Nootropic activity of Emblica officinalis G against Scopolamine induced amnesia in Albino Rats

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Abstract

Learning refers as the ability to change behavior based on past experiences whereas memory is a process of remembering. *Emblica officinalis* is also known as rejuvenatorand has been widely used in traditional medicine. The present study was designed as pharmacological screening of ethanolic extract of *Emblica officinalis* plant on learning and memory using modular food maze chamber. The whole plant was collected from the Prayagraj region, Uttar Pradesh. After, it was dried and herbarium was developed of the *Emblica officinalis.*. The extraction was performed through Soxhlet apparatus using ethanol solvent. The extract was evaluated for its phytoconstituents i.e., alkaloids, glycosides etc. All the rats were divided into 5 groups i.e., group 1 was given distilled water, group 2 was given Piracetam, group 3 administered ethanolic extract of *Emblica officinalis* (50mg), group 4 given administered Ethanolic extract of *Emblica officinalis* (100mg) and group 5 was treated with Ethanolic extract of *Emblica officinalis* (200mg). Modular Food Maze apparatus, EPM and blood glucose level parameters were evaluated.

Keywords: *Emblica officinalis* , learning & memory, modular food maze apparatus, blood glucose level, EPM

INTRODUCTION

Cognitive impairment and memory deficits, particularly in the context of neurodegenerative diseases and aging, continue to pose significant challenges to public health and quality of life. Nootropics, also known as smart drugs or cognitive enhancers, are substances that have gained attention for their potential to improve cognitive functions, including memory and learning. The search for effective nootropic agents has led researchers to explore natural compounds with purported cognitive-enhancing properties. Emblica officinalis (E. officinalis), commonly known as Amla or Indian gooseberry, is one such natural substance with a rich history in traditional medicine for its potential health benefits. (1,2)

E. officinalis is a deciduous tree native to the Indian subcontinent and is renowned for its high antioxidant content, primarily ascorbic acid (vitamin C). However, recent studies have extended their focus beyond its antioxidant properties, aiming to elucidate its neuroprotective and cognitive-enhancing potential. While various studies have suggested that E. officinalis exhibits promising neuroprotective properties, including its ability to scavenge free radicals and protect against oxidative stress, its specific role in ameliorating cognitive impairments, particularly in cases of memory deficits, remains a subject of exploration.(3,4)

Scopolamine, a muscarinic receptor antagonist, is commonly employed to induce memory impairment in animal models, making it a valuable tool for investigating potential nootropic agents. Studies have demonstrated that scopolamine administration can lead to cholinergic dysfunction, oxidative stress, and neuroinflammation, which collectively contribute to memory deficits in animal models. As such, scopolamine-induced amnesia serves as a relevant and reproducible model for assessing the efficacy of potential nootropic agents in memory enhancement.(5,6,7)

This research paper aims to investigate the nootropic activity of E. officinalis extract against scopolamine-induced amnesia in albino rats. We hypothesize that E. officinalis, with its antioxidative and neuroprotective properties, may counteract the detrimental effects of scopolamine on memory and cognition. A successful outcome of this study could shed light on the potential use of E. officinalis as a natural nootropic agent, offering a safer and more accessible alternative to pharmaceutical interventions for cognitive enhancement.(8,9,10)

Cognitive functions, particularly memory and learning, play an integral role in our daily lives, shaping our ability to acquire, process, and retain information. Cognitive deficits, such as memory impairment, pose significant challenges to individuals' well-being and quality of life. Moreover, as the global population ages, the prevalence of neurodegenerative disorders, such as Alzheimer's disease, has surged, emphasizing the pressing need for effective interventions to preserve and enhance cognitive function. One avenue of research that has gained considerable attention in recent years is the development and investigation of nootropic agents, often referred to as "smart drugs" or cognitive enhancers, which are substances that have the potential to improve cognitive performance, including memory and learning.(11,12)

The pursuit of nootropic agents has been a subject of great interest among scientists, clinicians, and researchers, as it holds the promise of improving cognitive abilities in both healthy individuals and those suffering from cognitive decline. In this context, natural

substances with purported cognitive-enhancing properties have garnered significant attention due to their potential efficacy, safety, and relatively low cost compared to synthetic pharmaceutical interventions. Emblica officinalis, commonly known as Amla or Indian gooseberry, is one such natural substance that has captured the curiosity of researchers and traditional healers alike. (13,14)

