



ANTI-ALZHEIMER POTENTIAL OF ORANGE JUICE

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ABSTRACT

Orange (*Citrus sinensis*) is well known for its nutritional and medicinal properties throughout the world. This study was undertaken to test the effect of orange juice on the memory of mice. A total of 228 mice divided in 38 groups were employed in this study. Orange juice (2.5%, 5%, 7.5%, 10% v/v) was administered orally to mice with the help of an oral feeding needle. Morris water maze, elevated plus maze and object recognition task served as exteroceptive behavioral models, whereas scopolamine-induced amnesia and alprazolam-induced amnesia served as interoceptive memory models. Brain AchE activity was measured using Ellman's method. Orange juice significantly ($p < 0.01$) reduced transfer latency of mice in Elevated plus maze model. In another experimental model, orange juice significantly ($p < 0.01$) increased discrimination index in object recognition task, revealing its memory enhancing potential. Furthermore, orange juice in different concentrations enhanced the time spent by mice in target quadrant, when tested using Morris water maze. In the present study, Brain AChE activity was significantly reduced by orange juice, thereby suggesting pro-cholinergic mechanism for orange juice. These findings, when taken together indicate that orange juice possesses promising memory enhancing potential.

Key words: *Citrus sinensis*, Memory, Morris water maze

INTRODUCTION

Orange (*Citrus sinensis*) is well known for its nutritional and medicinal properties throughout the world. No wonder that oranges are one of the most popular fruits in the world. From times immemorial, whole orange plant including ripe and unripe fruits, juice, orange peels, leaves and flowers are used as a traditional medicine. The major medicinal properties of orange fruit include anti-bacterial, anti-fungal, anti-diabetic, cardio-protective, anti-cancer, anti-arthritis, anti-inflammatory, anti-oxidant, anti-asthmatic and anti-hypertensive activity. Learning and memory are two fundamental cognitive functions that confer us the ability to accumulate knowledge from our experiences¹. Learning refers to the information that living beings acquire about their surroundings². Learning is the acquisition of any new information and skills about the events occurring in surroundings. Subsequent retrieval of this information is referred to as memory³. Thus memory is the ability of an individual to record the information and recall it whenever needed. Central cholinergic pathways play a crucial role in learning and memory processes and the degree of cholinergic neurodegeneration correlates positively with severity of memory impairment^{4, 5}. Benzodiazepines (BZ) such as diazepam and alprazolam have been reported to induce memory impairment or anterograde amnesia in rodents and human beings⁶. Dementia is a syndrome of failing memory and other intellectual functions with little or no disturbance in consciousness⁷. It is a brain disorder that seriously affects a person's ability to carry out daily activities. Alzheimer's disease (AD) is the most common form of senile dementia⁸. AD is a genetically heterogeneous, progressive and neurodegenerative disorder, which is associated with aphasia, apraxia and agnosia, with loss of memory being the cardinal symptom⁹. **Objective:** In the light of above facts, the present study was undertaken to explore the effect of orange (*Citrus sinensis*) juice on memory using various exteroceptive and interoceptive behavioral models.

MATERIALS AND METHODS

Plant material

The fresh Orange fruits were purchased from local market of Hisar and got authenticated from Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (Ref. NISCAIR/RHMD/Consult/-2011-12/1895/195). Orange juice was administered in different concentrations (2.5%, 5%, 7.5% and 10% v/v p.o.) daily for a duration of 10 days to mice with the help of an oral feeding needle.

Animals

Adult (6 months old) mice, of either sex, weighing around 20-25g were procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary Sciences, Hisar. A total of 228 Swiss mice divided into 38 groups were employed in the present study. Each group comprised of a minimum of 6 animals. The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Normal saline (vehicle, p.o), Piracetam (400mg/kg), Donepezil (1mg/kg) and *Citrus sinensis* (2.5%, 5%, 7.5% and 10% v/v p.o.) were administered for 10 successive days to mice. Scopolamine (0.4mg/kg) and Alprazolam (0.5mg/kg) were administered on 9th day. Biochemical studies were carried on 10th day after drugs/vehicle/CS administration. Effect on locomotor activity of mice was studied using photoactometer.

