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Research Article
Evaluation of Nootropic Activity of *Spinacia oleracea* in Scopolamine Induced Cognitive Decline Mice

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Abstract

Background and Objective: Alzheimer disease (AD) is reflected by progressive cognitive debility usually start with impairment in the capability to form new memories, but unavoidably disturbing all knowledgeable tasks. The present study was aimed at investigating the neuroprotective effect aqueous extract of *Spinacia oleracea* (AESO) in scopolamine induced cognitive decline mice. **Materials and Methods:** Memory impairment was produced by administration of Scopolamine (1.4 mg kg^{-1} i.p.) in albino mice. Nootropic activity in mice with the treatment of AESO (200-400 mg kg^{-1}) and donepezil (5 mg kg^{-1}) were administered to different groups of mice. Effect of extract on learning and memory of mice was evaluated using elevated rectangular maze, pole climbing and morris water maze test and also estimated the brain acetylcholinesterase (AChE) concentration and the percentage of inhibition of AChE. **Results:** AESO showed significantly improved in learning and memory of mice, as indicated by the decline in transfer latency using rectangular maze test, decrease in escape latency during training, retrieval using morris water maze, pole climbing test and neuroprotective activity through reduced brain AChE concentration and increased the percentage of inhibition of AChE activity in rat brain. **Conclusion:** Thus, aqueous extract of *Spinacia oleracea* showed memory enhancing and neuroprotective activity in mice probably by inhibiting brain AChE activity.

Key words: Nootropic, acetylcholinesterase, donepezil, *Spinacia oleracea,* scopolamine, neuroprotective

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease of undetermined etiology, seen in the elderly although in frequently earlier 60 years excluding when its inheritance is autosomal leading[1,2]. Combined existence of amyloid beta (Aβ) and tau (τ) stands out as the hallmark of progressive AD and the basis of most disease-modifying therapy[3,4]. Initial stage of disease is characterized by the injury of current memory which is followed by damage of cognitive aptitudes, vocabulary and concepts[5]. Early diminishing of recent memory is due to participation of median temporal lobe and hippocampus which controls recent memory[6]. Then, participation of other parts of brain may manifest as sleep disturbances, problems in judgment, psychological changes, pyramidal and extrapyramidal motor signs[7]. According to Prince *et al.*[8] global prevalence of dementia rise from 30 million (2010) to 46.8 million and global expenditure on dementia rose from US$ 604 million (2010)-US$ 818 million (2015). In India, the prevalence of dementia was 33.6 in every 1,000 people of which 54% were cases of AD[8].

The *Spinacia oleracea* (spinach) belongs to family Chenopodiaceae is a leafy vegetable with broad, crisp, dark green leaves and is the greatest widespread of all greens. It is a fast growing, cool season annual crop and is cultivated for its green leaves. The leaves are alternate, succulent, fleshy, very smooth and dark green in colour. They are generally 5–8 cm in length and one to one and half cms in width. The bottom of the leaf is shiny, with thick veins running across[9].

Spinach-derived phytochemicals and bioactives are able to scavenge reactive oxygen species and prevent macromolecular oxidative damage, modulate expression and activity of genes involved in metabolism, proliferation, inflammation and antioxidant defense and also curb food intake by inducing secretion of satiety hormones[9]. The leaves of spinach are reported many activities like antidiabetic[10], antioxidant[11] spatial memory[12], atischizoprinic[13], anti-inflammatory[14], anticancer[15] and antibacterial[16].

Herbal medications are ahead extensive approval globally in more than 80% of the world population, due to their higher biosafety profile over the synthetic medications. The present study was aimed at investigating the neuroprotective effect aqueous extract of *Spinacia oleracea* (AESO) in scopolamine induced cognitive decline mice. In this research, the *S. oleracea* was evaluated as a nootropic herb that can serve as a promising agent in AD owing to its antioxidant safety profile.

MATERIALS AND METHODS

Research duration: The Research study was conducted in Department of Pharmacology, Vaagdevi College of Pharmacy, Warangal and India for the period of 6 months (July- December, 2018).

Chemicals: Scopolamine purchased from Cadila Healthcare Ltd., Donepezil gift sample from hetero drugs. All other chemicals and reagents are analytical grade.

Plant material: The plant material *Spinacia oleracea* leaves were collected from local areas of Hanamkonda, Warangal District, Telangana, India during the month of April, 2018.

Preparation of plant extract: Fresh leaves of *Spinacia oleracea* were collected, washed and air dried. Leaves were taken into mixer grinder. Juice was prepared by using distilled water. Than juice was filtered and evaporated. The obtained powder was in green colour.

Drugs: Scopolamine purchased from Cadila Healthcare Ltd., Donepezil gift sample from hetero drugs.