Notably, Amla is believed to modulate various molecular pathways implicated in cognitive processes. These potential mechanisms include its ability to scavenge free radicals, thereby protecting against oxidative stress, reducing inflammation, and regulating neurotransmitter systems that are essential for learning and memory. However, the specific effects of Amla on memory and cognition, particularly in cases of memory deficits, have yet to be comprehensively explored. This gap in our understanding serves as the impetus for the current research endeavor. Scopolamine, a tropane alkaloid and muscarinic receptor antagonist, is a well-established pharmacological tool in the study of memory impairment. Its utility as an amnestic agent in animal models is widely recognized, providing a reliable means to investigate the effects of potential nootropic agents on memory enhancement. Scopolamine administration is known to induce cholinergic dysfunction, increase oxidative stress, and trigger neuroinflammatory responses, collectively contributing to memory deficits in animal models. Consequently, the scopolamine-induced amnesia model has become an invaluable tool for assessing the efficacy of prospective cognitive enhancers and nootropic agents.(15)

In addition to its cholinergic effects, scopolamine also induces oxidative stress and neuroinflammation. These processes contribute to the disruption of synaptic plasticity and neurodegenerative changes in the brain. The resulting memory deficits observed in scopolamine-treated animal models parallel the cognitive impairments seen in neurodegenerative conditions, making scopolamine a valuable tool for studying potential cognitive enhancers.The scopolamine-induced amnesia model offers several advantages for studying nootropic agents, the model reliably induces memory deficits in animals, ensuring consistency in experimental outcomes.The cholinergic system is a key player in learning and memory processes, and scopolamine-induced amnesia mirrors the effects of cholinergic dysfunction, which are observed in conditions like Alzheimer's disease. Scopolamine administration provides researchers with a controlled method for inducing memory impairment, allowing for the systematic evaluation of potential cognitive enhancers. As scopolamine-induced amnesia shares features with cognitive deficits in human conditions,

findings from this model have implications for the development of interventions for memory disorders. Given the potential cognitive benefits of Amla, its rich phytochemical profile, and traditional use, there is a compelling rationale for investigating its nootropic properties, especially in the context of scopolamine-induced amnesia. Several key factors underscore the significance of this research: The pursuit of nootropic agents from natural sources aligns with the growing interest in holistic and complementary approaches to health and well-being. Amla has the potential to offer cognitive enhancement along with its established health benefits. If Amla is confirmed to possess nootropic properties, it may serve as a safe, cost-effective, and readily available option for individuals seeking to improve their cognitive abilities, whether they are experiencing age-related cognitive decline or simply looking to optimize their mental performance.(16)

Amla's historical use in traditional systems of medicine, such as Ayurveda, underscores the potential value of this fruit as a cognitive enhancer. These traditional systems have a wealth of empirical knowledge that can guide modern scientific inquiry.

MATERIALS AND METHODS

Plant Identification

The whole plant was collected from the Prayagraj region, Uttar Pradesh. After, it was dried and herbarium was developed of the E.officinalis. It was identified and authenticated by the botanist.

Preparation of plant extract

The collected parts were firstly peeled off, then with the help of blender convert it into powdered form, about 200 gm of powdered was subjected it into defatting with the help of petroleum ether and then subjected to filter with the help of musclein cloth and then filtrate cake was subjected to dried at 50 degree for 10 min in hot air oven in order to remove moisture and make it dry. Take the deffated powdered and then subjected to extract with the help of soxhlet apparatus using mixture of 40% methanol+ 20% ethanol+ 40%water and the resulting obtained solution was placed in water bath (45^0C)(17,18)

Animals

Animals were purchased From CDRI (Central drug research institute) animal house having weight ranges in between 150-210 gm and 10-12 weeks old of albino wistar rats., for our

research purpose. Storage condition maintained at 12hr dark condition and 12hr light condition, maintained at $24 \pm 2°C$ conditions is maintained. During research protocol diet was pellet and ad libitum water. Accommodation of the experimental animals were kept in isolation specified area in animal house at-least before the 14 days prior to start the protocol for the experiment, which is approved by I.A.E.C

Drugs and Chemicals and Experimental design

Scopolamine hydrobromide 98% purchased from Yarrow Chem. Products (Mumbai Standard drug piracetam was obtained as a gift sample)

Experimental design

Wistar albino rats (either sex) weighing (150-250gm) were divided into 5 groups with 6 animals each as mentioned below in the table. All the test solutions were freshly preparedprior administration.

Ethanolic extract of *Emblica officinalis* and Piracetam were administered once a day forup to 8 days. Diazepam will be used to induce amnesia in animals (1mg/kg, i.p.) 30 minutes prior to acquisition trial (19).

