparison test); Gg –Glycyrrhiza glabra.

Table-4: Probe Trail (Retention) of the Morris water maze task for 4 Weeks duration.

Groups	Treatment	Probe Trail (5th Day)
Group –I	Control	20.42± 1.88
Group –II	Diazepam7mg/kg/i.p	3.54±0.32
Group –III	Gg75mg/kg/p.o	25.22±1.19*
Group –IV	Gg150mg/kg/p.o	44.66±0.76**
Group –V	Gg225mg/kg/p.o	44.20±0.82**
Group –VI	Gg300mg/kg/p.oGg150mg+	25.29±1.28*
Group –VII	Diazepam7mg/kg/i.pGg 225mg+	44.94±0.59**
Group –VIII	Diazepam7mg/kg/i.p	43.73±1.07**

n=6; values are expressed as Mean ± SEM in seconds; *p<0.05, **P<0.01 (ANOVA followed by Dunnett's multiple comparison test); Gg– Glycyrrhiza glabra.

Graph-1: Probe Trail (Retention) of the Morris water maze task for 4 Weeks duration:



n=6; values are expressed as Mean ± SEM in seconds; A- Control; B- Diazepam 7mg/kg/i.p;

C- Gg 75mg/kg/p.o; D- Gg 150 mg/kg/p.o; E- Gg 225mg/kg/p.o; F- Gg 300mg/kg/p.o;

G - Gg150mg + Diazepam7mg/kg/i.p; H-Gg 225mg+Diazepam7mg/kg/i.p.

6.Discussion

Dementia is collection of symptoms that include decreased intellectual functioning that interferes with normal life functions and is usually used to describe people who have two or more major life functions impaired or lost such as memory, language, perception, judgment or reasoning; they may lose emotional and behavioral control, develop personality changes and have problem solving abilities reduced or lost. Dementia may be static, the result of a unique global brain injury, or progressive, resulting in long-term decline in memory due to damage or disease.

Nootropics also referred to as smart drugs, memory enhancers, neuro enhancers, cognitive enhancers, and intelligence enhancers, are drugs, supplements, nutraceuticals, and functional foods that improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration. [18]

As for date only limited herbal drugs the effect learning and memory are available with valid scientific data especially as a monotherapy. An ayurvedic preparation called Abana contains various herbal ingredients among which the one of the ingredient is Gg. Hence present study was selected to evaluate the contribution of aqueous root extract of Gg for its learning and memory enhancing activity in one month old male Wistar albino rats.

The stimulus lie outside the body in exteroceptive behavior models (Elevated plus maze, Hebb-William maze and Morris water maze), whereas, it lies within the body in the case of interoceptive models (Diazepam). In the present study aqueous root extract of Gg administered orally for 6 weeks improved learning and memory of one month old rats significantly in both the exteroceptive and interoceptive behavioral models employed. All the doses of aqueous root extract of Gg (75, 150, 225 and 300mg/kg, p.o) given for 4 weeks in one month old rats improved the memory as reflected by diminished TL compared to control animals. Additionally, the aqueous root extract of Gg in the dose of 150 and 225mg/kg significantly ($p<0.01$) increased learning and memory in rats compared to control. Furthermore pretreatment with aqueous root extract of Gg (150 and 225mg/kg, p.o) given for 4 weeks protected the animals from learning and memory impairment produced by interoceptive stimuli (Diazepam).

Antioxidant-based drugs or formulations are used for the treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease [19]. In view of this, the neuroprotective effect aqueous root extract of Gg may be attributed to its antioxidant and anti-inflammatory action by the virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

In conclusion, based on our results obtained, the aqueous root extract of Gg has shown cognitive enhancing activity in all the selected doses, but it is more significant in the dose of 150 and 225mg/kg. However further extensive studies are needed to know the exact mechanism of action as a potent and efficacious nootropic agent.

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