"Effect of ethanolic extracts on scopolamine induced Alzheimer in rats by spatial navigation task"

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ABSTRACT

Alzheimer's disease is a neurodegenerative disorder characterized by the presence of excessive amount of specific protein and loss of neurotransmitter. Traditional folklore remedy has constantly played a key role in the management of innumerable illnesses across the world. Meanwhile prehistoric times man has depend on the plant kingdom for the cure to ailments. Present study data reveals the memory enhancing property through spatial navigation task, there are medicinal plants were used namely Bacopa monniera , Centella asiatica, Melissa officinalis. Bacopa monniera has been utilized in the Ayurvedic arrangement of medication for quite a long time. Mostly, it was utilized as a cerebrum tonic for upgrading memory improvement, learning. Centella asiatica is one of the central herbs for treating skin issues, to mend wounds, for reviving the nerves and cerebrum cells, thus essentially known as a "Brain food" in India. Melissa officinalis, is a therapeutic plant just as most critical business plants amid the ongoing decades. It is utilized in customary medication for different illnesses like a plant juice, cream or tea mixture is utilized for anxious fomentation, and for advancing rest, and improves useful gastrointestinal grumblings, delirium etc. The main objective of this study is to perform a spatial navigation task of ethanolic extract of all three medicinal Plants in Morris water maze. In present investigation, memory performance was carried out in terms of latency.

Keywords*: Brahmi, Lemon balm, latency, Morris water maze, Alzheimer etc.*

INTRODUCTION-

Alzheimer's disease is named after Dr. Alois Alzheimer, a German doctor, (1907) he was noticed changes in the brain tissue of a woman who had died of an unusual mental illness. It is characterized by the presence of excessive amount of neuritic plaque containing amyloid β protein and abnormal tau protein filaments in the form of neurofibrillary tangle, loss of cholinergic cells, particularly in the basal forebrain is accompanied by loss of neurotransmitter acetylcholine.[1]

Since ancient times man has relied upon the plant kingdom for the cure to ailments. Traditional folklore medicine has always played a major role in treatment of various ailments across the world.[2] even in modern eras; the importance of traditional medicines cannot be overlooked. Often, many potent chemical compounds have been isolated from various plant parts. There are many compounds which, in use today, have been built on the structural backbone of a naturally occurring potent chemical.[3]

Centella asiatica L. has been utilized as a restorative herb for a great many years in India, China, Srilanka, Nepal and Madagascar. *Centella asiatica* is one of the central herbs for treating skin issues, to mend wounds, for reviving the nerves and cerebrum cells, thus essentially known as a "Brain food" in India. *Centella asiatica* (Linn.)Urban system equivalent word Hydrocotyle asiatica Linn., commonly known as Indian Pennywort, has a place with the family Apiaceae (recently known as Umbelliferae).[4]

Bacopa monniera (likewise alluded to as Bacopa monnieri, Herpestis monniera, water hyssop, and "Brahmi) has been utilized in the Ayurvedic arrangement of medication for quite a long time. Generally, it was utilized as a cerebrum tonic for upgrading memory improvement, learning, and fixation and to furnish alleviation to patients with nervousness or epileptic issue. [5,6]The plant has likewise been utilized in India and Pakistan as a heart tonic, stomach related guide, and to improve respiratory capacity in instances of bronchoconstriction. [7]

Melissa officinalis L. otherwise called lemon balm, honey balm, nectar salve, is a perennial herb having a place with Lamiaceae family. It is local to southern Europe and northern Africa, Caucasus and northern Iran, the Eastern Mediterranean area and Western Asia, just as tropical nations (Brazil). In Iran, this plant is known locally by the names Badranjbooye, Varangboo and Faranjmoshk. Lemon balm is a therapeutic plant just as most critical business plants amid the ongoing decades. It is utilized in customary medication for different illnesses like a plant juice, cream or tea mixture is utilized for anxious fomentation, and for advancing rest, and improves useful gastrointestinal grumblings, delirium, sadness, endless bronchial catarrh, headache, toothache, ear infection, migraine and hypertension and, remotely, for stiffness, nerve agonies and firm necks. In Unani arrangement of prescription its grass and seeds are utilized in it is utilized in epilepsy, loss of motion, ringers palsi, joint inflammation, Mastitis, halitosis, its syrup and distillate are made for various ailments.[8]

MATERIALS AND METHOD-

Collection and identification of plant material

The entire plant materials of, *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* were gathered from Agra, Uttar Pradesh (India) and recognized by Dr. SatendraYadav, A. K. College, Shikohabad, Firozabad, Uttar Pradesh (India). After authentication, the plant materials were gathered in mass, washed, shade dried and extricated with ethanol for 48 hrs in a Soxhlet assembly.