Group	Treatment		Route of administration
Group 1 (Control)	Distilled water		Intraperitoneal
Group 2 (Standard)	Piracetam (200mg/kg)		Intraperitoneal
Group 3 (Test group I)	Ethanolic extract of *E.officinalis*(50mg)		Intraperitoneal
Group 4 (Test group II)	Ethanolic extract of *E.officinalis* (100mg)		Intraperitoneal
Group 5 (Test group III)	Ethanolic (200mg)	Extract of E.officinalis	Intraperitoneal

Administration of Test and Drug compounds

Administration of ScHBr (1mg/kg.,b.w., i.p) was 60 min before the acquistion trials in group 3,4 and 5. PDWA extract at a dose of 250 and 500mg/kg were given for subsequent days respectively and acquistion trial was carried out 60 min after the last dose. Standard Nootropic medication-Piracetam was administered by using normal saline solution at dose of 1mg/kg. Standard drug, Plant extract was administered 30 min before the conducting of the experiments.(20,21)

Modular Food Maze apparatus

Modular Food Maze Chamber was used to evaluate learning and memory enhancing activity of ethanolic extract of whole plant of *Emblica officinalis* plant. It is a combination of modular maze and food chamber together, hence named as Modular Food Maze Chamber. The apparatus consists of a wooden chamber [100cm(L)×120cm(B)×30cm(H)] with a start box [12cm(L)×20cm(B)]. A modular (complex) maze is fitted inside the chamber which has opening (15cm) to the start box and has an end towards the food boxes [20cm(L)×20cm(B) each]. There are 6 food boxes inside this chamber with two-way open doors [12(L)×12 cm(B) each]. Out of 6 food boxes some food boxes contain food and some are empty. Animal will be placed in start box will allow to explore the maze and reached to the food boxes. The time to cross the maze and to reach the food boxes will be calculated. Number of errors will also be noted down (22).

Each tested or treated animal were placed in start box of Modular Food Maze Chamber and were monitored for crossing of maze and reach to food boxes. Time to cross the mazeand accessibility to food boxes will be calculated.

Elevated Plus Maze

The Elevated Plus Maze test is used to measure anxiety, learning and memory behaviour. EPM consists 2 enclosed quadrants (50×40×10cm)and 2 open quadrants (50×10cm). It was elevated 70cm from the ground. Time taken by rat to move from open arm and to cross the line marked in central and enclosed arm with all 4 paws is taken as transfer latency time (LT). The no. of entries and time spent in open arm is recorded in 5 min (23).

RESULTS
Phytochemical constituents

Chemical Constituents	Types of tests	Ethanolic extract of *T. cordifolia*
Saponins		
	Foam test	+
Alkaloids		
	Dragendroff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Carbohydrates		
	Fehling's test	+
	Benedict's test	+
Tannins		
	Ferric chloride test	-
	Shinoda test	-
	Alkaline reagent test	-
	Lead acetate solution test	-
Proteins		

	Biuret test	+
	Million's test	+
	Xanthoprotein test	+
	Ninhydrine test	+
Glycosides		
	Keller-killiani test	+
	Bromine water test	+
	Legal's test	+
Amino acids		
	Ninhydrine test	+
	Test for tyrosine	+
Terpenes		
Flavonoids	Lead acetate	+

Pharmacological activity

Modular Food Maze Chamber Test

A marked no. of right entries was observed as 1.93**, 2.28** and 3.44** in ethanolic extract of *Emblica officinalis* treated animals at 50mg/kg, 100mg/kg and 200mg/kg, respectively. Similarly, no. of wrong entries was got decreased as 2.62**, 2.17*** and 1.98*** in *Emblica officinalis* treated animals at the dose of 50mg/kg, 100mg/kg and 200mg/kg, respectively that was significantly different from the control group.No. of wrong entries was highest in control group (3.42***) while minimum in test group treated with ethanolic extract of *Emblica officinalis* at the dose of 200mg/kg (1.98***) after standard drug treated group (1.75**).

The effects were seen as dose-dependent and comparable to standard drug- Piracetam treated rats. Thus, it exhibited that ethanolic extract of *Emblica officinalis* has positive

learning (taming) and memory in rodent model used in this study.

