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ORIGINAL ARTICLE

Study of *Emblica officinalis* and Scopolamine on High Fat Diet Induced Memory Loss in Rats

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ABSTRACT
Alzheimer is considered to be one of the most prominent diseases of modern day world specially in elderly patients. With the progression in age it is found that there is the formation of neurofibrillary tangles which causes the disruption in normal functioning of neurotransmitters like Acetylcholine, Dopamine which are responsible for learning and memory functions. The current study establishes a relation between increased cholesterol content and lack of learning and memory process and effect of Embellica Officinalis on it. Increased cholesterol or obesity also gives rise to condition like dementia and loss of learning and memory functions which is similar to Alzheimer's disease. In the current study group of rats given high fat diet was first let to gain the weight and after this their performance was checked on Morris Water Maze and EPM they showed a considerable decrease in Learning and Memory functions when compared with Scopolamine treated group and Control group. In contrast to it the animal group treated with Emblica officinalis and Piracetam showed considerable increase in learning and memory functions. Giving a support to our study objective that high cholesterol levels or obesity causes decrease in learning and memory functions like in Alzheimer's.
Key words: - Dementia, Morris water maze, EPM, Cognitive functions, *Emblica officinalis*,

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INTRODUCTION

The acquisition of different types of knowledge is known as learning. New capacities, skills values, understanding references, and subsequent retention of that information are called memory. One of the most basic mental processes is memory. Scientists use a lot of different strategies to study this process. There are two different approaches to understanding learning [1]. Learning is defined as the acquisition of different types of knowledge supported by perceived information. It leads to the development of new capacities, skills, values, understandings and preferences. Learning functions can be performed by different brain. Learning processes which depend on mental capacities of leaning subject the type of knowledge which has to be acquitted, as well as on socio-cognitive and environmental circumstances. Habituation and sensitizations are common type of learning. In habituation type there is a progressive diminution of behavioural response probably with repetition of stimulus. It is another form of integration. An animal first response to stimulus but if it is neither rewarding neither harmful animal reduces subsequent response [2]. In the sensitization learning, there is progressive amplification of a response follows repeated administration of stimulus [3]. The recall in to present consciousness of past experience of knowledge acquired through past experience [4]. Memory is based upon duration, nature, and retrieval of information. From information processing, there are three main stages in the formation and retrieval of memory: -

- Encoding or registration (processing and combination of received information)
- Storage (creation of permanent recorded information).

- Retrieval of recall (calling back the stored in formations in response to some activity or process). Memory types are three different types of memories- Sensory memory, Short term memory, Long term memory

SENSARY MEMORY: Sensory memory is the ability to look at an item and remember what it looked like with just a second of observation or memorization. Because this form of memory degraded very quickly so this type of memory can't be prolonging via rehearsal [5].

LONG TERM MEMORY: This memory can store much larger quantity of information for potentially unlimited duration (sometimes a whole life span [6] The hippocampus is essential to the consolidation of information from short term to long term [7].

SHORT TERM MEMORY: This type of memory that allows one to recall something from several seconds to as long as a minute without rehearsal. Its capacity is also very limited.

