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Original Article

Pharmacology Section

Effect of *Convolvulus pluricaulis* and Omega-3 Fatty Acid alone and in Combination on Learning and Memory

RAJENDRA NATH[1], RISHI PAL[2], RAHUL KUMAR[3], PRAMOD KUMAR SINGH[4], RAKESH KUMAR DIXIT[5], AMOD KUMAR SACHAN[6]

ABSTRACT

Introduction: Mental disorders contribute to a significant load of morbidity and disability. In neurological disorders, alternative medicine use is common due to their fewer side-effects. There are scant evidences regarding the role of *Convolvulus pluricaulis (C. pluricaulis)* and Omega (ω)-3 fatty acids in learning and memory, and none for their combined effect.

Aim: To evaluate the effect of *C. pluricaulis* alone and in combination with Omega-3 fatty acids.

Materials and Methods: The present study was an animal study done on Wistar rats. *C. pluricaulis* whole plant powder in doses of 100 mg/kg body weight (b.w.) and 400 mg/kg b.w. was used. Omega-3 fatty acid (500 mg/kg b.w.) was administered orally alone and along with a higher dose of *C. pluricaulis*. Scopolamine induced model for amnesia in rats was used in the study and donepezil was taken as standard. Apart from the behavioural analysis, Acetylcholinesterase (AchE) estimation in rat's whole brain tissue was done using spectrophotometry.

Results: *C. pluricaulis* showed significant memory enhancement in a dose dependent manner (100 mg/kg b.w. and 400 mg/kg b.w.) alone and in combination (*C. pluricaulis* 400 mg/kg b.w.) with ω-3 fatty acid in dose of 500 mg/kg b.w.

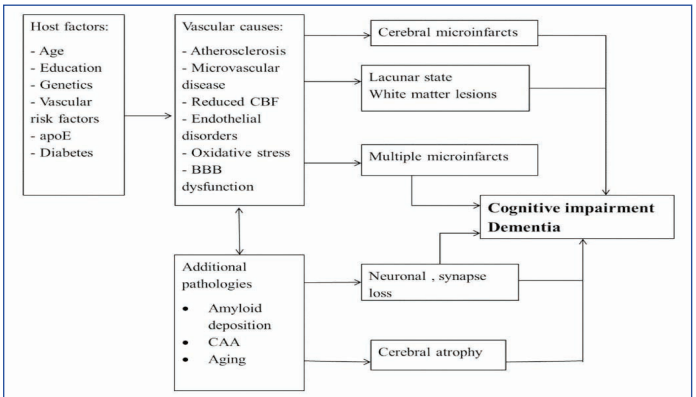
Conclusion: The combination of *C. pluricaulis* and ω-3 fatty acid showed greater significant effect as compared to alone which was comparable to standard drug.The encouraging results reveal the importance of herbal drugs and nutrients in cognition improvement.

Keywords: Alzheimer's disease, Cognition, Complementary and alternative medicine, Cook's pole climbing test, Morris water maze test

INTRODUCTION

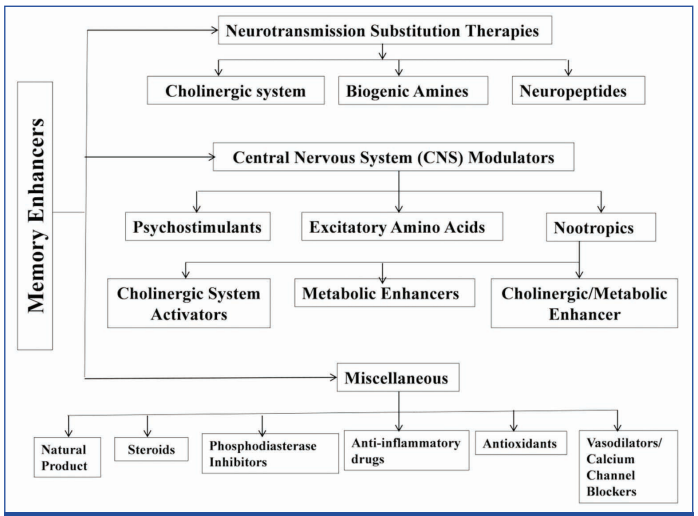
It has been proven in our ancient Ayurveda medical system that good memory and knowledge are very much associated with a long life. Memory is a data registration, storage and retrieval mechanism due to changes in neurotransmission at the synapse [1]. Learning is the core mechanism by which our mind set, emotional speech, behaviour, and activities are created. Latest neuroimaging studies have shown that amygdala and prefrontal cortex coordinate with the temporal medial cortex [2]. Dementia means acquired deficiency of intelligence, memory, and personality without gross clouding of consciousness. Factors affecting cognitive dysfunction and dementia are represented in [Table/Fig-1].