Experimental Design

Exteroceptive models : Elevated plus maze, Object recognition task (ORT) and Morris water maze (MWM) served as the exteroceptive behavioral models to test memory in mice. The procedure, technique and end point for testing memory was followed as described in earlier studies^{10, 11, 12}.

Interoceptive models : Scopolamine induced amnesia and Alprazolam induced amnesia served as interoceptive behavioral models^{13, 14} in the present study.

Biochemical Estimation

Brain acetylcholinesterase enzyme activity was estimated using the method of Ellman et al¹⁵.

Statistical Analysis

All the results were expressed as mean $\pm$ standard error (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. *p*-Values < 0.05 were considered as statistically significant.

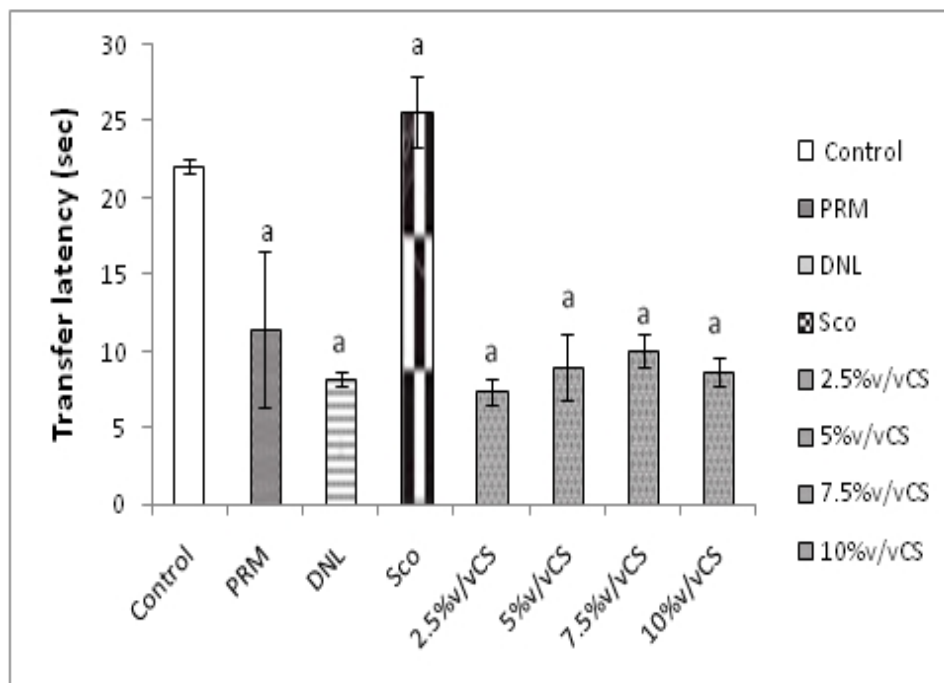


Fig. 1: Effect of *Citrus sinensis* on Transfer latency of mice using Elevated plus maze.

Donepezil (1 mg/kg i.p.) and piracetam (400mg/kg i.p.) were used as standard drugs. Values are expressed as mean $\pm$ SEM (n = 6). a denotes *p*<0.01 as compared to group. One way ANOVA followed by Dunnett's *t*-test.

