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Nootropic activity of *Punica granatum* fruit extract on scopolamine-induced cognitive impairment

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Abstract

Dementia is a prevalent cognitive condition associated with aging and serves as a distinctive indication of several neurodegenerative disorders, such as Alzheimer's disease. The impairment observed in the aging process may be attributed to brain cell's increased oxidative stress vulnerability. Because the synthetic drugs used to treat these challenges have detrimental impacts on health, natural treatments are a good, focused way to treat these issues. As an alternative, fruits and other biochemicals—a significant part of the diet—can be used for therapeutic purposes. *Punic granatum* (Pomegranate) is a fruit rich in antioxidants.

The purpose of this study was to investigate the impact of fruit extract from *Punica granatum* on learning and memory using two models, namely, elevated plus maze and shuttle box in scopolamine-induced cognitive impairment. *Punica granatum* extract was administered in 100mg/kg and 200mg/kg to Wistar rats for 6 weeks. The extract produced significant improvement in learning and memory in rats. Further, they also reversed scopolamine-induced amnesia. Significantly less acetylcholinesterase activity was seen in the brain, which may have increased cholinergic transmission and improved animal memory. These results demonstrate that Punica *granatum* can improve cognitive impairment, cholinergic dysfunction, and oxidative stress damage and may be promising in the pharmacotherapy as well as prophylactics of Alzheimer's disease.

Keywords: *Punica granatum*, learning, memory, cognitive function

1. Introduction

Memory is the capacity to store sensory information, experiences, and knowledge, store it for a short or long period of time, and retrieve it later when needed. Learning involves acquiring information that alters behavior and memory. The outcome of neuronal processes, like long-term potentiation, causes persistent behavioral changes. Slow learning, poor memory, slow retention, and recall speed are prevalent in today's demanding and cutthroat world.[1]

Cognition involves the processing of information. It indicates a comparatively high degree of processing of information, encompassing language, logic, skilled motions, motivation, memory, and thinking. All conscious and unconscious activities—such as seeing, recognizing, conceiving, and reasoning—that lead to the accumulation of knowledge are categorized as cognitive processes.[2]

Dementia, a major health problem in the 21st century, is one of the most functionally debilitating disorders. Alzheimer's disease is the most common type of dementia. While specific symptoms of dementia can be managed for a limited duration, dementia cannot currently be cured or significantly stabilized over time. However, the control of possible risk factors may be able to alter the onset of dementia.

These days, memory loss and cognitive decline are frequently linked to several environmental and natural causes, including high carbon dioxide and carbon monoxide levels, aging, and physical and mental stress.[3]

It is also crucial to remember that oxidative stress is thought to be a significant contributing element to AD and that immunological and inflammatory processes may aid in the disease's degenerative process. Oxygen free radicals may contribute to the age-related decline in cognitive performance and potentially lead to Alzheimer's disease in the elderly. Free radicals cause oxidative stress, which damages and peroxides lipids in healthy human cells by upsetting the stability of macromolecules in biology, including proteins, lipids, and DNA, leading to conditions like diabetes, chronic inflammation, cardiovascular disease, aging, neurodegenerative disorders, and cancer.[4]

Even with significant advances in treating Alzheimer's disease (AD), a cure for this degenerative neurological condition is still unattainable. The two drug classes that the FDA has approved are cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists; however, both groups of drugs only provide symptomatic improvement in a small percentage of people with early-stage AD.

Traditionally, herbal drugs are used to enhance cognitive functions. Plants like *Nardostachys jatamansi, Bacopa monnieri, and Withania somnifera have already proven memory-enhancing* properties.[5,6]

As an alternative, fruits and various biochemicals—a significant part of the diet—can be utilized therapeutically. Owing to the antioxidant qualities of polyphenolic compounds, numerous businesses use this characteristic to promote beverages high in polyphenols. One such fruit well-known for its medicinal uses because of its antioxidant qualities is the pomegranate (Punica granatum L.). Pomegranate extract is proven to have significant bioactive characteristics, including antioxidant and anti-inflammatory actions.

Punica granatum, or Pomegranates, has been extensively used as a traditional medicine source in the old Ayurvedic medical system. It is used in ancient cultures as food and as a medicinal remedy. Pomegranate comes from the Latin *pomum,* which means "apple," and granatum, which means "seeded." Numerous phytochemicals, including flavonoids and polyphenols, which may have antioxidant and potential neuroprotective properties, are abundant in Punica granatum.[7]

Therefore, the main objective of this research was to examine the effect of *Punica granatum* fruit extract on memory and learning processes of both standard and scopolamine-induced amnesic rats.