The prevalence of all forms of dementia in South East Asian countries is about 2.7% [3]. The proportion of patients with early onset dementia is high (49.9%) in a developing country as opposed to (7-30%) in developed world [4].



[Table/Fig-1]: Factors causing cognitive impairment and dementia.
apoE: Apolipoprotein E; CBF: Cerebral blood flow; BBB: Blood brain barrier; CAA: Cerebral amyloid angiopathy

Many compounds influence learning by influencing the mechanism involved in memory storage and some other compounds impact output by altering attention or motivational mechanism [5]. Various ways to improve memory have been shown in [Table/Fig-2].



[Table/Fig-2]: Approach to enhance memory.

Donepezil reversibly inhibits AchE by preventing the hydrolysis of the neurotransmitter acetylcholine and, therefore, increasing its function [6]. Its key clinical application is in the treatment of Alzheimer's disease, to increase acetylcholine in Central Nervous System (CNS).

In the last few years, there has been an increased interest in the field of herbal medicine, because of its natural origin and fewer side effects. The World Health Organisation (WHO) estimates that 80% of the population uses herbs and as high as 95% in developing countries [7]. Several plants, collectively called "Medhya" plants (intellect promoting), like *Convolvulus microphyllus, C. pluricaulis, Bacopa*

monnieri, Acorus calamus, Zingiber officinale, Centella asiatica and Celastrus paniculatus are beneficial in memory disorders [8].

C.pluricaulis Choisy (Shankhpushpi) is a perennial herb that is used in Indian and Chinese medicine for chronic cough, sleeplessness, anxiety, epilepsy, hallucinations, etc. *C.pluricaulis* Choisy, CP of family Convolvulaceae is considered as Medhya Rasayana (memory enhancer) in Ayurvedic texts [9], and has been used as a rejuvenator, antiageing, mental stimulant, tranquilizer, anticonvulsant, and anxiolytic [10]. As per Ayurvedic Pharmacopoeia of India, all parts of *Convolvulus pluricaulis* are approved for medicinal use [11]. Some other plants like *Clitorea ternatea Linn (C. ternatea), Evolvulus alsinoides Linn* and *Canscora decussata Schult* are also known under the name Shankhpushpi.

The role of nutrition is increasingly being recognised and recently been stated that 'nutrition and nutraceuticals should now be considered as mainstream elements of psychiatric practice' [12-14]. The ω-3 fatty acid shows a beneficial role in the neurodegenerative disorder, mood disorder, and psychiatric disorder. The ω-3 fatty acids, Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA) are incorporated into neuronal phospholipids of neuronal membranes, influences neurotransmission electrical signal transduction mechanisms [15]. Due to the adverse effects of the drugs available presently for dementia, people are switching to complementary therapies which include nutritional supplements like ω-3 fatty acids and herbal drugs, one of which is *C.pluricaulis* [16].

Scopolamine is an alkaloid obtained from Solanaceae, especially *Datura metel L.* and *Scoopula carniolica* causes amnesia in humans and also impairs learning in animals [17]. Scopolamine induced amnesic rodent model is one of the well-established animal models for memory dysfunction.

Since, the literature shows that there is ample evidence regarding the role of *C.pluricaulis* and ω-3 fatty acids in cognition improvement, but only a few scientific studies have been done in this regard and none have been done to see the combined effect of *C. pluricaulis* and ω-3 fatty acids. Therefore, the present study was planned to evaluate the effect of *C.pluricaulis* alone and with ω-3 fatty acid.