NEUROTRANSMITTERS IN MEMORY AND LEARNING

Acetylcholine (Ach), Monoamines, Amino acids, Lipids, Peptides and Neurotropin are in volved in most of the behavioural features like falling asleep, getting off from sleep maintaining emotional behaviour and ability to summerise a particular incident or thing and being able to recall it when its needed. [8] **Cholinergic system** ·Various studies have been performed to clear cut stablish a link between Acetylcholine along with learning and memory processes. In neurodegenerative disorders like Alzheimers centrally acting cholinergic drugs are used for the treatment due to this cholinergic activity of Ach [9]. **Glutamatergic pathway** The most abundant neurotransmitter in the central nervous system which have evident effect on learning memory functions is the excitatory glutamate [10]. Among the glutamatergic receptors, N-methyl d-aspartate (NMDA) is the most important receptor involved in generation of LTP [11]. Nitric oxide acts as a messenger and plays an adjuvant role in mediating synaptic changes. Drugs [12]. **GABAergic System** has a very important role in the processes of learning and memory as it is distributed widely in the vital parts of CNS. Gaba is found in two forms GABA-A which is responsible for the stopping of information from being transferred to short term from long term memory specially due to alcohol [13]. While GABA-B is directly involved in learning and memory and synaptic plasticity [14]. **Endorphins The** two components having most important role in learning, memory of hippocampal basis are Dynorphins and Nociception [15]. It was reviewed that endorphins mediate learning process especially in association with stress [16]. **Dopaminergic System** Dopamine is a neurotransmitter so its role is much more than being precursor of nordadrenaline in brain [17]. Serotonergic System Deficiency of 5-HT in regions such as hippocampus can impair memory [18]. 5-HT interacts with its receptors and has a role in tasks of passive avoidance retention, and LTP [19].

ALZHEIMER DISEASE

Alzheimer. Alzheimer's disease is a progressive, degenerative, and irreversible neurological disease with no cure. Brain tissue shows "neurofibrillary tangles" (twisted fragments of protein within nerve cells that clog up the cell), "neuritic plaques" (abnormal clusters of dead and dying nerve cells, other brain cells, and protein), and "senile plaques" (areas where products of dying nerve cells have accumulated around protein. The correct balance of neurotransmitters is critical to the brain. Three neurotransmitters commonly affected by AD are acetylcholine, serotonin, and nor epinephrine. AD is characterized histologically by the presence of intercellular and extracellular amyloid deposits in the brain, together with widespread neuronal cell loss. The neuronal cell loss is found to be associated with inflammation and oxidative stress markers extracellular amyloid deposits are known as neutic of senile plaques, while amyloid deposits within and around blood vessels are referred to as congophilic amyloid angiopathy.APP gene is found on chromosome 21, and down syndrome, otherwise known as trisomy 21, is a well-known genetic causes of an early onset from of AD [20]. A number of factors that appears to play a major role in the development if this disorder (AD). The major genetic risk factor for AD is the possession of one or both E4 alleles of the apotipoprotein gene. Dietary risk factor includes a high cholesterol diet and high dietary fat intake [21]. High fat diets and obesity pose serious health problems, such as type II diabetes and cardiovascular disease. Impaired cognitive function is also associated with high fat intake. High fat diet is directly linked with the high deposition of cholesterol in body specially in brain that causes Alzheimer's Disease [22]. It has been identified as a protein misfolding disease due to the accumulation of abnormally folded Amyloid β protein in the brains of AD patients [23]. Amyloid β, is a short peptide that is an abnormal proteolytic by-product of the transmembrane protein amyloid precursor protein (APP), which is mainly involved in neuronal development [24]. High fat diet modulates the risk of developing AD as it increases the accumulation of β Amyloid.

MATERIAL AND METHODS

Drug collection-The fresh fruits of *Emblica officinalis* were collected from the local market as the fruits are easily available. After authentication the fruits were purchased from Himgiri Traders, Dehradun in bulk, from local market they were than dried in sun and turned in to coarsed powder by grinding with the help of a mechanical grinder.

Extraction of fruits-300 gm of powdered fruit was extracted with methanol in soxlet apparatus. After the complete extraction the extract was concentrated on a water bath and finally solvent was removed under pressure. The weight of extract was recorded.

Animals: -Wister rats of either sex weighing 40-60 gm considered as young were procured from Jeeva Life sciences, Registration Number CCSEA/IAEC/JLS/19/02/23/173**.** Animals were fed standard pallet diet supplied by Aashirwad industries, Punjab. The animals were housed, 12hours light and 12 hours' dark in the department animal house.