Memory impairment in animals has been studied to identify potential treatment targets and learn more about its molecular causes. The rodent model of scopolamine-induced amnesia is a widely recognized animal model for memory impairment.[8]

2. Materials & methods

2.1 Animals

Male adult Wistar albino rats (180-250g) were used for this research study after approval from the Institutional Animal Ethics Committee (IAEC) (KSHEMA/IAEC/14/2017). The animals were kept in polypropylene cages under standard conditions in the animal house, Department of Pharmacology, K S Hegde Medical Academy, Nitte (Deemed to be University). Water and food were freely available to them.

2.2 Preparation of extract

The fruit was peeled, and its edible portion was cut, dried, and coarsely powdered. A weighed quantity (500g) of powder was exposed to a 72-hour Soxhlet extraction process using ethyl alcohol at 78°C. After the extracts were concentrated and the solvent was entirely eliminated using a rotary evaporator, they were refrigerated for later use. The extract from Punica granatum had a yield percentage of 13%. The extract was dissolved in distilled water and orally administered to the rats using a rat feeding tube.

2.3 Experimental protocol

The animals were segregated into six groups. They were adjusted to the lab environment for a minimum of three days.

Group 1 - Control - received distilled water orally

Group 2 - Scopolamine (0.4 mg / kg i.p.)

Group 3 - *Punica granatum* extract 100mg/kg

Group 4 - *Punica granatum* extract 200mg/kg

Group 5 – *Punica granatum* extract 100mg/kg + Scopolamine

Group 6 - *Punica granatum* extract 200mg/kg + Scopolamine

N= 6 rats PG- *Punica granatum*

Punica granatum extract was given orally daily for six weeks. *Then,* learning and memory of *Punica granatum* extract were evaluated by two behavioral paradigms - elevated plus maze and shuttle box. Scopolamine was dissolved in normal saline and administered intraperitoneally at 0.4mg/kg 30 minutes before the trial.[9]

2.4 Elevated plus maze

The elevated plus-maze apparatus comprises two opposing open arms and two closed arms connected by the central platform of five centimeters. Each arm measured 50 cm in length and 10 cm in width. The closed arm has a high wall measuring 40 cm in length.

The apparatus was raised from the ground at 50 cm height. To test its memory and learning skills, the rat was placed in the middle of the maze, facing away from the center, at the end of the open arm. Transfer latency (TL) was defined as the amount of time it took to enter any one of the closed arms. All four legs inside the closed arm were recorded as entries. The rat was given another three minutes to investigate the maze before being allowed to return to its home cage. The maze was cleaned with 70% alcohol to prevent odor cues from previous trials.

Retention was evaluated 24 hours after the learning trial, where the transfer latency was noted.[10]

2.5 Shuttle box

It is a foot-shock-motivated two-way shuttle box. This apparatus consists of a rectangular box with electrifiable grid floors. Stainless steel bars of 2 mm diameter spaced one centimeter apart covered the floor of the two compartments. A manually actuated doorway separated the box into two compartments. A 5 W lamp positioned on the wall of the compartment provided illumination. The animal's latency from the shock zone to the non-shock zone was observed.

The rats were allowed to explore the box for 5 minutes without shock, and the connecting doors opened. The trial starts by placing the rat in the compartment. A buzzer is presented, and 5 seconds later, electric shocks (1mA; 50 Hz) are given and continued until the rat jumps into the shock-free chamber. In response to the buzzer, animals can learn to avoid shock by shuttling from one compartment to another. It is repeated till the criterion of 9 out of 10 consecutive trials is reached. The rat was subjected to another trial after 24 hours to assess the retention of the previously learned active avoidance response. The time needed by the rat to reach the safe area was measured.[11]

2.6 Assay of acetylcholinesterase activity

Ellman’s method was used to assess the activity of acetylcholinesterase. After the behavioral experiments, the brain was quickly removed, the hippocampus was isolated, and homogenized in 0.1 M phosphate buffer at pH 8. The reaction mixture comprised 2.6 ml of phosphate buffer (0.1 M, pH 8.0), 0.4 ml of homogenate aliquot, and 0.1 ml of 0.01 M dithiobisnitrobenzoic acid

