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Studies on nootropic activity of roots of *Tylophora indica* in mice

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Abstract

The present work was designed to assess the potential of aqueous extract of *Tylophora indica* on cognitive functions in mice using elevated plus maze model and Morris water maze model. Piracetam (200 mg/kg) was used as a standard nootropic agent for comparison. The results of elevated plus maze model showed that, *Tylophora indica* extract treated mice at 250 mg/kg and 500 mg/kg, significantly ($p<0.01$) reduced the time required to find the closed arm compared to untreated mice. Further, the extract treatment at 250 mg/kg and 500 mg/kg, showed significant ($p <0.01$) improvement in scopolamine-impaired performance with respect to acquisition and retention of memory in spatial and working memory tests, in Morris water maze model. In conclusion, *Tylophora indica* appears to be a promising candidate for improving memory and it would be worthwhile to explore the potential of this plant in management of dementia and Alzheimer's disease.

Keywords: *Tylophora indica*, nootropic, elevated plus maze model, Morris water maze model.

1. Introduction

An enhanced life expectancy all over the world has been accompanied by an increased number of people suffering from age-associated dementia. This syndrome not only causes a terrible reduction in the quality of life of the sufferer, but also places tremendous burden on both the career and the welfare system [1]. Such cognitive disorders are prevalent due to various factors such as natural (aging, stress), environmental (excess levels of carbon monoxide and carbon dioxide) and diseases (Alzheimer's disease, Huntington Chorea) [2]. Dementia is a syndrome of failing memory and other intellectual functions with little or no disturbance in consciousness [1]. Alzheimer's disease (AD) is a leading cause of dementia in developed countries [3]. Alzheimer's disease is a progressive neurodegenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and ultimately death [4]. It is a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgement, orientation,

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comprehension, learning capacity and language [5]. Dysfunction of cholinergic neurotransmission in the brain contributes to the salient cognitive decline in AD [3].

Although there is no cure for dementia of AD type at present, alternative pharmacologic treatment modalities can reduce the symptoms of cognitive impairment and slow disease progression [6]. Nootropic agents like piracetam and cholinesterase inhibitors like Donepezil® are commonly used for improving memory, mood and behavior. However the resulting adverse effects of these drugs such as diarrhoea, nausea, insomnia, bronchitis, loose stools, muscular cramps and other known side effects [7, 8] have made their use limited. Hence, it is worthwhile to explore the utility of traditional medicines in treatment and management of various cognitive disorders.

The plant *Tylophora indica* Burm.f. (Family: Asclepiadaceae) is distributed in the plains of southern, central and eastern India. Dried root of the plant is useful in treatment of dysentery, asthma and bronchitis [9]. However, there is lack of scientific data regarding effect of root of plant on learning and memory in preclinical animal studies. Hence, it was thought prudent to investigate nootropic potential of aqueous/ water extract [WE] of *Tylophora indica* [TI] *in vivo*, using standard animal models.

2. Materials and methods

2.1. Plant material

The fresh roots of *T. indica* were obtained from local source and were identified and authenticated by Department of Botany, Ruia College, Mumbai. Dried powdered roots were defatted with petroleum ether (60-80°C) and successively extracted with water. The extract so obtained was dried at 40°C using a vacuum evaporator and used for further studies.

2.2 Drugs

Scopolamine butyl bromide (Buscopan®, German Remedies Ltd, Mumbai) and piracetam (Nootropil®, UCB India Pvt Ltd, Gujarat) were used in the study.

2.3 Experimental Animals

Adult male Swiss Albino mice (20-25 gm) were used for the study. Animals were housed under standard hygiene conditions, 12/12 h light and dark cycle and fed with standard pelleted diet and water *ad libitum*. The animals were acclimatized to the laboratory environment for atleast a week before experimentation. All the experiments were performed in accordance with the Institutional Animal Ethics Committee (IAEC) approved protocol as per directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of animal welfare division, Government of India, New Delhi.

