

Inhibitory effect of *Capparis zeylanica* Linn. on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia

Amrendra Kumar Chaudhary^{1*}, Ruchi Solanki², Vandana Singh³, Umesh Kumar Singh¹

¹Kharvel Subharti College of Pharmacy, S.V. Subharti University, Meerut-250005, UP, India; ²School of Pharmaceutical Sciences, Shobhit University, Meerut-250110, UP, India; ³Translam Institute of Pharmaceutical Education and Research, Meerut, -250001, India

ABSTRACT

Capparis zeylanica Linn. a 'Rasayana' drug is used for its memory enhancing effects in the traditional Ayurvedic system of medicine. The aim of this study was to evaluate acetylcholinesterase (AChE) inhibitory and memory enhancing activities of *Capparis zeylanica* Linn. The *in-vitro* and *ex-vivo* models of AChE inhibitory activity were used along with Morris water maze test to study the effect on memory in rats. The anticholinesterase effect of methanolic and aqueous extracts of *Capparis zeylanica* was measured by spectrophotometric Ellman method at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml and brain monoamine oxidase (MAO-A and MAO-B) activity was assessed by Naoi's method. The results of *in-vivo* and *ex-vivo* AChE assay revealed that methanolic and aqueous extracts of *Capparis zeylanica* inhibit AChE activity, whereas these extracts did not alter MAO activity at any concentration tested as compared to moclobemide and L-deprenyl. The results indicate that *Capparis zeylanica* improves scopolamine-induced memory deficits through inhibition of AChE activity, and not by direct MAO inhibition.

Keywords *Capparis zeylanica* Linn, Rasayana drug, anticholinesterase, MAO activity, amnesia, scopolamine

INTRODUCTION

Capparis zeylanica Linn. belonging to family *Capparidaceae*, is commonly known as Indian caper; found throughout India and has been used as a 'Rasayana' drug in the traditional Ayurvedic system of medicine. Rasayana plants are said to prevent ageing, re-establish youth, strengthen life, generate power, impart intelligence, aid improper digestion, clear complexion, enhance brain power, vital energy, eye sight, memory and prevent diseases, all of which imply that they increase the resistance of the body against any onslaught (Govindrajan et al., 2005; Puri et al., 2003). The plant *Capparis zeylanica* is reported to have alkaloids, triterpenes, flavanoids, steroidal substances and saponins. Phytochemical screening of the leaf extracts showed the presence of alkaloids, flavonoids, saponin glycosides, terpenoids, tannins, proteins and carbohydrates (Sharaf, 1997; Satyanarayan et al., 2008). The roots of *Capparis zeylanica* contain alkaloid, phytosterol, acids and mucilage. Fatty acids like ricinolenic acid, malvalic acid, sterculic acid, linoleic acid were also obtained (Mahmood et al., 1991; Haque et al., 2004). In Northern India, the leaves of *Capparis zeylanica* are widely used as counter-irritant, febrifuge and as a cataplasm in swellings, boils and piles (Chopra, 1969; Kirtikar et al., 1987). In Ayurveda, the *Capparis zeylanica* is used traditionally as cooling, cholagogue, bitter, stomachic, sedative, anti-hydrotic and also used in cholera, neuralgia, hemiplegia and rheumatism. Recent *in-vivo* and

in-vitro studies have indicated antioxidant (Agarwal et al., 2009), anti-microbial (Chopade et al., 2008), analgesic (Upaganlawar et al., 2008), antipyretic (Ghule et al., 2007), anti-inflammatory (Chaudhary et al., 2004), anti-spasmodic (Mishra et al., 2007), immunomodulatory (Ghule et al., 2006) activities of *Capparis zeylanica*.

In a previous work we have evaluated the effect of methanolic and aqueous extracts of *Capparis zeylanica* Linn. leaves on spatial learning and memory in rats using Morris water maze task. Results of the previous studies indicated that scopolamine-induced amnesia was reversed by *Capparis zeylanica*, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in rat brain (Solanki et al., 2012). However till now, no scientific data is available in support of acetylcholinesterase (AChE) inhibitory and memory enhancing activities of *Capparis zeylanica*.

