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RESEARCH ARTICLE

Nootropic activity of *Celastrus paniculatus* seed

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Abstract

The effect of *Celastrus paniculatus* Willd. (Celastraceae) seed aqueous extract on learning and memory was studied using elevated plus maze and passive avoidance test (sodium nitrite induced amnesia rodent model). The aqueous seed extract was administered orally in two different doses to rats (350 and 1050 mg/kg) and to mice (500 and 1500 mg/kg). The results were compared to piracetam (100 mg/kg, p.o.) used as a standard drug. Chemical hypoxia was induced by subcutaneous administration of sodium nitrite (35 mg/kg), immediately after acquisition training. In elevated plus maze and sodium nitrite-induced amnesia model, *Celastrus paniculatus* extract has showed statistically significant improvement in memory process when compared to control. The estimation of acetylcholinesterase enzyme in rat brain supports the plus maze and passive avoidance test by reducing acetylcholinesterase activity which helps in memory performance. The study reveals that the aqueous extract of *Celastrus paniculatus* seed has dose-dependent cholinergic activity, thereby improving memory performance. The mechanism by which *Celastrus paniculatus* enhances cognition may be due to increased acetylcholine level in rat brain.

Keywords: *Acetylcholinesterase; Celastrus paniculatus; elevated plus maze; nootropic; passive avoidance*

Introduction

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative function on the central nervous system, particularly on intellectual performance, learning capacity and memory (Giurgea, 1973). Indian medicinal plants have been used in the treatment of cognitive dysfunction, insomnia, and epilepsy (Chopra, 1958). One popular plant is *Celastrus paniculatus* Willd. (Celastraceae), which is known for its ability to improve memory (Nadkarni, 1976). Ayurveda, the ancient Indian traditional system of medicine, has used this plant seed for prevention and treatment of various diseases (Vaidyaratnam, 1997).

Pharmacological studies suggest that the oil obtained from the seed of *C. paniculatus* possesses sedative and anticonvulsant properties (Gaitonde et al., 1957). Analgesic and anti-inflammatory effects of a *C. paniculatus* seed methanol extract have been reported in mice and rats (Ahmad et al., 1994). *C. paniculatus* seed oil has a long history and has been reported to improve memory processes in rats (Karanth et al., 1981), beneficial to psychiatric patients (Hakim, 1964), and increased the intelligence quotient (IQ) of mentally retarded children (Nalini et al., 1986). More recently, rats treated with *C. paniculatus* seed oil for 15 days exhibited a significant decrease in the levels of norepinephrine, dopamine, serotonin and their respective metabolites in both brain and urine (Nalini et al., 1995). Chronic treatment with *C. paniculatus* seed oil reversed scopolamine-induced deficits in navigational memory performance of rats (Gattu et al., 1997). A methanol extract of *C. paniculatus* seed oil exhibited free radical scavenging effects (Russo et al., 2001).

There is a lack of scientific data regarding the effect of aqueous extract of *C. paniculatus* seed on learning and memory. The present study was therefore carried out to explore nootropic effect of aqueous extract of *Celastrus paniculatus* seed using two animal models, namely elevated plus maze (EPM) and passive avoidance (sodium nitrite-induced amnesia model) followed by

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determination of acetylcholinesterase enzyme (AChE) activity in rat brain.

Materials and methods

Animals

Adult Wistar rats (weighing 200-250 g) and Swiss albino mice (weighing 20-25 g) of either sex were obtained from Drugs Testing Laboratory, Palace Road, Bangalore. The animals were maintained under standard nutritional and environmental conditions, having access to food and water *ad libitum*. The experiments were carried out with the approval of the Institutional Animal Ethics Committee (Proposal no. GCP/CPCSEA/03/2006-07).

Drugs and chemicals

The aqueous extract of *Celastrus paniculatus* seed (from Shrusti Herbal Pharma, Bangalore), piracetam tablets (UCB India, Vapi, Gujarat), acetylthiocholine iodide (Himedia RM 770), 5,5'-dithiobis-2-nitrobenzoic acid (Himedia RM 1677), potassium dihydrogen phosphate (Reachem Laboratory Chemicals), sodium hydroxide (Qualigens Laboratory reagents) and sodium bicarbonate (Burgoyne Laboratory reagents) were used for the study.

Preparation of extract

The coarse powder of the seed was extracted with 6 parts boiling water for 5 h and filtered to yield the extract. The extract was then concentrated and finally dried to a powder.

