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ORIGINAL ARTICLE

Effect of *Emblica officinalis* on Various Learning and Memory Processes

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ABSTRACT
*Alzheimer's and similar memory disorders increase with age, with neurofibrillary tangles disrupting neurotransmission, impairing learning and memory. Fat deposition during aging contributes to these tangles. This study aims to explore the link between constant fat accumulation and memory loss, a key characteristic of Alzheimer's disease. For measuring the effects of fat accumulation on learning and memory, we induced high cholesterol levels in animals by feeding them a high-fat diet with free access to water. Another group was treated with scopolamine. These two models were used to compare the effects of memory impairment. The memory outcomes were evaluated in animals treated with *Emblica officinalis* (Amla), a crude drug in this study, and Piracetam, a known memory enhancer. The Morris water maze was used as the experimental model. The control group spent the most time in the target quadrant (Q4), followed by the high-fat diet group, indicating some memory retention despite fat accumulation. The scopolamine-treated group spent the least time in Q4, showing memory impairment. Time spent in the target quadrant was highest in the control group and the high-fat diet + Piracetam group, followed by high-fat diet + Amla group. The scopolamine-treated group spent the least time, confirming memory disruption. This suggests that scopolamine and high-fat diet treatments impair memory, while Piracetam and Amla help enhance memory, The Morris water maze experiment shows that animals treated with scopolamine or a high-fat diet spent less time in the target quadrant (Q4), indicating memory dysfunction. In contrast, animals treated with Piracetam and *Emblica officinalis* (Amla) spent more time in the target quadrant, suggesting that these substances improve memory and learning.*
KEYWORDS: *Emblica officinalis*, Learning and Memory, Cognitive functions, Neurotransmission

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INTRODUCTION

We have acquired many special things in our life-time, such as, we have knowledge of how to read, how to express in writing and how to carry out other functions in the society in a well-accepted manner. We have learned these skills by difference range of experiences in our life time. These skills help you in adjusting in life in appropriate manner, you behave in descent manner with others in social situations and in personal life. How have we acquired such behavior patterns? This is by the procedure of learning. Learning procedure is vital to all living beings which give the ability to adapt accordingly in every situation. Similar to that we also memorize and remember a lot of things, episodes of events that has happened with us or around us and by the help of this we plan and manage our current planning's and may be future also. We are able to recall the incidences of past because of a special function called Memory which helps us in connecting with past as well as making new strategies and planning for current and future. A few things you retain for a short time and others for a long time in your memory. How this retention process takes place? Forgetting is opposite to memory. Description of memory functions will give us a definite idea about process of forgetting as well. We learn various skills and adapt to varying and ever changing conditions of the environment around us. Our learning helps in making our behavior accordingly to the conditions around us. This experience we get through the process of learning. Since birth we learn new things on regular basis and this helps us to modify our behavior according to the situation and addition of certain new behavioral features makes a deep impact on our life. Other interesting fact about learning is