Therefore, with the aim to investigate the possible mechanism of its memory enhancing activity, in the present study methanolic and aqueous extracts of *Capparis zeylanica* Linn. leaves was further evaluated using *in-vitro* and *ex-vivo* models of AChE inhibitory activity and brain monoamine oxidase (MAO-A and MAO-B) activity along with Morris water maze test to study the effect on memory.

MATERIALS AND METHODS

Drugs and Chemicals

Scopolamine, galantamine, DTNB (dithiobisnitrobenzoic acid), electric eel acetylcholinesterase, acetylthiocholine iodide, serotonin, benzylamine, L-deprenyl and moclobemide were obtained from Sigma-Aldrich, U.S.A. Piracetam was purchased

*Correspondence: Amrendra Kumar Chaudhary

E- mail: amrendrapharma@gmail.com

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from UCB India Pvt. Ltd, India. Petroleum ether, methanol, chloroform, ammonia, sulphuric acid, fehling solution B, sulphur powder precipitated, glacial acetic acid, butanol, fuming nitric acid, mercury and hydrochloric acid were procured from Rankem, New Delhi, India.

Animals

Adult (7 months of age) Wistar rats (160 - 240 g) and Swiss albino mice (20 - 40 g) of either sex were obtained from Indian Veterinary Research Institute, Bareilly, U.P, India. The animals were housed in polypropylene cage under standard conditions ($25 \pm 2^\circ\text{C}$, 12 h light and dark cycle) with free access to standard pellet feed (Ashirwad Industries, Mohali, Punjab) and water *ad libitum*. All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethical Committee (Registration number: 1279/ac/09/CPCSEA) and were in accordance with CPCSEA guidelines.

Collection and authentication of plant material

The leaves of *Capparis zeylanica* were obtained from a commercial supplier and authenticated by Prof. H. B Singh, Scientist F and Head, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at Raw Materials Herbarium & Museum (NISCAIR/RHMD/consult/-2009-10/1305/108 dated November 03, 2009).

Preparation of extracts

The powdered leaves (150 g) were placed in Soxhlet apparatus and extracted successively using petroleum ether (40 - 60°C) and methanol for approximate 3 h. The solvent was completely removed under reduced pressure till the semi solid mass was obtained. After extraction with petroleum ether and methanol, remaining residue of leaves was air-dried. The dried marc was cold macerated at room temperature for 7 days with constant stirring and then filtered through a filter paper to obtain water extract. The extracts were stored in the refrigerator and a weighed amount was suspended in distilled water with 1% Tween 80 prior to administration (Ghule et al., 2006).

Preliminary phytochemical testing

Preliminary phytochemical screening of the methanolic and aqueous extract of the leaves of *Capparis zeylanica* was done to test the presence of the active chemical constituents such as alkaloids, glycosides, flavonoids, tannins, amino acid, carbohydrates, phenolic compounds, fats and fixed oils (Khandelwal, 2004).

Acute toxicity study

The *Capparis zeylanica* extract was administered orally in the dose of 50, 100, 150, 200, 400 and 800 mg/kg to different groups of mice (n = 6). After administration of extracts, mice were allowed food and water *ad libitum*. All the animals were observed for possible mortality cases and behavioral changes for 24 h (Lorke, 1983).

In-vitro acetylcholinesterase assay

The methanolic and aqueous extracts of leaves of *Capparis zeylanica* were tested for their *in-vitro* acetylcholinesterase inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Ellman et al., 1961). Electric eel acetylcholinesterase was used, and acetyl thiocholine iodide (ATCI) was used as a substrate of the reaction. 5, 5-dithiobis (2-nitrobenzoyl) acid (DTNB) was used for the measurement of AChE activity. Briefly, 150 μl of 0.1 M sodium phosphate buffer (pH 8.0), 10 μl test compound solution (in ethanol), and