Animals: Thirty Albino mice (20-25 g) are used to study neuroprotective activity. The animals were procured from Mahaveer Enterprises, Hyderabad. They are housed into group of six mice per cage and maintained at 24±1°C with relative humidity 45-55% and 12:12 h dark/light cycle. The animals had free access to food (standard chew pellets) and water *ad libitum*. The Institutional Ethics Committee approved all the experimental procedures. The experimental protocol was approved by Institutional Animal Ethics Committee (IACE) and care of animals was taken as per guidelines of CPCSEA, department of animal welfare and government of India.

Experimental design: Selected animals were divided into 5 groups each group consist of 6 animals each. Freshly prepared test extract were given daily for 9 days. The training sessions for all the animals were given on day 1. Test extracts were given, after 1 h the Retention time (RT) was recorded. This is followed by successive 1, 3, 5, 7 and 9 days. Group 1 served as normal control and received orally 2% gum acacia. Group 2 served as disease control (Scopolamine 1.4 mg kg^{-1} i.p.) was given 2% gum acacia. Group 3 received standard memory enhancing drug donepezil 5 mg kg^{-1}. Group 4 fed

with 200 mg kg^{-1} of aqueous extract of *Spinacia oleracea* leaves and group 5 fed with 400 mg kg^{-1} of aqueous extract of *Spinacia oleracea* leaves for 9 days[17].

Rectangular maze test: The maze consists of completely closed rectangular box with an entry and reward chamber partitioned with wooden slats into blind passages leaving just twisting corridor leading from the entry to the reward chamber. On the first day all the mice were familiarized with rectangular maze for a period of 10 min. This was known as training session. On the 3rd day the mice was placed in the entry chamber and the timer was activated as soon as the mouse leaves the entry chamber. The time taken for the rats to reach the reward chamber was taken as the latency time. Four readings are taken and average of reading gives learning score. Lower scores indicate efficient learning and higher scored indicates poor learning in animals[18].

Morris water maze test: Method was carried out in a circular pool (90 cm in diameter and 50 cm in height) of water with a featureless inner surface[19]. The first day of the experiment was dedicated to swimming training for 60 sec in the absence of the platform. During the 4 consecutive days the rats were given the trail session with the platform in place. Once the rats located on the platform, it was permitted to remain on it for 10 sec, if the rats did not locate the platform within 120 sec, it was placed on the platform for 10 sec and then removed from the pool. One day after the final training trial sessions (on day 5). Rats were individually subjected to a probe trial session in which the platform was removed from the pool and rats were allowed to swim for 120 sec to search for it and the latency time was determined[20].

Pole climbing test in mice: Training and testing was conducted in a 25×25×40 cm chamber that was enclosed in a dimly lit, attenuated box, Scrambled shock was delivered to grid floor of the chamber. A 2.8 KHZ speaker and a 28 v light were situated on the top of the chamber. A wooden pole 2.5 cm in a diameter was suspended by a counter balance weight through a hole in the upper layer center of the chamber. The response was recorded when a mice jumps on the pole and activates micro switch, the activation of light and speaker together used as conditioned stimulus[18].

Estimation of cholinergic status in brain: After *in vivo* studies (On day 9), animals were sacrificed and the brain tissues were quickly removed, cleaned with ice-cold saline and stored at -20°C for estimation of cholinesterase. For preparation of homogenate, the fresh whole brain was weighed and transferred to homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% sodium chloride solution and phosphate buffer (0.1M, pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of cholinesterase levels. The cholinergic marker, cholinesterase was estimated in the whole brain according to Zhao *et al.*[21] method. The end point was formation of yellow color due to reaction of thiocholine form of acetylthiocholine iodide in presence of dithiobis nitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The sample was first treated with 5, 5′-dinitrobenzoic acid (DTNB) and the Optical density (OD) of yellow color compound formed during the reaction at 412 nm every min was measured[21].

Statistical analysis: All data were expressed as Mean±SD. The data were analyzed by using ANOVA, followed by Newman-keul's multiple comparison tests. The value $p<0.05$ were considered to be a significant.

RESULTS

Rectangular maze test: The transfer latency was measured for all the groups' day 1-9 when compared with disease control group. Figure 1 showed that there is significant ($p<0.001$) decreases the transfer latency time on day 5, 7 and 9 with group 3, 4 and 5 when compared with group 2.

Morris water maze test: Table 1 shows the decreased significantly ($p<0.001$) in transfer latency of group 3, 4 and 5 on day 5, 7 and 9 day but on day 3 also shows significant effect ($p<0.01$) when compared with group 2.

