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# Impact of Rotatory Vestibular Stimulation and *Centella asiatica* on Spatial Learning and Memory in Wistar Albino Rats.

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**ABSTRACT**

There are several methods to enhance cognition via altering brain plasticity and the present study was undertaken to observe the effect of Rotatory vestibular stimulation and *Centella asiatica* on cognition in healthy Wistar albino rats. A total of 72 adult male Wistar albino rats were randomly assigned into four groups. For Group A (Control Group) neither Vestibular Stimulation, nor the *Centella asiatica* was administered, Group B (Vestibular stimulated group), Rotatory Vestibular Stimulation was given for 5 minutes in a Rotatory vestibular apparatus at a rate of 50 revolutions per minute in clock wise direction for 30 days, Group C (*CeA* alone) treated with 2mg/kg of *Centella asiatica* for 30days, Group D (*CeA*+Vestibular), treated with 2mg/kg of *Centella asiatica* followed by 5 minute of Rotatory Vestibular Stimulation for 30days. It has been concluded that *Centella asiatica* in combination of rotatory vestibular stimulation provides significant improvement in cognition than vestibular stimulation and *Centella asiatica* alone. However no reports are available on the combined action of both Rotatory vestibular stimulation and herbal extracts on brain plasticity, the present investigation was undertaken to evaluate the Cognition enhancement activity of combined Rotatory vestibular stimulation and *CeA* extract in rats and recommend further detailed study on combination of Rotatory vestibular stimulation and *Centella asiatica* to explore the mechanism of action of the interventions and therapeutic validity to recommend this intervention for treatment of cognitive disorders.

**Keywords:** *Centella asiatica*, Hippocampus, Learning and Memory, Rotatory Vestibular Stimulation

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## INTRODUCTION

Vestibular stimulation is one of most popular therapies applied not only as an intervention for learning disability in developmentally delayed children but also to enhance cognition. Various aspects of cognition, like spatial memory and spatial perception are linked to vestibular function. Vestibular stimulation contributes to cognitive functions through its extensive connections with hippocampus and various other areas of the brain [1]. Vestibular stimulation activates a large neural network, in various areas of brain and the neural networks shared in various cognitive processes might be co-activated and hence various vestibular stimulation techniques may be used for manipulating various cognitive tasks [2].Vestibular stimulation enhances hippocampal long-term potentiation via activation of cholinergic septohippocampal cells [3]. Vestibular stimulation reduces stress via providing postural security and balance, calming effects. It reduces abnormal muscle tone at slow speeds and increases alertness at high speeds and increases attention span and concentration. [4]. Hence the speed of vestibular stimulation is crucial as it causes both positive and negative impact on the organism. Earlier studies at our research centre provided preliminary evidence of vestibular enhancement of cognition in scopolamine induced amnesia model of Wistar albino rats [5]. Spinning or rotation results in vestibular stimulation, it may increases the release of hippocampal acetylcholine levels which in turn enhances hippocampal basal dendritic LTP [6]. Vestibular inputs are necessary for path integration [7]. Rotatory vestibular stimulation is a safe and noninvasive method of stimulating the brain areas related to cognition. Among the several thousand drug yielding plants, *Centella asiatica* is an important constituent of the Ayurvedic medicine [8]. The primary active constituent and the major bioactive compounds of this plant is triterpenoid compounds, saponins known as asiaticoside, madecassoside and madasiatic acid , oxyasiaticoside, centelloside, brahmoside, brahminoside, thankunoside, isothankunoside and related sapogenins. *Centella asiatica* extract is found to be very effective in such manipulations leading to dendritic alteration [9]. *Centella asiatica* has the potential to attenuate the age-related decline in cognitive function in healthy middle age and elderly adults [10]. *Centella asiatica* juice is found to be very effective to enhance the dendritic arborization [11]. The present study was undertaken to observe the combined effect Rotatory Vestibular Stimulation and *Centella asiatica* on cognition in healthy Wistar albino rats, to provide further evidence for therapeutic validity of vestibular stimulation.