MATERIALS AND METHODS

This pre-clinical animal study was conducted in the Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India during April 2018 to September 2018. Amnesia inducing drug scopolamine hydrochloride obtained from Sigma Aldrich (USA), administered in a dose of 0.3 mg/kg by intraperitoneal injection [18]. Test drugs:

a. *C.pluricaulis* whole plant powder was purchased from International Organisation for Standardisation (ISO) and Good Manufacturing Practices (GMP) Certified Company which is also recognised by LACON India and United States Department of Agriculture (USDA), powder given in doses of 100 mg/kg b.w. and 400 mg/kg b.w [19-21] orally with the help of a feeding cannula.

b. Omega-3 Fatty Acids capsules (Maxepa) obtained from Merck (Germany) administered in a dose of 500 mg/kg b.w. [22] orally with the help of a feeding cannula in Tween 20 as a vehicle. Standard drug donepezil hydrochloride was obtained from Sigma Aldrich (USA) given in a dose of 0.75 mg/kg b.w. administered through intraperitoneal injection [23].

Toxicity studies were not done by authors as according to one previous study, there is no mortality amongst the graded dose of *C.pluricaulis* in albino Wistar rats up to a dose of 5000 mg/kg for a duration of 72 hours [24]. Therefore, the two doses of *C.pluricaulis*, 100 mg/kg b.w. and 400 mg/kg b.w. [19-21] were chosen for the evaluation of effect in learning and memory in present study which can be safe for both the experimental animals and human consumption.

Animals: Adult healthy albino Wistar rats of either sex, weighing 160-200 gm were obtained from Committee For the Purpose of Control and Supervision on Experimental Animals (CPCSEA) certified animal house. Animals were given normal rat pellet diet and water ad libitum and kept under temperature 25±2°C with 12 hours light-dark cycle. The animals were acclimatised for one week. Study protocol was approved by Institutional Animal Ethics Committee before conducting the study (Ref. no.– 92/IAEC/2018/dated 16/04/2018). All procedures were performed in accordance with the recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Procedure

A total number of 48 Wistar rats were used in the study. Rats were randomly allocated into seven groups (n=6) using stratified randomisation and no blinding. Every group contains the animals of same age and sex to reduce confounding bias. Different groups in present study were as follows:

Group I: Rats were administered distilled water per oral (p.o.) 1 mL.

Group II: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. intraperitoneally) [25]

Group III: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. i.p.) and *C.pluricaulis* whole plant powder (100 mg/kg b.w. p.o.) [19]

Group IV: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. intraperitoneally) and *C.pluricaulis* whole plant powder (400 mg/kg b.w. p.o.) [20,21]

Group V: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. i.p.) and ω-3 FA (500 mg/kg b.w. p.o.) [22]

Group VI: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. i.p.), ω-3 F A (500 mg/kg b.w. p.o.) and *C.pluricaulis* whole plant powder (400 mg/kg bw p.o.)

Group VII: Rats were administered scopolamine hydrochloride (0.3 mg/kg b.w. intraperitoneally) and standard drug donepezil (0.75 mg/kg b.w. intraperitoneally) [23].

Animal model for learning and memory: Scopolamine induced model for amnesia in rats was used in a single dose of 0.3 mg/kg intraperitoneally [18].

Behavioural Animal Tests

1. Effect on learning and memory in rat using Cook's Pole climbing test: Cook and Weidley's pole climbing apparatus was used to study cognitive function [18]. The apparatus has an experimental chamber (25×25×25 cm) with the floor grid in a sound proof enclosure. Scrambled shock (6 mA) was delivered to the grid floor of the chamber composed of stainless steel rods. The study rats were allowed to explore for 45 seconds. After 5 seconds of Conditioned Stimulus (CS) i.e., buzzer signal was turned on then Unconditioned Stimulus (US) i.e., electric shock was delivered through grid floor.

Assessment of Conditioned Avoidance Response (CAR): The Avoidance Response (AR) for 10 trials was noted. The rats who were found sensitive to foot shock were included. Vehicle and test drugs were administered for 14 days once daily according to study design (group I to VII). A Training Trial (TT) of all animals was conducted after administering drugs for seven days. A Relearning Trial (RT) was conducted on 8th day. On day 9th, all the animals except control (group I) were administered single dose of scopolamine (0.3 mg/kg intraperitoneally) 30 minutes before the administration of the extracts. This schedule was continued till 15 days for evaluation of retention trial.

