EFFECT OF DEGLYCYRRHINATED LICORICE ON LEARNING AND MEMORY IN RATS ASSESSED BY HEBB WILLIAM MAZE AND ELEVATED PLUS MAZE

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ABSTRACT

Context: The glycyrrhiza glabra(licorice) has shown a neuroprotective role. Deglycyrrhinated licorice (DGL) preparations is made from licorice from which the glycyrrhizin has been removed. **Aims:** To evaluate the effects of Deglycyrrhinated licorice (DGL) in diazepam induced impairment of learning & memory in rats. **Settings and Design:** Animals were grouped into 6 groups(n=6), Group 1(control) received the vehicle, group 2 & group 3 animals received DGL a licorice root extract of 10mg/kg & 13.5mg/kg respectively. Group 4 diazepam of (1mg/kg i.p) was given. Group 5 & group 6 received DGL 10mg/kg & 13.5mg/kg respectively for 10 days and on 10th day diazepam 1mg/kg was administered. **Methods and Material:** The transfer latency (TL), time taken to reach reward chamber (TRC) using EPM and HWM respectively, was evaluated on 10th & 11th day. **Statistical analysis used:** Analysed using Non-parametric measure – Median ± Interquartile range (IQR), where P value <0.05 was considered significant. **Results:** The TL & TRC was significantly reduced in DGL (13.5mg/kg) & even diazepam induced amnesia was reversed showing memory enhancing activity. **Conclusions:** In our study DGL has shown a memory enhancing property in laboratory models employed.

KEYWORDS: Deglycyrrhinated, licorice, learning, memory, Diazepam, amnesia.

INTRODUCTION

Learning is a process of procuring information and skills, while ensuing the retention of the acquired information is called memory.[1] The pervasiveness in impairments of learning & memory in cognitive disorders like Alzheimer's disease, amnesia, delirium, depression & Schizophrenia is still at large.[2] For the management of these disorders, new memory enhancers are being constantly explored, of which herbs play a vital role.[3]

Glycyrrhiza glabra, is also known as licorice and sweet wood, which is grown in Mediterranean and certain areas of Asia.[4] For centuries together the roots and rhizomes of glycyrrhiza glabra (family: Leguminosae) are in use. The roots have antiulcer, expectorant, diuretic, laxative, sedative[5], antipyretic[6], antimicrobial and anxiolytic activities[7] The Glycyrrhiza glabra, important constituent is glycyrrhizin which has antiviral[8] anti-inflammatory[9] and antioxidant action.[10] In Ayurveda, roots and rhizomes of glycyrrhiza glabra is known as a medhya rasayana which means a substance optimizing the brain functions.[11]

One of the side effects is licorice-induced hypertension and edema which occurs due to *glycyrrhizic acid* a component of licorice. In deglycyrrhinated licorice the glycyrrhizin component is eliminated to an extent.[4]

Deglycyrrhinated licorice preparations are useful in treating various types of ulcer, while topical licorice preparations have been used to sooth & heal skin eruptions, such as psoriasis & herpetic lesions.[4]

The administration of benzodiazepines before the learning of a list of words or geometrical patterns impairs the recall of these lists in the later test. Similar findings have also been found in animals in a wide variety of tasks and species evidencing anterograde amnesia, benzodiazepines would impair acquisition processes mainly by disrupting the ability to build new associations between events.[13]

Hence, present study is taken up to investigate and validate the learning and memory enhancing activity of deglycyrrhinated licorice (DGL) in rats.

Objective of the study: To evaluate the effects of deglycyrrhinated licorice (DGL) in diazepam induced impairment of learning & memory in rats using Hebb William maze & elevated plus maze.

MATERIAL AND METHODS

Animals

Male Albino rats *Wistar* strain of 4 – 6 weeks, weighing (200-250g) was obtained from central animal house. The animals were grouped and housed in poly propylene cages and maintained under standard laboratory conditions (temperature 25 ± 2^0) with dark and light cycle (12h/12h). The rats had free access to standard dry pellet diet and water *ad libitum*. The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Chemicals

Diazepam - diazepam injection, Calmpose®, Ranbaxy, India.

Plant material

Deglycyrrhinated licorice(DGL) a licorice root extract (GutGard®) obtained from Natural remedies, Bengaluru, India. DGL composition contains less than 0.2% of glycyrrhizin, safety studies by Natural remedies claims that acute oral toxicity study with a higher dose of 5000mg/kg did not manifest any toxicological signs in rats and was found to be non-mutagenic in Ames test.

