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Neuroprotective effect of Emblica on enhancing memory in scopolamine induced memory impaired Long Evans Rats

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Abstract

Background: Alzheimer's disease is the commonest cause of progressive loss of memory and dementia due to oxidative stress. Various constituents of Amloki (*Phyllanthus emblica* or PE) fruit including phenols, tannoid,tannin and vitamin C counteract the oxidative stress and protect memory. **Objective**: To investigate the capacity of *Phyllanthus emblica* to resist the scopolamine induced memory loss in male Long-Evans rats. **Method**: This animal behavioral study had been done on 18 male Long-Evans rats (body weight of 200-300 gm) divided into control group (normal saline 5ml/kg for 26 days), memory impaired group (scopolamine 2mg/kg for 5 days) and PE group (*Phyllanthus emblica* 400mg/kg for 26 days+scopolamine 2 mg/kg for 5 days) each consisting of 6 rats. Working memory (short term) reference memory of all groups were tested by Morris Water Maze (MWM) test assessing escape latency time and target crossing. Rats of all groups were first room acclimatized for first 7 days without treatment. Then during day 8-33 normal saline EEPE (ethanolic extract of PE) was given to the respective group and during day 22-33 scopolamine was also given to the respective group. Then habituation,acquisition and probe trial was done between day 19 to day 28. In day 22-27(acquisition phase) reference memory was assessed and in day 30-33(training and test phase) working memory was assessed. Statistical analysis was done by ANOVA followed by Bonferroni's post hoc test. **Result**: Escape latency time in memory impaired group was significantly higher compared to control (normal memory) group and PE pretreated group, but there was no significant difference in escape latency time between control group and PE pretreated group. **Conclusion:** *Phyllanthus emblica* can effectively prevent scopolamine induced memory loss in Long Evans rats.

Introduction

Memory is a set of programmed neural connections in the brain, to encode, store, and recover information. Memory plays a critical role in many aspects of our daily existence[1]. Without memory many other important capacities (such as language, the identification of familiar objects or the maintenance of social relationships) would not be possible[2].Alzheimer's disease is the most common cause of gradual loss of memory and dementia in elderly[3]. Dementia is not curable and available therapies are unsatisfactory[4]. Thirty five million of global population are affected by AD including 5.5 million Americans and it is projected that in 2050 more than 115 million people will have dementia[1]. In Bangladesh prevalence of dementia is 3.7%,approximately 1.9% of total death in Bangladesh is due to dementia or Alzheimer's disease[5].

This disease is characterized by the formation of senile plaques, amyloid β (Aβ) deposits, and neurofibrillary tangles (NFTs) in the hippocampus and cortex. Senile plaques are spherical collections of dilated, tortuous, dystrophic neuritis around a central amyloid core, microglia and reactive astrocytes at the periphery[1].

Among many naturally occurring antioxidant fruit,PE has been well investigated against oxidative stress in animal model experiment. It is a local plant of tropical regions of Southeast Asia including Bangladesh where it is known as 'Amloki' and is found in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Tangail, Dinajpur, Sylhet and villages of Bangladesh. The fruit is spherical, smooth, hard, light greenish yellow in colour have 6 vertical streaks[1] possessing many pharmaceutical properties and therapeutically used in snakebite and scorpion sting. Fruits and its leaves are rich source of vitamin C and used as aphrodisiac, antipyretic, biliousness, and in asthma, bronchitis, leucorrhoea, vomiting, chronic dysentry[6].

Scopolamine is tropane alkaloid with antimuscarinic activity[7-8]. It is often administered intraperitoneally in experimental animals to study cognitive deficits. It has structural similarity with acetylcholine and blocks muscarinic receptor which causes cholinergic dysfunction and cognitive impairment[9].

Methods

Study design and Place

This was an experimental animal model study which was conducted in KM Fariduddin animal research lab of the Department of Physiology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh between 2020 to 2021.

Experimental Animal

For this study 18 healthy adult male Long- Evans rats weighing 200-300 grams were purchased from central animal house of BSMMU. The rats were kept in group of 6 in number per animal case and remained under standard environmental conditions (27° -28°C temperature, 60.5% relative humidity with a half day light and dark cycle). Proper laboratory food and water were given. The care and use of the animals were monitored according to the guideline of animal experimentation ethies committee of International Center of Diarrheal Disease of Bangladesh. Ethical clearence of this experiment was given by the Institutional Review Board of BSMMU, Dhaka, Bangladesh.

Drugs and Chemicals

i.p injection of Scopolamine butyl bromide(Sanofi Aventis Limited)dose 2 mg/kg , [8]Normal saline (Beximco Pharma Ltd) at 5 ml/kg and 400mg/kg EEPE was used.[3]

Preparation of ethanolic extract of Phyllanthus emhlica (PE)

PE leaves were identified by a taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A voucher specimen was preserved in the herbarium with an Accession number DACB-65751 PE.

