Effect of *Tribulus Terrestris* on Learning and Memory in Wistar Rats

Natesh Prabhu, Sanjay Hadigal, Ullal Sheetal D*, Sushma DS and Ashok Shenoy K

Department of Pharmacology, Kasturba Medical College, Light House Hill Road, Mangalore-575001, Manipal University, Manipal, Karnataka, India

ABSTRACT

Objectives: The present study was designed to evaluate the effect of aqueous extract of fruits of *Tribulus terrestris* on learning and memory in rodents. **Materials and Methods:** Thirty wistar rats were divided in 5 groups of 6 rats each. Baseline values for the time taken to reach reward chamber (TRC) in the Hebb William Maze and transfer latency (TL) in the T-maze were recorded on Day 1. Mean of 5 sessions was calculated for each rat. Group I was normal control, group II piracetam standard, group III, IV and V received *Tribulus terrestris* orally at 100mg/kg, 200mg/kg and 400mg/kg respectively for 14 days. At the end of 14 days, each rat was tested for TRC and TL and compared with the control group. **Results:** Group IV showed a significant decrease in TRC when compared to group I in Hebb William Maze ($p < 0.0001$). Group IV also showed a significant decrease in TL when compared to group I in T-maze ($p < 0.0001$). Group III showed a significant decrease in TL when compared to group I in the T-maze ($p = 0.035$), however there was no decrease in TRC in this group. **Conclusions:** The aqueous extract of fruits of *Tribulus terrestris* showed a dose dependent beneficial effect in learning and memory models in rats, with 200mg/kg being most beneficial.

Keywords: Hebb William Maze, Learning, Memory, T Maze, Tribulus terrestris

INTRODUCTION

Learning is defined as the acquisition of information and skills and subsequent retention of that information is called memory. Understanding the cellular basis of learning and memory is clearly one of the most challenging problems in science and is of enormous interest to every person. The possible benefits to everyone in general are almost unlimited. They might range from combating diseases such as Alzheimer's disease to helping an individual, store and recall information better. A number of agents have been tried on various behavioural and pharmacological models for evaluation of the process of learning and memory.

***Corresponding author:**
Dr. Ullal Sheetal D
Associate Professor
Department of Pharmacology, Kasturba Medical College, Light House Hill Road, Mangalore-575001, Manipal University, Manipal, Karnataka, India
E-mail: sheetal.ullal@manipal.edu

DOI: *10.5530/pj.2014.4.10*

The models can be broadly classified as exteroceptive and interoceptive aversion stimuli models[1]. The exteroceptive models consist of behaviour on mazes such as T maze and avoidance behaviour on shuttle box. Interoceptive models include scopolamine induced amnesia, electroshock induced amnesia and hypoxic stress induced learning deficits.

Tribulus terrestris is a flowering plant from the family Zygophallaceae and is native to warm and tropical regions such as southern Asia. The flowers are 4–10 mm wide, with five lemon yellow petals. A week after each flowerblooms, it is followed by a fruit that easily falls apart into four or five single-seeded nutlets. It is used as a tonic, aphrodisiac, analgesic, astringent, anti-hypertensive, diuretic, and urinary anti-infective[2]. Its aqueous extract has shown diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization in vitro[3]. Tribulosin, a methanolic extract of *Tribulus terrestris* protects rat heart from ischemia/perfusion injury[4]. Despite its aphrodisiac activity, it has shown no effect on endocrine sensitive tissues like prostrate, seminal vesicle, uterus and vagina in wistar rat pointing to the lack of androgenic and estrogenic activity in vivo[5].

Since its effect on learning and memory hasn't been explored, the present study was designed to evaluate the effect of aqueous extract of fruits of *Tribulus terrestris* on learning and memory in rodent behavioral models of learning and memory.

MATERIALS AND METHODS

Experimental animals

Wistar albino male rats weighing 180–220 grams inbred in the institutional animal house were used for the study. Rats were housed in clean polypropylene cages, three rats were kept in each cage, in a controlled environment (22°–24°c) with a 12 hour light and dark cycle with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum*. The rats were allowed to acclimatise to these conditions for one week. Experiments were performed during the light phase of the cycle (10:00–17:00) in the Department of Pharmacology, Kasturba Medical College, Mangalore.

Study material

Tribulus terrestris fruit powder, obtained from Himalaya Health Care, Bangalore (gift sample), was dissolved in distilled water and administered to the rats in the doses of 100 mg/kg, 200 mg/kg and 400mg/kg.

Study procedure

The rodent behavioural models of learning and memory used for the study were Hebb William Maze and the T maze.

