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Effect of *Argyreia speciosa* extract on learning and memory paradigms in mice

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ABSTRACT
The objective of the present study was to investigate the effect of hydroalcoholic extract of *Argyreia speciosa* (AS) on learning and memory paradigms in mice. Radial arm maze and morris water maze were the screening tests used to assess the activity of the extract. Piracetam served as a reference standard. The mice pretreated with AS 200 or AS 400 mg/kg or Piracetam (150mg/kg) showed a decrease in number of days required to make the mice learned, time taken to find food by the learned mice in radial arm maze. In morris water maze, the mice treated with the above mentioned doses showed a significant reduction in the number of days required to make the mice learned, escape latency of learned mice and number of circles completed before it escapes on to the platform. The results suggest facilitation of spatial learning and memory processes and thereby validated its traditional claim of being tonic in dullness of intellect.
KEY WORDS- Spatial Memory, Radial arm maze, Morris water maze, *Argyreia speciosa*.

INTRODUCTION

Learning is defined as the acquisition of information and skills, while subsequent retention of that information is called memory (1). Poor learning abilities, impaired memory, lower retention and slow recall are the common problems in stressful situations. Moreover, age, stress and emotions are conditions that may lead to impaired learning, memory loss, amnesia, and dementia or to more ominous threats like Schizophrenia and Alzheimer's disease (2). As memory involves many interwoven brain functions, it results in different types of memories and virtually any type of brain damage can result in one or other type of memory loss (3). Working memory is one of the type of memories which refers to a brain system that provides storage and manipulation of the information necessary for complex cognitive tasks like language, comprehension, learning and reasoning (4).

Argyreia speciosa (AS) is commonly grown as an elephant creeper which is a woody climber found throughout India up to an altitude of 1000 ft. The roots are claimed to be a tonic in dullness of intellect (5), however, it has not been scientifically documented so far for its effect of spatial learning and memory. The present study was therefore carried out for confirming veracity of aforementioned traditional claim of AS using two animal models, namely radial arm maze and morris water maze.

MATERIALS AND METHODS

Preparation of AS extract

Hydroalcoholic extract of AS was received as a gift sample (No: ARG/4019) from Green Chem Bangalore, India. *A. speciosa* roots were extracted with 50% aqueous alcohol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. This mass was diluted with 25% aqueous alcohol, filtered and concentrated and dried to get a fine powdered form of the extract. This powdered extract was dissolved in an appropriate quantity of distilled water and administered orally with oral feeding needle. The standard Piracetam (Nootropil, UCB, Batch no- V007003)) suspension was purchased from local market.

Animals

Male Swiss albino mice (18-22 g) were used. These mice were housed in standard laboratory conditions of temperature, relative humidity, lighting with adequate

food and water. The mice were transferred to the laboratory at least one hour before the start of the experiment and all experiments were carried out form 8.00-1600 hr. The study was approved by Institutional Animal Ethical Committee.

Treatment

Mice were divided into six groups of six animals each. Group I animals served as control and orally received distilled water (10 ml/kg). Group II, III, and IV received an oral dose of 100, 200, 400 mg/kg of AS extract respectively. Group V animals received piracetam (150 mg/kg) orally for comparison. These mice were tested everyday for either radial arm maze task performance or morris water maze task performance along with the above mentioned drug treatment.

Radial arm maze task

Locally fabricated wooden radial arm maze elevated 50cm above the floor consisting of an octagonal central hub 36cm in diameter with eight radial arms was used. Each arm 43 cm long, 15cm wide with 12 cm sides, had small black plastic cups mounted at 30cm from the central hub (6, 7).

The mice were trained for radial maze task performance by conducting daily training trial which consisted of two sessions wherein one food pellet was placed in a fixed arm and then in the variable arm to record the effect of extract on spatial reference and spatial working memory respectively. Mice maintained at 85% of their total diet were placed individually in the central hub and were allowed to choose the arm freely to get the food with upper cut off limit of 300 sec. The time taken by each mouse to find the food along with number of reentries was considered to assess radial maze task performance. Mouse was considered to be learned when he found the food with maximum one reentry for three consecutive days. The number of days required for making the mice learned and the latency to find the food along with number of initial correct entries (i.e. before first reentry) of learned mouse was recorded as the effect of the drug on learning and memory process. One-hour interval was kept between the spatial reference and spatial working memory evaluation. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli (6, 7, 8).