Preparation of plant extract

The entire plant materials were washed altogether under running tap water pursued by sterile distilled water and dried under shade. They were ground into coarse powder by utilizing mechanical pulveriser. The powder, around 100 g, were weighed and more than once extracted with ethanol in a 500 mL round base flask containing 250 ml solvent independently. The reflux time for solvent was fluctuating with 25 to 40 cycles for complete extraction in soxhlet mechanical assembly .[9,10]

Animal Experimentation

Animals-

Young male wistar rat (180– 230 g) acquired from IVRI, Izatnagar, Bareilly (U. P.), and the animal experimentation was performed in Teerthanker Mahaveer College of Pharmacy, Moradabad (U. P.) India. Animals were acclimatized to research centre conditions at room temperature before experimentation. Following medical procedure, animals were held under standard states of a 12-hour light/dim cycle with sustenance and water not obligatory in gatherings of 2, in plastic cage with delicate bedding. All experiments were done between 9.00 AM and 3.00 PM. The trial convention was endorsed by the Institutional Animals Ethics Committee and the consideration of lab animal was taken according to the rules of CPCSEA, Ministry of Forests and Environment, Government of India.

Acute toxicity studies

Acute oral toxicity studies were performed by OECD-423 rules. Young male wistar rat chosen by random sampling strategy were utilized in this examination. The animals were fasted for 4 h with free access to water as it were. The plant extract was regulated orally at a portion of 5 mg/animal at first and mortality was watched for 3 days. In the event that the mortality was seen in 2/3 or 3/3 animal, at that point the portion regulated was considered as a dangerous portion. Be that as it may, if the mortality was seen in just a single rat out of three animals then a similar dose was repeated to affirm the toxic effect. On the off chance that mortality was not watched, the method was then repeated with higher dosages, for example, 50, 100 and 2000 mg/kg.

Experimental design

Male wistar rats were randomly divided into seven groups (n = 6 per group) .The portions of concentrates were chosen dependent on the past examinations in the lab and those detailed in the literature. [11]

Spatial Navigation Task

The acquisition and retention of a spatial navigation task was assessed by utilizing Morris Water Maze. [12] Animals were prepared to swim toward a noticeable stage in a roundabout pool (180 cm in diameter and 60 cm in height) situated in a test room. On a fundamental level, rats can escape from swimming by climbing on to the stage and after some time the rats clearly get familiar with the spatial area of the stage from any beginning

position at the outline of the pool. In this way the stage offers no local cues to direct the escape behavior of the rats. The main spatial signs are those outside of the tank basically the viewable signs. The pool was loaded up with water (28±2◦C) to a height of 40cm, a movable roundabout platform (9 cm diameter), mounted on a segment, and was put in a pool 2 cm over the water level during the acquisition phase. A similar stage was set in the pool 2 cm beneath the water level for the maze retention phase. The water was made opaque by including a nontoxic color. Four similarly dispersed locations around the edge of the pool (N, S, E, and W) were utilized as beginning points and this partitioned the pool into four equivalent quadrants.

(1) *Maze acquisition phase (training)*- Animals got training session comprising of 4 preliminaries on day 13. In each of the 4 preliminaries, the beginning position was different. A preliminary started by releasing the animal into the maze facing towards the wall of the pool. The latency to find the escape stage was recorded to a maximum of 90 seconds. On the off chance that the rat did not escape onto the stage inside this time, it was guided to the stage and was permitted to stay there for 20 seconds. The time taken by rat to achieve the stage was taken as the initial acquisition latency (IAL).

