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Memory enhancing activity of *Glycyrrhiza glabra* in mice

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Abstract

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities. The present study was undertaken to investigate the effects of *Glycyrrhiza glabra* (popularly known as liquorice) on learning and memory in mice. Elevated plus-maze and passive avoidance paradigm were employed to test learning and memory. Three doses (75, 150 and 300 mg/kg p.o.) of aqueous extract of *Glycyrrhiza glabra* were administered for 7 successive days in separate groups of animals. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by diazepam (1 mg/kg i.p.) and scopolamine (0.4 mg/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice may be contributing favorably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by liquorice, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in mouse brain. However, further studies are necessitated to identify the exact mechanism of action. In the present investigation, *Glycyrrhiza glabra* has shown promise as a memory enhancing agent in all the laboratory models employed.

Keywords: Liquorice; Amnesia; Learning; Memory

1. Introduction

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it 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. The central cholinergic pathways play a prominent role in learning and memory processes (Nabeshima, 1993). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (Higashida and Ogawa, 1987) and human beings (Sitaram et al., 1978). Epidemiological studies of Indian population reveal that dementia is largely a hidden problem (Shaji et al., 2002). Prevalence rates for dementia increase exponentially with advancing age (Kawas et al., 2000; Vas et al., 2001). Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients.

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been in clinical use for centuries. The roots have antiulcer, expectorant, diuretic, laxative, sedative (Hikino, 1985), antipyretic (Lata et al., 1999), antimicrobial and anxiolytic activities (Ambawade et al., 2001). The main constituent of *Glycyrrhiza glabra* is glycyrrhizin which has antiviral (Ceremelli et al., 1996) anti-inflammatory (Yokota et al., 1998) and antioxidant action (Ju et al., 1989). In Ayurveda, it is used extensively to relieve "Vata" and "Kapha" inflammations. Ayurvedic system of medicine is based on three fundamental principles or doshas called Vata, Pitta and Kapha. These doshas govern all cellular processes responsible for healthy life. Vata governs all movements/activities, Pitta governs heat/energy levels and regulates various transformations whereas, Kapha controls growth, structural modifications and lubrication. When these principles, which guide the processes of our body/mind get disturbed in an individual due to bad environment or poor diet the individual starts suffering from some disease. For instance, when, Vata gets out of balance, the consequences are hyper-active mind, circulatory disorders, poor neurotransmission, irreg-

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ular elimination and uncomfortable menses. If Pitta is disturbed, we observe excessive acidity resulting in heartburn, peptic ulcers, hot temper and inflammations. Whereas, if Kapha gets out of balance, the result is chronic congestion, weight gain, high cholesterol levels and acne.

1.1. Objective

The present study was undertaken to investigate the effects of *Glycyrrhiza glabra*, popularly known as liquorice on learning and memory in mice.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (3 months old), weighing around 25 g and procured from disease free small animal house, CCS Haryana Agriculture University, Hisar (Haryana) were used in the present study. They had free access to food and water, and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. Food given to mice consisted of wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The animals were acclimatized for at least 5 days before behavioral experiments. Experiments were carried out between 09:00 and 14:00 h. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines (Reg. No. 0436).

2.2. Drugs

Liquorice powder (Himalaya Drug Company, Bangalore, India), scopolamine hydrobromide (Sigma-Aldrich, USA) and diazepam (Ranbaxy, India) were used in the present study.

2.3. Laboratory models for testing learning and memory

(i) Scopolamine-induced amnesia (Interoceptive Behavior Model).
(ii) Diazepam-induced amnesia (Interoceptive Behavior Model).
(iii) Elevated plus-maze (Exteroceptive Behavior Model).

Elevated plus-maze served as the exteroceptive behavior model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as per the parameters described by the investigators working in the area of neuropsychopharmacology (Itoh et al., 1990; Reddy and Kulkarni, 1998; Dhingra et al., 2003; Parle and Dhingra, 2003). The apparatus consisted of two open arms (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was the time taken by mouse with all its four legs to move into one of the enclosed arms. TL was recorded on the first day. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for another 10 s and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial. Another laboratory model, viz. passive avoidance apparatus was employed to substantiate the findings and overcome the limitations of elevated plus-maze.

(iv) Passive avoidance paradigm (Exteroceptive Behavior Model).

Passive avoidance behaviour based on negative reinforcement was used to examine the long-term memory (Reddy and Kulkarni, 1998; Parle and Dhingra, 2003). The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V ac) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 s and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wood platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range (2–15 s) during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 s, electric shocks were delivered for 15 s. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 s.