that every experience or change in behavior is not learning the change should be permanent that's the most important condition required to be called a learning. To be precise if we go according to the proper definition than learning is Change in behavior which is there for a limited period of time is not learning ii) the behavioral changes due to maturation process does not form part of learning. A study was conducted on children which concluded with a finding that children learn to behave according to their surroundings. If their surrounding is filled with aggressive behavior they tend to follow the same likewise if their surrounding is peaceful and happily set up they learn to behave in the same way[1]. Atkinson and Shiffrin (1968) were the two scientists who divided the processing of memory in humans in to three stages namely sensory memory, short term memory and long term memory[2]. Learning and memory are interrelated and without memory there can't be any learning of newer things. Generally, this king of experiences is a part of Long term memory which stays and registered for a longer time in our brain. William James (1890) described this kind of memory as "the knowledge of a former state of mind after it has once dropped from consciousness. "Progress in understanding long-term memory has come from behavioral investigations of people with intact memories as well as of patients with memory deficits. Insights into the operation of memory also have come from lesion and recording studies in animals and neuroimaging studies in humans [3]. The Long term memory is generally divided in to two forms Declarative memory (Explicit memory) and Non declarative memory (Implicit memory) Declarative memory as the name suggest is the memory that can be told to others meaning that it's the description of an incidence happy or sad or some data's in form of numbers or signs or our future plans or our successful, unsuccessful ideas.[4]. Declarative memory is further divided in to Episodic memory where the episode of past time is stored. It can be past memories past events anything that has happened with us in past and Semantic memory as knowledge about words and concepts, their properties, and interrelations on Declarative (Implicit memory) stands for the certain events that causes the change in once behavior suddenly without any prior signs of it. These changes are sudden and nobody notices the exact reason behind the change [5].Among other things, neurotransmitters are crucial for behavior, memory, and learning. One type of chemical substance that transmits signals between neurons is called a neurotransmitter. Generally, a sending neuron releases little amounts of a neurotransmitter, which causes the receiving cell's receptors to become active. When enough receptors are triggered, the receiving neuron may become active and transmit the information on its own. Receptor activation then starts a sequence of chemical changes in the receiving neuron. Numerous neurotransmitters, including as norepinephrine, acetylcholine, dopamine, and serotonin, have been found. Neuronal receptors are often exclusive to a single class of neurotransmitter. This enables a high level of specialization in the transmission of signals between neurons: a single neuron may react potently to release of a specific neurotransmitter, even though its neighbor might not be as responsive. The brain chemicals known as neurotransmitters are responsible for information transmission throughout the body and brain. They serve as a messenger between "neurons," or nerve cells [6].One can reduce neurotransmitter levels in a variety of ways. Neurotransmitters come in two varieties: excitatory and inhibitory. Excitatory neurotransmitters are what stimulate the brain, although they are not inherently exciting. Inhibitory substances are those that serve to establish equilibrium and calm the brain. When excitatory neurotransmitters are excessive, inhibitory neurotransmitters, which regulate mood, are quickly depleted.

ACETYLCHOLINE:The first neurotransmitter identified was acetylcholine. A large portion of muscle activation, including that of the gastrointestinal tract, is attributed to it [7]. The excitatory neurotransmitter acetylcholine is a highly common neurotransmitter any disorder leading to degeneration of Acetylcholine can cause Alzheimers [8].

NOREPINEPHRINE:One neurotransmitter that is essential for learning, emotions, emotion regulation, sleep, and dreaming is norepinephrine[9]. Additionally, norepinephrine is released into the bloodstream as a hormone, which raises heart rate and causes blood vessels to constrict. It is well known that norepinephrine causes our neurological systems to go into "high alert." It is crucial for memory formation and is widely distributed in the sympathetic nervous system [10].

DOPAMINE:Dopamine is another relative of adrenaline and norepinephrine. It's an inhibitory neurotransmitter, which means that when it attaches itself to a receptor site, it prevents the neuron from firing. Dopamine has a close relationship with the brain's reward systems. Nicotine and drugs such as cocaine, opium, heroin, and alcohol raise dopamine levels [11]. So does nicotine. Dopamine neurons are most likely implicated if it feels nice. One neurotransmitter that regulates posture and movement is dopamine. In addition, it regulates mood and is essential for both reliance and positive reinforcement. Parkinson's disease is characterized by the stiffness of muscles due to dopamine depletion in certain brain regions [12].

SEROTONIN:It has been shown that the inhibitory neurotransmitter serotonin plays a crucial role in mood and emotion. It has been demonstrated that insufficient serotonin causes sadness, obsessive-compulsive disorder, difficulties controlling anger, and suicidal thoughts [13]. Not enough also increases the need for carbohydrates, or starchy foods, and makes it difficult to fall asleep, two further symptoms linked to sadness and other mental disorders. It has already been linked to IBD and migraine [14].

ENDORPHIN:It performs comparable functions to opioids (opium, morphine, heroin, etc.) and is structurally quite similar to them [15]. Opioid medications function by binding to endorphin receptor sites; endorphin is an inhibitory neurotransmitter that is involved in both pain relief and pleasure. Bears and other animals can hibernate because of this neurotransmitter as well [16]. Nerve impulses are transmitted by nerve cells or neurons to convey messages. The neurotransmitters' mobility carries the impulses from one neuron to another or to a bodily cell. A nerve impulse eventually reaches the axon terminal after passing through the axon, which is a long, thin projection that emerges from the nerve cell. The slightly enlarged terminals of the branches that emerge from an axon are known as synaptic knobs. The neurovesicles, which hold and release neurotransmitters, are found within them. Neurons unite to form specialized structures known as synapses, which are used to transfer impulses. It acts as a connection point for information or impulses to go between neurons [17].