Table Time spent in maze, No. of wrong/right entries in modular food maze

Treatment	Time spent in maze	No. of wrong entries	No. of right entries
Distilled water	11.34*	3.42***	1.72**
Piracetam (200mg/kg)	10.50**	1.75**	3.67*
Ethanolic extract of *Emblica officinalis* (50mg/kg)	11.29***	2.62**	1.93**
Ethanolic extract of *Emblica officinalis* (100mg/kg)	11.86***	2.17***	2.28**
Ethanolic extract of *Emblica officinalis* (200mg/kg)	11.75**	1.98***	3.44**

Level of significance denoted by*; n= 6 Values were exhibited in Mean ± SEM
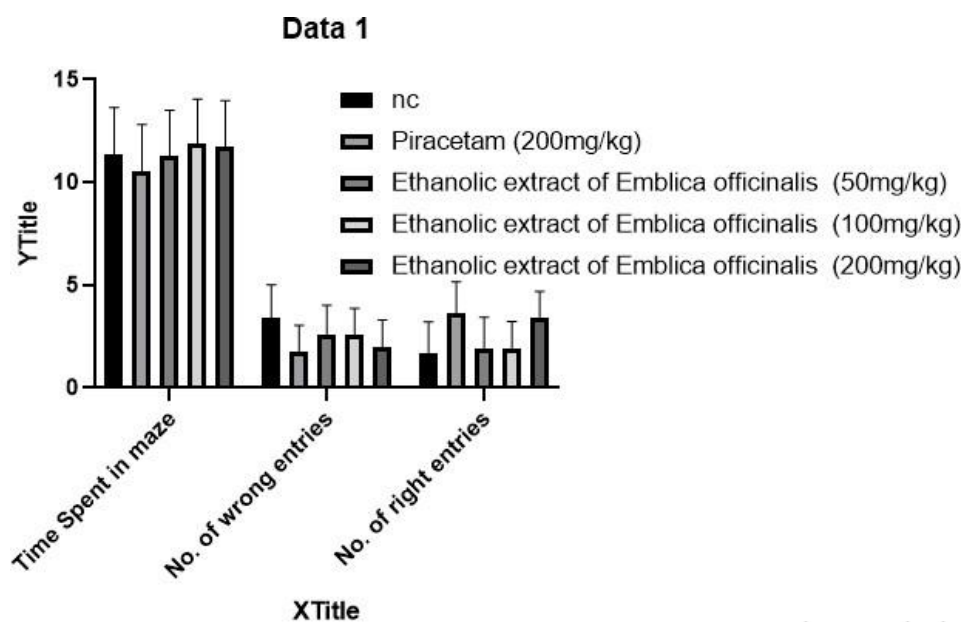
Elevated plus maze test

No. of entries was observed as 9.17 ± 0.26*** in standard group and ethanolic extract of

Emblica officinalis demonstrated no. of entries as 5.42±0.27***, 6.42±0.20*** and 8.52±0.67*** at the dose of 50mg/kg, 100mg/kg and 200mg/kg, respectively. Whereas, distilled water treated rats shown minimum no. of entries in open quadrant of EPM. Time spent was observed as 31.34±0.13***%, 48.34±0.18**% and 69.67±0.31***% in ethanolic extract of *Emblica officinalis* treated rats at doses of 50mg/kg, 100mg/kg and 200mg/kg, respectively.

Percentage of time spent was better increased in *Emblica officinalis* treated rats when compared with control group that indicated for its modulating behaviour in taming and memory in rodent model.

Table No. of entries and % of time spent in open arm in EPM

Treatment	In open arm (in seconds)		
	No. of entries	*Time spent*	*time spent (%)*
Distilled water	4.13±0.19*	68.40±0.50	22.67±0.12*
Piracetam (200mg/kg)	9.17±0.26***	234.27±0.26	78.00±0.20**
Ethanolic extract of *Emblica officinalis* (50mg/kg)	5.42±0.27***	94.62±0.16	31.34±0.13***
Ethanolic extract of *Emblica officinalis* (100mg/kg)	6.42±0.20***	145.10±0.27	48.34±0.18**
Ethanolic extract of *Emblica officinalis* (200mg/kg)	8.52±0.67***	209.38±0.19	69.67±0.31***