(2) *Maze retention phase (testing for retention of the learned task)*- Following 24 hour (day 14) and 8 days (day 21) after IAL, the rat was released randomly from one of the edges confronting the wall of the pool. The time taken to locate the hidden stage was recorded and named as first retention latency (first RL) and second retention latency (second RL) on day 14 and day 21 following central administration of scopolamine, separately.[13]

Statistical Analysis

Values are communicated as mean ± SEM. The behavioral assessment data were analysed by a repeated measures two-way ANOVA with drug-treated groups as between and sessions as the within subjects factors. The esteem P < .05 was viewed as significant.

RESULTS AND DISCUSSION-

a. Effect of *Centella asiatica* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats.

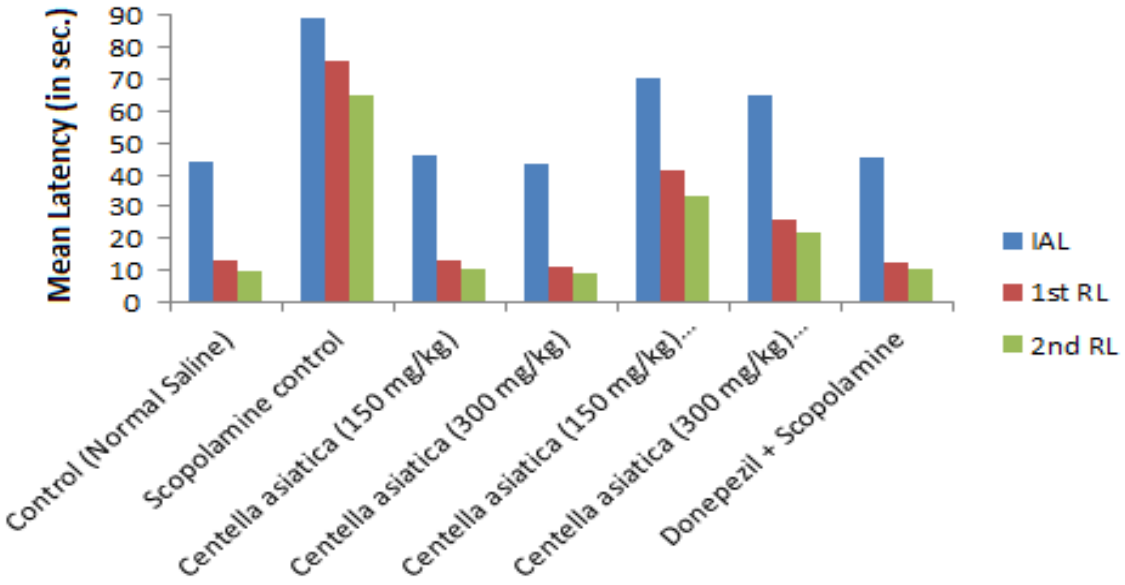

Figure 1: Effect of *Centella asiatica* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats.

In this study it was observed that control group of animals quickly learned to swim directly to the platform in the Morris water maze on day 13. Scopolamine-treated rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 13. *Centella asiatica* (150 and 300 mg/kg, PO) group of rats was also performed similarly during the acquisition of a spatial

navigation task on day 13. In contrast, *Centella asiatica* (150 and 300 mg/kg, PO) treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to scopolamine treated rats on day 13 following scopolamine administration. Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform were significantly decreased in control rats on days 14 and 21, respectively, as compared to IAL on day 13 following colchicine administration. On the contrary, the performance in the scopolamine-treated rats was changed after initial training in the water maze on days 14 and 21, with significant increase in mean retention latencies compared to IAL on day 13. The results suggest that scopolamine caused significant cognitive impairment. However, chronic *Centella asiatica* treatment (150 and 300 mg/kg, PO) starting before scopolamine administration showed a significant decline in the1st and 2nd RL as compared to scopolamine-treated rats on days 14 and 21, respectively, following scopolamine administration and improved the retention performance of the spatial navigation task. It was also found that the effect of *Centella asiatica* (150 and 300 mg/kg, PO) on the said is treatment less when compared with standard drug donepezil.

b. Effect of *Bacopa monnieri* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats.