Morris water maze

Morris water maze was employed to evaluate learning and memory. It consisted of a circular water tank (diameter 150 cm and height 45 cm) and was filled with water up to 30 cm (at 25^{o}c). The tank was divided in to four quadrants with the help of two threads, fixed at right angles to each other on the rim of the pool. A platform (10cm^{2}) of 29cm, height was located in the centre of one of these four quadrants. The position of the platform and clues were kept constant through the training session. In the present study the target quadrant was Q4. Each animal was subjected to four trials each day with an interval of 5 mints, during which they were allowed to stand on the platform for 20 seconds. In case if the animal was unable to locate the hidden platform in 120 seconds, the animal was gently guided with hands to platform. Escape latency time to locate the hidden platform in water maze was noted as an index of acquisition. Rats were subjected to acquisition trial for four consecutive days. On the 5th day the platform was removed and the time spent by each animal in searching for the platform in each quadrant and Q4 was noted. This time spent by the animal in target quadrant and Q4 in search of missing platform was noted as an index of retrieval.

Acquisition trial

Each mouse was subjected to four consecutive trials each day (after 16 days of drug treatment). A rest interval of 5 mint was allowed in between each trial. Four trials trials per day were repeated for four consecutive days. Starting position for each day to conduct four-acquisition trials was changed as followed and Q4 was maintained as target quadrant in all acquisition trials

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

Mean escape latency time calculated each day during acquisition trial was used as an index of acquisition.

Retrieval trial

On 5th day the platform was removed. Each mouse was placed in the water maze and allowed to explore the maze for 120 seconds. Each mouse was subjected to four such trials each day starting from different quadrants. Mean time spent in target quadrant Q_4 was considered as the Index of retrieval, care was taken that the relative location of the water maze was not disturbed by other factors like changing of the background or any other changing visual clues during the total duration of study.

Submission of experimental protocol to IACE for approval

The experimental protocol was approved by Institution Animal Ethical committee

Estimation of blood glucose

Blood sample was collected from retro orbital plexus then; blood glucose was determined by the glucose oxidase peroxidase (GOD-POD). This diagnostic reagent kit (AGAPPE Diagnostics Kerala, India) is intended for simpler determination of glucose in serum/plasma/urinate samples. The intensity of the colour is directly proportional to the amount of glucose present in the sample and it is measured spectrophotometrically at 530nm.

Procedure

Table 1. Estimation of blood glucose

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

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

Calculation

$$Blood\ glucose = \frac{\text{Blood glucose}}{\text{OD standard}} \times n$$

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^{0}C, measure the absorbance of sample and standard against the reagent blank.

Cholesterol conc. = Absorbance of sample x 200
Absorbance of standard

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 retrograde (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 anterograde (Animals were kept on free access to standard food pallet chow diet and water, scopolamine was given for 4 consecutive days 30 minutes before the trial, and on 5th day it was replaced by normal saline). Group 4: Scopolamine anterograde + Standard (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). Group 5: Scopolamine retrograde + Amla (Animals were given standard food pallets chow diet and water, normal saline was administered firstly through i.p route followed by Amla through oral route after 15 mints for 4 consecutive days and on 5th day saline was exchanged with scopolamine, and then trials were performed 30 mints later). Group 6: High Fat diet (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 7: 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 8: High fat diet + Standard (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 AND DISCUSSION

The graph represents the time spent by animals treated with different medicaments in 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.