Morris water maze task

The apparatus used was a circular tank (100 cm in diameter) filled to a depth of 30 cm with water (25^{o} C $\pm$ 2^{o} C). The tank was divided arbitrarily into four equal quadrants with a small removable platform (5 cm in width) located at a central fixed position and then in different quadrants to assess effect of AS on spatial reference and spatial working memory respectively. Optimum quantity of fresh milk was used to make the water opaque (6, 7).

The mice were tested by conducting a pre selection trial; wherein mice were released into the tank individually to find the hidden platform. Those mice that could not escape on the hidden platform in the allotted time of 90 seconds or refused to search and float on the water were excluded (9).

The aforementioned selected mice were released individually into the tank and allowed to find the hidden platform placed at either fixed or different quadrants with upper cut off of 90 sec. The escape latency (the latency to find hidden platform) and the number of circles completed by the mice (number of incomplete circles were converted to round figure based upon either less or more than half the distance traveled) were recorded everyday. These sessions were continued along with the respective drug treatment until escape latency was below 10 sec for three consecutive days. The mice meeting this criterion were labeled as learned mice. The number of days required to make the mice learn, escape latency and number of circles completed by the learned mice were considered to evaluate the effect of drug on learning and memory processes. Experiments were performed between 08:00-1600 hr everyday. One hour interval was kept between the sessions of spatial reference and spatial working memory assessment (6-9).

Statistical analysis

The data was expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnetts test. The level of significance was set at $p< 0.05$.

RESULTS

Radial arm maze

AS 200 and 400 mg/kg showed significant reduction in number of days required to make the mice learned in both spatial reference (11.50 ±0.42, 09.66 ± 0.33) as well as spatial working memory (12.66 ±0.49, 11.66 ± 0.21). The effect was found to be dose dependent in the former model only. On the contrary, similar doses showed dose dependent reduction in latency to find the food by the learned mice only in spatial working memory (57.48 ±1.34, 50.31 ± 1.40) when compared to vehicle treated control mice (64.50 ±2.68). AS pretreatment did not show any significant change in the number of initial correct entries in both the models at any dose level (Table 1).

Table 1. Effect of AS extract and piracetam on radial maze task performance in mice.

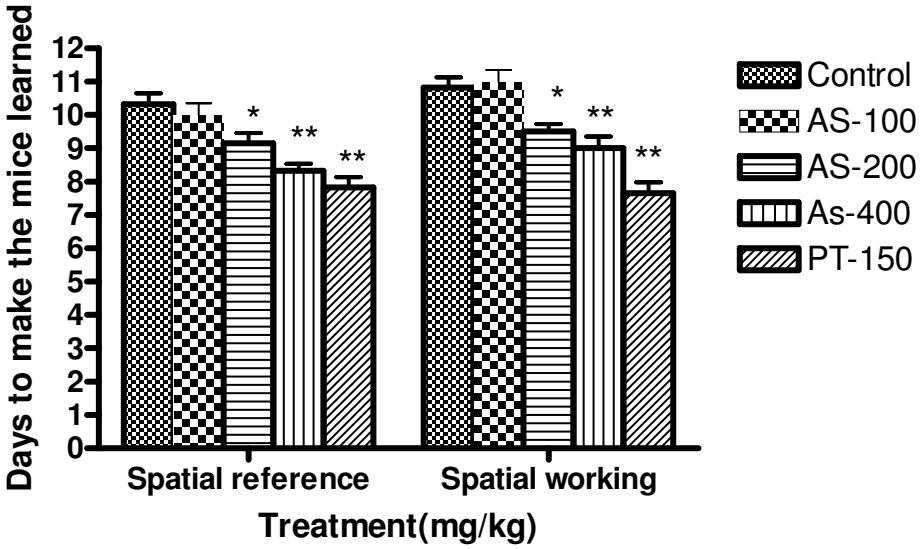
Treatment(mg/kg)	Spatial reference			Spatial working		
	Days to make mice learned	Latency to find food (second)	Number of initial correct entries	Days to make mice learned	Latency to find food (second)	Number of initial correct entries
Control	13.16 ± 0.40	48.57 ± 2.02	4.83 ± 0.30	14.83 ± 0.30	64.50 ± 2.68	5.00 ± 0.36
AS-100	13.16 ± 0.30	42.25* ± 1.39	4.66 ± 0.33	15.16 ± 0.30	66.16 ± 2.10	5.33 ± 0.49
AS-200	11.50* ± 0.42	38.25**± 1.41	5.33 ± 0.55	12.66** ± 0.49	57.48* ± 1.34	4.66 ± 0.33
AS-400	9.66** ± 0.33	33.61**± 1.55	5.66 ± 0.33	11.66** ± 0.21	50.31** ± 1.40	5.00 ± 0.36
Piracetam-150	7.16** ± 0.47	24.74**± 1.00	4.66 ± 0.49	9.33** ± 0.33	35.57** ± 1.24	4.5 ± 0.42