2.4 Elevated plus maze model

Mice were divided randomly into five groups of six animals. Group I comprised of saline- treated control animals; Group II animals received piracetam 200 mg/kg, orally; and Groups III and IV were fed orally with TI[WE] at doses 250 and 500 mg/kg respectively. The elevated plus maze apparatus used in the study consisted of two open and two closed arms facing each other. The maze was elevated at a height of 50 cm from the ground. The animals were placed individually 30 min after oral administration of either vehicle or test drug at the end of either of the open arms and the time taken by the animal to move from open to closed arm (transfer latency) was noted on the first day. The time elapsed between the time that the animal was placed on the open arm and the time at which all four legs were inside the enclosed arms, was noted as the transfer latency. The transfer latency was again recorded 24 hr after the first exposure [10].

2.5 Morris water maze model

The experimental groups used for spatial and working memory testing in Morris water maze model were similar to that used in elevated plus maze model described earlier. The Morris water maze consisted of a large water tank (48cm x 28cm x 18cm) filled with water, which was made opaque by adding milk. Water provided a uniform intramaze environment, thus eliminating any olfactory interference. A 7x7 cm rectangular escape platform was constructed of water-resistant material and covered with material that allows the animal to remain on top when it is submerged. The platform was 10 cm in height so that it could be submerged 2 cm below the level of water surface. The water temperature was maintained at 26°C [11].

2.6 Study protocol for spatial memory test

In this test, mouse was released with its head pointed towards side of the water pool. The time taken by the mouse to find a hidden platform in water pool after previous exposure to the set up, using only available external cues was determined as a measure of spatial memory. The mouse was allowed to remain on platform for 10 sec and later placed in holding cage for 15 sec. Again the mouse was released from same place and time for reaching the submerged platform was recorded. Likewise, ten trials were conducted and average time to reach the submerged platform was recorded, keeping similar experimental conditions. Drugs were administered orally and after 90 min all the groups were exposed to the training schedule. This procedure was repeated at 24 hr interval for two more days until each subject acquired minimum time interval to reach the submerged platform in the pool. On fourth day, after complete training, all groups were treated with scopolamine butyl bromide (2 mg/kg, *i.p.*). 30 min later, they were treated with test extract/ std, and were tested for spatial memory after further 90 min [11]. Mice with memory impairment took more time to reach the platform [12]. The animals were also tested for spatial memory on fifth day to check the ability of test extract to restore scopolamine-induced amnesia.

2.7 Study protocol for working memory test

This test was applied after the acquisition phase of testing was completed. It is important that the mice demonstrate that they know the location of hidden platform before beginning the test. This method has been referred as the reversal test. As in spatial memory test, time for the mouse to reach the submerged platform was recorded. The submerged platform was then moved to a new location. Then, the mouse was released from the same place and time for reaching the submerged platform was recorded. In this manner, the hidden platform was changed to all four quadrants and average time to reach the submerged platform in each quadrant was recorded [11].

2.8 Evaluation of spatial memory and working memory tests

Latency to reach the platform in sec (mean values) was recorded on day 0, day 1, day 2, day 4 and day 5. Day 1 is the day from which animals were treated with the test extract/std. Day 4 is the day on which animals were treated with scopolamine butyl bromide 2mg/kg, i.p [11].

2.9 Statistical analysis

All the results were statistically interpreted as mean ± SD. Data were analyzed using One Way ANOVA followed by Dunnett's test. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1 Effect of TI[WE] on transfer latency of mice in elevated plus maze model

TI[WE] treatment at doses of 250 mg/kg and 500 mg/kg, showed significant ($p<0.01$) decrease in transfer latency to find closed arm

on day two, compared to vehicle treated mice (Table 1).

3.2 Effect of TI[WE] on scopolamine- induced spatial memory impairment

Scopolamine-treated mice without any treatment, took longer time to reach the platform due to memory impairment on day four and five. However, TI[WE] at 250 mg/kg and 500 mg/kg, significantly ($p<0.01$) reduced the time required by mouse to reach the platform after scopolamine treatment on day four and day five (Table 2).