2. Effect on learning and memory in rat using the Morris Water Maze test (MWM): The acquisition and retention related to spatial

orientation were examined through MWM test [26]. The rats were placed in a circular pool (180 cm diameter×60 cm height) and required to find an invisible platform. The pool was filled with water (28±2ºC) to a depth of 40 cm and water was made opaque by adding a non toxic water dispersible emulsion to prevent visibility. On 1st day, four swimming training sessions of the 60 seconds were done with platform. On 2nd day, four trial sessions were performed with the platform in place. The time interval between each trial session was 30 minutes. During each trial session, the time was taken to find the hidden platform (transfer latency) which was recorded. After the last TT session, on the 15th day, the platform was removed from the pool, and rats were allowed to swim for 60 seconds to search for it. The test drugs and the vehicle were administered according to study design (group I to VII) once daily for 14 days. On the 14th day, a single dose of scopolamine (0.3 mg/kg intraperitoneally) was administered 45 minutes before the test. All the groups, except control group I, were received scopolamine. On the 15th day, the time spent in the target quadrant (where the platform was placed) was recorded.

Assessment of transfer latency on day 14th and time spent in target quadrant on day 15th: On the last day of behavioural study for learning and memory, the animals of group-I to group-VII were sacrificed by pentobarbitone-100 mg/kg as a method of euthanasia for the evaluation of biochemical parameters.

Biochemical Parameter

Colourimetric determination of AchE activity: AchE activity was measured according to Ellman's method [27]. AchE activity was measured by increased yellow colour produced from thiocholine as it reacts with dithiobisnitrobenzoate ion.

- Acetylthiolcholine $\xrightarrow{\text{enzyme}}$ thiocholine+acetate
- Thiocholine + dithiobisnitrobenzoate $\longrightarrow$ yellow colour

A colourimetric AchE assay kit was used to measure the AchE activity according to the manufacturer's instructions (Abcam, Cambridge, UK) and the data were expressed in mU/g of brain tissue. The absorption intensity of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) adduct was used to measure the amount of thiocholine formed, which was proportional to the AchE activity. On the last day, brain tissues were collected after sacrificing rats.

STATISTICAL ANALYSIS

Data were expressed as mean±Standard Error of the Mean (SEM). Statistical analysis was carried out by one-way Analysis of Variance (ANOVA) followed by Dunn's multiple comparison test to compare the different parameters like AR on day 7, transfer latency, time spent in the target quadrant. The ARs from 9 to 15 days were compared using two-way analysis of variance followed by Bonferroni's post-hoc test. A probability level of less than 0.05 ($p<0.05$) was accepted as being significant in all types of statistical tests.

RESULTS

Behavioural Test

Cook's pole climbing test: In [Table/Fig-3]- number of ARs in TT and RT on Day 7 have been summarised. *C.pluricaulis* (100 mg/kg, 400 mg/kg) (CP-100, CP-400), ω-3 fatty acids (500 mg/kg) (ω-3 FA) and combination of *C.pluricaulis*- 400 mg/kg and ω-3 fatty acids (500 mg/kg) (CP-400+ω-3 FA) were administered for 7 days. CP-400 and ω-3 FA showed significant increase ($p<0.05$) in ARs than control. CP (400)+ω-3 FA and donepezil showed significant ($p<0.05$) increase in ARs as compared to CP-400 and ω-3 FA alone. CP (400)+ω-3 FA and donepezil showed comparable response.

Group	Treatment (Dose- mg/kg p.o.)	No. of Avoidance Responses (AR)	
		Training Trial (TT)	Relearning Trial (RT)
I	Control	6.17±0.31	7.01±0.58
II	Scopolamine (0.3 mg/kg)	1.89±24	1.98±18
III	CP (100)	6.63±0.32	7.16±0.70
IV	CP (400)	8.50±0.42[a]	9.15±0.22[a]
V	ω-3 FA	8.17±0.40[a]	8.82±0.31[a]
VI	CP (400) + ω-3 FA	9.09±0.31[b]	9.62± 0.26[b]
VII	Donepezil	9.37±0.31[b]	9.81±0.21[b]

[Table/Fig-3]: Effect of shankhpushpi (*Convolvulus pluricaulis*), omega-3 fatty acids and combination treatment on learning memory in rats when administered for 7 days using Cook and Weidley's pole climbing apparatus.
All values are mean±SEM (n=6); [a]$p<0.05$ compared with control; [b]$p<0.05$ compared with group IV and group V; One-way ANOVA followed by Dunn's multiple comparison test

Scopolamine produced amnesia as exhibited by the reduction in the number of ARs. However, CP produced better retention and recovery in a dose-dependent manner as compared to vehicle. CP-100 showed no significant response in improving learning and memory impairment caused by scopolamine as compared to CP-400. CP-400 and ω-3 FA took 4-5 days to reach the point of complete retention of AR and reversal of learning memory impairment induced by scopolamine indicating better retention and recovery also showed comparable response as donepezil. The values have been summarised in [Table/Fig-4].