20 μl of enzyme solution (0.09 units/ml) were mixed and incubated for 15 min at 25°C. 10 μl of DTNB (10 mM) was then added and reaction was initiated by the addition of substrate (10 μl of ATCI, 14 mM solution). The hydrolysis of the ATCI was measured by the formation of the colored product 5-thio-2-nitrobenzoate anion formed by the reaction of DTNB and thiocholine, which is released by the hydrolysis of enzyme. The formation of the colored product was measured at 410 nm after 5 min. Galantamine, a standard AChE inhibitor was used as positive control, which was dissolved in ethanol. Percent acetylcholinesterase inhibition was calculated (Ahemed et al., 2009).

$$\text{Percentage inhibition} = (E - S) / E \times 100$$

Where, E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. All the experiments were done in triplicate (Orhan et al., 2009).

Ex-vivo estimation of acetylcholinesterase activity

Animals were pre-treated with methanolic and aqueous extracts of *Capparis zeylanica* (50, 100 and 150 mg/kg) orally for 7 days and on the day of experiment the same doses were administered 1 h before the experiment. To assess the anti-AChE activity of methanolic (MCZ) and aqueous (ACZ) extract of the leaves of *Capparis zeylanica*, all groups of rats were decapitated for enzymatic estimation, after the probe trial. The animals were sacrificed by means of quick decapitation without anaesthesia and the brain was isolated immediately. The dissection for discrete regions of brain (frontal cortex and hippocampus) was carried out. AChE inhibitory activity of MCZ and ACZ was measured as described in *in-vitro* assay method (Ellman et al., 1961) in frontal cortex and hippocampus. The discrete brain regions were homogenized in ice cold 0.1 M phosphate buffer (pH 8.0) using homogenizer. The homogenates were centrifuged at $1000 \times g$ for 10 min at 4°C, and supernatant was used as a source of enzyme in AChE assay. The total acetyl cholinesterase activity in the aliquot of the homogenate was estimated. The aliquot was mixed with phosphate buffer (pH 8.0). To this, the substrate acetyl thiocholine iodide and dithiobisnitrobenzoic acid (DTNB) reagent were added. Acetylthiocholine iodide was hydrolyzed to thiocholine and acetate by AChE. Thiocholine reacted with DTNB reagent to produce a yellow colour. The rate of formation of thiocholine from acetylthiocholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The rate of colour (yellow) development is the measure of the AChE activity. Change in absorbance per minute of the sample was read at 410 nm. The enzyme activity is expressed as the 'n' moles of substrate hydrolyzed/minute/mg of protein (Ahemed et al., 2009). The protein contents were determined in the brain samples using Lowry (1951) method.

$$R = \delta\text{OD} / E \times \text{mg of protein}$$

Where R is the rate of enzyme activity in 'n' mole of acetylthiocholine iodide hydrolyzed per minute per mg of protein. δOD is the change in absorbance per minute and E is the extinction coefficient, which is $13600 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of MAO-A and MAO-B activity

Monoamine oxidase (MAO) preparation from mouse brain
The crude MAO was prepared using Naoi's method. The male mice (20 - 25 g) were decapitated, and the brains were homogenized in an ice cold 20 ml of 0.25 M sucrose solution containing a 10 mM potassium phosphate buffer (pH 7.4) and