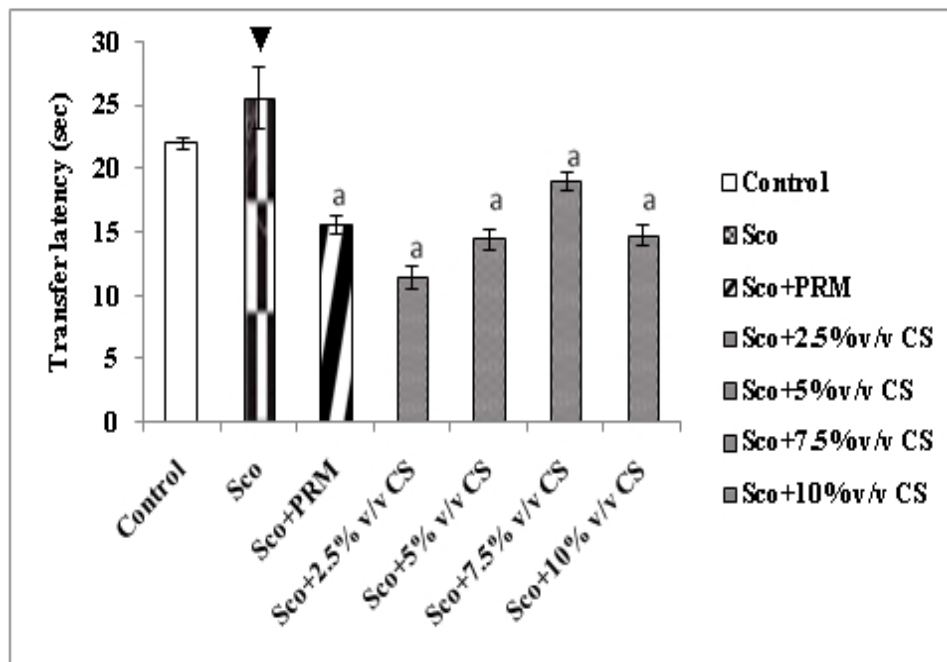


Fig. 2: Reversal of Scopolamine induced Amnesia by *Citrus sinensis*

Piracetam (400 mg/kg, i.p.) was used as a standard drug. Values are expressed in mean $\pm$ S.E.M (n=6). a denotes *p*<0.01 as compared to Scopolamine group, ▼ denotes *p*<0.01 as compared to Control group. One way ANOVA followed by Dunnett's *t*-test.

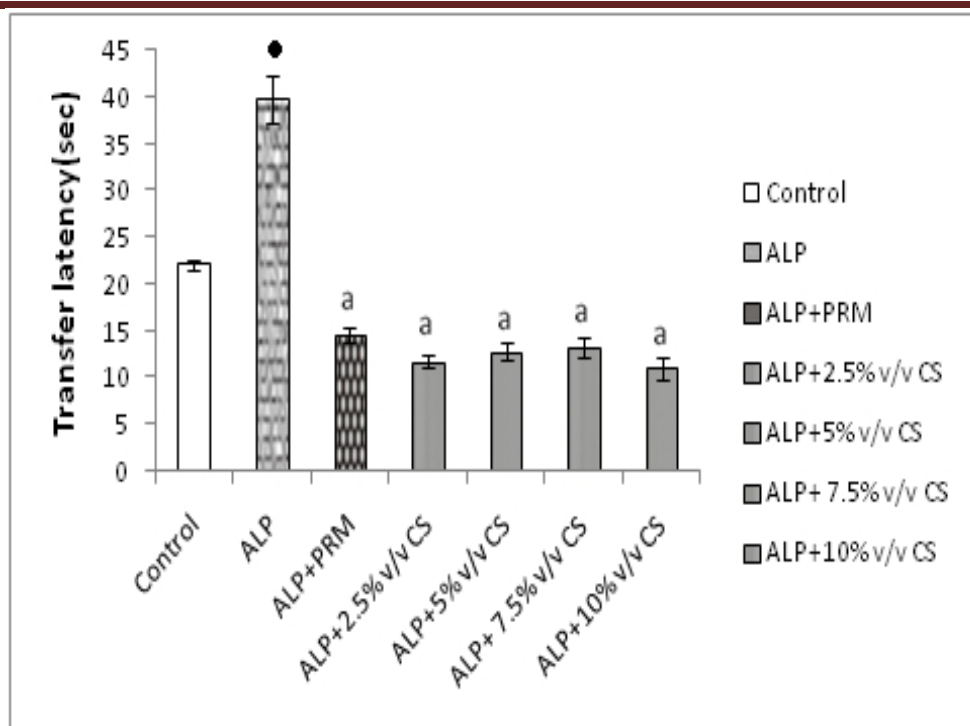


Fig. 3: Reversal of Alprazolam (0.5 mg/kg) induced Amnesia by *Citrus sinensis*

Values are expressed in mean $\pm$ S.E.M (n=6). a denotes $p < 0.01$ as compared to Alprazolam group, • denotes $p < 0.01$ as compared to Control group. One way ANOVA followed by Dunnett's t-test.