3.3 Effect of TI[WE] on scopolamine- induced working memory impairment.

TI[WE] at both the doses (250 mg/kg and 500 mg/kg) significantly ($p<0.01$) improved scopolamine-impaired performance in all four quadrants on day four and day five, compared to vehicle control group (Table 3).

Table 1. Effect of TI [WE] on transfer latency of mice in elevated plus maze model.

Experimental groups	Transfer latency (sec)	
	Day 1	Day 2
Vehicle control	28.9 ± 7.01	16.42 ± 1.90
Positive control	20.93 ± 8.65	10.75 ± 0.23 **
TI [WE] 250 mg/kg	27.00 ± 2.79	9.83 ± 0.94 **
TI [WE] 500 mg/kg	22.80 ± 2.65	8.80 ± 1.30 **

*P < 0.05, ** P < 0.01, *** P < 0.001, when compared to control group on each day by one-way ANOVA followed by Dunnett's test.

4. Discussion

Learning is defined as the acquisition of information and skills, while subsequent retention of that information is called memory. One of the challenging tasks for neuroscientist is to elucidate the biochemical and molecular mechanisms under-lying learning and memory [10]. To assess the learning and memory paradigms in laboratory animals, mazes are used conventionally. The elevated plus maze is used to measure the anxiety state in animals, however transfer latency i.e. the time elapsed between the movement of the animal from an open to an enclosed arm is markedly shortened, if the animal has previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. In elevated plus maze model, acquisition (learning) can be considered as transfer latency on first day trials and the retention/consolidation (memory) is examined 24 hr later [10]. The animals treated with TI[WE] at 250 mg/kg and 500 mg/kg, showed significant ($p<0.01$) decrease in transfer latency on day two compared to vehicle control group, indicating cognitive enhancing effect of the extract in rodents.

Table 2. Effect of TI [WE] on scopolamine-induced spatial memory impairment in Morris water maze model.

Experimental groups	Latency to reach the platform (sec)				
	Day 0	Day 1T	Day 2	Day 4 #	Day 5
Vehicle Control	8.24 ± 3.99	6.32 ± 0.43	5.40 ± 1.89	12.05 ± 4.57	6.25 ± 1.62
Positive Control	8.90 ±1.97	5.86 ± 1.17	4.52 ± 0.94	4.05 ± 1.11 **	3.32 ± 1.46 **
TI[WE] 250 mg/kg	8.17 ± 2.27	7.16 ± 2.22	5.08 ± 1.51	4.41 ± 1.04 **	4.19 ± 1.06 *
TI[WE] 500 mg/kg	7.12 ± 0.96	5.33 ±1.93	4.99 ± 1.84	5.10 ± 1.06 **	3.98 ± 0.98 *

T Day from which animals was treated with TI[WE], # Day on which animals was treated with scopolamine (2 mg/kg, i.p.), *P < 0.05, ** P < 0.01, *** P < 0.001, when compared to control group on each day by one-way ANOVA followed by Dunnett's test

The water maze task was introduced by Morris (1981) and colleagues as a spatial localization or navigation task [10]. Since then, Morris water maze model has been extensively used to study the neurological mechanisms that underlie spatial navigation to influence special cognitive processes [14,15]. The same task can also be used to test working memory by changing the hidden platform from one quadrant to another quadrant. This model throws light on ability of the agent in improving cognitive functions impaired by scopolamine (centrally acting acetylcholine blocker), thereby emphasizing its utility in cognitive disorders involving dementia. The extract treatment at 250 mg/kg and 500 mg/kg, showed significant ($p<0.01$) improvement in scopolamine-impaired performance with respect to acquisition and retention of memory, in both spatial and working memory tests, thereby reinforcing the nootropic potential of TI[WE].

