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## *Allium Sativum* Improves Short-Term Memory In Mice

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### ABSTRACT

Alzheimer's disease is a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language. Nootropic agents like, piracetam, and cholinesterase inhibitors like, Donepezil are commonly used for improving memory, mood and behavior but their adverse side effects have made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. In the present study we evaluated the effectiveness of aqueous extract of *Allium sativum* Linn. On aging, scopolamine and diazepam induced amnesia in mice. Elevated plus maze was employed to assess short-term memory. In order to delineate the possible mechanism through which *A.sativum* elicits the anti-amnesic effects, the whole brain acetyl cholinesterase activity (AChE) was also assessed. Three doses (7.5, 10, and 15mg/kg, p.o.) of aqueous extract of *A.sativum* were administered for 6 successive days to both young and aged mice. The 7.5, 10, and 15mg/kg, p.o aqueous extracts of *A.sativum* elicited profound neuroprotective effect on scopolamine (0.4mg/kg i.p.) and diazepam (1mg/kg, i.p.) treated and older mice compared to control and piracetam (200mg/kg, i.p.) treated mice. Aqueous extract of *A.sativum* significantly inhibited acetyl cholinesterase (AChE) activity in the whole brain homogenate of mice indicating its potential in the attenuation of learning and memory deficits especially in the aged mice. Hence *A.sativum* can be highly useful in the management of cognitive disorders.

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### KEYWORDS

Amnesia;  
Acetyl cholinesterase;  
*Allium sativum*;  
Piracetam.

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### INTRODUCTION

Alzheimer's disease is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgments, communicate and carry out daily activities and ultimately leads to death<sup>[1]</sup>. Alzheimer's is the most common form of dementia, a group of conditions that all gradually destroy brain cells and lead to progressive decline in mental function. The prevalence of mental and cognitive disorders, referred to as cognitive deficits which impinge on daily life and are thus of consequence to both those affected and society doubles every five years between the ages of 60 and 95<sup>[2,3]</sup>. Epidemiological studies have revealed that between two-thirds and Alzheimer's disease entirely or significantly causes three quarters of all cognitive deficits. Modulation of brain aging with complex extracts containing active phytochemicals has been useful in the aging of wild type rodents with encouraging results<sup>[4]</sup>. Ayurvedic medicinal plants had successfully attenuated memory dysfunction induced by scopolamine, ethanol and diazepam<sup>[5]</sup>.

Recent advancements in the treatment of Alzheimer's disease and controlled studies have demonstrated that cholinesterase inhibitors delay cognitive decline by 6-10 months<sup>[6]</sup>. AChE-is indicated for probable Alzheimer's disease(mild to moderate stage). Three AChE-inhibitor agents are available in Switzerland: tacrine, Donepezil and rivastigmine. Tacrine is a first-generation AChE-inhibitor. Donepezil and rivastigmine are second-generation AChE-inhibitors, which are selectively centrally active. Despite their relatively selective and central action, second generation AChE- inhibitors may still cause dose-dependent cholinergic adverse effects<sup>[7,8]</sup>. Thus the treatment of Alzheimer's disease has become a demanding interdisciplinary undertaking. The crucial breakthrough in the treatment of dementia, namely the definitive halt of the progressive degenerative process, has yet to be achieved.

*A. sativum* Linn. (Liliaceae) is commonly known as garlic. It is also known by several names like lahsuan, bellulli in India. The root is made up of group of small bulbs surrounded by a dry, whitish covering that holds them together. Bulb is the part

used in herbal medicine. It contains many active constituents, most importantly a sulphur-containing compound called allicin, amino acids like arginine, methionine<sup>[9]</sup>. Lysine and threonine, tannins, calcium. It also consists of selenium<sup>[10]</sup>. Germanium, essential oil, ajoene, glucokinins, B group vitamins, vitamin C, flavanoids<sup>[11]</sup> and lectins<sup>[12]</sup>. The bulb is pungent, tonic, aphrodisiac, digestive, antihelminthic; useful in the disease of eyes and heart. Garlic has been reported to lower the blood glucose, cholesterol and triglycerides levels; therefore it plays an important role in the prevention of atherosclerosis. It has anticancer<sup>[13,14]</sup>, antioxidant<sup>[15]</sup>, antifungal activity<sup>[16]</sup>, antidiabetic<sup>[17]</sup> and antibacterial activity<sup>[18]</sup>. Garlic juice has been used for blood pressure<sup>[19]</sup>. However, there are no empirical data or scientific reports to support the effect of this plant on neurodegenerative disorders.