Drug treatment

Deglycyrrhinated licorice, which is a licorice root extract preparation (GutGard®) was obtained by Natural remedies, India. The DGL dose 10mg/kg and 13.5mg/kg was suspended in distilled water and administered orally to rats.

Injection of diazepam (Calmpose®) was diluted in normal saline. Volume of oral administration and i.p. injection was 1 ml/100 g of rat.

Laboratory models for testing learning and memory:

(i) Interoceptive behavioral models:

-   Diazepam-induced amnesia

(ii) Exteroceptive Behavior Model:

-   Hebb William maze

-   Elevated plus maze

Diazepam-induced amnesia

Diazepam has well-known amnestic properties. To demonstrate the amnesic effects in rats, diazepam 1-2mg/kg, intraperitoneal (i.p) is administered using a 1ml syringe & 23G needle.[14]

Hebb William Maze (HWM)

HWM is an incentive-based exteroceptive behavioural model useful for measuring spatial working memory of rodents. Briefly, HWM consists of mainly three components, (a) animal chamber (or start box), which is attached to (b) the middle chamber (or exploratory area) and (c) a reward chamber at the other end of the maze in which reward (food) is kept. The box is partitioned with wooden slats into blind passages leaving just one twisting corridor leading from the entry to the reward chamber.[15] On the first day (i.e.,10th day of DGL administration), the mouse is placed in the animal chamber and the door is opened to facilitate the entry of animal into the next chamber. The door of the start box is closed immediately after the animal moved into the next chamber so as to prevent back entry. Time taken by the animal to reach Reward Chamber (TRC) from start box on first day reflects the learning index. Later Diazepam (1mg/kg) is injected i.p. after 90min of administration of DGL extract on tenth day. Each animal is allowed to explore the maze for three minutes with all the doors opened before returning to home cage. Retention (memory score) of this learned task will be examined 24h after the first-day (i.e., 11th day). Significant reduction in TRC value indicated improvement of memory.

Elevated plus maze (EPM)

Elevated plus-maze served as the exteroceptive behaviour model to evaluate learning and memory in rat. 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 (i.e., 10th 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 (i.e., 11th day) trial.

Experimental protocol

The male wistar rats were divided into 6 groups, where each group had 6 rats. The drugs were administered for 10 consecutive days to all groups and with the help of experimental models - Elevated plus maze (EPM) & Hebb William maze (HWM) the transfer latency (TL) & time take to reach the chamber (TRC) was recorded on 10th day and after 24hrs again.

Group 1 – Animals served as control, received vehicle i.e., distilled water through oral feed.

Group 2 & 3- Here the animals received deglycyrrhinated licorice (DGL) of 10mg/kg and 13.5mg/kg respectively through oral feed.

Group 4 – Animal received diazepam 1mg/kg intra peritoneal (i.p)

Group 5 & 6– Rats received DGL of 10mg/kg & 13.5mg/kg respectively for 10 days. For both the groups on 10th day after 90mins of administration of oral feed, diazepam 1mg/kg i.p was given and the TL & TRC was recorded after 45mins, similar parameters were checked again after 24 hrs.

Statistical Analysis

The data obtained was analysed using non-parametric measure – median ± interquartile range (IQR), Kruskal Wallis test, Mann-Whitney U test with Bonferroni correction, where P value <0.05 was considered significant.

RESULTS

Effect of TL using EPM

The effect of control, DGL (10mg/kg and 13.5mg/kg), diazepam control, DGL both doses along with diazepam were evaluated at 1st day (i.e., 10th day) & after 24hrs of administration of drugs. Transfer latency in group 2 on the first day didn't show much improvement & even on 2nd day as compared to the control group, this indicates significant impairment in learning. The higher dose of DGL 13.5mg/kg significantly reduced the TL on 1st day & after 24hrs when compared with control group, which proves that the DGL extract improved learning & memory. When diazepam was injected to diazepam control group, it impaired the memory both on first day & after 24hrs. In group 5 the TL didn't show improvement in comparison to diazepam group, while DGL extract (13.5mg/kg) in group 6 showed improvement in TL, which indicates DGL protected the animal from diazepam induced impairment in learning & memory.

(Refer table-1)

Table 1: Effect of Deglycyrrhinated licorice(DGL) on Transfer latency (TL) in rats using elevated plus maze.