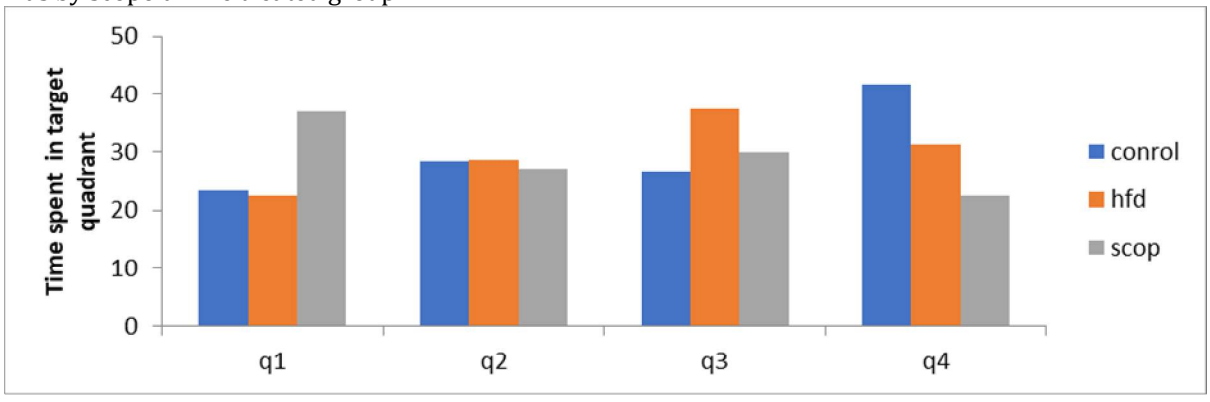

Figure 1: Time spent by drug treated animals in target quadrant

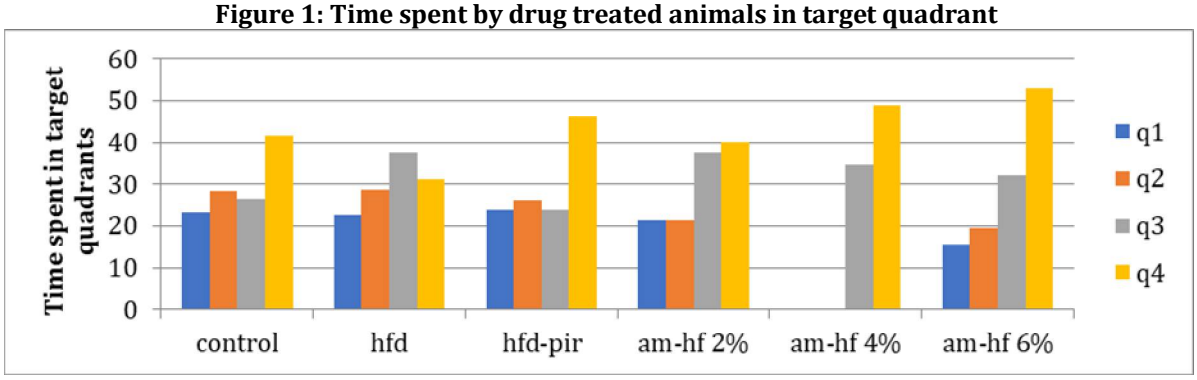

Figure 2: Highest and Lowest Time spent by drug treated animals in target quadrant

Figure 2 shows the highest time spent by control group in target quadrant 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.