At first 5kg fruits of PE were purchased, washed and sun dried for 7 days. It was then dried in an oven for 24 hours followed by grinded into powder. Five hundred gram of this powder was soaked in 2.5 liter of ethanol (98%) and kept for 7 days and then filtered. Filtrate was dried by evaporating at 50°C temperature by using rotary evaporator at reduced pressure to get the crude extract of PE (10 gram). Then this extract was stored at 4°C for further use[1].

Experimental Design

3 experimental groups (6 rats/group) formed by randomly selected Animals.

1) Control group (Normal saline 5 ml/kg for 26 consecutive days) served as control
2) Memory impaired group (Scopolamine 2 mg/kg for 5 consecutive days)
3) PE Experimental group (ethanolic *Phyllanthus emblica* extract 400 mg/kg for 26 days consecutive days and scopolamine 2 mg/kg for 5 consecutive days)

Morris Water Maze test

Apparatus

Morris water maze (MWM) is a circular pool, 150 cm in diameter and 50 cm high. This pool was arbitrarily divided into four quadrants: north-west (NW), north-east (NE), south-east (SE) and south-west (SW) quadrants. A black round platform (15 cm in diameter) was placed at the center of any one quadrant. To determine the start locations, eight points north (N), south (S), east (E), west (W), north-east (NE), north-west (NW), south-east (SE) and south-west (SW) of MWM were labelled (Figure 1). The whole inner wall of the pool and platform were painted in black to avoid visual clue in the pool. During the test, the maze was filled with water to a depth of 30 cm with 24°-26°C water. As a result, the platform was submerged 2 cm under the water and was the only escape place from the water for the rat. MWM test was conducted in well illuminated room which contained numerous extra maze cues such as racks, a window, a door, shelves, computer etc[10-11].

Figure-1: Morris water maze without water. NE : North-east; NW : North-west; SW : South-west; SE : South-east.

Test was done according to methods of previous study[10-12]. Selected rats were room acclimatized for 7 days in lab. Rat had to swim to escape at a hidden platform located 2 cm below the water surface.This test has reference and working memory component.

Reference memory test

This test was undertaken in 3 phases

Habituation phase(day 19-day 21): Each rat was allowed to swim for three minutes in these 3 days without platform which was considered as acclimatization peroid.

Acquisition phase(day 22-day 27):

Here rat had to swim to a prefixed platform irrespective of its start location in the maze. Four trials were done on every rat at 30 minute interval for 6 days. In this test platform was in same position in all trials of these 6 days but sequence of start locations were changed daily. Trial 1 was started 30 minutes after taking its assigned treatment and was allowed to swim in the pool after releasing from its start location. The rat searched the platform to escape and in failure of finding it within 60 minutes rat was gently guided to the escape platform in which rat was allowed to stay there for 20 seconds. Then rat was placed in a case with fresh newspaper which dried the rat and newspaper was replaced as necessary and rat was remained there for 30 seconds. In this way 2nd,3rd and 4th trial were also done. Sequence of start location was according to table I. Data was recorded as escape latency in each trial.

Probe trial phase: This test was done 24 hours after completion of trial 4 of acquisition phase. Rat had to swim to a point from which platform was removed.

Start location was at distant most spot from platform site (SW) and platform was removed from NE quadrant. Data was recorded as number of target crossing in 60 seconds

Working memory test

It has pretraining (6 days) and training phase (4 days).

Pretraining phase(day 22-day 27): Previous training of acquisition phase of reference memory test is the pretraining phase of working memory test.

Training phase (day 30-day 33): Here rat had to swim to a prefixed platform that was changed daily. This phase was conducted at 30 minute interval for consecutive 4 days for 4 trials (trial 1,2,3,4). Trial 1 was started 30 minutes after giving the specific treatment. Platform was under the water surface that was changed daily and its position and start location was according to table II. In these trials procedure of acquisition phase of reference memory test was followed. Escape latency time of the trials were recorded.

Table I: Platform position and sequence of start locations in acquisition phase of reference memory test in Morris water maze

	Sequence of start locations				
Day	1st trial	2nd trial	3rd trial	4th trial	Platform
Day 22	SW	SE	S	W	
Day 23	SE	S	W	SW	
Day 24	S	W	SW	SE	NE
Day 25	W	SW	SE	S	
Day 26	SE	SW	W	S	
Day 27	W	SE	S	SW	

Table II: Platform position and sequence of start locations of working memory test in Morris water maze test

	Sequence of start locations				
Day	1st trial	2nd trial	3rd trial	4th trial	Platform
Day 30	SE	SW	S	W	NE
Day 31	NE	NW	N	E	SW
Day 32	SE	E	NE	S	NW
Day 33	NW	W	N	NE	SE

Table III: Treatment plan in Morris Water Maze test

Phase	Duration	Days of experiment	Treatment	With/ Without Platform
Roomacclimatization	7	Day1-7	No treatment	Without platform
	11	Day8-18	I-NS, III-*EEPE*	Without platform
Habituation	3	Day19-21	I-NS, III-*EEPE*	Without platform
Acquisition	6	Day22-27	I-NS, II-Sco, III-Sco + *EEPE*	With platform
Probe trial	1	Day28	I-NS, III-*EEPE*	Without platform
Training and test	4	Day30-33	I-NS, II-Sco, III-Sco + *EEPE*	With platform

Evaluation of memory

Working memory test was done at training and test phase and reference memory test was done at acquisition phase of Morris water maze test.Time required for rat from entry into water to escape into platform is escape latency time and number of passing the area from which platform was removed is called target crossing.