Pole climbing test: The plant extracts memory enhancing activity was evaluated by using pole climbing test in scopolamine induced amnesic model. The escape latency was measured for all the groups on day 1-9 showed in Table 2. The escape latency is more significantly ($p<0.001$) decreased in group 3, 4 and 5 on day 3, 5, 7 and 9 when compared with group 2.

Table 1: Effect of *Spinacia oleracea* on memory impairment in morris water maze test in scopolamine induced cognitive decline in mice

	Transfer latency (sec) (Mean±SD)				
Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
Group 1	48.37±3.22	47.22±2.76	45.98±1.32	47.16±2.73	46.70±1.93
Group 2	47.45±2.19	53.87±1.98	57.13±2.31	64.72±3.03	79.55±2.74
Group 3	50.92±2.97	48.69±1.32**	45.32±2.14***	42.93±3.82***	36.70±1.87***
Group 4	49.35±1.93	47.61±2.66**	46.97±1.03***	44.82±3.91***	42.93±3.12***
Group 5	51.68±3.02	49.73±2.91**	46.38±3.16***	42.27±2.06***	39.22±1.98***

Values are Mean±SD, n = 6, **p<0.01, ***p<0.001, compared to disease control group

Table 2: Effect of *Spinacia oleracea* on memory impairment in pole climbing test in scopolamine induced cognitive decline in mice

	Escape latency (sec) (Mean±SD)				
Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
Group 1	19.13±0.98	18.78±1.63	17.42±1.25	17.97±0.87	18.18±1.26
Group 2	18.29±1.73	22.51±1.97	24.66±0.93	27.10±1.02	35.88±1.32
Group 3	19.82±1.67	18.53±2.03***	16.89±0.95***	14.73±1.28***	13.29±2.57***
Group 4	18.52±2.18	18.18±1.06***	17.32±1.93***	16.93±2.08***	16.19±1.19***
Group 5	17.96±1.73	17.19±0.29***	16.62±1.33***	15.34±2.42***	14.47±2.10***

Values are Mean±SD, n = 6, ***p<0.001, compared to disease control group

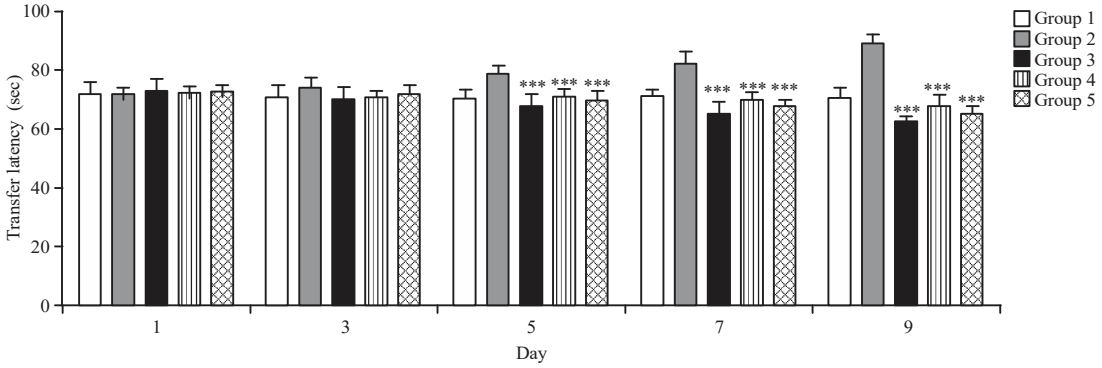

Fig. 1: Effect of *Spinacia oleracea* on memory impairment in rectangular maze test in scopolamine induced cognitive decline in mice

***p<0.001, compared to disease control group

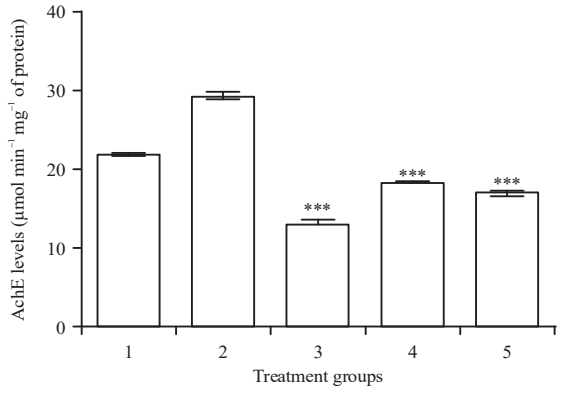

Fig. 2: Effect of *Spinacia oleracea* on AchE levels in scopolamine induced cognitive decline in mice

***p<0.001, compared to disease control group

AchE estimation: In group 2 significantly increased the brain AchE levels after treatment, group 3 (donepezil) and group 4 and 5 (*S. oleracea* leaves extracts) significantly (p<0.001) inhibited the brain AchE levels when compared with group 2 (Fig. 2).