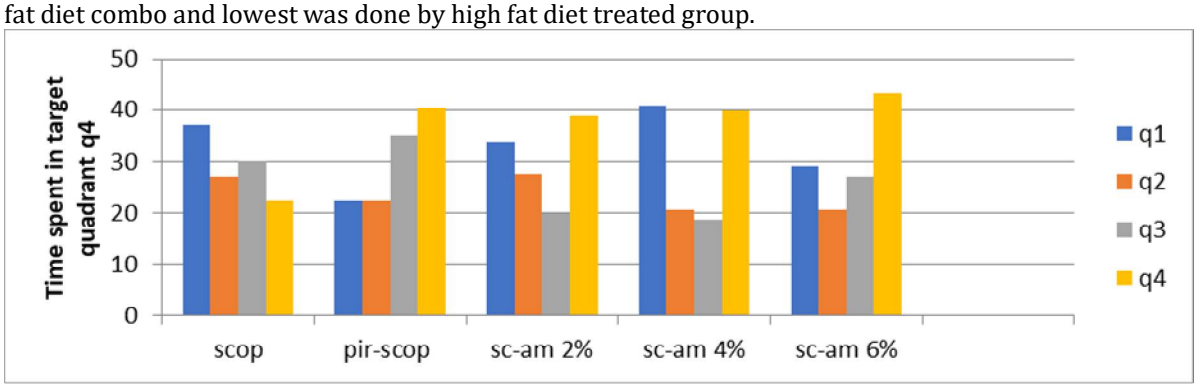

Figure 3: Time spent by drug treated groups in q4

The graph indicates highest time spent in the target quadrant was by scopolamine and Amla treated group and lowest time spent in the target quadrant was by scopolamine treated group

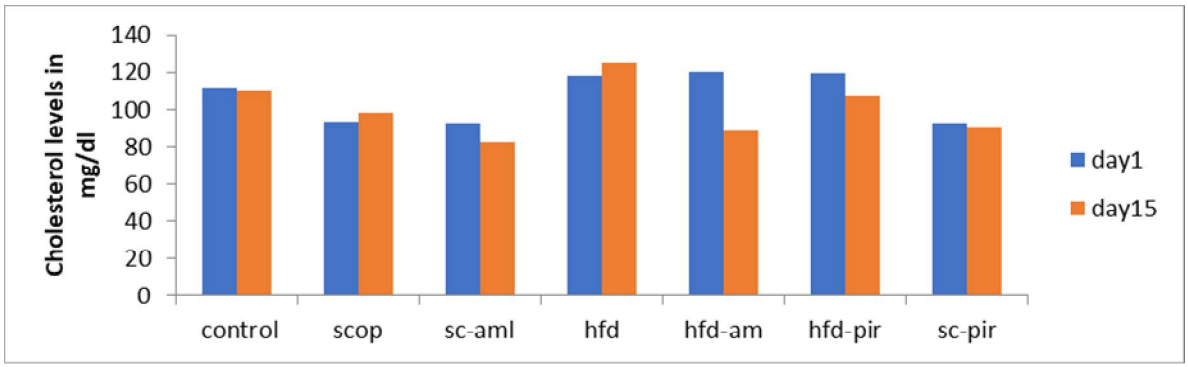

Figure 4: This figure shows the cholesterol levels of all the groups under study. The samples were taken on day 1 and day 15

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 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 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 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 scopolamine and Amla treated group and 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 showed improved memory functions because it has reduced the fat levels. This figure shows the cholesterol levels of all the groups under study. The samples were taken on day 1 and day 15. The study shows that there were not much changes in the cholesterol levels of the control group as the group was not treated by any drug while maximum difference was seened in high fat diet and Amla treated group. The group showed considerable decreased levels of Cholesterol on day 15 after being treated with Amla. This figure shows the glucose levels of all the groups under study. The samples were taken on day 1 and day 15. The study shows that there were not much changes in the Glucose levels of the control group as the group was not treated by any drug while maximum difference was seened in Scopolamine and Piracetam treated group. The group showed considerable decreased levels of Cholesterol on day 15 after being treated with Amla.

CONCLUSION

The above studies indicated that memory loss is prominent in groups treated with High Fat Diet and Scopolamine treated groups which was proved by lesser time spent groups in the target quadrant q4. Scopolamine being anticholinergic in action has a property of slowing the neuronal signalling, thus causing impaired memory functions and delayed learning. The study has showed that each group treated with High Fat Diet and Scopolamine showed loss in memory and impaired learning functions which was determined by spending lowest time in the target quadrants whereas group treated with Piracetam which is a nootropic and is used in Alzheimer's and Parkinson's disease related dementia and *Emblica officinalis* which is a potent antioxidant and rich source of vitamin C showed highest time recorded in the target quadrant giving clear result that *Emblica officinalis* is capable of curing the memory loss induced by high cholesterol deposition.

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