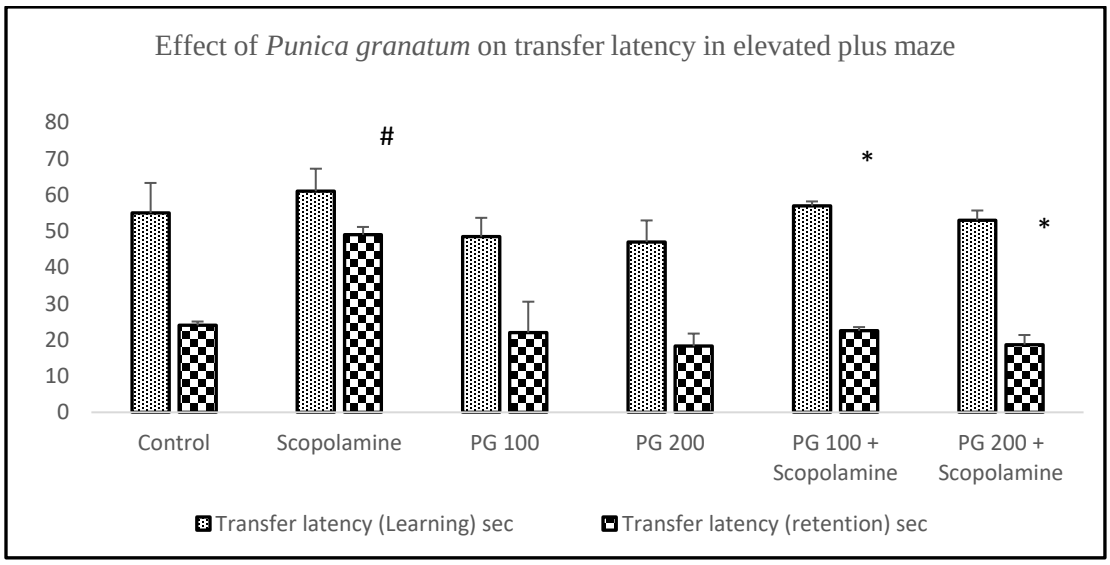
(DTNB). Using a spectrophotometer, changes in the absorbance were noted every two minutes for ten minutes at 412 nm after adding the substrate, acetylthiocholine iodide (0.075 M). The activity was expressed as micromoles hydrolyzed per minute per gram of tissue[12].

2.7 Statistical analysis

The data are reported as mean± standard deviation. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by Tukey's test. $P<0.05$ was considered significant.