Hebb-William Maze is an incentive based exteroceptive behavioural model useful for measuring spatial and working memory of rats. It mainly consists of three compartments: chamber B, in which the rat is placed and has a sliding door that is opened to allow the rat to enter the middle chamber, (exploratory area of maze), the door of which is closed to prevent its back entry to chamber B. The animal has to explore the middle chamber and reach the reward chamber A, at the other end of the maze in which the reward (food) is kept. An electrical system provides indication when the rat is placed in chamber B, when it leaves it to enter the maze i.e. middle chamber and when it enters reward chamber A, thus enabling the reaction time to be noted without observing the animal. Time taken to reach the reward chamber (TRC) was recorded by the digital timer of the Hebb William Maze. Each rat was allowed to explore the maze for an additional 20seconds with all the doors opened before returning to its cage. Twelve hours fasted rats were employed in the study and the average of 5 sessions were recorded for each rat[6].

T - Maze serves as an exteroceptive behavioural model to evaluate learning and memory in rats. The apparatus consists of one open arm (50cm x 10cm) and two covered arms (50cm x 10cm x 40cm). The central platform (10cm x 10cm) and the maze is elevated to a height of 50cm from the ground. Each rat was placed at the end of the open arm, facing away from the central platform. Rats prefer to be in the covered arms. Transfer latency (TL) was taken as the time taken by the rat to move into the covered arms with all its four limbs. The rat was allowed to explore the maze for an additional 20 seconds before returning to its home cage[7–9]. The average of TL of 5 sessions was recorded.

Thirty wistar rats were divided in 5 groups of 6 rats each. Baseline values for the time taken to reach reward chamber (TRC) by Hebb William Maze and transfer latency (TL) by T-maze were recorded on Day 1. A mean of 5 sessions was calculated for each rat. Group I was normal control, group II piracetam standard intraperitoneally at 200mg/kg, group III, IV and V received *Tribulus terrestris* orally at 100mg/kg, 200mg/kg and 400mg/kg respectively for 14 days as shown in Table 1. At the end of 14 days, each rat was tested for TRC and TL and compared with the control group.

Statistical Analysis

The results were expressed as Mean ± Standard Deviation (SD). Statistical analysis was done using one way ANOVA followed by Tukey post hoc test, using SPSS version 11.5. The value of $p<0.05$ was considered significant. Analysis within groups between day 1 and day 14 in Hebb William Maze and T Maze was done by paired t test.

RESULTS

Hebb William Maze

In the analysis of the TRC in Hebb William Maze as shown in Table 2, all groups showed reduction compared to baseline. Group II receiving piracetam showed a significant reduction in TRC when compared to Group I, III and V ($p<0.001$). But no significant difference was seen when compared with Group IV. Group IV showed significant reduction in TRC when compared with Group

I

Table 1: Drugs along with the respective route administered to the animals

Group No.	Group	Test/standard drug treatment	Dose	Route	Duration of treatment
I	Normal control	2% Gum acacia	10 ml/kg	Oral	14 days
II	Piracetam control	Piracetam	200mg/kg	i.p	14 days
III	TT1	*Tribulus terrestris*	100 mg/kg	Oral	14 days
IV	TT2	*Tribulus terrestris*	200 mg/kg	Oral	14 days
V	TT3	*Tribulus terrestris*	400 mg/kg	Oral	14 days

TT- *Tribulus terrestris*. *Tribulus terrestris* was given at 3 different doses i.e. TT1, TT2 and TT3. i.p- intraperitoneal, ml- milli litre, mg- milli gram and kg- kilogram.

II

Table 2: Results of Hebb William Maze and T Maze

Group No.	Hebb William Maze: TRC (sec)		T Maze: TL (sec)	
	Day 1	Day 14	Day 1	Day 14
I	120.27 ± 14.51	76.63 ± 4.88	133.71 ± 7.93	139.46 ± 6.31
II	115.39 ± 4.18	47.46 ± 3.61*	132.85 ± 3.50	94.53 ± 2.04$^{£}$
III	123.42 ± 6.13	74.75 ± 5.86	133.95 ± 2.52	130.36 ± 7.39$^{€}$
IV	122.92 ± 10.89	54.18 ± 3.86$^{\$}$	135.16 ± 5.32	126.56 ± 3.29$^{\#}$
V	121.59 ± 9.30	73.19 ± 5.56	131.42 ± 5.43	133.40 ± 4.49

The data are expressed as Mean ± SD ; TRC- Time to reach Reward Chamber, TL- Transfer Latency ; *p<0.001 vs Group I, III, V ; $^{\$}$p<0.001 vs Group I, III, V ; $^{\#}$p<0.001 vs Group I ; $^{€}$p<0.05 vs Group I ; $^{£}$p<0.001 vs Group I, III, IV, V

III

Table 3 : Results of analysis within groups for the difference between Day 1 and 14 in Hebb William Maze and T maze

Group No.	Hebb William Maze: TRC (sec)	T Maze: TL (sec)
I	43.63 ± 13.68**	-5.75 ± 12.09
II	67.93 ± 2.93**	38.31 ± 2.65**
III	48.67 ± 9.77**	3.59 ± 7.57
IV	68.74 ± 13.61**	8.59 ± 6.93*
V	48.39 ± 6.42**	-1.98 ± 3.80

The data are expressed as Mean ± SD ; TRC- Time to reach Reward Chamber, TL- Transfer Latency ; **p<0.001, *p<0.05

I, III and V ($p<0.001$). As shown in Table 3, difference between day 1 and day 14 of TRC in all the groups was found to be statistically significant ($p<0.001$).