Table 1. *In-vitro* acetylcholinesterase and MAO inhibitory activity of extracts

	Conc. (mg/ml)	Parameter	Treatment				
			MCZ	ACZ	Galantamine	Moclobemide	L- deprenyl
% Enzyme inhibition \pm SEM ^a	0.1	MAO-A	1.58 \pm 0.480	1.67 \pm 0.511	—	—	—
		MAO-B	1.48 \pm 0.607	1.48 \pm 0.607	—	—	—
		AChE	13.27 \pm 1.958	10.55 \pm 0.092	—	—	—
	0.3	MAO-A	2.68 \pm 0.096	2.22 \pm 0.912	—	—	—
		MAO-B	1.91 \pm 0.817	1.91 \pm 0.817	—	—	—
		AChE	21.03 \pm 0.806	17.46 \pm 0.929	—	—	—
	1.0	MAO-A	3.25 \pm 0.553	3.42 \pm 0.585	—	57.86 \pm 1.027	—
		MAO-B	2.75 \pm 1.020	2.75 \pm 1.020	—	—	61.82 \pm 3.578
		AChE	31.42 \pm 1.979	27.17 \pm 1.861	81.75 \pm 2.059	—	—
	3.0	MAO-A	3.01 \pm 0.911	3.01 \pm 0.911	—	—	—
		MAO-B	3.24 \pm 1.211	3.24 \pm 1.211	—	—	—
		AChE	38.35 \pm 1.194	33.86 \pm 0.990	—	—	—
	10.0	MAO-A	4.25 \pm 0.210	4.25 \pm 0.210	—	—	—
		MAO-B	7.28 \pm 2.254	7.28 \pm 2.254	—	—	—
		AChE	50.80 \pm 0.912	46.78 \pm 3.074	—	—	—
	30.0	MAO-A	4.85 \pm 0.921	4.85 \pm 0.921	—	—	—
		MAO-B	7.55 \pm 0.843	7.55 \pm 0.843	—	—	—
		AChE	55.22 \pm 0.492	52.96 \pm 2.188	—	—	—
IC ₅₀ (mg/ml)	MAO-A	— ^b	— ^b	—	—	—	
	MAO-B	— ^b	— ^b	—	—	—	
	AChE	12.32	19.49	—	—	—	

^aValues are expressed in mean \pm SEM^bNot calculated due to its less activity.

centrifuged at 1,200 g for 5 min at 4°C. The supernatant was collected and further centrifuged at 16,000 g for 20 min at 4°C. The crude mitochondrial pellet was washed using a 10 mM sodium phosphate buffer (pH 7.4) and suspended in the same buffer.

In-vitro assay for MAO activity

The monoamine oxidase type A and B, with serotonin and benzyl amine as a substrate, were assayed spectrophotometrically (Naoi et al., 1987; Ro et al., 2001). The activity of monoamine oxidase-A was determined by quantifying the oxidative product of 5-hydroxytryptamine (serotonin), which has an absorbance peak at 280 nm. Similarly, the activity of monoamine oxidase-B was determined by quantifying the oxidative product of benzyl amines, which has an absorbance peak at 242 nm (Nag et al., 2001). MAO-A and MAO-B activities in the mouse brain were measured in the presence of L-deprenyl (MAO-B inhibitor) and moclobemide (MAO-A inhibitor), respectively. Percent MAO inhibition was calculated (Woo et al., 2005).

For MAO-A: sodium phosphate buffer (2 ml) + 50 μ l mitochondrial fractions + 50 μ l drug solution + 50 μ l serotonin (substrate) and the absorbance was taken at 280 nm.

For MAO-B: sodium phosphate buffer (2 ml) + 50 μ l mitochondrial fraction + 50 μ l drug solution + 50 μ l benzyl amine (substrate) and the absorbance was taken at 242 nm.

$$\text{Percentage inhibition} = (E-S) / E \times 100$$

Where, E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. All the experiments were done in triplicate.

Statistical analysis

Data are expressed as mean \pm standard error of the mean

(SEM). The data were analyzed with Graph Pad Prism 3.0 computer software statistical analysis using Two-way ANOVA followed by Bonferroni post tests. Median inhibitory concentrations (IC₅₀ values) are represented with 95% confidence intervals (CI). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Yield of plant extract

The yield of petroleum ether, methanol and aqueous extracts of *Capparis zeylanica* was found to be 3.07, 9.03 and 12.17%, respectively.

Phytochemical testing of *Capparis zeylanica*

Preliminary phytochemical screening indicated presence of alkaloid, flavonoids, saponins, terpenoids, steroids and tannins were present in methanolic extract of *Capparis zeylanica*. Whereas aqueous extract showed the presence of alkaloid, flavonoids, saponins, terpenoids, steroids and carbohydrates.