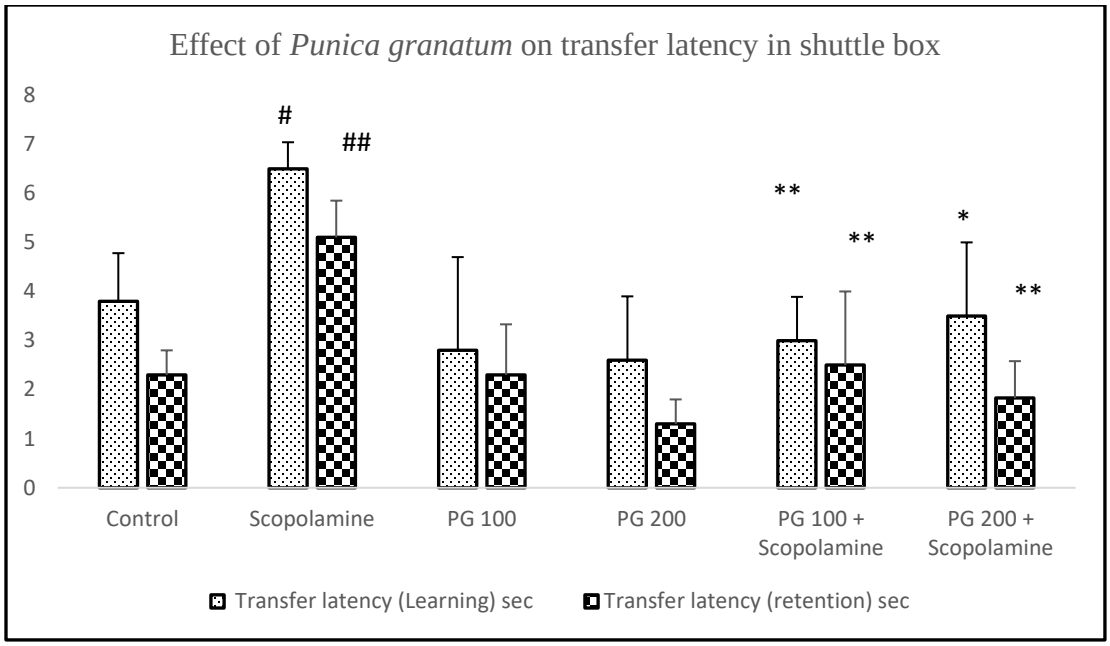
3. Results

Figure.1



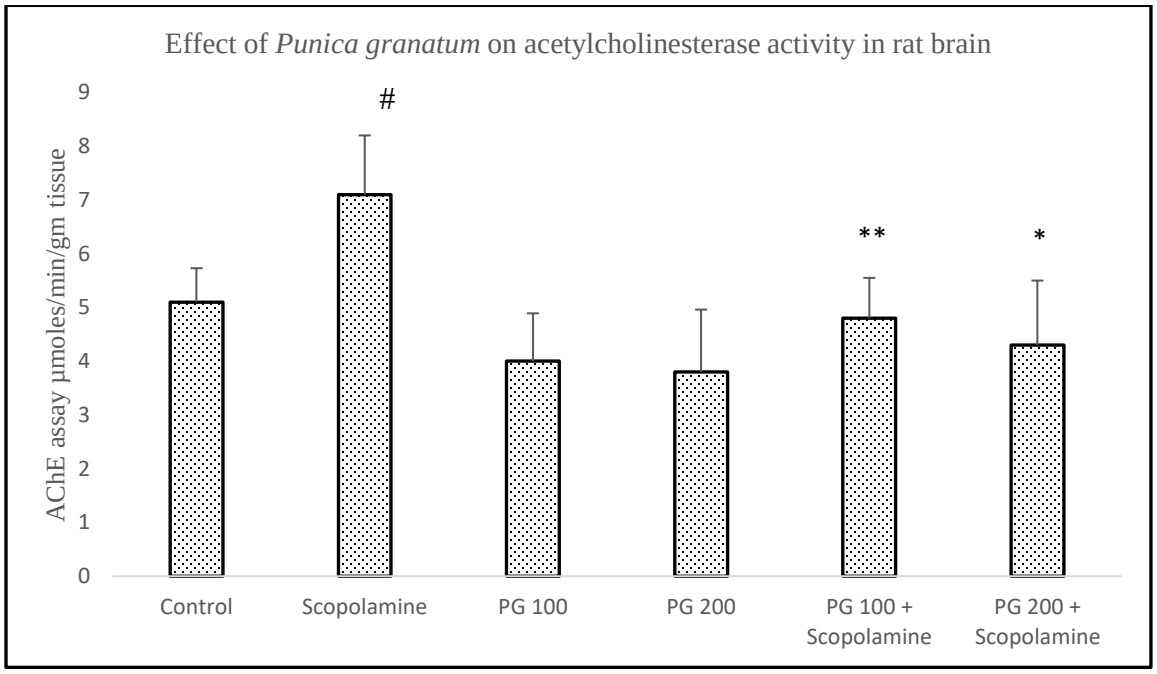
n = 6, Values are expressed as Mean ± Standard deviation. # $p<0.001$ vs control, *$p<0.001$ vs scopolamine

Figure 2



n = 6, Values are expressed as Mean ± SD. # p<0.05 vs control, ## p<0.001 vs control, *p<0.01 vs scopolamine, **p<0.001 vs scopolamine

Figure 3



n = 6, Values are expressed as Mean ± SD. # $p<0.01$ vs control, *$p<0.01$ vs scopolamine and **$p<0.001$ vs scopolamine

3.1 Effect of *Punica granatum* on scopolamine-induced memory impairment in the elevated plus maze

The Scopolamine group showed a significant ($p<0.05$) increase in transfer latency values on learning and retention compared to control rats. The *PG extract* at a dose level of 100 and 200 mg/kg orally demonstrated a significant ($p<0.001$) decrease in transfer latency on retention day in the elevated plus maze test as compared to the scopolamine group. It indicated a reversal of memory deficit induced by scopolamine.

3.2 Effect of *Punica granatum* on scopolamine-induced memory Impairment in Shuttle Box

In the shuttle box, the scopolamine group showed a significant ($p<0.05$) increase in transfer latency values on learning and retention compared to control rats, indicating impairment in learning and memory, as shown in Figure 2. PG extract significantly reversed the scopolamine-induced amnesia by producing a memory-enhancing effect, evident by decreased transfer latency in the shuttle box.

3.3 Effect of *Punica granatum* on acetylcholinesterase activity

There was a significant increase in acetylcholinesterase activity in the brains of scopolamine-treated rats compared to the control group (*$p<0.01$). *Punica granatum* pretreatment significantly reduced the acetylcholinesterase activity in the hippocampus of amnesic rats compared to scopolamine, as shown in Figure 3.