In the present study *A. sativum* was investigated for its potential as a nootropic agent. Elevated plus maze, a neutral exteroceptive behavioural model was employed to access short-term memory and to delineate the mechanism by which *A. sativum* exerts nootropic action; its effects on brain cholinesterase levels were also determined.

### MATERIALS AND METHODS

#### Preparation of garlic extract

Fresh garlic (*A. sativum*) bulbs were purchased from the local market. It was identified and authenticated by the first author at Department of Pharmacognosy, SET's college of Pharmacy, Dharwad, Karnataka, India. The garlic bulbs were peeled off. An aqueous extract was prepared by homogenizing the bulbs in pestle and mortar using normal saline (0.9% w/w). It was then filtered with the help of muslin cloth and Whatman filter paper and stored in refrigerator at 4°C (yield: 34.8%).

#### Drugs and chemicals

Scopolamine (Sigma Aldrich, USA), Diazepam (Valium<sup>®</sup>, Ranbaxy laboratories Ltd, Mumbai, India), Piracetam (Nootropil<sup>®</sup> UCB India pvt. Ltd., Vapi, India) and Phenytoin (Zydus Neurosciences, Ahmedabad, India). Scopolamine hydrobromide and

Diazepam injection were dissolved separately in normal saline. The volume of i.p.injection was 1ml/100g of mouse.

### Animals

Young(3-4 months old) mice weighing around 20g and older(12-15 months old) mice weighing around 35g were used in the present study. Animals were procured from disease free animal house, Venkateshwara Enterprises, Bangalore. They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and maintained under 12:12h light and dark cycles. All experiments were carried out during daytime from 0900 to 1900 hours. The Institutional Animals Ethics Committee(AEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, and Govt. of India.

### Acute toxicity studies

Garlic extract at different dose(5-1000mg/kg) was administered to young and older mice. During the first four hours after the drug administration the animals were kept for observation of gross behavioral changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, diarrhea and mortality were observed. GE at doses more than 500mg/kg produced profuse watery stools. The doses selected were 7.5mg/kg, 10mg/kg and 15-mg/kg b.w/day/mouse.

## MEMORY MODELS

### Elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model(where in stimulus existed outside the body) to evaluate learning and memory in mice<sup>[20, 4]</sup>. The apparatus consists of two open arms (16cm×5cm) and two covered arms(16cm×5cm×12 cm). The arms extended from a central platform (5cm×5cm), and maze is elevated to a height of 25cm from the floor. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered

arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec, it is gently pushed into one of the two covered arms and the TL was assigned as 90 seconds. The mouse was allowed to explore the maze for 10 seconds and then returned to its home cage. Memory retention was examined on the second day, 24 hours after the first day's trial.

### Locomotor function

Locomotor activity of control and drug treated animals with the help of photoactometer (INCO, Ambala, India).

Estimation of brain Acetyl Cholinesterase (AChE) activity: Mice of either sex weighing around 25g were used. Group I(n=5), served as control and was treated with saline. Group II(n=5), was treated with phenytoin(1mg/kg, i.p.), Group III with piracetam (200mg/kg, i.p.), group IV, V and VI(n=5) were treated with garlic extract (7.5mg/kg, 10mg/kg and 15mg/kg, p.o.) respectively for 6 days. On the 7<sup>th</sup> day animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured. This was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate 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'-dithionitrobenzoic acid(DTNB) and the optical density(OD) of the yellow color compound formed during the reaction at 412nm every minute for a period of three minutes was measured<sup>[21]</sup>. Protein estimation was done using Folin's method. AChE activity was calculated using following formula:

$$R = \frac{\delta O.D \times \text{Volume of Assay}(3\text{ml})}{E \times \text{mg of protein}}$$

Where R=Rate of enzyme activity in 'n' mole of Acetylcholine iodide hydrolyzed/minute/mg protein,  $\delta O.D$ =change in absorbance/minute, E=Extinction coefficient=13600/M/cm.

### Statistical analysis

All the results were expressed as mean( $\pm$ SEM). The data from elevated plus maze and Actophotometer were analyzed using ANOVA fol-

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lowed by Student's (Unpaired) 't' test. Kruskal Wallis one-way ANOVA followed by SPSS software was used for the analysis of non-normally distributed data of whole brain AChE activity.  $P < 0.10$  was considered significant.

### RESULTS

#### Effect on locomotor activity

Garlic extract (7.5, 10 and 15 mg/kg, p.o.) did not exhibit any significant change in the locomotor functioning of the animals (Score:  $218.2 \pm 11$ ,  $222 \pm 6$  and  $211 \pm 15$ ) when tested on actophotometer as compared to control group (Score:  $216.3 \pm 13$ ).