Acute toxicity

The mice treated orally with 50, 100 and 150 mg/kg of methanolic and aqueous extracts of leaves of *Capparis zeylanica* were found to be normal. The acute toxicity study revealed that the extracts have good margin of safety and did not show lethal effects on the animals up to the doses of 800 mg/kg.

Effect on brain MAO activity

The methanolic and aqueous extracts of leaves of *Capparis zeylanica* (MCZ and ACZ respectively) were tested for their *in-vitro* MAO inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Table1). The extracts did not inhibit

Table 2. Effect of MCZ on acetylcholinesterase activity in different brain regions of rat

Location	Acetylcholinesterase activity (μ mole of substrate hydrolyzed/minute/mg of protein)					
	Control	Scopolamine	MCZ-50	MCZ-100	MCZ-150	P-500
Frontal cortex	35.98 \pm 0.792	40.92 \pm 2.214 ^a	29.93 \pm 0.262 ^{a,c}	24.14 \pm 0.990 ^{a,c,d}	19.60 \pm 0.624 ^{a,c,d,e}	13.14 \pm 0.439 ^{a,c,d,e,f}
Hippocampus	34.32 \pm 0.391	39.03 \pm 0.784 ^b	28.82 \pm 0.220 ^{a,c}	23.41 \pm 1.20 ^{a,c,d}	18.43 \pm 0.972 ^{a,c,d,e}	12.88 \pm 0.391 ^{a,c,d,e,f}

After probe trial in Morris water maze test, AChE assay was carried out in frontal cortex and hippocampus. Piracetam was used as the standard drug. The results are expressed as Mean \pm Standard Error Mean (SEM). The data were analyzed with Graph Pad Prism statistical analysis using Two-way ANOVA followed by Bonferroni post tests. A value < 0.05 was considered statistically significant. $p^a < 0.001$ compared with control, $p^b < 0.01$ compared with control, $p^c < 0.001$ compared with scopolamine, $p^d < 0.001$ compared with MCZ-50, $p^e < 0.001$ compared with MCZ-100 and $p^f < 0.001$ compared with MCZ-150.

MAO-A and MAO-B as compared to moclobemide and L-deprenyl, respectively.

Effect on *in-vitro* acetylcholinesterase activity

The methanolic and aqueous extracts of leaves of *Capparis zeylanica* were tested for their *in-vitro* acetylcholinesterase inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Table 1). The extracts showed a mild inhibition as compared to galantamine. The most active one was noted to be methanolic extract having IC₅₀ value at 12.32 mg/ml, aqueous extract displayed a similar activity with IC₅₀ 19.49 mg/ml.

Ex-vivo acetylcholinesterase assay in frontal cortex and hippocampus

After testing the spatial reference memory by Morris water maze test, *ex-vivo* assay was performed for assessing the AChE activity. The analysis showed that brain AChE activity was different between groups [$F_{5, 60} = 232.72$, $p < 0.0001$] in different brain regions [$F_{1, 60} = 4.41$, $p < 0.05$] (Table 2 and 3). Further, post-hoc analysis revealed that both the extracts of *Capparis zeylanica* (50, 100 and 200 mg/kg) dose-dependently decreased AChE activity in prefrontal cortex and hippocampus compared to control.

DISCUSSION

Cholinergic neurons in the central cholinergic system possess an important function in the process of learning and memory which is influenced by drugs effective in this system (Everitt et al., 1997). Therefore, the measurement of acetylcholinesterase activity has been extensively used as an experimental model to screen pure molecules or extracts with possible efficiency against dementia.

Many anti-cholinergic drugs, such as scopolamine induces a transient disruption of memory in humans and experimental animals by blocking postsynaptic muscarinic receptors (Drachman et al., 1974). This effect can be antagonized by cholinomimetics, such as physostigmine (Megazzini et al., 1965) which increase brain ACh contents. To find out whether *Capparis zeylanica* has any cholinergic activity, the MCZ and

ACZ were evaluated for anti-AChE activity in *in-vitro* and *ex-vivo* models. In the present study, MCZ and ACZ pre-treatment for 7 days (50, 100 and 150 mg/kg) inhibited AChE activity in the frontal cortex and hippocampus regions. Anti-cholinesterase activity of both the extracts of *Capparis zeylanica* may facilitate cholinergic neurotransmission which is important for learning and memory functions.

