

EFFECT OF VARIOUS SPECIES OF SHANKHPUSHPI ON SPATIAL MEMORY IN MORRIS WATER MAZE TASK IN EXPERIMENTAL ANIMALS

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ABSTRACT

The main objective of the present study was to investigate and compare the neuropsychopharmacological effects of various reported species of Shankhpushpi - *Evolvulus alsinoides* Linn., *Convolvulus pluricaulis* Sieb. and *Clitorea ternatea* Linn. on learning and memory processes by Morris water maze paradigm. Shankhpushpi is reported to fall in the category of "controversial drug" since it has more than one botanical source. Pharmacological studies on Shankhpushpi have been of interest on account of the different plant which go by that name or by related names. Morris Water maze was employed to evaluate learning and memory parameters. Alcoholic extract of *Evolvulus alsinoides* Linn., *Convolvulus pluricaulis* Sieb. and *Clitorea ternatea* Linn. were prepared and administered to rats per oral, at a dose of 250 and 500 mg/kg body weight. Piracetam (200 mg/kg body weight ip) was used as standard drug. The animals were subjected to training for eight days. At the end of trial session the animals were subjected to spatial memory test by measuring the time spent in the target quadrant. Amongst the three species of Shankhpushpi ethanolic extract of *Evolvulus alsinoides* seemed to be the best since time spent in target quadrant after the training session was the maximum. Results indicate that all the three species exhibited a dose dependent nootropic effect.

Keywords: Nootropic, cognitive, water maze, shankhpushpi, target quadrant, spatial memory.

INTRODUCTION

Treatment of cognitive disorders such as amnesia, attention deficit and Alzheimer's diseases still seems to be a herculean task in the field of modern medicine. Alzheimer's is a progressive neurodegenerative disorder of brain. The onset is slow but repercussions nasty. It gradually leads to dementia, behavioral and personality changes finally leading to death.¹ In present times its treatment seems to be a challenging task. Deterioration in memory in humans is part and parcel of normal aging process too.

Nootropics are a new class of psychotropic agents having ameliorating effect on learning capabilities and memory.^{2,3} Nootropic agents such as piracetam, aniracetam, pramiracetam, donezipil are presently used for improving memory, mood and behavior.⁴ However, the associated adverse effect act as a deterrent to their use.

Shankhpushpi is a well known ayurvedic drug spoken of as a brain tonic, laxative and alterative.^{5,6,7} Since times immemorial, Shankhpushpi is known to be beneficial in cognitive disorders. It is a *medhya rasayan*. In Ayurveda, herbs that promote intelligence are called *medhya herbs*.⁸ Rasayanas are Ayurvedic preparations that promote

resistance against infections and other diseases by maintaining equilibrium of *Vatta*, *Pitta* and *Kapha*. They improve memory intelligence and promote youthfulness and efficiency.^{9,10}

The descriptions of Shankhpushpi in usual reference books are not adequate to conclude definitely which one of the following plants is the correct source of Shankhpushpi^{5,6,11} -

- Evolvulus alsinoides* Linn.
- Convolvulus pluricaulis* Sieb.
- Clitorea ternatea* Linn.

It seems obvious that the term Shankhpushpi has been applied loosely to these three plants.^{12,13} Hence, it seems of interest to evaluate the nootropic potential of all these plants and attempt to establish the best taxa amongst the three reported species.

Numerous models are available for evaluation of nootropic (cognitive) activity for example, elevated plus maze, jumping box, Morris water maze etc. Morris water maze is a commonly used paradigm for studying spatial memory in animals. It was first described in 1981. Water maze has been used extensively to study the neurobiological mechanisms that underlie spatial learning and memory, age associated changes in spatial navigation and ability of nootropic agents to influence specific cognitive processes.^{14,15}

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MATERIALS AND METHOD

Animals

Wistar albino rats (80-110 gm body weight) of either sex were selected. The animals were housed in groups of five under standard laboratory conditions. Animals were fed on standard pellet diet and water made freely available. The animals were acclimatized to the environment for a week prior to the studies. All experiments were performed in the forenoon. The institutional animal ethical committee (CPCSEA Reg No. 1156/ac/07/CPCSEA) approved the protocol. Experiments were performed in accordance with the guidelines of the institutional animal ethical committee.