MATERIAL AND METHODS

Collection of fruits: Since fresh *Embellica Officinalis* fruits are readily available, they were gathered from the neighbourhood market. Following validation, the fruits were bought in bulk from Hangiri Traders in Dehradun. They were then ground with the aid of a machine to create a coarse powder after being sun-dried.

Fruit extraction: Using a Soxlet apparatus, 300 gm of powdered fruit were extracted using methanol. Following full extraction, the extract was concentrated in a water bath before the solvent was eventually withdrawn under pressure. The extract's weight was noted.

Animals: Jeva Life Sciences, Registration Number CCSEA/IAEC/JLS/19/02/23/173, provided us with young wistar rats of either sex, weighing 40–60 grams. The regular pallet food provided by Aashirwad Industries was fed to the animals.

Morris water maze

The Morris water maze was used to assess memory and learning. It was composed of a 45-centimeter-high, circular water tank with a diameter of 150 cm and a water level of 30 cm (at 25 °C). With the aid of two threads that were fastened at right angles to one another on the pool's rim, the tank was divided into four quadrants. In the middle of one of these four quadrants stood a platform (10 cm2) with a height of 29 cm. Throughout the training session, the platform's location and the clues' locations remained consistent. The target quadrant in the current investigation was Q4. Every animal had four trials a day, separated by five minutes, in which they were permitted to stand on the small platform. If after 120 seconds the animal still couldn't find the secret platform, it was gently led there by hands. In the water maze, the escape latency time to find the hidden platform was observed as an acquisition index. An acquisition study was conducted on rats for four days in a row. The platform was taken down on the fifth day, and it was recorded how long it took each animal to look for it in each quadrant and Q4. The amount of time the animal spent searching for the missing platform in the target quadrant and Q4 was recorded as a retrieval index.

Acquisition trial

Every mouse received four consecutive trials every day (after 16 days of medication administration). Between each trial, there was a 5-minute rest period. For four days in a row, four trials were conducted each day. Q4 was kept as the target quadrant in all acquisition trials, and the starting position for each day's four acquisition trials was altered as follows.

Day 1 Q_1 Q_2 Q_3 Q_4
Day2 Q_2 Q_4 Q_3 Q_1
Day3 Q_4 Q_3 Q_1 Q_2
Day4 Q_3 Q_1 Q_2 Q_4

An acquisition index was created using the mean escape latency time that was determined daily during the acquisition trial.

Trial of retrieval

The platform was taken down on day five. After being dropped into the water maze, each mouse was given 120 seconds to investigate. per mouse was put through four of these trials per day, beginning in various quadrants. The index of retrieval was determined by calculating the mean time spent in target

quadrant Q4. Throughout the investigation, attention was made to ensure that the relative location of the water maze was not affected by any external influences, such as shifting background or other visual clues.

Experimental protocol

The procedure methodology for experiment was submitted for approval to IAEC. As we know that without this approval we can't complete the experimentation part.

Calculating blood glucose

Blood was drawn from the Retro orbital plexus, and blood glucose was measured using the Glucose oxidase peroxidase test(GOD-POD). This diagnostic reagent kit from AGAPPE Diagnostics in Kerala, India makes it simpler to measure the amount of glucose present in urine, plasma, or blood samples. The intensity of the colour, which is measured spectrophotometrically at 530 nm, is directly associated with the amount of glucose present in the sample.

Procedure

Mix and read the optical density (OD) after 10 minutes incubation

Table 1. Calculating blood glucose

Reagent	Procedure for 1 ml		
	Blank	Standard	Test
Working	1 ml	1ml	1ml
Distilled water	10µl	------	------
Standard	------	10µl	------
Sample	------	-------	10µl

Calculation

Blood glucose $= \frac{\text{OD sample}}{\text{OD standard}} \text{Xn}$

n= standard concentration

Estimation of Serum Cholesterol

The estimation is done on the basis of CHOD-PAP methodology. The enzymatic determination of cholesterol is done.