Behavioral studies

Elevated plus maze

The elevated plus maze was used to assess the retention of learning and memory (Itoh et al., 1990). The plus maze consists of two opposite open arms (50 × 10 cm) crossed with two enclosed arms of the same dimensions with 40 cm high walls. The arms were connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. Rats were randomly divided into 4 groups of 10 rats each. Group 1 received distilled water to serve as control. Standard drug piracetam (100 mg/kg, p.o.) was given to group 2. Groups 3 and 4 were administered the extract at a dose of 350 and 1,050 mg/kg, respectively for 30 successive days. Lower dose selection was based on Kumar and Gupta (2002) and three times the lower dose was taken as the higher dose. The maze was kept in a dimly lit room elevated 50 cm above floor level. On day 1, a rat was individually placed on the end of one of the open arms, facing away from the centre, and the time taken by the animal to enter one of the closed arms (transfer latency (TL) on day 1) was recorded. The rat was left in the enclosed arm for 10–15 s and returned to its home cage. On day 2, the procedure was repeated and TL was recorded. Similarly, after an interval of 30 days drug treatment, on day 30 TL was again recorded.

Passive avoidance test

Sodium nitrite-induced amnesia is a type of interoceptive aversive stimuli model. Sodium nitrite (35 mg/kg, subcutaneously) was administered immediately after the learning trial on day 1 to induce amnesia in mice (Bhattacharya, 1994). Mice were randomly divided into 4 groups of 10 mice each. Group 1 received distilled water to serve as control. Standard drug piracetam (140 mg/kg, per os) was given to group 2. Groups 3 and 4 were administered the extract at a dose of 500 and 1500 mg/kg respectively for 15 successive days. The passive avoidance apparatus consisted of a Plexiglas box (30 × 30 × 40 cm) with a steel rod grid floor (29 parallel steel rods, 0.3 cm in diameter set 1 cm apart). A wooden platform (8 × 8 × 5 cm) was placed in the center of the grid floor. Intermittent electric shocks (1 Hz, 0.5 s, 60 V DC) were delivered to the grid floor by an isolated stimulator. Each mouse was trained by gently placing it on the platform. When the animal stepped down from the platform and placed all its paws on the grid floor, shock was delivered for 15 s. Animals showing a step-down latency (SDL) of more than 15 s in the first training session were excluded from the experiment. Animals had a training session and then 24 h later each mouse was placed on the platform and the SDL was measured as a passive avoidance behavior. The latencies indicated memory levels. An upper cut-off time of 300 s was set. Similarly after an interval of a fortnight of drug treatment, on day 15 SDL was again recorded.

Biochemical studies

Estimation of acetylcholinesterase enzyme activity in rat brain

Acetylcholinesterase activity was measured by the method of Ellman et al. (1961). The rats were decapitated after drug administration and the brain was removed. The brain tissue was homogenized in 0.1 M-phosphate buffer, pH 7.2, in a Teflon glass homogenizer. The reaction mixture consisted of a 0.4 mL aliquot of homogenate, 2.6 mL phosphate buffer (0.1 M, pH 8) and 0.1 mL of dithiobisnitrobenzoic acid (DTNB, 0.01 M), incubated at room temperature for 5 min. After the addition of the substrate acetylthiocholine iodide (0.075 M), the absorbance was measured every min for 5 min at 412 nm using a spectrophotometer (Elico SL 159 UV-VIS spectrophotometer).

Statistical analysis

Results were expressed as mean ± SEM. The data were evaluated by one-way ANOVA followed by Student Newman-Keuls post-test using GraphPad Prism 4. Values of $p<0.05$, $p<0.01$ and $p<0.001$ were considered statistically significant.

Results

In the elevated plus maze paradigm, the rats treated with the standard drug piracetam and the *C. paniculatus* extract showed a decrease in transfer latency (TL) on day 30 when compared to control (vehicle-treated rats) (Table 1). The effect of the extract of *C. paniculatus* (350 and 1050 mg/kg, p.o.) on TL revealed significant reduction by 37.46 and 55.33%, respectively. Thus the significant decrease in TL of *C. paniculatus* extract as compared with the control group indicates the enhancement of cognitive function in rats.

Sodium nitrite-induced cognitive deficit in mice was indicated by a decrease in SDL during retention (at 24 h and on day 15) trials. The mice treated with the standard drug piracetam and the extract showed an increase in SDL on day 15 when compared to control ($NaNO_2$-treated mice) (Table 2). Piracetam and both doses of *C. paniculatus* significantly reversed the hypoxic deficits of retention on day 15.