## MATERIALS AND METHODS

**Animals**: A total of 72 adult male Wistar albino rats 120 ± 30 g, were used for the study and each group consists of 18 no. of rats (n=18). The rats were bred and maintained at the central animal research facility (Rodent house Register number: 496/01/a/CPCSEA) of the Little Flower Medical Research Centre (LFMRC), Angamaly. They were housed in groups in polypropylene cages in an acclimatized (25-27$^0$ c) room and were maintained on a 12 hr light /dark cycle. Food and water was given ad libitum. They were randomly assigned into four groups. Group A: Control group - Without any interventions. Group B - Rotatory Vestibular Stimulation was given for 5 minutes in a Rotatory vestibular apparatus at a rate of 50 revolutions per minute for 30 days. Group C - Treated with 2mg/kg of *Centella asiatica* for 30days. Group D- Treated with 2mg/kg of *Centella asiatica* followed by 5 minutes of Rotatory Vestibular Stimulation in a Rotatory vestibular apparatus at a rate of 50 revolutions per minute for 30days.

**Apparatus used for the study: Radial Arm Maze**: The behavioral experiments included in the study were Radial Arm Maze Task. The details of the procedure and apparatus used are same as described in the previous papers from our research centre [12]. However in the present study instead of score and error the numbers of trials taken for attaining the correct entries were recorded.

**Rotatory Vestibular Stimulation Instrument**: Rotatory Vestibular stimulation was applied by using a device, designed at our research centre. This instrument was made out of fiber frame with three fibre cages with it. The fibre cages were of about 15cm length and 10cm width. Only one animal can occupy comfortably in one cage without any entrapment stress. The device works on electricity and speed of rotation was fixed at 50 revolutions per minute by trial and error method.

**Experimental Design**: All the rats were subjected for Behavioral studies after 30days of rotatory vestibular stimulation and *CeA* administration, in Radial Arm Maze. The behavioral experiments were carried out in three phases, viz; Orientation and Training Session, Learning Performance Test (Acquisition Test), and Memory

Performance Test (Retention test).The rats were semi starved for 48 hrs before the start of behavioral experiments, conducted in the same room, with the same allocentric cues such as doors, windows, posters, and the experimenter. Experimenter always maintained same position throughout the whole of the experiment. During the three days of orientation the semi starved rats were allowed to familiarize themselves with the radial maze. After the orientation phase, the behavioral task was performed, where all the eight arms of the maze were baited with food pellets and then the rat was placed in the center of the maze and allowed to freely explore the maze. The rats were required or trained to take the food pellet from each arm without making a reentry in to the already visited arm. The training or trial was terminated when the animal takes the food reward from the all eight arms, or after 10minutes if all the eight arms were not visited. Six trials per day was given with an inter trial interval of 1hour. After acquisition phase all the trained rats were kept for consolidation of the learned task for 10 days. After 10 days of acquisition, the retention test was carried out until the rats attaining the learning criteria. For the assessment of learning and memory the no. of trails taken for attaining the task were recorded. For analyzing the Long Term Potentiation (LTP), the retention test was repeated for 7 times with 10 days of gap in between each test. Control group rats were under gone the same procedure of behavioral task without providing any drug or stimulation. Rats of Group B received Rotatory Vestibular Stimulation for 30 days before the beginning of the behavioral task and also 15 minutes prior to the start of acquisition phase as well as each retention test, the rats of Group C was administered with *CeA* orally for 30 days before the beginning of the behavioral task and also 15 minutes prior to the start of acquisition phase as well as each retention test. Rats of Group D were provided with vestibular stimulation after 15 minutes of *CeA* administration for 30 days continuously before the behavioral task and also before each acquisition and retention test.

**Ethical approval**: The present study was approved by Institutional Animal ethical committee of Little Flower Medical Research Centre in 2012.

**Neuromorphological analysis of Pyramidal Neurons for Dendritic Quantification:** From each group six rats were sacrificed after behavioral experiments, and processed for the neuromorphological analysis of the pyramidal neurons randomly from the Hippocampus. The animal was perfused after anesthetized with anesthetic ether and thereafter decapitated and the brain was shelled out and the hippocampus dissected and processed through Rapid Golgi staining method. Briefly, the tissues for fixed for 5 days in Golgi fixative and impregnated with 0.75% aqueous silver nitrate solution for 48hours, sections of 120µm thickness were taken with microtome, dehydrated, cleared and mounted with Distrin plasticizer xylene mounting media. Then 10 pyramidal neurons were randomly selected from Hippocampal area and traced using mirror type camera Lucida and the dendritic arborization was studied using Sholl Analysis method.