Morris Water Maze (MWM) test: [Table/Fig-5] showed a significant increase in mean transfer latency time ($p<0.001$) in group II (scopolamine treated) as compared to group I (control) measured on day 14th. CP-400 and ω-3 FA treatment showed less increase in mean transfer latency ($p<0.05$) as compared to the scopolamine treated group. CP-400+ω-3 FA showed significant protective effect as less increase in transfer latency ($p<0.05$) than CP-400 and ω-3 FA alone. CP-400+ω-3 FA showed comparable response as compared to donepezil. The values have been summarised in [Table/Fig-5].

On the 15th day, scopolamine treated group showed significantly less time spent ($p<0.001$) in the target quadrant as compared with the vehicle treated. However, the rats pretreated with CP-400

Days	Number of Avoidance Responses (AR)						
	Group I Control (1 mL dist. water)	Group II Scopolamine (0.3 mg/kg)	Group III Scopolamine+ CP-100 mg/kg	Group IV Scopolamine+ CP-400 mg/kg	Group V Scopolamine+Omega-3 fatty acid (500 mg/kg)	Group VI Scopolamine+CP (400)+Omega-3 fatty acid	Group VII Scopolamine+ Donepezil
9	7.67±0.32	2.02±0.32[a]	2.63±0.32	3.85±0.36[b]	3.56 ±0.22[b]	5.65±0.32[c]	6.03 ±0.31[c]
10	8.42±0.31	2.63±0.22[a]	3.25±0.33	5.64±0.58[b]	4.91±0.32[b]	6.43±0.32[c]	7.21±0.31[c]
11	8.86±0.22	3.56±0.42[a]	3.96±0.48	7.43±0.37[b]	7.29±0.22[b]	8.45±0.22[c]	8.65±0.67[c]
12	9.04±0.42	4.02±0.58[a]	4.63±0.23	8.46±0.66[b]	8.08±0.31[b]	9.46±0.67	9.64±0.22
13	9.26±0.52	4.86±0.54[a]	5.91±0.33	9.23±0.33[b]	8.98±0.67[b]	9.53±0.31	9.75±0.67
14	9.35±0.47	5.62±0.42[a]	6.85±0.13	9.42±0.48[b]	9.29±0.22[b]	9.76±0.22	9.85±0.32
15	9.89±0.36	6.52±0.22[a]	7.93±0.25	9.67±0.42[b]	9.54±0.31[b]	9.82 ±0.48	9.88±0.22

[Table/Fig-4]: Effect of shankhpushpi (*Convolvulus pluricaulis*), omega-3 fatty acids and combination treatment on learning memory in scopolamine induced amnesia model of rats when administered from 7th to 15th day in Cook's Pole climbing test.
All values are mean±SEM (n=6); [a]$p<0.001$ as compared to control group, [b]$p<0.05$ as compared to group II, [c]$p<0.05$ as compared to group IV and V; Two-way ANOVA followed by Bonferroni's post-hoc test.

Group	Groups	Transfer latency (mean±SEM)
I	Control	23.17±1.65
II	Sco	103.33±2.07[a]
III	Sco+CP (100)	100.67±2.84
IV	Sco+CP (400)	52.83±1.9[b]
V	Sco+ω-FA	60±1.82[b]
VI	Sco+CP (400)+ω-FA	35.67±1.63[c]
VII	Sco+Donepezil	34.83±1.87[c]

[Table/Fig-5]: Effect of shankhpushpi (*Convolvulus pluricaulis*), omega-3 fatty acids and combination treatment of both on scopolamine induced learning memory impairment using Morris Water Maze (MWM) test. Time taken by rats to reach platform (transfer latency) on day 14.
Results are expressed as mean±SD (n-6); [a]p<0.001 compared to control, [b]p<0.05 compared to group II, [c]p<0.05 compared to group IV and V; One-way ANOVA followed by Dunn's multiple comparison test

and ω-3 FA spent more time in the target quadrant (p<0.05) as compared with scopolamine treatment. CP-100 showed no significant protective effect as compared to CP-400. CP-400+ω-3 FA showed a significant increase in time spent in the target quadrant (p<0.05) compared when both were given alone. Also, CP-400+ω-3 FA showed a comparable effect to donepezil. The values have been summarised in [Table/Fig-6].