Statistical analysis

Results were presented as mean±SEM (Standard error of mean). Statistical analysis was done with version 16 of SPSS. ANOVA followed by Bonferroni's post hoc test was done as statistical tests. P≤0.05 was considered as statistically significant.

Results

Reference memory

Escape latency (EL) time of acquisition phase of reference memory test shows that with progression of days EL time was gradually reduced in control and PE group but EL time was increased in memory impaired group during early part of acquisition phase. EL time in PE pretreated group is more than normal control group (Figure 2).

Working memory

In the line chart of escape latency(EL) of training and test phase of working memory test we observed that among the three groups (Ib,IIb,IIIb) escape latency time is progressively decreasing with repeated trial. EL time is higher in memory impaired group than others (control and PE pretreated group). EL time in PE pretreated group was more compared to normal controll group (Figure 3).

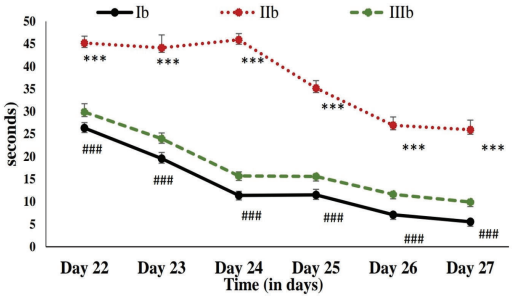

Figure 2: Escape latency (in acquisition phase) in different days of Morris Water Maze test in different groups of rats. Ib-controll, IIb-memory impaired group, IIIb-PE group

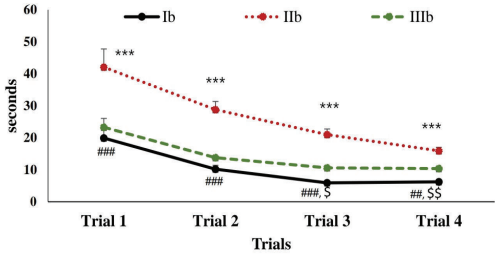

Figure 3: Escape latency (in training and test phase) in different trials of Morris Water Maze test in different groups of rats

Target crossing:

Ín addition target crossing in scopolamine treated rat is less than control group and PE group (Figure 4).

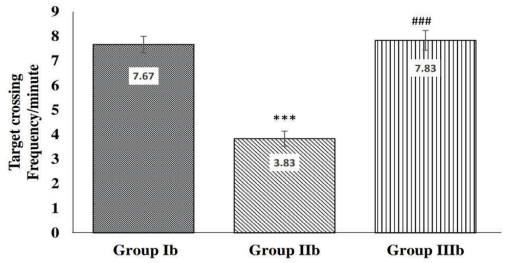

Figure 4: *Target crossing in Morris water maze test in different groups of rats. Ib, IIb, IIIb.*

Discussion

Amla-berry has memory enhancing activity and helps in mental functioning. It is useful in remedy of Alzheimer's disease as it causes memory improvement and reversal of memory deficit[13].

This study was designed to elicit the response of Long-Evans rats taken PE against adverse effect of memory imparing activity of scopolamine.

Results of this study demonstrated that with progression of days and trials EL time was gradually decreasing which reveals that/might be due to familiarization and memorization escape time required by rat was reduced. Higher EL time in scopolamine treated memory impaired rat suggests that scopolamine prevented building up of memory of rats required for escape learning.

EL time was more in PE group than normal memory (control) group which indicates that PE prevented memory impairment induced by scopolamine to some extent but not completely.

Target crossing in scopolamine group is less than PE and control group which indicates scopolamine prevented acquisition of memory in its group but PE had overcome this diminishing effect so that it returned almost to normal level.

So, we see that Amloki to some extent is effective to protect memory and learning process.

Scopolamine cause cholinergic receptor blockade which is the csuse of memory impairment. It also causes oxidative stress which is another cause of memory loss[14].

Among the bioactive chemicals of *Phyllanthus emblica* phenols, tannin, vitamin C etc. are important[14]. Memory enhancing effect of Amloki is due to acetylcholinesterase inhibition[3].

According to previous studies, memory impairment in scopolamine induced animal model occurs due to oxidative stress in brain as a result of altered brain oxidative enzymes. In Alzheimer's disease(AD) free radical is responsible for oxidative stress[1].

Conclusion

Hence, it can be concluded that extract of *Phyllanthus emblica* has neuroprotective effect by protecting neuron from organic reactions.

Ethical aspect

Ethical clearance was obtained from institutional review board of BSMMU.

Conflict of interest

Authors Declare no conflict of intest.

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