2.4. Preparation of liquorice extract

Liquorice extract was prepared (Indian Pharmacopoeia, 1966) by extracting liquorice powder with chloroform water (0.1%) in the ratio of 1:8 by double maceration (each maceration for 24 h). The aqueous extract obtained was passed through muslin cloth and the filtrate was boiled for 5 min. This extract was then set aside for 18 h and was filtered again using filter paper. The extract was concentrated until the density of the liquid extract became 1.06 g/ml. The solid contents of the aqueous extract were 0.03 g/ml. The yield of

the extract was 34.6%. The extract was administered orally to separate groups of mice in three different doses 75, 150 and 300 mg/kg (equivalent to 2.6, 5.19 and 10.38 g, respectively, of dried plant material). The response of animals to these doses was observed after 90 min. These doses were selected on the basis of our pilot study and earlier reports (Al-Qarawi et al., 2002).

2.5. Vehicle

Liquorice extract was diluted in distilled water. Scopolamine hydrobromide was dissolved in normal saline. Injection of diazepam (Calmpose®) was diluted in normal saline. Volume of oral administration and i.p. injection was 1 ml/100 g of mouse.

2.6. Drug protocol

Animals were divided into 14 groups and each group comprised of a minimum of five animals. Groups I to X represent observations on elevated plus-maze and groups XI to XIV represent observations using passive avoidance paradigm.

2.6.1. Using elevated plus-maze

Group I: control group for elevated plus-maze ($n = 6$): distilled water (1 ml/100 g) was administered p.o. for 7 days. After 90 min of administration on 7th day, transfer latency was recorded. Retention of learned task was examined after 24 h.

Groups II, III and IV ($n = 5$ each): liquorice aqueous extract (75, 150 and 300 mg/kg, respectively) was administered orally for 7 days. TL was noted after 90 min of administration on 7th day and after 24 h.

Groups V and VI ($n = 5$ each): scopolamine hydrobromide (0.4 mg/kg i.p.) and diazepam (1 mg/kg i.p.), respectively, were injected before training. TL was recorded after 45 min of injection. Retention was examined after 24 h.

Group VII ($n = 5$): TL was recorded on first day. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording TL on second day.

Group VIII ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. after 90 min of administration of liquorice extract on 7th day. TL was recorded after 45 min of injection and after 24 h.

Group IX ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. TL was recorded after 90 min of administration of extract. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording TL on 8th day.

Group X ($n = 5$): liquorice extract (150 mg/kg) was administered orally for 7 days. Diazepam (1 mg/kg) was injected i.p. after 90 min of administration of liquorice extract on 7th day. TL was recorded after 45 min of injection and after 24 h.

2.6.2. Using passive avoidance paradigm

Group XI: control group for passive avoidance paradigm ($n = 6$): distilled water (1 ml/100 g) was administered p.o. for 7 days. After 90 min of administration on 7th day, SDL was recorded during both the sessions of training. Retention of learned task was examined after 24 h.

Group XII ($n = 5$): liquorice extract (150 mg/kg) was administered orally for 7 days. SDL was recorded after 90 min of administration on 7th day and after 24 h.

Group XIII ($n = 5$): animals were trained on first day and SDL was recorded during both sessions of training. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording SDL on second day.

Group XIV ($n = 5$): liquorice extract (150 mg/kg) was administered for 7 days p.o. SDL was recorded after 90 min of administration of liquorice extract. Scopolamine hydrobromide (0.4 mg/kg) was injected i.p. 45 min prior to recording SDL on 8th day.

2.7. Statistical analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's '*t*' test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect on transfer latency (using elevated plus-maze)

TL of first day reflected learning behavior of animals whereas, TL of second day reflected retention of information or memory. Liquorice extract (75 mg/kg) administered for 7 days orally did not have any significant effect on TL of first day of training and on second day as compared to control. The higher dose (150 mg/kg) of the extract significantly decreased TL on first day as well as on second day, indicating significant improvement of learning and memory (Table 1). Surprisingly, the highest dose (300 mg/kg) of the extract significantly increased TL of first day, indicating significant impairment in learning. The dose-selection of scopolamine hydrobromide and diazepam was based on our earlier studies (Parle and Dhingra, 2003). Scopolamine hydrobromide (0.4 mg/kg) injected before training impaired learning significantly as indicated by increased TL. While scopolamine injected after training impaired memory significantly. Diazepam (1 mg/kg) injected before training impaired learning significantly. Liquorice extract (150 mg/kg) administered orally for 7 days protected the animals from scopolamine- and diazepam-induced impairment in learning and memory.