Group	Groups	Time spent in target quadrant (s) (mean±SEM)
I	Control	81.33±1.54
II	Sco	20.83±0.79[a]
III	Sco+CP (100)	24.17±1.17
IV	Sco+CP (400)	50±0.93[b]
V	Sco+ω-FA	48±1.88[b]
VI	Sco+CP (400)+ω-FA	70.67±1.2[c]
VII	Sco+Donepezil	72.05±0.99[c]

[Table/Fig-6]: Effect of shankhpushpi (*Convolvulus pluricaulis*), omega-3 fatty acids and combination treatment of both on scopolamine induced learning memory impairment using Morris Water Maze (MWM) test. Time spent in the target quadrant on day 15.
Results are expressed as mean±SD (n=6); [a]p<0.001 compared to control; [b]p<0.05 compared to group II; [c]p<0.05 compared to group IV and V; One-way ANOVA followed by Dunn's multiple comparison test

Biochemical parameter: AchE estimation by spectrophotometry-Ellman's assay- scopolamine administration (group II) alone significantly increased (p<0.001) the activity of AchE when compared to the control (group I), CP-100 and CP-400 showed a decrease in the level of AchE in a dose-dependent manner as compared to scopolamine. CP-400 and ω-3 FA significantly (p<0.05) decreased levels of AchE as compared to the scopolamine-treated group. CP-400+ω-3 FA and donepezil treatment (group VII) significantly (p<0.05) decreased the levels of AchE as compared to when given alone. CP-400+ω-3 FA showed a comparable effect to the standard donepezil treatment group. The values have been summarised in [Table/Fig-7].

Group	Treatment	Acetylcholinesterase (mU/g tissue)
I	Control	485.10±14.27
II	Sco	886.25±25.00[a]
III	Sco+CP (100)	781.46±18.29
IV	Sco+CP (400)	607.69±15.39[b]
V	Sco+ω-3 FA	630.31±14.52[b]
VI	Sco+CP (400)+ω-3 FA	525.62±13.08[c]
VII	Sco+Donepezil	500.60±15.62[c]

[Table/Fig-7]: Effect of scopolamine, shankhpushpi (*Convolvulus pluricaulis*), omega-3 fatty acids and combination treatment on acetylcholinesterase (AChE) activity (mU/g of tissue protein) in rat brain tissue.
All values are Mean±SEM (n=6)
[a]p<0.001 compared to control (Group-I); [b]p<0.05 compared to Group II; [c] p<0.05 compared to group IV and V; One-way ANOVA followed by Tukey Kramer's multiple comparisons test

DISCUSSION

In modern life, the prevalence of mental illness is increasing due to changing lifestyle, competitive environment, and stressful activities. The stress of modern life might affect learning and memory processes by suppressing adult neurogenesis. Various synthetic compounds for the treatment of dementia are costly and have many side effects [16]. So, there is increased demand for Complementary Alternative Medicine (CAM). Herbal medicines cause the prevention of mental illnesses, rejuvenate the body system, harmonise and extend the lifespan, and are also safer, more affordable, easily accessible. In 1993, the WHO invited experts to develop principles/criteria for guiding herbal medicines related research (WHO, 1993) [28].

Therefore, the present study was designed to evaluate the learning and memory enhancement effect of *C. pluricaulis* and ω-3- FA alone and in combination treatment.

C. pluricaulis contains many chemical constituents such as scopolin, ayapaninscopoletin-glycoside, fatty acids, kaempferol-glycoside, β-sitosterol, aliphatic compounds, and secondary metabolites having several types of alkaloids, flavonoids, and coumarins e.g., Shankhapushpine, convolamine, convoline, convolidine, convolvine, confoline, convosine with beneficial action as anxiolytic, antidepressant, antioxidant, hypolipidemic, immunomodulatory, analgesic, antibacterial, antidiabetic, etc., [19,29,30]. The ω-3 fatty acids present in the brain exert neuroprotective effect by regulating neurotransmission, cell survival, and nullifying neuroinflammation, and resulting in cognition enhancement. Very few literatures are documented showing the effect of *C. pluricaulis* in various mental disorders. So in present study, authors used it to elaborate on its effect on learning and memory.