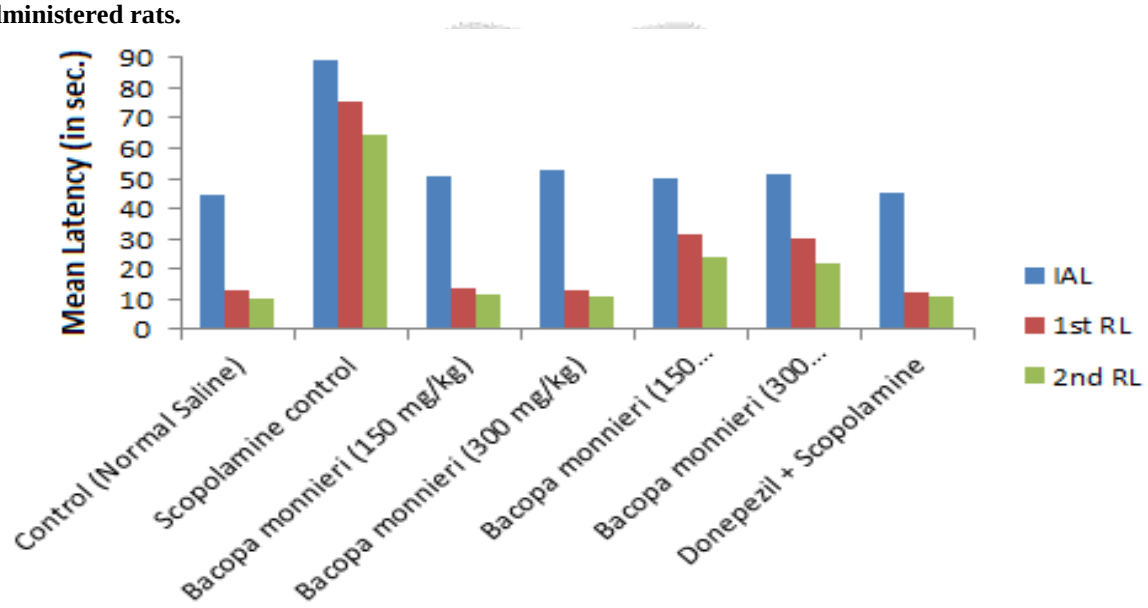

Figure 2: Effect of *Bacopa monnieri* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats.

In this study it was observed that control group of animals quickly learned to swim directly to the platform in the Morris water maze on day 13. Scopolamine-treated rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 13. *Bacopa monnieri* (150 and 300 mg/kg, PO) group of rats was also performed similarly during the acquisition of a spatial navigation task on day 13. In contrast, *Bacopa monnieri* (150 and 300 mg/kg, PO) treatment significantly decreased the IAL to reach the platform in the pretrained rats as compared to scopolamine treated rats on day 13 following scopolamine administration. Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform were significantly decreased in control rats on days 14 and 21, respectively, as compared to IAL on day 13 following colchicine administration. On the contrary, the performance in the scopolamine-treated rats was changed after initial training in the water maze on days 14 and 21, with significant increase in mean retention latencies compared to IAL on day 13. The results suggest that scopolamine caused significant cognitive impairment. However, chronic *Bacopa monnieri* treatment (150 and 300 mg/kg, PO) starting before scopolamine administration showed a significant decline in the 1st and 2nd RL as compared to scopolamine-treated rats on days 14 and 21, respectively, following colchicine administration (Table) and improved the retention performance of the spatial navigation task. It was also found that the effect of *Bacopa monnieri* (150 and 300 mg/kg, PO) on the said is treatment less when compared with standard drug donepezil. It was also observed that similar effects were seen in the groups which are treated with *Bacopa monnieri* 150 mg/kg and 300 mg/kg, respectively.

c. Effect of *Melissa officinalis* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats.