*AS: Argyreia speciosa. ; Results are expressed as mean ± SEM. (n = 6).Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *p<0.05, **p<0.01.*

Table 2. Effect of AS extract and piracetam on number of circles completed by the learned mice.

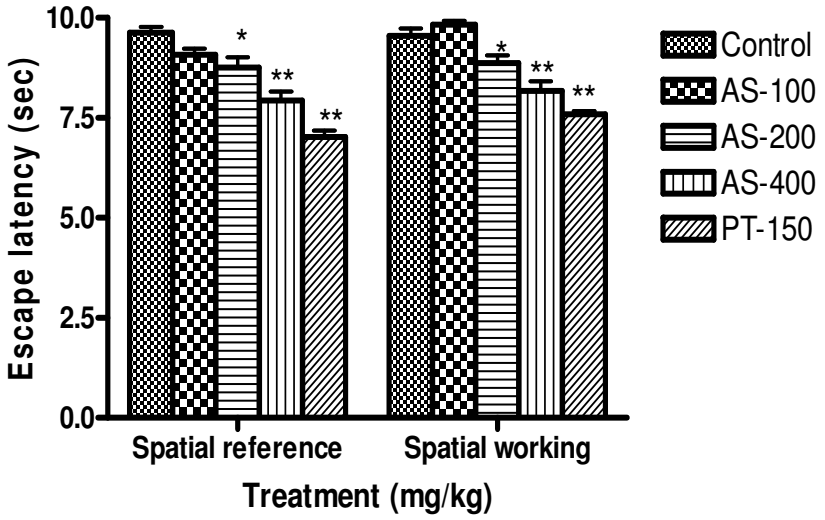
Treatment(mg/kg)	Number of circles completed	
	Spatial reference	Spatial working
Distilled water	6.33 ± 0.33	6.66 ± 0.21
AS- 100	6.00 ± 0.36	5.66 ± 0.33
AS- 200	5.00* ± 0.36	5.33* ± 0.21
AS- 400	4.83* ± 0.30	4.16** ± 0.30
Piracetam-150	4.33** ± 0.21	4.00** ± 0.36

*AS: Argyreia speciosa.; Results are expressed as mean ± SEM. (n = 6) .Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *p<0.05.*



AS: Argyreia speciosa.

Figure 1. Effect of AS extract and piracetam on days required to make the mice learned (mean. ± SEM)



AS: Argyreia speciosa.
Figure 2. Effect of AS extract and piracetam on escape latency (Mean. ± SEM).

Morris water maze

The AS 200 and 400 mg/kg pretreatment significantly reduced the number of days required to make the mice learned in both spatial reference (9.16 ±6.30, 8.33 ± 0.21) and spatial working memory (09.50 ±0.22, 09 ± 0.36) when compared against respective control (10.33 ±0.33, 10.83 ± 0.30). Furthermore, these two doses dose dependently reduced the escape latency in both spatial reference (8.76 ±0.25, 7.94 ± 0.22) as well as spatial working memory model (08.87 ±0.19, 8.18 ± 0.23) when compared to vehicle treated respective controls (Figure 1, 2).