Authors used a purified dried powder form of *C. pluricaulis* in the study as it contains most of the active constituents. The scopolamine model is used to induce amnesia such as showed in previous studies [31]. On systemic administration, it is centrally acting anticholinergic and leads to a reversible reduction in learning and retention processes. In Cook's Pole Climbing test, on the 7th day, a low dose of *C. pluricaulis* 100 mg/kg b.w. group achieved less AR in TT and in RT. A higher dose at 400 mg/kg b.w. of *C. pluricaulis* achieved comparatively more AR in TT and RT than the lower dose and of control. Thus, it showed the dose-dependent action of this herbal drug. Similarly, ω-3 fatty acids (500 mg/kg b.w.) achieved more AR in the TT and in the RT compared to control. The maximum acquisition was achieved earlier in the treatment group as compared to a control group which is represented by the increase in ARs. These results are in accordance with the previous study done so far [18]. Combination of *C. pluricaulis*, 400 mg/kg, and ω-3 fatty acids (500 mg/kg b.w.) achieved a greater significant (p<0.05) increase in AR in TT and in RT. On the 9th day when treated with scopolamine, the amnesia was less in *C. pluricaulis*- 400 mg/kg, ω-3 fatty acids, and a combination of these two showing better retention as in the control group than scopolamine treated group. As evident with results, combination of *C. pluricaulis* and ω-3 fatty acids treated group achieved maximum acquisition earlier than all other groups, less decreased in ARs on scopolamine administration and achieved maximum ARs early than both of these were given alone. These parameters have shown better retention and recovery of learning and memory by combined treatment [Table/Fig-3,4]. In the MWM test, on the 14th day, scopolamine showed an increase in transfer latency time (103.33±2.07) as compared to control (23.17±1.65), and on the 15th day without a platform, there was a decrease in time spent in the target quadrant as compared to control group. *C. pluricaulis*- 400 mg/kg b.w. showed significantly (p<0.05) less transfer latency time (52.83±1.9) on 14th day and more time spent in the target quadrant (50±0.93) on 15th day as compare to scopolamine treated group. But lower dose (100 mg/kg b.w) of *C. pluricaulis* did not produce a significant ameliorating effect on amnesia caused by scopolamine. A higher dose produced a significant (p<0.05) response which represented the dose-dependent

action of *C. pluricaulis*. Omega-3 fatty acids- 500 mg/kg b.w. also produced significant response i.e., decrease in transfer latency on 14th day and increase in time spent in target quadrant on 15th day as compared to scopolamine treated group. Thus, *C. pluricaulis* and ω-3 fatty acids have produced significant ($p<0.05$) ameliorating effect of cognitive impairment produced by scopolamine. This replicates the result which has been shown in other previous studies [26,32]. Combined treatment of both *C. pluricaulis*- 400 mg/kg b.w. and ω-3 fatty acids- 500 mg/kg b.w. produced greater significant ($p<0.05$) response on 14th day, increase in time spent in target quadrant 15th day than given alone. Combination of *C. pluricaulis* and ω-3 fatty acids treatment, therefore, showed the most significant ($p<0.05$) decrease in time latency and increase in the time spent in the target quadrant in the MWM test, suggesting its ameliorative potential against scopolamine induced cognitive dysfunction and these effects are comparable to standard drug donepezil [Table/Fig-5,6].

Scopolamine induced memory impairment manifested as increased AchE level in rat brain as compared to the control group. In this study, the scopolamine treated group has shown an increase in AchE activity but *C. pluricaulis* (100 mg/kg and 400 mg/kg), ω-3 fatty acids (500 mg/kg b.w.), and a combination of these two drugs reversed the effect of scopolamine resulting into the decreased level of AchE. A greater decrease in AchE at the higher dose (400 mg/kg) of *C. pluricaulis* than the lower dose (100 mg/kg) suggested its dose-dependent action. The higher dose of *C. pluricaulis* showed a significant ($p<0.05$) decrease in AchE level as compared to the scopolamine treatment group. Similarly, ω-3 fatty acids also exhibited a significant ($p<0.05$) decrease in AchE level in rat whole brain which was increased by scopolamine treatment. The results of the present study were in accordance with studies done previously [33,34]. Combination of *C. pluricaulis*– 400 mg/kg b.w. and ω-3 fatty acids exhibited greater significant ($p<0.05$) decrease in AchE level than treated alone. Combination treatment of *C. pluricaulis*- 400 mg/kg b.w. and ω-3 fatty acids showed a comparable effect compare to donepezil treatment [Table/Fig-7].