The results of estimation of acetylcholinesterase enzyme activity in rat brain are summarized in Table 3. Piracetam and *C. paniculatus* (both higher and lower dose) significantly decreased acetylcholinesterase activity as compared with control by 24.20, 13.17, and 19.33%, respectively. Therefore, these drugs improved the memory of rats by inhibiting acetylcholinesterase enzyme, thereby increasing acetylcholine level in rat brain.

Table 1. Effect of *C. paniculatus* on transfer latency (TL) of rats using elevated plus maze.

Group	Treatment	TL after 24 h (s)	TL on day 30 (s)
1	Control	17.1 ± 2.63	29.1 ± 5.87
2	Piracetam	20.6 ± 3.66	10.2 ± 1.19*
3	CP (350 mg/kg)	24.2 ± 5	18.2 ± 3.43*
4	CP (1050 mg/kg)	25.4 ± 5.41	13 ± 3.08*

CP, *Celastrus paniculatus.*
Values are mean ± SEM; *p<0.05.

Table 2. Effect of *C. paniculatus* on step-down latency (SDL) of cognitive deficit mice using step-down (passive avoidance) apparatus.

Group	Treatment	SDL after 24 h (s)	SDL on day 15 (s)
1	Control ($NaNO_2$ and distilled water)	160.9 ± 38.93	48.25 ± 10.18
2	$NaNO_2$ and Piracetam	157.2 ± 47.75	270 ± 30**
3	$NaNO_2$ and CP (500 mg/kg)	159.6 ± 41.4	243.4 ± 31.72**
4	$NaNO_2$ and CP (1500 mg/kg)	227.4 ± 34.28*	180.8 ± 47.31*

$NaNO_2$, sodium nitrite.
Values are mean ± SEM. *p<0.05, **p<0.001.

Table 3. Effect of *C. paniculatus* on brain acetylcholinesterase activity.

Group	Treatment	Cholinesterase activity (μM/min/g of tissue)	Inhibition of cholinesterase activity (%)
1	Control	39.64 ± 1.89	
2	Piracetam	30.05 ± 1.92**	24.2
3	CP (350 mg/kg)	34.42 ± 1.36*	13.17
4	CP (1050 mg/kg)	31.98 ± 1.91**	19.33

Values are mean ± SEM, *p<0.05, **p<0.01.

Discussion

The elevated plus maze is used to measure the anxiety state in animals (Anita & Kulkarni, 1991), however, transfer latency, i.e. the time elapsed between the movement of the animal from an open arm to an enclosed arm, was markedly shortened if the animal had previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related to memory processes. Recent studies of several nootropics and amnestic agents on EPM made this model a widely accepted paradigm to study learning and memory processes in rodents (Itoh et al., 1990; Achliya et al., 2004). In EPM, acquisition (learning) can be considered as transfer latency on first day trials and the retention/consolidation (memory) is examined 24 h later and on day 30. The animals treated with extract of *C. paniculatus* at both the dose levels showed a significant decrease in transfer latency as compared with the control group, which is an indication of the cognitive enhancer effect of *C. paniculatus* in rodents.

The term "passive avoidance" is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behavior. The step-down latency, i.e. the time taken to step down from the shock free zone to steel-rod grid floor, was markedly increased if the animal had previously experienced the electric shock grid floor, and this increased step-down latency has been shown to be related to memory processes (Bhattacharya, 1994). In the passive avoidance test (sodium nitrite-induced amnesia model), acquisition (learning) can be considered as step-down latency on

first day trials and the retention/consolidation (memory) is examined 24 h later and on day 15. The animals treated with *C. paniculatus* (both the doses) showed a significant increase in step-down latency as compared with the control group, which is an indication of the cognitive enhancer effect of *C. paniculatus* in rodents.

AChE activity was measured in rat brain as a marker enzyme for cholinergic function (Sudha et al., 1995). The estimation of AChE enzyme supports the plus maze and passive avoidance (sodium nitrite-induced amnesia) test by statistically reducing AChE activity, which leads to increased acetylcholine (ACh) level in brain, which helps in memory performance. The present study therefore demonstrates that the aqueous extract of *C. paniculatus* seed has dose-dependent cholinergic activity and hence improves memory performance by increasing ACh level in rat brain.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

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