Table 2. Estimation of Serum Cholesterol

	Blank	Standard	Sample
Working reagents	1000µl	1000µl	1000µl
Standard	---------	10µl	---------
Sample	---------	--------	10ul

Mix and incubate for 5 minutes at 37^0C, measure the absorbance of sample and standard against the reagent blank.

Cholesterol conc. $= \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$

Experimental protocol

Group 1: Control (Animals were kept on free access to standard food pallets chow diet and water and normal saline was administered 30 mints before trials). Group 2: Scopolamine Treated Group (Animals were kept on free access to standard pallets chow diet and water, normal saline was administered for 4 days 30 mints before trials and scopolamine was given on the 5th day). Group 3: *Scopolamine + Piracetam* treated group (Animals were kept on free access to standard food pallet chow diet and water, Scopolamine was administered firstly through i.p route followed by Piracetam after 15 mints for 4 consecutive days and on 5th day Scopolamine was exchanged with normal saline and then trials were performed 15 mints later). Group4: High Fat diet Group (animals were fed on high fat diet for fifteen days with fee access to water and normal saline was administered through i.p route 30 mints before the trials). Group 5: High fat diet + Amla (Animals were fed on high fat diet for fifteen days with fee access to water and Amla was administered through i.p route 30 mints before the trials through oral route). Group 6**:** High fat diet + Piracetam(Animals were fed on high fat diet for 15 days with fee access to water and Piracetam was administered through i.p route 30 mints before the trials through i.p route)

RESULTS

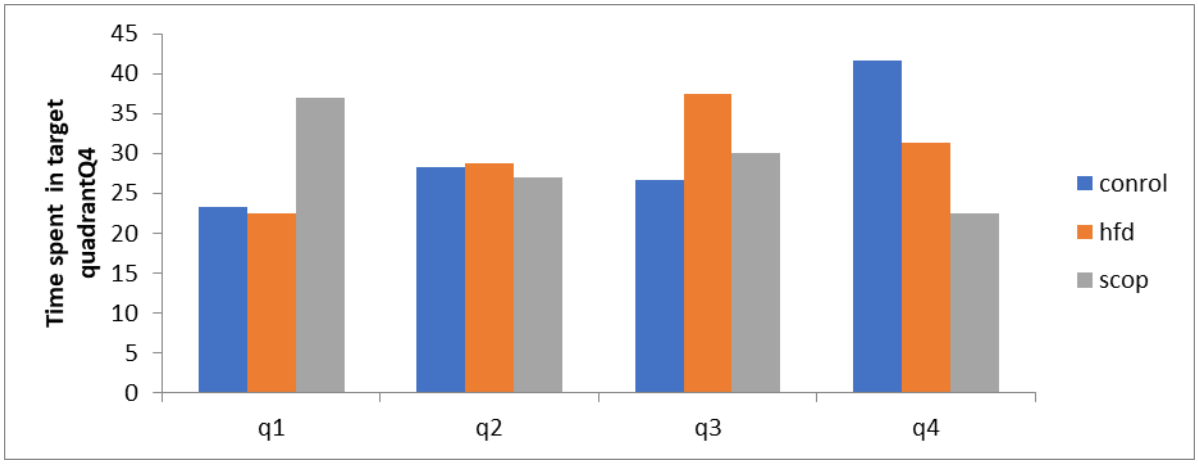

Figure 1. Time spent by different drug treated groups in target quadrant (Q4)

The above findings represent the time spent by drug treated animals in different quadrants q1 q2 q3 q4 (Morris water maze). The results indicate that highest time spent in the target quadrant Q4 as by control group and secondly was by High fat diet treated group. Lowest time spent in the target quadrant was by *Scopolamine* treated group. Our aim of the study is to see how much memory loss has been created by Scopolamine which is anticholinergic in action and High fat diet treated group as increased cholesterol also decreases memory and learning process.