Level of significance denoted by *; n= 6Values were exhibited in Mean ± SEM

Data 1
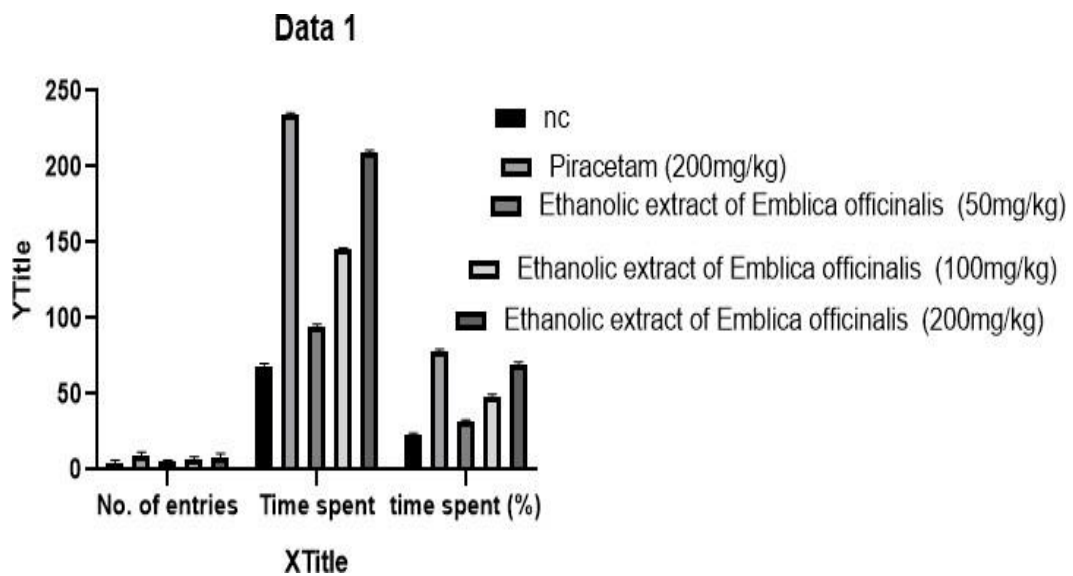
Histopathological Slide
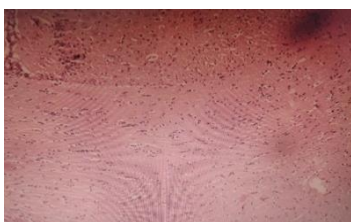
a) Normal

b) Toxic

c) Standard

d) Treatment group 100mg/kg

e) Treatment group 200mg/kg.

Fig: Shows effect of drug at neurons of various treated group in which toxic group shows degradation of neuron which is recovered by drugs as seen in standard group and treated group.

Discussion

It is normal for people to experience disability in proper learning and maintaining the memory. As for many, it is a persistent problem that interferes with work and family obligations. The motivation, energy, and pleasure needed to support and recover from the memory loss. It is a disability with many faces that can manifest at any age, be chronic or waxing and waning, and frequently coexists with a wide range of other problems, including anxiety disorders, substance abuse, loss of special person and behavioural disorders. There is a lot of information on the prevalence and symptoms of hindrance in learning and memory in the general population. It is frequently held responsible for or a contributing factor in medical disorders. In this study, *Emblica officinalis* significantly demonstrated the increased no. of right entries, minimized no. of wrong entries in modular food maze chamber that indicated for its positive impact on the learning (taming) and memory response in rats when compared with the control group. In EPM, *Emblica officinalis* was found to modulate (increase) the no. of time spent and no. of entries in open quadrants comparable to close quadrants when compared with control group. While, the blood sugar level was not get affectedwith the treatment of *Emblica officinalis* plant extract and observed as normal amongall the groups of rats. Disturbances that result in oxidative stress and high cortisol levels might promote neuro-degeneration, which may then cause a decline in cognitive function. Clinical relevance for any chemical, organic, or synthetic agent that improves executive functioning of the brain is enormous. Studies on the effects of NS seeds and/or its contents on the CNS and on behavioural actions are scarce in comparison to studies involving other plant materials, with the majority of these studies concentrating on spatial memory. The process by which the brain recognises, encodes, stores, and retrieves knowledge about items or pathways is known as spatial memory. It has elements of working memory and reference memory andis typically linked to curiosity and exploratory behaviour, which represent the demand for information when confronted with unfamiliar environments (Mohammad, et al; 2016). As acetylcholine (ACh), a neurotransmitter that aids in learning and memory, is known to degenerate in cholinergic neurons in Alzheimer's disease (AD), its decreased release will lead to memory impairment. Therefore, one method of managing ACh is to increase it by preventing acetylcholinesterase from degrading it. Pharmacological research has shown that NS plays a role in the suppression of AChE, the main enzyme responsible for Ach hydrolysis, which keeps the effects of *Emblica officinalis* in the encoding of new memories.

Conclusion

The most frequent symptoms of Alzehimer's and associated problems include memory loss, dreamy mood, lack of focus, attention-deficient persons with an increased degree of activity, and other neuro-related diseases. If such a disease is not dealt with, it might lead to death. Unfortunately, no precise treatment has yet been discovered for such a disease. This has led to initiatives to study and cure these diseases. *Emblica officinalis* contains polyphenolic compounds and other chemical components (L). Many animal models were used to measure such conditions. Seed extracts have been shown to lower latency times in the elevated plus labyrinth and the Morris water labyrinth. Additional biochemical data showed that AChE activity has declined and TBARS levels have declined, indicating that this medication might be used to treat cognitive impairment in the future.

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