T Maze

As shown in Table 2, Group IV also showed a significant decrease in TL when compared to Group I in T-maze ($p<0.001$). Group III showed a significant decrease in TL when compared to Group I for the T-maze ($p=0.035$). Group II was found to be superior to all the other groups with regard to TL ($p<0.001$). As shown in Table 3, difference between day 1 and day 14 of TL in Group I ($p<0.001$) and Group IV ($p=0.029$) was found to be statistically significant.

DISCUSSION

Tribulus terrestris commonly known as "Gokshura" in India is a plant with multiple uses. It has been found to be useful in multiple conditions from erectile dysfunction and kidney dysfunction to urinary infections.

Hebb William Maze and T Maze behavioural models have been utilized for the assessment of learning and memory in rats in this study. A fall in TRC and TL on subsequent exposures to the Hebb William Maze and the T maze respectively, was taken as an index of successful retention[9].

Piracetam showed significantly reduced values of TRC when compared with all the groups except, the group that received *Tribulus terrestris* at a dose of 200mg/kg. This indicates that *Tribulus terrestris* at a dose of 200mg/kg was on par with piracetam with regard to reducing the TRC in the Hebb William Maze after 14 days. *Tribulus terrestris* at a dose of 200mg/kg also showed improved results when compared to the same being given at doses of 100mg/kg, 400mg/kg and also with the normal control.

Piracetam showed significantly reduced values of TL when compared to all the other groups. *Tribulus terrestris* at a dose of 200mg/kg showed significantly better results when compared to the normal control. *Tribulus terrestris* at a dose of 400mg/kg did not show significant difference in the reduction of TL. But, *Tribulus terrestris* at a dose of 100mg/kg also showed improved results when compared to normal control, but no decrease in TRC was noticed with this group. This indicates that *Tribulus terrestris* only at a dose of 200mg/kg could significantly reduce both TRC and TL.

In the study done by Martino Andrade AJ *et al*, *Tribulus terrestris* was used at a dose of 11, 42 and 110mg/kg/day and was unable to stimulate the endocrine sensitive tissues like prostate, seminal vesicle, uterus and vagina[5]. Another study, done by Jungmo Do *et al*, *Tribulus terrestris* showed a dose dependent increase in the Intracavernous Pressure after being administered for one month at a dose of 2.5, 5, 10, 50 and 100mg/kg in rabbits, thus demonstrating its use as a potential agent for erectile dysfunction[10].

Our study, is the first study exploring the effect of *Tribulus terrestris* on learning and memory in rats. The aqueous extract of fruits of *Tribulus terrestris* showed a dose dependent beneficial effect in learning and memory models in rats, with 200mg/kg being most beneficial.

REFERENCES

1. Kulkarni SK, Reddy, Meth DS. Find Exp Clin. Pharmacol. 1996; 18: 219–30.
2. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activities of *Tribulus Terrestris* L. growing in Iraq. J Zhejiang Univ Sci B. 2008; Feb. 9(2): 154–9.
3. Aggarwal A, Tandon S, Singla SK, Tandon C. Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization invitro by aqueous extract of plant. Int Braz J Urol. 2010; Jul-Aug. 36(4): 480–8.
4. Zhang S, Li H, Yang SJ. Tribulosin protects rat hearts from ischemia/perfusion injury. Acta pharmacol sin. 2010; Jun. 31(6): 671–8.
5. Martino-Andrade AJ, Morais RN, Spercoski KM, Rossi SC, Vechi MF, Golin M, et al. Effects of Tribulus terrestris on endocrine sensitive organs in male and female wistar rats. J Ethnopharmacol. 2010; Jan 8. 127(1); 165–70.
6. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electro convulsive shock. Psychopharmacology, 1990; 101: 27–33.
7. Parle M, Dhingra D, Kulkarni SK. Improvement of mouse memory by Myristica fragrans seeds. J Med Food. 2004; 7: 157–61.
8. Joshi H, Parle M. Brahmi rasayana improves learning and memory in mice. Evid Based Complement Alternat Med. 2006; 3(1): 79–85.
9. Milindparle, Nirmalsingh, Mani Vasudevan. Regular rehearsal helps in consolidation of long term memory. Journal of sports science and medicine. 2006; 5: 80–88.
10. Jungmo Do, Seemin Choi, Jaehwi Choi,Jae Seog Hyun. Effect and mechanism of action of a *Tribulus terrestris* extract on penile erection. Korean J Urol. 2013; 54: 183–88.