Alterations in the levels of various neurochemicals have found to play a crucial role in the pathophysiology of memory deficits of laboratory animals and patients with Alzheimer's disease [16]. However, the central cholinergic pathways play a prominent role in the learning and memory processes. The degree of cholinergic neurodegeneration correlates positively with severity of memory impairment [17]. Recent studies suggest that the deficits

Table 3. Effect of TI [WE] on scopolamine-induced working memory impairment in Morris water maze model.

Quadrant	Experimental groups (mg/kg)	Latency to reach the platform (sec)				
		Day 0	Day1T	Day 2	Day 4 #	Day 5
1	Vehicle ctrl	8.24 ± 3.99	6.32 ± 0.43	5.40 ± 1.89	12.05 ± 4.57	6.25 ± 1.62
	Pos ctrl	8.90 ± 1.97	5.86 ± 1.17	4.52 ± 0.94	4.05 ± 1.11**	3.32 ± 1.46 **
	TI [WE] 250	8.17 ± 2.27	7.16 ± 2.22	5.08 ± 1.51	4.41 ± 1.04**	4.19 ± 1.06 *
	TI [WE] 500	7.12 ± 0.96	5.33 ± 1.93	4.99 ± 1.84	5.10 ± 1.06**	3.98 ± 0.98 *
2	Vehicle ctrl	7.62 ± 2.02	5.21 ± 1.88	6.81 ± 1.72	8.32 ± 1.51	6.87 ± 1.52
	Pos ctrl	7.50 ± 1.98	4.67 ± 1.41	4.69 ± 2.33	4.21 ± 2.35**	2.78 ± 0.81 **
	TI [WE] 250	9.77 ± 1.59	4.54 ± 1.43	4.16 ± 2.70	3.96 ± 1.69**	3.46 ± 0.65 **
	TI [WE] 500	10.8 ± 3.78	4.99 ± 2.79	4.08 ± 2.19	4.60 ± 1.12**	3.60 ± 0.98 **
3	Vehicle ctrl	7.82 ± 2.92	7.22 ± 1.41	4.43 ± 1.68	10.00 ± 2.65	6.29 ± 1.98
	Pos ctrl	7.33 ± 2.17	6.32 ± 2.14	3.59 ± 1.28	3.58 ± 0.20**	3.29 ± 0.88 **
	TI [WE] 250	10.49 ± 2.86	4.70 ± 2.40	4.66 ± 1.85	4.11 ± 0.91**	3.41 ± 1.25 **
	TI [WE] 500	8.87 ± 3.90	3.59 ± 1.8 *	3.71 ± 0.87	3.99 ± 1.69**	2.94 ± 0.75 **
4	Vehicle ctrl	9.18 ± 1.80	7.63 ± 2.36	5.16 ± 2.05	9.01 ± 2.11	6.25 ± 1.17
	Pos ctrl	7.12 ± 2.08	6.62 ± 2.68	5.37 ± 1.44	4.21 ± 0.75**	4.02 ± 1.29 **
	TI [WE] 250	11.58 ± 3.2	4.05 ± 0.7 *	3.41 ± 0.95	3.92 ± 1.94**	3.12 ± 1.25 **
	TI [WE] 500	8.17 ± 1.03	4.36 ± 2.68	3.39 ± 0.97	4.81 ± 0.50**	3.67 ± 0.89 **

T Day from which animals was treated with TI[WE], # Day on which animals was treated with scopolamine (2 mg/kg, i.p.), *P < 0.05, **P < 0.01, *** P < 0.001, when compared to control group on each day by one-way ANOVA followed by Dunnett's test

in the swimming maze are antagonized by centrally acting cholinergic drugs as physostigmine, oxotremorine and tacrine [18]. The improved spatial and working memory observed in the treatment groups, suggests that TI[WE] might act on the central cholinergic system for reversing the scopolamine- impaired memory in Morris water maze model.

On the basis of these findings, it can be concluded that aqueous extract of T. indica acts as a memory enhancer agent, possibly through modulation of the cholinergic system. However, investigations using more experimental paradigms may be warranted for further confirmation of nootropic potential of *T. indica* in the treatment of dementia and Alzheimer's disease.

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