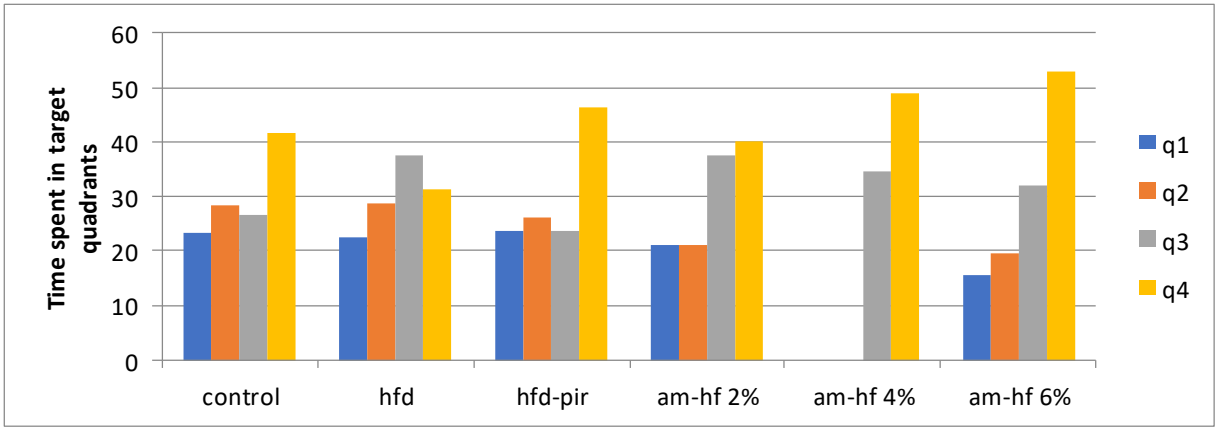

Figure 2. Time spent in each quadrant by drug treated animals

The above findings represent the time spent by drug treated animals in different quadrants q1 q2 q3 q4 (Morris water maze). The above findings shows that the highest time spent by control group was in the target quadrant q4 than in q2 thirdly in q3 and then in q1, over all indicating than in the target quadrant highest time spent was done by Amla and high fat diet combo and lowest was done by high fat diet treated group. Showing that there was a memory abduction in the high fat diet treated group due to the slowing of neurotransmission which effects the memory and learning function.

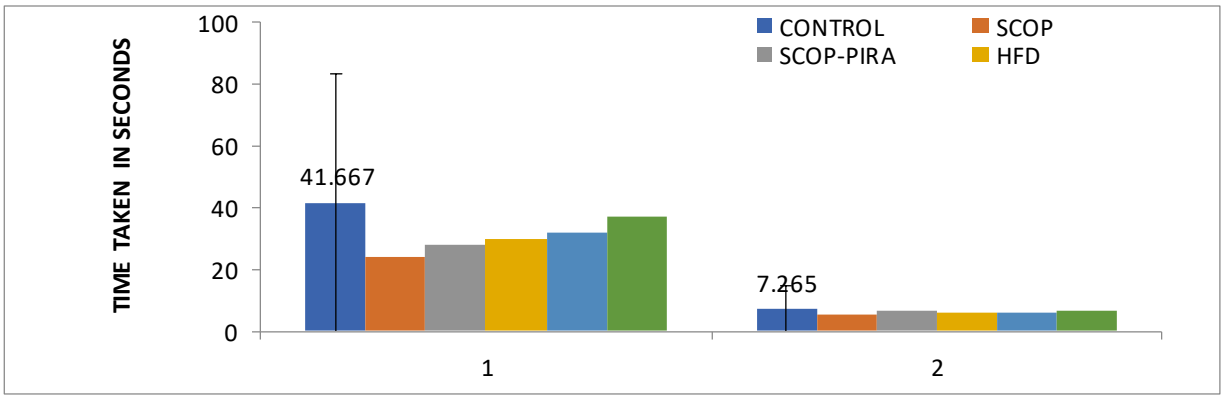

Figure 3. Time spent in the target quadrant by drug treated animals (in seconds)

The above findings indicate highest time spent in the target quadrant was by Control group and High fat diet-Piracetam treated group and third was High fat diet and Amla treated group while lowest time spent in the target quadrant was by scopolamine treated group. The above finding indicates that Scopolamine a well known anticholinergic has well stablished action of shutting memory functions due to breaking of neuronal signaling has the lowest time spent in the target quadrant q4. While Piracetam is a well-known memory enhancer has second most time spent in the target quadrant and it neutralized the effects of memory abolishment caused by High fat diet as indicated above in figure.