#### Effect on transfer latency using elevated plus maze

Aged mice showed higher transfer latency values on day 1 and day 2 (after 24 hours) as compared to young mice, indicating impairment in learning and memory (aging induced amnesia). Pretreatment for 5 days with piracetam (200 mg/kg, i.p.) significantly decreased ( $P < 0.001$ ) transfer latency on 6<sup>th</sup> day and on 7<sup>th</sup> day as compared to control group indicating improvement in both learning and memory. Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg i.p.) significantly ( $P < 0.001$ ) increased transfer latency in young mice on day 6<sup>th</sup> and day 7<sup>th</sup> indicating profound loss of memory (amnesia). Garlic extract (7.5 mg/kg and 10 mg/kg i.p.) decreased transfer latency significantly ( $P < 0.001$ ) when compared to control group of young mice. However the higher dose of garlic extract (15 mg/kg, p.o.) profoundly decreased transfer latency compared to control, scopolamine, diazepam and piracetam treated groups indicating potential improvement in learning and memory.

Higher dose of GE (15 mg/Kg, p.o.) significantly improved learning and memory in aged mice followed by GE (10 and 7.5 mg/kg, p.o.) compared to control group of aged mice.

#### Effect on whole brain acetylcholinesterase (AChE) activity

Phenytoin (12 mg/kg, p.o.) significantly elevated the whole brain AChE activity compared to control and piracetam (200 mg/Kg, p.o.) treated groups. High-

est dose of garlic (15 mg/kg, p.o.) significantly reduced AChE activity followed by garlic (7.5 and 10 mg/Kg, p.o.).

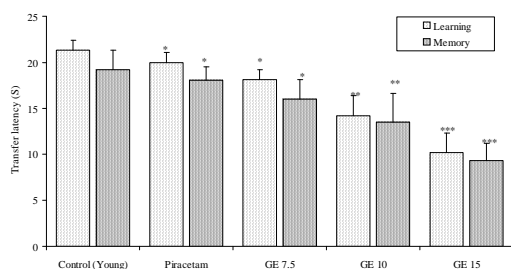
### DISCUSSION

Alzheimer disease (AD) is the fourth leading cause of death in adults. The incidence of the disease rises steeply with age. AD is twice more common in women than in men. AD affects the parts of the brain that control memory, thought and language, resulting in progressive intellectual deterioration<sup>[22]</sup>. Some of the most frequently observed symptoms of the disease include a progressive inability to remember facts and events and later, to recognize friends and family<sup>[23]</sup>. Oxidative damages, impaired neurotransmission and degeneration of neurons lead to irreversible decline in cognitive abilities, treatment with nootropics such as piracetam, pramiracetam, aniracetam and cholinesterase inhibitors like Donepezil® and tacrine have not been successful for long term therapy due to their adverse effects<sup>[24, 25]</sup>.

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. The Research and Development thrust in the pharmaceutical sector is focused on development of new drugs, innovative/indigenous processes for known drugs and development of plant-based drugs through investigation of leads from the traditional systems of medicine.

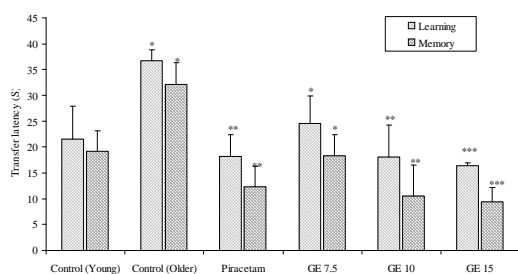
AD is a progressive, degenerative disease characterized by memory loss, language deterioration, poor judgment, impaired visuospatial skills etc. Dysfunction of cholinergic neurotransmission in the brain contributes to the salient cognitive decline in AD. Loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine. One of the most accepted strategies in AD treatment is the use of cholinesterase inhibitors. Their clinical efficacy is thought to result from prolonging the half-life of acetylcholine through inhibition of AChE<sup>[26]</sup>.

In the present study *A. sativum* commonly known as garlic was screened for its potential as a nootropic agent. Elevated plus maze, a neutral exteroceptive behavioral model was employed to assess short-term



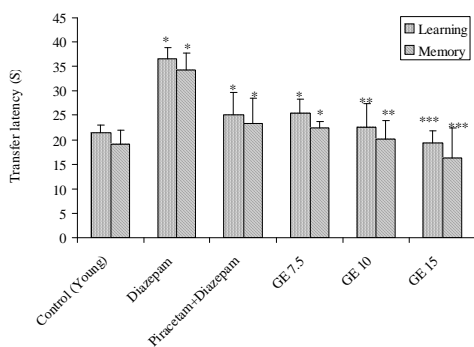
\*denotes P<0.05 as compared to control group of young mice.  
 \*\*denotes P<0.001 as compared to control group of young mice.  
 \*\*\*denotes P<0.001 as compared to control group of young mice (One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Figure 1: Effect of *A.sativum* on transfer latencies of young mice