Possible explanations and the mechanism for the effect on learning and memory enhancement, authors got in present study with *C. pluricaulis* are:

1) The most significant mechanism is the increase in acetylcholine content in the brain especially the hippocampal region which is the neurochemical basis for their effect in the improvement of learning and memory [35] and this has been evaluated and proved in the present study by estimating the AchE level in the brain [Table/Fig-7];
2) Wide range of secondary metabolites in *C. pluricaulis*, including convolvine, triterpenoids, flavonol glycosides, anthocyanins, and steroids which may be responsible for learning and memory enhancing properties as well as in other pharmacological activities [18,26,36];
3) It also possesses significant antioxidant activity when tested in vitro [33] and this may be the other mechanism for a possibly beneficial effect on learning and memory by scavenging free radicals. In a study, immunohistochemical and histopathological findings also supported the protective effect of *C. pluricaulis* [21].

An explanation for the learning and memory enhancing effect of ω-3 fatty acids in present study may be due to, (as reported in various previous studies): 1) Enhancement of the spontaneous and evoked release of acetylcholine by the hippocampus [34]; 2) Changes in neuronal lipid composition could result in an increased spontaneous release of acetylcholine [34]; 3) Increase in the cortico-hippocampal DHA/arachidonic acid molar ratio; decrease in neuronal apoptotic products [37]; 4) The reduction of DHA decreases hippocampal phosphatidylserine and increases neuronalapoptosis [38]; 5) Omega-3 fatty acids supplementation promotes neurite extension, cell survival, and synaptic plasticity and also prevent dendritic atrophy [39].

In the present study, it may be due to the combined effect of the proposed above mechanisms of both. Combination of *C. pluricaulis*-whole plant powder, dose- 400 mg/kg b.w. p.o.) along with ω-3 fatty acid (500 mg/kg b.w.) has not been studied previously therefore with the above combination, this is the first study of its kind to evaluate its effect in learning and memory enhancement.

Limitation(s)

Details of the complete mechanism have not explored in this study. Therefore, further experiments are required to elucidate the exact mechanism of action. Also, more specific and longer duration animal and human studies are required to further substantiate the findings of the present study.

CONCLUSION(S)

C.pluricaulis has shown learning and memory enhancement effect. A comparatively higher dose (400 mg/kg b.w. p.o.) has been found to be more effective than a lower dose (100 mg/kg b.w. p.o.) in a dose-dependent manner. The ω-3 fatty acids (500 mg/kg b.w. p.o.) also has shown learning and memory enhancement effect which was comparable to the effect of *C. pluricaulis* at a dose of 400 mg/kg b.w. Combination treatment of both have shown a greater and significant effect on learning and memory than when both drugs were given alone and which was comparable to standard drug donepezil. Scopolamine induced memory impairment exhibited an increase in the level of AchE. *C. pluricaulis* and ω-3 fatty acids have reversed this increase in the level of AchE, which was again maximum with the combination treatment and was comparable to standard drug donepezil.

The results that were obtained in the present study, reveal the importance of the herbal drugs and nutrients in impaired cognition (learning and memory impairment) state and shows that *C. pluricaulis* alone in a higher dose and in combination with ω-3 fatty acids might have the potential usefulness in the management of learning and memory impairment as an alternative/add-on, preventive as well as therapeutic treatment. These drugs and their combination can be used for a longer duration, in any age group of the patient without causing any serious side effects. Further experiments of longer duration are required for substantiating the findings and exploring detailed mechanism.

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PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
2. Additional Professor, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
3. Additional Professor, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
4. Post Doc Fellow, Department of Biochemistry, Veer Bahadur Singh Purvanchal University, Jaunpur, Uttar Pradesh, India.
5. Professor, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
6. Professor, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rajendra Nath,
Department of Pharmacology, King George's Medical University,
Lucknow, Uttar Pradesh, India.
E-mail: rajendranath013@gmail.com

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