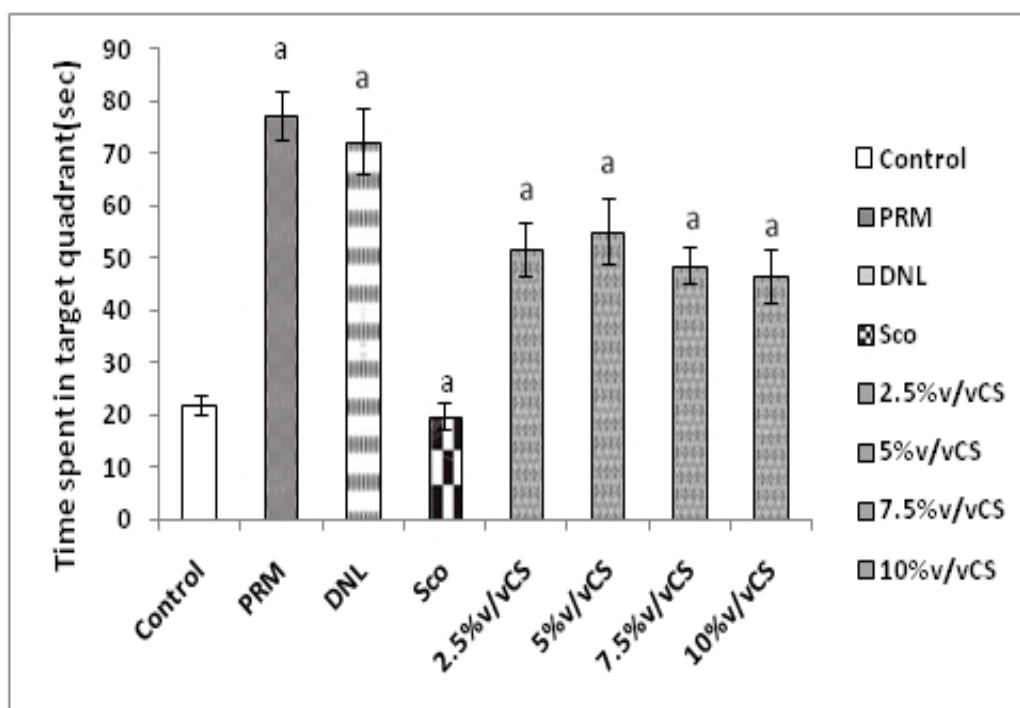


Fig. 4: Effect of *Citrus sinensis* on Time spent in target quadrant (TSTQ) by mice using Morris water maze.

Donepezil (1mg/kg i.p.) and piracetam (400mg/kg i.p.) were used as standard drugs. Values are expressed as mean $\pm$ SEM (n = 6). a denotes $p < 0.01$ as compared to group. One way ANOVA followed by Dunnett's t-test.