Therefore, cholinergic hypofunction represents as one of the major problems resulting in cognitive impairment. The results of *ex-vivo* AChE assay revealed that *Capparis zeylanica* extracts possess AChE inhibitory activity in hippocampus and frontal cortex, which suggests their ability to cross blood brain barrier and by inhibiting AChE enzyme providing longer time for acetylcholine to stimulate postsynaptic muscarinic receptors. Enhancing cholinergic transmission is likely to offer beneficial effects which may improve patient's conditions. Taking these facts together anti-AChE activity of *Capparis zeylanica* may be an important mechanism associated with improvement of learning and memory functions in rats.

Studies have suggested subtle functions of the different aminergic systems in spatial memory formation. Under certain circumstances, stimulation of the noradrenergic system enhances MWM acquisition. The selective monoamine oxidase B (MAO-B) inhibitor L-deprenyl thus alleviated scopolamine-induced acquisition and probe trial performance deficits (Yavich et al., 1993). The results from the present study show that *Capparis zeylanica* extracts did not alter MAO activity at any concentration tested as compared to moclobemide and L-deprenyl. Thus, it can be concluded that the nootropic activity of *Capparis zeylanica* is not a result of direct MAO inhibition but may be, at least in part, mediated by AChE inhibition. However, these contentions need to be further explored before coming to any conclusion on precise mechanism of action.

Preliminary phytochemical screening showed that flavonoids and terpenoids were the main chemical constituents of *Capparis zeylanica* extracts. Flavonoids and terpenoids contribute synergistically to the neuroprotection mainly via antioxidant activity (Mu et al., 2007). Probably, these compounds are considered to play major role in memory improvement effect of this plant. The present study suggests that *Capparis zeylanica* may possibly play an important role in

Table 3. Effect of ACZ on acetylcholinesterase activity in different brain regions of rat

Location	Acetylcholinesterase activity (μ mole of substrate hydrolyzed/minute/mg of protein)					
	Control	Scopolamine	ACZ-50	ACZ-100	ACZ-150	P-500
Frontal cortex	35.99 \pm 0.792	40.92 \pm 2.214 ^a	28.45 \pm 0.176 ^{a,c}	23.43 \pm 0.879 ^{a,c,d}	17.95 \pm 0.576 ^{a,c,d,e}	13.14 \pm 0.439 ^{a,c,d,f,g}
Hippocampus	34.32 \pm 0.391	39.03 \pm 0.784 ^b	26.65 \pm 0.142 ^{a,c}	21.83 \pm 0.957 ^{a,c,d}	16.75 \pm 0.867 ^{a,c,d,f}	12.88 \pm 0.391 ^{a,c,d,f,g}

After probe trial in Morris water maze test, AChE assay was carried out in frontal cortex and hippocampus. Piracetam was used as the standard drug. The results are expressed as mean \pm standard error mean (SEM). The data were analyzed with Graph Pad Prism statistical analysis using Two-way ANOVA followed by Bonferroni post tests. A value < 0.05 was considered statistically significant. $p^a < 0.001$ compared with control, $p^b < 0.01$ compared with control, $p^c < 0.001$ compared with scopolamine, $p^d < 0.001$ compared with ACZ-50, $p^e < 0.01$ compared with ACZ-100, $p^f < 0.001$ compared with ACZ-100 and $p^g < 0.001$ compared with ACZ-150.

preventing cholinergic dysfunctions. To our knowledge this is the first report providing evidence for the AChE inhibitory and memory enhancing effect of the *Capparis zeylanica* in scopolamine-induced amnesia in rats.

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CONFLICT OF INTEREST

The authors have no conflicting financial interests.

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