DISCUSSION

In the current study, a sequence of investigates were intended in edict to explore the cognitive enhancement of the aqueous extract from *S. oleracea* leaf in a scopolamine induced a mice model of cognitive impairment *in vivo*.

Alzheimers disease (AD) is a neurodegenerative disease triggering memory loss and dementia, which typically affects

the aging people. It is characterized by aphasia, apraxia and amnesia with the loss of memory as the main symptom. AD is accompanying with a loss of memory, language, learning, attention and non cognitive symptoms such as depression and psychosis in individual's life[22,23].

Scopolamine is a muscarinic acetylcholine receptor antagonist identified to block signals essential memory[24]. Present results are support with previous data displaying that the mice with a model of scopolamine impaired memory significantly reduced their scores during training sessions within Y-maze and radial arm-maze tests[25].

Learning method and memory in rodents is mostly assessed by using rectangular model. It is reflected as the authenticated model for the assessment of memory. Transfer latency (TL) of this model is establishing to be useful parameter for the assessment of cognition.

Administration of the aqueous extract *S. oleracea* leaf at both doses enhanced the diminishing effect of scopolamine on memory formation, suggesting that the aqueous extract could act as an unspecific enhancer of the cholinergic activity.

S. oleracea significantly reduced the initial transfer latency (ITL) on 5th day of the treatment. Besides, *S. oleracea* also significantly reduced the retention transfer latency (RTL) on 5th day of the treatment after administration of scopolamine for the impairment of memory, indicating that *S. Oleracea* improved the learning task and retained the information compared with previous studies[26,27].

In Morris water maze, a decrease in escape latency (EL) during training and an increase in time spent in the target quadrant during retrieval indicated improvement of learning and memory respectively and vice versa.

The promising fundamental mechanism of the aqueous extract action could be the rise of the brain cholinergic receptor sensitivity or the reduction of the AChE activity. Sugisaki *et al.*[28] stated that hippocampal-dependent memory is reliant by the increasing of extracellular acetylcholine (ACh) level. Also, the cholinergic synaptic transmission could by decreased by an over expression of AChE activity persuaded lessening of ACh level[29]. AChE action assessed in the rat hippocampal homogenates was suggestively augmented by scopolamine as compared to control group. The aqueous extract administration much reduced the AChE activity in the scopolamine-treated mice, proposing that the aqueous extract may deliberate anti-amnesic effects. Likewise, robust inhibition of the brain AChE activity was showed by administration of different herbal extracts in the scopolamine treated mice[30]. Therefore, the current study suggests that the aqueous extract *S. oleracea* has dose dependent potential on cholinergic neuronal system and this resulted in elevating the brain acetylcholine level which resulted in improvement of memory function[26].

The aqueous extract of *S. oleracea* leaves administration for 9 days improved learning and memory of mice significantly in both the models employed. Oxygen free radicals are implicated in the process of age related decline in cognitive performance might be responsible for development of Alzheimer's disease in elderly persons. *S. oleracea* has been reported to possess antioxidant property. The neuroprotective effect of *S. oleracea* may be attributed to its antioxidant property by the virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function. From the behavioral test, that is rectangular maze and morris water mazetests, it is clearly seen that there was a general decrease in the transfer latency in *S. oleracea* equipotent to extracts of *C. rotundus* and *Z. officinale*, is a potential supplement to improve neurodegeneration and memory impairment[31].

In case of *in vitro* paradigms the *S. oleracea* inhibited the acetyl cholinesterase enzyme there by stimulating acetylcholine concentration in the brain homogenate. By the *in-vivo* and *in-vivo* studies it was demonstrated that the *S. oleracea* has memory improving effect against scopolamine induced amnesia and neuroprotective effect by inhibiting AchE enzyme.

A limitation of present study is that the efficacy of aqueous extract of *S. oleracea* not determined. Based on previous studies, it has been demonstrated that *Ocimum sanctum*[32] and *Withania somnifera*[33] exerts protective effects against oxidative stress-related neurodegeneration. Hence, aqueous extract of *S. oleracea* produced significant memory enhancing activity observed 5th day of treatment but equipotent, that they might be partly responsible for the neuroprotective effect of in this study. However, further investigations are necessary to provide better understanding concerning the possible active ingredients.

CONCLUSION

In the present study it was demonstrated that aqueous extract of *S. oleracea* had a potential therapeutic effect in improving the memory in mice by a decrease in transfer latency time in case of morris water maze, rectangular maze and pole climbing and neuroprotective action through inhibition of acetylcholinesterase enzyme.

SIGNIFICANCE STATEMENT

This study finds out the promising memory enhancing effect of *S. oleracea* that can be helpful for memory impairment rats. This study will assistance the researcher to explore the plant extracts shows significant effect with fewer side effects.

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