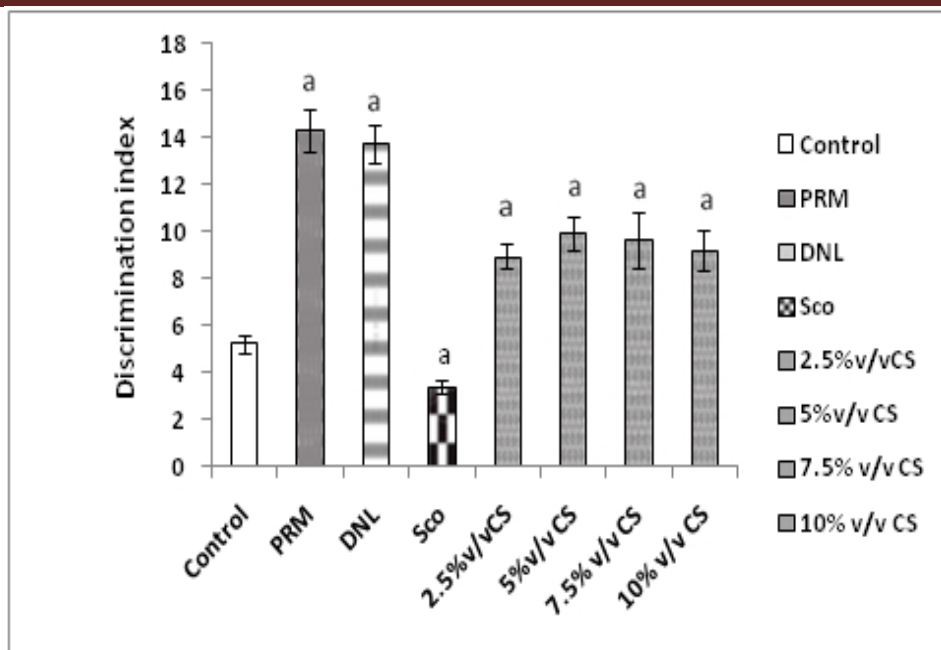


Fig 5: Effect of *Citrus sinensis* on Discrimination index of mice

Donepezil (1 mg/kg i.p.) and piracetam (400mg/kg i.p.) were used as standard drugs. Values are expressed as mean ± SEM (n = 6). a denotes p<0.01 as compared to group. One way ANOVA followed by Dunnett's t-test.

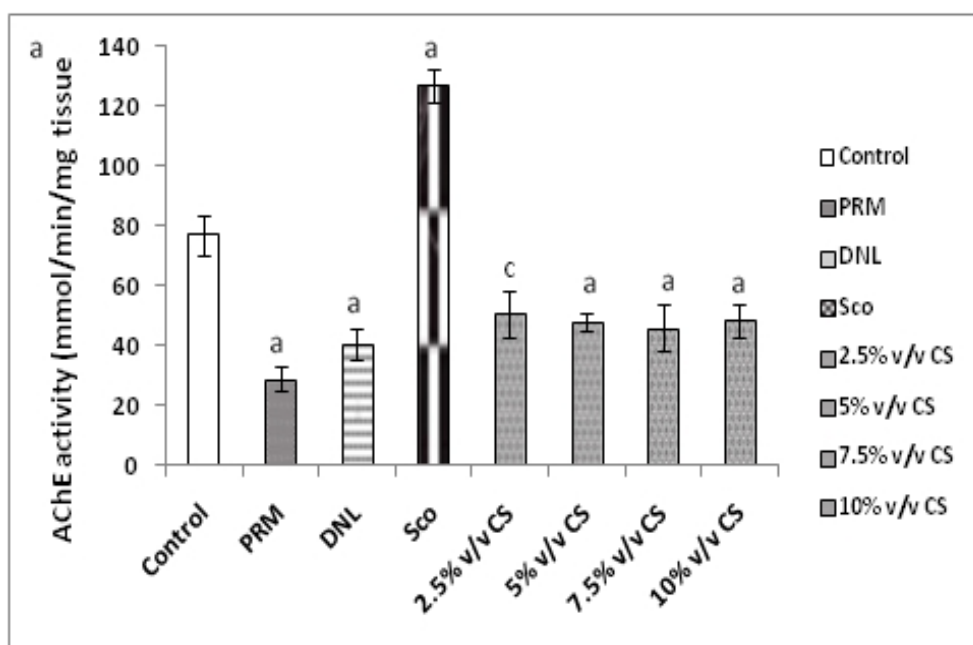


Fig. 6: Inhibition of Brain Acetylcholinesterase activity by *Citrus sinensis* juice

Piracetam (400 mg/kg, i.p.) and Donepezil (1mg/kg) were used as standard drugs. Values are expressed in mean ± S.E.M (n=6). c denotes p<0.05 as compared to control group, a p<0.01 as compared to control group. One way ANOVA followed by Dunnett's t-test.