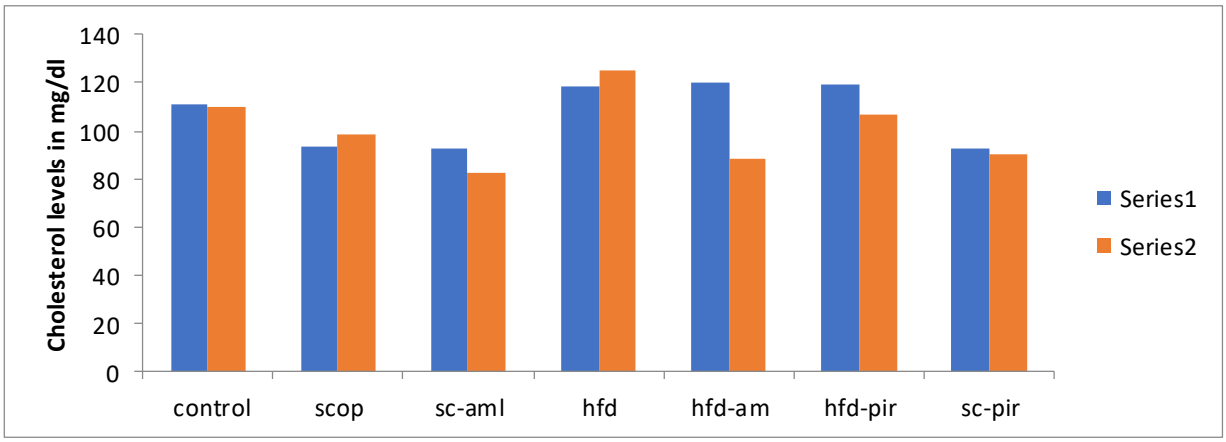

Figure 4. Cholesterol levels in drug treated groups

This above result shows the cholesterol levels of all the groups under study. The samples were taken on day 1 and day 15. Indicating that highest level of cholesterols was found in the group fed with high fat diet. The lowest levels were found in the groups treated with Amla.

DISCUSSION

The graph represents the time spent by animals treated with different drugs in quadrants q1 q2 q3 q4 (Morris water maze). The results indicate that in Graph 1 the highest time spent in the target quadrant Q4 as by control group and secondly was by High fat diet treated group. Lowest time spent in the target quadrant was by scopolamine treated group. This study is among one of the parameters where we try to establish a relation between memory loss and time spent in the target quadrant. The study shows that maximum time was spent by control group which was not treated with any drug while minimum time spent was by Scopolamine treated group indicating a total memory loss that's why the animals were not able to remember the position of platform placed at quadrant q4. Figure 2 shows the highest time spent by control group in target quadrant, over all indicating than in the target quadrant highest time spent was done by Amla and high fat diet combo and lowest was done by high fat diet treated group. Showing that high fat diet has caused memory loss that's why the animals were not able to remember the position of platform placed at quadrant q4. The graph indicates highest time spent in the target quadrant was by Control group and High fat diet-Piracetam treated group and third was High fat diet and Amla treated

group while lowest time spent in the target quadrant was by scopolamine treated group showing that scopolamine being anti cholinergic caused memory loss that's why the animals were not able to remember the position of platform placed at quadrant q4. And the group treated with Amla and HFD showed improved memory functions because it has reduced the fat levels and improved the neurotransmission.

CONCLUSION

As people age, fat accumulation disrupts neuronal signaling, leading to difficulties in learning and memory—symptoms typical of Alzheimer's disease. This study explores this connection by inducing memory loss in animals with Scopolamine, which disrupts neuronal transmission, and by increasing fat consumption through a high-fat diet. Results showed that fat deposition significantly impaired cognitive functions. However, treatment with *Emblica officinalis* and Piracetam improved memory and learning abilities, suggesting that reducing fat content may help mitigate memory loss in the elderly. The study highlights the link between fat accumulation and cognitive decline in aging.

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