Plant Extracts

The dried plants of the three reported species- *Evolvulus alsinoides* Linn. *Convolvulus pluricaulis* Sieb. *Clitorea ternatea* Linn. were procured from Tirunelveli, Tamil Nadu (M/s Chelladurai) and authenticated at Department of Botany, Forest Research Institute, Dehradun. The plant materials were then individually extracted with ethanol (95%) using soxhlet extractor. The crude extracts thus obtained were dried to obtain thick viscous residues for further use.

Experimental

Rats were grouped into 8 groups of 6 animals each. Group I animals served as control and received the vehicle only. Group II animals received standard drug Piracetam (200 mg/kg body weight i.p.). Group III, IV and V received ethanolic extracts of *Convolvulus pluricaulis*, *Evolvulus alsinoides* and *Clitorea ternatea* respectively at 250 mg/kg body weight p.o. Group III a, Group IV a and Group V a received ethanolic extracts of *Convolvulus pluricaulis*, *Evolvulus alsinoides* and *Clitorea ternatea* respectively at 500 mg/kg body weight p.o.

The apparatus used to evaluate the cognitive function was a circular tank (100 cm diameter) filled with water to a depth of 30 cm. The medium was made opaque by adding milk to it. The tank was equally distributed into four points along the perimeter which served as starting locations. The tank was arbitrarily divided into four equal quadrants and a small platform (5 cm wide) was placed in the center of one of the quadrants. The water maze was always located in a large room with minimal disturbance and the observer always sat in the same position. Experiments were carried out between 9.00 hrs to 12.00 hrs. During the training session the animals were released into the water and allowed 120 seconds to find the platform. Each animal received four trials on day one and subsequently eight trials per day for eight days. The trials sessions had a five minute inter trial interval.¹⁵ The test extracts were administered sixty minutes prior to the first trial daily.

Twenty four hours after the last training trial the animals were subjected to spatial memory test whereby the time spent in the target quadrant was measured and taken as an indicator for spatial memory.

Statistical analysis

The results are reported as mean \pm SEM and were analyzed by one way ANOVA followed by Tukey-kramer multiple comparisons test.

RESULTS

The time spent in the target quadrant in the water maze is depicted in Table 1 (Figure 1). All the three species of

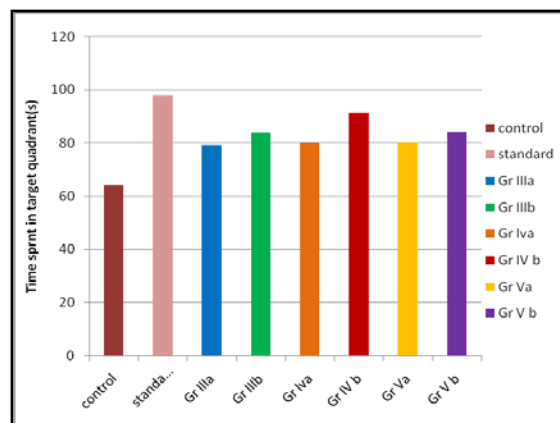
Shankhpushpi exhibited an increase in time spent in target quadrant which was statistically significant when compared to control. This implies that all have the potential to improve spatial memory in animals. When compared to the reference standard *Convolvulus pluricaulis*, *Evolvulus alsinoides* and *Clitorea ternatea* at a dose of 250 mg/kg body weight were highly significant. An increase in dose from 250 mg/kg body weight to 500 mg/kg body weight depicted a marked increase in time spent in target quadrant which was not statistically significant, in the case of *Convolvulus pluricaulis* and *Clitorea ternatea* ($p > 0.05$). This dose dependant pattern was statistically significant in case of *Evolvulus alsinoides* ($p < 0.05$).

Table 1. Effect of various species of Shankhpushpi on spatial memory in Morris water maze task in rats.

S no	Treatment	Group	Dose (mg/kg)	Time spent in target quadrant(s)
1	Control	I		64 \pm 5.31
2	Standard	II	200	98 \pm 1.065***
3	<i>Convolvulus pluricaulis</i>	III a	250	79.3 \pm 1.23**
		III b	500	84 \pm 1.15***
4	<i>Evolvulus alsinoides</i>	IV a	250	80.06 \pm 1.89***
		IV b	500	91.3 \pm 1.01***
5	<i>Clitorea ternatea</i>	V a	250	80.3 \pm 2.51***
		V b	500	84.3 \pm 1.66***

Values are in mean \pm SEM (n=6) *** p < 0.001, **p < 0.01, *p < 0.05, ns p > 0.05

Figure 1. Effect of various species of Shankhpushpi on spatial memory in morris water maze task in rats.