Group No (n=6)	Treatment	Dose (/kg)	TL (on 1st/ 10th day) secs	TL after 24hrs
1	Control (Vehicle)	10ml	35.50(18.50-34.50)	20.00(3.75-10.75)
2	DGL- I for 10 days	10mg	43.50(35.75-54.25)	26.50(19.00-41.50)
3	DGL- II for 10 days	13.5mg	22.50(20.75- 47.75) [a]	13.50(12.50-29.75) [a]
4	Diazepam i.p (before training)	1mg	39.00(24.25 – 42.00)	23.50(19.00-32.00)
5	DGL-I for 10 days + Diazepam (before training)	10mg, 1mg	42.50(34.50- 52.00)	15.00(7.50-18.25) [b]
6	DGL-II for 10 days + Diazepam (before training)	13.5mg, 1mg	24.50(14.50- 32.25) [b]	12.00(5.50-17.50) [b]

Values – Median (Inter-quartile range)

a- $P < 0.05$ as compared to control group. b-$P < 0.05$ as compared to diazepam group

Effect of TRC using HWM

Here changes in time taken by the rats to reach the reward chamber from the entry chamber in DGL 13.5mg/kg treated animals showed a significant reduction in TRC on both 1st & 2nd day in comparison to control group. The time taken by animals to reach reward chamber in DGL 10mg/kg treated animals didn't show much reduction in TRC, indicating no significant improvement in learning & memory. In group 5 animals the TRC didn't show much improvement when compared to diazepam group, While group 6 animals showed significant reduction in TRC when compared to diazepam group. (Refer table-2).

Table-2: Effect of Deglycyrrhinated licorice (DGL) on time taken to reach reward chamber (TRC) by rats using Hebb-William maze.

Group No (n=6)	Treatment	Dose (/kg)	TRC (on 1st/ 10th day) secs	TRC after 24hrs
1	Control (Vehicle)	10ml	35.50 (25.75-74.75)	22.00 (16.0-55.00)
2	DGL- I for 10 days	10mg	39.50(32.25-105.25)	90.50 (31.25-138.75) [a]
3	DGL- II for 10 days	13.5mg	27.00(14.00-50.50) [a]	15.50 (13.25-25.75) [a]
4	Diazepam i.p (before training)	1mg	46.00(28.00-57.25) [a]	25.00 (14.75-31.00)
5	DGL-I for 10 days + Diazepam (before training)	10mg, 1mg	57.50(30.25-105.00)	33.50 (26.25-91.00)
6	DGL-II for 10 days + Diazepam (before training)	13.5m, 1mg	22.00(16.75-42.50) [b]	13.50 (9.25-22.25) [b]

Values – Median (Inter-quartile range)

a- $P < 0.05$ as compared to control group.

b- $P < 0.05$ as compared to diazepam group.

DISCUSSION

The present study evaluates the effect of Deglycyrrhinated licorice on learning and memory in rats using Hebb-William maze and elevated plus maze.

Use of herbal supplements as alternative sources has attracted the researchers worldwide over the past few years and several studies on medicinal plants have been undertaken to evaluate the learning and memory effects in animals.[15]

The DGL dose of 13.5mg/kg has an effect on the extroceptive behavioural models used in our study. The acquisition of learning skills and memory enhancing activity of DGL has been evidenced by transfer latency in elevated plus maze and time taken to reach reward chamber in Hebb-William maze.

Alzheimer's disease is a neurodegenerative disorder where the brain cells degenerate and die, leading to a steady decline in memory and mental function. Progressive memory loss, dementia, cognitive deficits are currently seen as medical & social problems.[16]

DGL is devoid of glycyrrhizic acid which causes inhibition of 11β-hydroxysteroid dehydrogenase leading to state of hypermineralocorticism which results in hypokalemia and mineralocorticoid-related hypertension.[17]

When GutGard, a deglycyrrhizinated root extract of *G. glabra* was prescribed as a remedy to patients with functional dyspepsia, it was safe and effective. In management of H. pylori also GutGard was an effective alternative treatment approach.[18,19]

Diazepam-induced anterograde amnesia in inhibitory avoidance task is mediated through influences on the basolateral amygdala nucleus.[20]

Therefore, DGL preparation 13.5mg/kg when administered orally for 10days significantly reversed the amnesia induces by diazepam which was shown by decrease in transfer latency and also reduction in time taken to reach the reward chamber, this is probably due to reversal action on basolateral amygdala.

Studies have shown basolateral amygdala nuclei and cholinergic neurotransmitter involvement in learning and memory. Acetylcholine is a major neurotransmitter in the brain

and progression of Alzheimer's disease is caused due to cholinergic deficit leading to cognitive dysfunction and decline.[21]

CONCLUSION

The findings of DGL at 13.5mg/kg revealed significant improvement in learning and memory in the models elevated plus maze and Hebb-William maze, DGL also reversed the diazepam-induced amnesia. Further studies are recommended using other suitable models in learning and memory. Thus, our results indicate that DGL at 13.5mg/kg has a beneficial role on learning & memory.

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