RESULTS

Effect of *citrus sinensis* juice on transfer latency (TL) of mice

TL is defined as the time taken by mice to move into one of the covered arms with all its four legs. *Citrus sinensis* juice (CS) reduced the Transfer Latency (TL) of mice remarkably (p<0.01) when administered in different concentrations (2.5%, 5%, 7.5%, 10% v/v p.o.) for 10 days to mice. Reduced TL of 10th day indicated improved memory of mice. Animals treated with Piracetam (nootropic agent) and Donepezil (Acetylcholinesterase inhibitor) too showed remarkable improvement (p<0.01) in memory (Fig. 1). Memory deficits induced by Scopolamine (Sco) and

Alprazolam (ALP) were reversed by orange juice administered chronically for 10 days. (Fig. 2, 3)

Influence of orange juice on time spent in target quadrant (TSTQ)

Enhancement of time spent in target quadrant in Morris water maze model indicated improvement of memory and *vice versa*. CS at different concentrations (2.5%, 5%, 7.5%, and 10% v/v p.o.) enhanced the time spent in TSTQ significantly (p<0.01), when administered for 10 successive days to mice. The effect of CS was found to be comparable to that of Piracetam (nootropic agent) and Donepezil (Acetylcholinesterase inhibitor) (Fig. 4).

Effect of *citrus sinensis* on discrimination index using Object recognition task

Discrimination index (DI) is the ratio of exploration time of novel object to the familiar object. CS at different concentration (2.5%, 5%, 7.5% and 10% v/v p.o.) increased the DI considerably ($p < 0.01$), when compared to control group animals (Fig. 5).

Effect of *citrus sinensis* juice on locomotor activity

No significant difference was observed in the locomotion scores of the control group and treated group

Inhibition of brain acetyl cholinesterase activity by orange juice.

Acetylcholine is considered to be the most important neurotransmitter involved in the regulation of cognitive functions. AChE enzyme controls the concentrations of acetylcholine in brain by degrading acetylcholine. CS juice, when administered for 10 days significantly ($p < 0.01$) reduced brain AChE (mmol/min/mg tissue) activity (Fig. 6).

DISCUSSION

Alzheimer's disease is a crippling neurodegenerative disorder characterized by progressive loss of memory, followed by complete dementia. Central cholinergic system plays a crucial role in the maintenance of memory function. Cholinomimetic drugs have been shown to enhance memory, whereas centrally acting cholinergic antagonists (Scopolamine) impair memory. Therefore, scopolamine is widely utilized as an experimental tool to study the anti-amnesic potential of new medicines. In the present study, scopolamine, an anti-cholinergic agent and alprazolam (Sedative), a benzodiazepine agonist produced memory deficits in mice. Interestingly, *Citrus sinensis*, when administered to mice for 10 days reversed these memory deficits as reflected by diminished TL and enhanced TSTQ values. These findings suggest a neuro-protective role for the nutrients present in *Citrus sinensis*. Acetylcholine is considered to be the most important neurotransmitter responsible for creating and maintaining long term memory. Selective loss of cholinergic neurons and increased acetyl cholinesterase (enzyme responsible for degradation of Ach) activity was also reported to be a characteristic feature of senile dementia of the Alzheimer's type. In the present study, orange juice administered for 10 days showed elevation of acetylcholine levels by significant reduction of acetyl cholinesterase activity in the brains of treated young mice. Oxygen free radicals and other by products of oxidative metabolism have shown neuro-toxic effects. Anti-oxidant rich diets improved cerebellar physiology and learning ability of aged mice. Orange juice contains free radical scavenging compounds like vitamin C, B1, B2, which might be contributing favorably in improving the brain function. It has been observed that elderly patients suffering from

Alzheimer's disease showed reduction in symptoms upon chronic use of anti-inflammatory drugs. Epidemiological studies have almost confirmed that non-steroidal anti-inflammatory drugs reduce the incidence of AD. Orange fruits contain polymethoxyflavones (nobiletin) as anti-inflammatory constituents, which might be preventing the development of inflammatory lesions in brain. Thus, improvement in memory shown by orange juice in the present study may be due to i) inhibition of AChE activity, ii) free radical scavenging activity and / or anti-inflammatory property of the orange juice.

CONCLUSION

In the present study, orange juice when administered orally to mice for 10 consecutive days showed remarkable improvement in memory scores in various experimental models. This memory enhancing effect shown by orange juice may be attributed to i) inhibition of AChE activity, ii) free radical scavenging activity and / or anti-inflammatory property of the orange juice. These findings highlight the anti- Alzheimer potential of orange juice.

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