DISCUSSION

Water maze task was introduced as a spatial localization or navigation task. The task has been used extensively to study the neurobiological mechanisms that underlie spatial learning and memory.

The different varieties of Shankhpushpi showed variation in the time spent in target quadrant, an indicator of spatial learning and memory. All the reported three species of Shankhpushpi did have a positive effect on cognitive functions as they all improved spatial memory. An increase in dose exhibited a corresponding increase in the time spent in target quadrant. From this it is reasonable to suggest that the three reported species of Shankhpushpi improve spatial learning and memory.

Evolvulus alsinoides exhibited to be the best amongst the three reported species. However, more detailed and well planned experimentation encompassing more parameters of learning and memory processes are necessary to evaluate the exact mechanism of action of various reported species of Shankhpushpi.

CONCLUSION

In conclusion, our results emphasize on the dose dependant effect of the three reported species of shankhapushpi on spatial memory using the water maze task. Further, *evolvulus alsinoides* promises to be the superior taxa amongst the three reported species of

shankhapushpi viz. *convolvulus pluricaulis*, *clitorea ternatea* and *evolvulus alsinoides*.

These research findings reinforce the need to identify and select the preferred species amongst the reported species of shankhapushpi, with maximal activity for cognitive behavior.

REFERENCES

1. Jewart R D, Green J, Lu C J, Cellar J, Tune L E; Cognitive, behavioral and psychological changes in Alzheimer's disease patients as a function of incontinence medication. *Am J Geriatric Psychiatry*. 2005; 13:324-328.
2. Joshi H, Parle M; Evaluation of nootropic potential of *Ocimum sanctum* Linn. In mice. *Ind J of Expt Biology*. 2006; 44:133-136.
3. Parle M et al. Neurochemical basis of learning and memory. *Indian journal of pharmaceutical sciences*. 2004; 66(4):371-376.
4. Joshi H, Parle M; Nootropic activity of calyces of *Hibiscus sabdariffa* Linn. *Iranian J of Pharmacology and Therapeutics*. 2006; 5(1):15-20.
5. Gian s aulakh, et al Phytochemistry and pharmacology of shankhapushpi-four varieties. *Ancient science of life*. 1988:vol vii(3&4):149-156.
6. Karandikar G K, Satakopan S; Shankhpushpi-A pharamacognostic study. *Ind J of Pharm*. 1959; 21:200-203.
7. Karandikar G K, Satakopan S; Shankhpushpi-A pharamacognostic study. *Ind J of Pharm*. 1959a; 21:327.
8. Rathee P, Chaudhary H, Rathee S, Rathee D; Natural memory boosters. *Pharmacognosy Reviews*. 2008; 2(4):249-256.
9. Sharma V N et al. Some pharmacological actions of *Convolvulus pluricaulis* choisy: an Indian indigenous herb. *Indian J med.res*. 1965; 53:871-876
10. Taranalli A D et al. Influence of *clitorea ternatea* extracts on memory and central cholinergic activity in rats. *Pharmaceutical biology*. 2000; 38:51-56.
11. Virbala C S Botanical identity of shankhapushpi. *Indian j pharm*. 1961; 23:223-224.
12. Singh, H B et al. Need for authentication of market samples of crude drug shankhapushpi *Convolvulus microphyllus*. *JMAPS*. 2000; 22(4A):23(1A)-2.1
13. Sinha P et al A comparative study on shankhapushpi- *Canscora decussata* Schultz., *Convolvulus pluricaulis* choisy and *Evolvulus alsinoides* linn. *B.M.E.B.R*. 1986; 7:62-73
14. Brandies R et al .The use of Morris water maze in the study of memory and learning .*international journal of neuroscience*. 1989; 48:29-69.
15. Kulkarni S K; *Handbook of Experimental Pharmacology*. 3rd ed. Vallabh Prakashan, Delhi, 1999, pp 43-84.