4. Discussion

Disorders of CNS can be associated with impaired mental function, memory loss, and cognitive deficits. Prevention and early detection of memory disorders will positively influence society by decreasing the disease burden and financial load.

As of date, there are only a handful of herbal drugs with memory-enhancing activity available with scientific data. Therefore, an attempt was undertaken to assess the impact of *Punica granatum* on learning and memory in the current study. The nootropic effect was evaluated using two models: elevated plus maze and two-way shuttle box. The two-way shuttle box was used to measure active avoidance, and the elevated plus maze model was used to evaluate the impact on learning and memory as an exteroceptive behavioral test.

While Alzheimer's disease appears to affect numerous neurotransmitter systems, cholinergic dysfunctions have garnered particular attention and are the focus of most AD therapeutics. Stress can impair the cholinergic system, which impairs learning.

As a neuromodulator of plasticity, arousal, and reward, acetylcholine is regarded as a significant neurotransmitter in controlling cognitive processes. Any damage to the cholinergic system leads to a deficiency of cholinergic function, which is associated with memory defects.[13] Hence, approaches to enhance cholinergic function by stimulation of cholinergic receptors or potentiation of Ach by inhibiting acetylcholine hydrolysis by cholinesterase enzyme. Acetylcholinesterase (AChE) is a crucial enzyme that hydrolyzes acetylcholine (ACh) into choline and acetate, controlling the neurotransmitter's concentration in the synaptic cleft and playing a role in memory and learning. Any change in acetylcholine levels or AChE activity can impact the cholinergic transmission mechanism, impairing learning and memory that resembles Alzheimer's disease.[14]

Scopolamine -A muscarinic receptor antagonist used to cause memory impairments in animal studies. The injection of scopolamine disrupts memory short-term retention and learning and acquisition.[7]

Punica granatum treatment for six successive weeks showed increased learning and memory, as seen by lower transfer latency than rats treated with scopolamine. Shortened transfer latency was considered an index of memory improvement. Transfer latency after medication treatment reflected the learning behavior of animals, whereas transfer latency of the next day reflected the retention of information or memory.[15] *Punica granatum* (100mg/kg, 200mg/kg orally) produced a memory enhancement effect in scopolamine-induced cognitive dysfunction when compared to normal rats, thus indicating the possibility of amnesia reversal.

Rats treated with *Punica granatum* for six weeks showed increased learning and memory, as seen by lower transfer latency than those treated with scopolamine. Reduced transmission latency was thought to be a sign of better memory. Transfer latency during medication treatment showed how the mice learned, whereas transfer latency the next day showed how the animals remembered or retained the knowledge.15 Compared to normal rats, animals given 100 mg/kg or 200 mg/kg of Punica granatum orally showed memory enhancement effects, indicating the possibility of amnesia reversal.

In the present study, the *Punica granatum* extract, when administered for six weeks, resulted in a considerable decrease in brain acetylcholinesterase activity, which likely facilitated cholinergic

transmission and improved animal memory. An increase in endogenous ACh at the cholinergic terminals has been demonstrated to modify long-term potentiation (LTP), the key neuronal mechanism underlying learning and memory, and synaptic plasticity.[16]

Antioxidant compounds derived from medicinally significant plants have great potential to treat oxidative stress and various degenerative illnesses. The antioxidant capacity of *Punica granatum* is responsible for its protective impact since it reduces oxidative stress on susceptible brain cells, which leads to less brain damage and improved neuronal function. The results of this investigation may support the usefulness of *Punica granatum* in scopolamine-induced amnesia in rats with possible therapeutic effects in dementia, as oxidative stress is connected to the etiology of dementia.

Results of preliminary phytochemical tests of *Punica granatum* indicated the presence of flavonoids, steroids, and tannins. Punica granatum is also said to be a rich source of polyphenolic compounds, and its flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallagic acid, and ellagic acid esters of glucose) are responsible for the antioxidant properties of pomegranates. [17,18]

The possible mechanism of nootropic activity may be due to essential phytoconstituents (flavonoids, steroids, and tannins) and its antioxidant properties [19, 20, 21].

Hence, these results suggest that the cognition-enhancing property of Punica granatum may be attributed to its pro-cholinergic, inhibition of the AChE enzyme, and antioxidant activity and may be used in delaying the onset and reducing the severity of Dementia. Therefore, incorporating *Punica granatum* into one's diet would be beneficial and can be used as an adjunct to current dementia therapy regimens.

However, further studies addressing the underlying detailed mechanism of cognitive enhancement of *Punica granatum* are required.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgment

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