The number of circles completed by the learned mice was significantly reduced with AS 200 and 400 mg/kg pretreatment. These doses were equipotent in spatial reference model while exhibited dose dependent effect in spatial working memory model (Table 2).

DISCUSSION

The importance of learning and memory process is greatly recognised with prevalence of Alzheimer's disease, which is a rapidly growing pathologic condition(10) and other related pathological states (11) wherein loss of cognitive function, weakened memory and impaired learning ability are the most common symptoms(12).Epidemiological studies of Indian population have also revealed that cognitive impairment is largely a hidden problem in the country and its prevalence rates increase exponentially with advancing age (13)

Since the last two decades the pharmacotherapy with psychoactive drugs like pyritinol, tacrine hydrochloride, anticholinesterase etc are available however they are not effective in all cases and exerts numerous side effects especially upon long term administration (8,14,) These reports suggest the need of alternative therapy.

In neuropharmacology, a lack of suitable model with one parameter to be observed in order to screen agents that affect learning and memory processes is a major limitation. Moreover memory performance depend upon the difficulty and the nature of task, when the tasks used to assess memory becomes more complex, the performance appears to be more impaired. Hence the evaluation is usually carried out using series of paradigms that work upon different mechanisms (15). Similar principle is applied in this study.

Various mazes are used conventionally to assess the learning and memory paradigms in laboratory animals (1, 9). Radial arm maze task performance is an appetitively motivated task and is also useful to assess the spatial reference as well as spatial working memory performance and agents that affect these processes (7).The Morris water maze works on spatial localisation or navigation task and is extensively used to study the neurological mechanisms that underlie spatial learning and memory, age- associated changes in spatial navigation and ability of nootropic agents to influence specific cognitive processes (6).

In this study, AS significantly decreased the number of days required to make the mice learned as per set criteria and time taken to find the food by the learned

mice in the radial arm maze model. Also a reduction in the number of days required to make the mice learned as per set criteria, escape latency, number of circles completed by the learned mice in morris water maze when compared to respective control mice was seen. These results support the traditional claim of AS being a tonic in dullness of intellect especially in spatial memory impairments (5). The difference in the significance of individual parameters of sessions do not necessarily relate particular aspect of spatial reference or spatial working memory because of the lack of suitable model with single parameter to be recorded which can precisely represent the human learning and memory processes (7). Hence significant improvement in most of the parameters is usually considered as effect of the drug (8, 16, 17), while the dose showing significant improvement in the maximum parameters could be considered as the most effective dose. The present findings when gauged on this basis revealed the facilitation of learning and memory. In addition the preliminary phytochemical investigation of AS showed the presence of saponins, alkaloids, flavonoids, steroids, glycosides and carbohydrates. These pharmacophores have been shown to possess nootropic activity and thereby support the aforementioned findings (18-20). The oxidative stresses, generation of free radicals and deprivation of oxygen are common causes for neurodegeneration and related cognitive impairments especially in spatial learning and memory deficit. (21, 22). The significant facilitation in spatial learning processes by the pretreatment with AS suggest it possible use to treat aforementioned conditions.

Thus, the present study documented significant improvement in radial arm maze and morris water maze performance and thereby facilitation of spatial learning and memory with pretreatment of AS. These results indicated possible use of the extract as a part of therapy to treat poor learners and patients with impaired spatial memory functions. Moreover, it may be employed as a buffer against rapid age related decline in mental functions observed with various neurological disorders (3). The reported difference of the effectiveness of the extract towards reference and working memory in these paradigms may be due to factors like experimental conditions, experimental protocol employed and modulation of specific neurotransmitters, neurochemicals involved (1,17). The exact mechanism of its action and phytochemicals responsible will be revealed after detailed biochemical and phytochemical investigations which is underway.

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