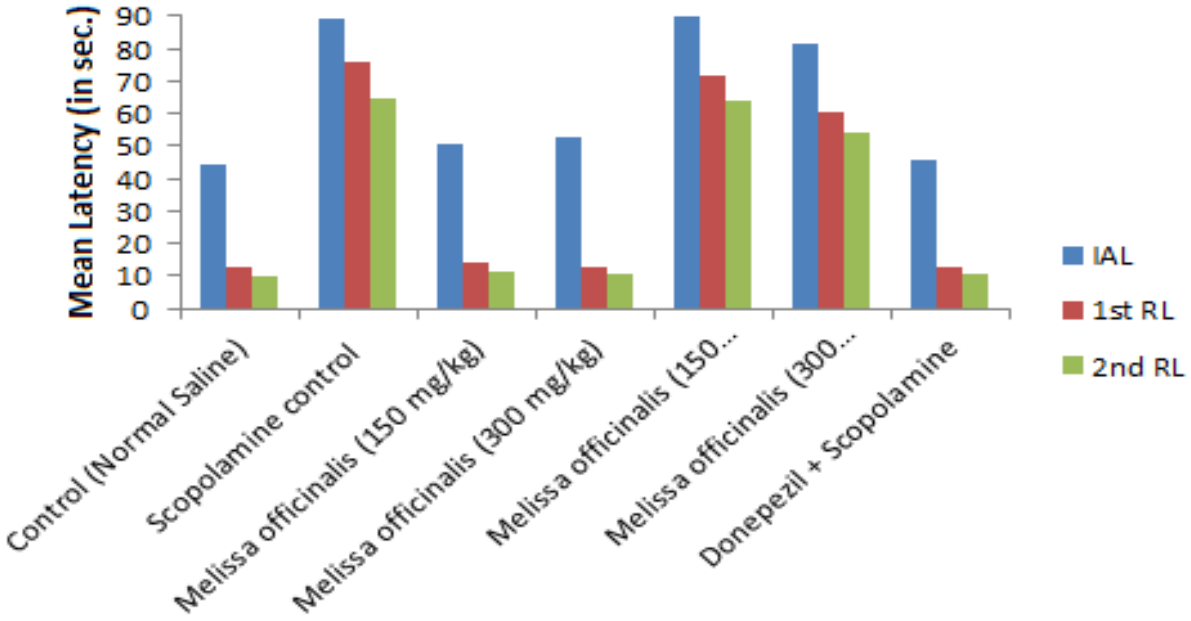

Figure 3: Effect of *Melissa officinalis* (150 and 300 mg/kg, PO) on spatial navigation task in scopolamine administered rats

In this study it was observed that control group of animals quickly learned to swim directly to the platform in the Morris water maze on day 13. Scopolamine-treated rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 13. *Melissa officinalis* (150 and 300 mg/kg, PO) group of rats was also performed similarly during the acquisition of a spatial navigation task on day 13. In contrast, *Melissa officinalis* (150 and 300 mg/kg, PO) treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to scopolamine treated rats on day 13 following scopolamine administration (Table). Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform were significantly decreased in control rats on days 14 and 21, respectively, as compared to IAL on day 13 following colchicine administration. On the contrary, the performance in the scopolamine-treated rats was changed after initial training in the water maze on days 14 and 21, with significant increase in mean retention latencies compared to IAL on day 13. The results suggest that scopolamine caused significant cognitive impairment. However, chronic *Melissa officinalis* treatment (150 and 300 mg/kg, PO) starting before scopolamine administration showed a significant decline in the 1st and 2nd RL as compared to scopolamine-treated rats on days 14 and 21, respectively, following colchicine administration (Table) and improved the retention performance of the spatial navigation task. It was also found that *Melissa officinalis* (150 and 300 mg/kg, PO) on the said is treatment less when compared with standard drug donepezil. It was also found that the performance of *Melissa officinalis* (150 mg/kg, PO) was less as compared to *Melissa officinalis* (300 mg/kg, PO) in the scopolamine-treated rats.

The comparative study of ethanolic extracts of *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* were performed by Moris Water Maze test. *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to scopolamine treated rats on day 13 following scopolamine administration. The mean ITL on day 13 for each rat utilized for *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* was relatively unstable and showed significant variation among different groups. The mean duration of initial transfer latency, 1st retention transfer latency and 2nd retention transfer latency for all the groups treated with *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* extracts were recorded. The duration was decreased with *Bacopa monnieri*, *Centella asiatica* and *Melissa officinalis* significantly ($p<0.001$).

CONCLUSION-

The present study demonstrates the comparative study of anti-Alzheimer activity of *Centella asiatica*, *Bacopa monnieri* and *Melissa officinalis. It was concluded that Bacopa monnieri, Centella asiatica & Melissa officinalis* extracts did not produce any mortality even at the highest dose of 2000 mg per animal. Both the doses of 150

mg/kg and 300 mg/kg were found to be non-toxic and hence selected for further psychopharmacological and biochemical studies.

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