Values are in mean±SEM. (n=6)  
 \* denotes P<0.05 as compared to control group of young mice.  
 \*\*denotes P<0.01 as compared to control group of older mice.  
 \*\*\* denotes P<0.001 as compared to control group of older mice (One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

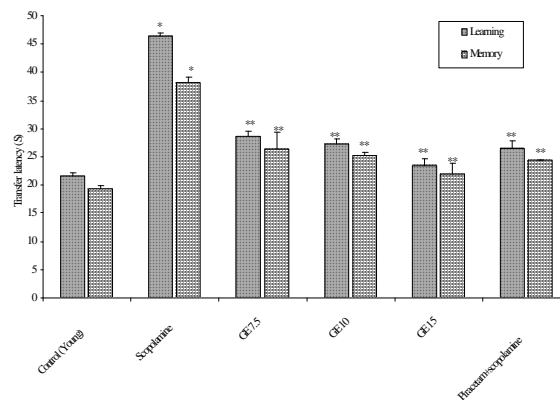
Figure 2: Effect of *A.sativum* on transfer latencies of aged mice



Values are in mean ± SEM. (n=6)  
 \*denotes P<0.05 as compared to control group of young mice.  
 \*\*denotes P<0.01 as compared to control group of diazepam mice.  
 \*\*\* denotes P<0.001 as compared to control group of diazepam mice (One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

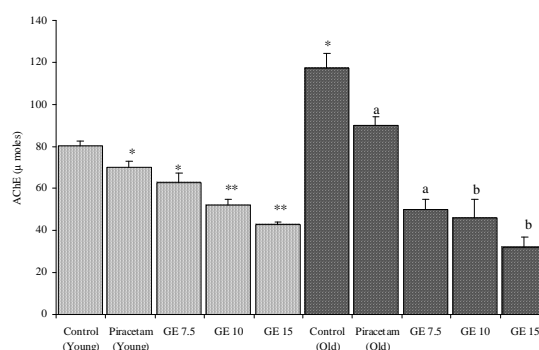
Figure 3: Effect of *A.sativum* on transfer latencies of Diazepam induced amnesic mice

memory. Both *A.sativum* aqueous extract and piracetam meet major criteria for nootropic activity, namely improvements of memory in absence of cog-



Values are in mean±SEM. (n=6)  
 \* denotes P<0.05 as compared to control group of young mice.  
 \*\* denotes P<0.001 as compared to scopolamine treated mice. (One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Figure 4 : Effect of *A.sativum* on transfer latencies of Scopolamine induced amnesic mice



Values are in mean±SEM. (n=6)  
 \* denotes P<0.01 as compared to control group of young mice.  
 \*\* denotes P<0.001 as compared to control group of young mice.  
 a denotes P<0.01 as compared to control group of older mice.  
 b denotes P<0.001 as compared to control group of older mice. (One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Figure 5: Effect of *A.sativum* on whole brain AChE activity

nitive deficits<sup>[27]</sup>. Pretreatment with aqueous extract of *A.sativum*(10mg/kg, p.o.) for 6 days significantly reduced transfer latency time of young mice and more profoundly in aged mice compared to control and piracetam treated standard groups of mice. The aqueous extract of *A.sativum* (7.5mg/kg, 10 mg/kg, 15mg/kg) elicited profound neuroprotective effect in scopolamine, diazepam treated and older mice compared to control groups and piracetam treated mice. Aqueous extracts of *A.sativum* significantly inhibited acetyl choline esterase(AChE) activity in



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the whole brain homogenate of mice indicating its potential in the attenuation of learning and memory deficits especially in the aged mice. *A.sativum* significantly reversed amnesia induced by scopolamine and diazepam(1mg/kg; i.p.), which probably indicated that *A.sativum*, potentiated memory and learning especially in scopolamine and diazepam induced amnesic mice.

Considering the lack and the need of the drugs with proven effectiveness in improving learning and memory the specific memory improving and anticholine esterase effect of *A.sativum* can be of enormous interest for further investigations.

### CONCLUSION

Since *A.sativum* extract(7.5, 10, 15mg/Kg) elicited profound neuroprotective effect in scopolamine and diazepam treated and aged mice compared to control groups and piracetam treated groups, it can be used for the management of AD and other neurodegenerative disorders. However further investigations using more experimental paradigms are warranted for further confirmation of the treatment of various cognitive disorders.

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