3.2. Effect on step-down latency (SDL)

Liquorice extract (150 mg/kg) administered for 7 days significantly increased SDL (242 ± 39.1) as compared to con-

Table 1
Effect of liquorice extract on transfer latency (TL) of mice using elevated plus-maze paradigm

Group no.	Treatment	Dose (kg^{-1})	TL on 1st/7th day	TL after 24 h
I	Control (vehicle)	10 ml	25.5 ± 3.0	18.5 ± 2.0
II	Liquorice extract for 7 days p.o.	75 mg	23.7 ± 3.5	14.8 ± 2.8
III	Liquorice extract for 7 days p.o.	150 mg	15.2 ± 1.1[a]	12.3 ± 1.2[a]
IV	Liquorice extract for 7 days p.o.	300 mg	34.6 ± 1.8[a]	24.4 ± 1.8
V	Scopolamine HBr (before training)	0.4 mg i.p.	52.2 ± 6.3[a]	30.4 ± 6.6
VI	Diazepam (before training)	1 mg i.p.	49.7 ± 7.0[a]	30.4 ± 6.8
VII	Scopolamine HBr (after training)	0.4 mg i.p.	24.7 ± 8.6	44.8 ± 13.2[a]
VIII	Liquorice extract for 7 days + scopolamine HBr (before training)	150 mg, 0.4 mg	15.6 ± 2.6[b]	10.7 ± 1.4[b]
IX	Liquorice extract for 7 days + scopolamine HBr (after training)	150 mg, 0.4 mg	15.1 ± 1.8	15.3 ± 3.1[b]
X	Liquorice extract for 7 days + diazepam (before training)	150 mg, 1 mg	16.7 ± 1.8[c]	10.5 ± 2.2[c]

Values are in Mean ± S.E.M.
[a] $P < 0.05$ as compared to control group.
[b] $P < 0.05$ as compared to scopolamine alone (before/after training).
[c] $P < 0.05$ as compared to diazepam alone (before training).

trol group (119.5 ± 35.7) on second day indicating improvement of memory. Scopolamine hydrobromide (0.4 mg/kg) significantly decreased SDL (15.6 ± 2.2) on second day indicating impairment of memory (amnesia). Liquorice extract (150 mg/kg) administered orally for 7 days significantly reversed amnesia (SDL = 23.4 ± 1.8) induced by scopolamine hydrobromide.

4. Discussion

In the present study, 150 mg/kg of liquorice extract (equivalent to 5.19 g of dried plant material) administered orally for 7 days improved learning and memory of mice significantly in both the exteroceptive behavioral models employed. The stimulus lie outside the body in exteroceptive behavior models, whereas, it lies within the body in the case of interoceptive models. This is the first research finding showing enhancement of learning and memory by liquorice. Furthermore, pretreatment with liquorice extract (150 mg/kg) for 7 days protected the animals from learning and memory impairment produced by interoceptive stimuli (scopolamine and diazepam). These findings suggested the possible neuroprotective role for liquorice. The impairment of learning due to the highest dose (300 mg/kg) of the extract probably represented the lethal effect of the extract. This paradoxical effect could also be due to the sedative property of the drug (Hikino, 1985). Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer's disease patients. Moreover, it has also been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms of Alzheimer's disease upon chronic use of anti-inflammatory drugs (McGeer and McGeer, 1999). Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia and also against amyloid deposits in the brain (Rao et al., 2002; Stephan et al., 2003). Anti-inflammatory action of liquorice (Yokota et al., 1998) might also be contributing to the observed memory-enhancing activity of liquorice. Oxygen free-radicals are implicated in the process of ageing and may be responsible for the development of Alzheimer's disease in elderly persons (Sinclair et al., 1998). Oxygen-free radicals and other products of oxidative metabolism have been shown to be neurotoxic (Sayre et al., 1997) and antioxidant-rich diets improved cerebellar physiology and motor learning in aged-rats (Bickford et al., 2000). The protective effect of liquorice extract may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function, thereby enhancing the memory. Furthermore, liquorice has been found to possess antioxidant property as well (Ju et al., 1989). Thus, a combination of anti-inflammatory, antioxidant and neuroprotective role could all be leading to the net memory-enhancing effect.

5. Conclusion

In the present investigation, *Glycyrrhiza glabra* has shown promise as a memory enhancing agent in mice in all the laboratory models employed.

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