**Biochemical analysis – Analysis of Acetylcholine esterase activity:** 12 rats from each group were used for the analysis of acetyl cholinesterase activity. After dissecting out the brain, the hippocampus were isolated and processed to estimate the activity of acetyl cholinesterase by Elman et al (1961) method [13].

## RESULTS

Data was analyzed by one way anova and followed by Bonferroni Post Hoc Test in SPSS 20.0. The level of significance was fixed at 5% ($p<0.05$) and 1% ($p<0.01$) level

**Behavioral Analysis: Acquisition:** In acquisition, the mean number of the trials in learning of Group B, C, D, were decreased significantly (P value<0.001) when compared with Group A. From the result it is observed that the no. of trials taken for acquisition decreased significantly in Group B when compare with Group C (p value<0.01) .The number of trials taken for acquisition in Group D decreased far better than Group B and C (p value<0.01). From the result of acquisition it is clear that the Rotatory vestibular stimulation along with *CeA* (Group D) improves learning with a significant decrease in the number of trials taken for acquiring the learning criteria. Results are shown in Fig.1.

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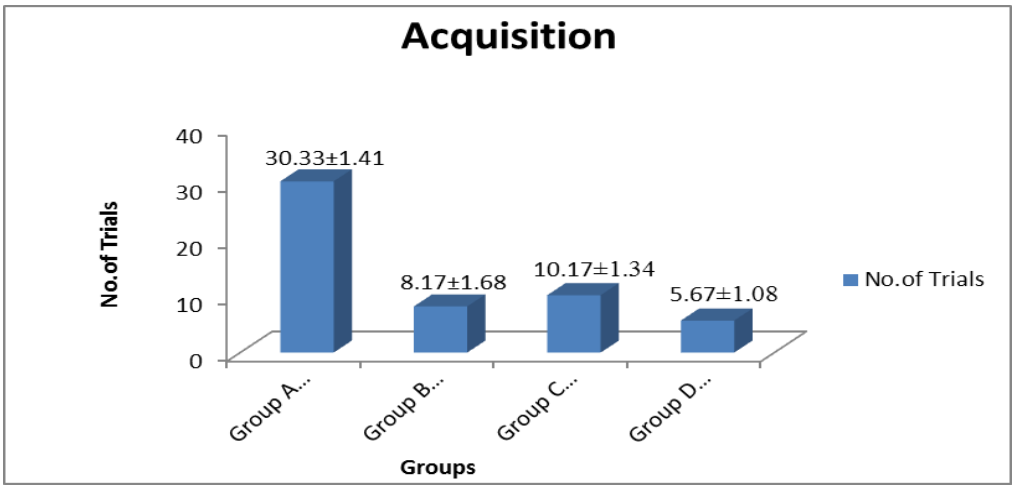


**Figure 1: No. of trails taken for acquisition in different treated groups comparing with control group. Data represented as Mean ±SD.**

R**etention:** All the treated rats showed a detectable decrease in number of trials taken when compared to the Control (Group A) (p value <0.01). Among the three treated groups, the Vestibular group (Group B), *CeA* group (Group C) and *CeA*+vestibular group (Group D) shows a better performance in retention on the 10$^{th}$ day with a non-significance decrease in number of trials respectively. Whereas, from the 20$^{th}$ day of retention onwards the Vestibular stimulated group shows a significant decrease in number of trials when compare with Group D (p value <0.01), and a non-significant decrease when compare with Group C. But on 60$^{th}$ and 70$^{th}$ day of retention *CeA* alone group shows much more better performance in memory than the other groups, however it shows a non-significant decrease in number of trials when compare with Group B. From the analysis it may be concluded that, either rotatory vestibular stimulation or the administration of *CeA* alone is good enough to enhance memory than combining both rotatory vestibular stimulation and *CeA* extract though it facilitates the enhancement of learning. Results are shown in Fig.2.

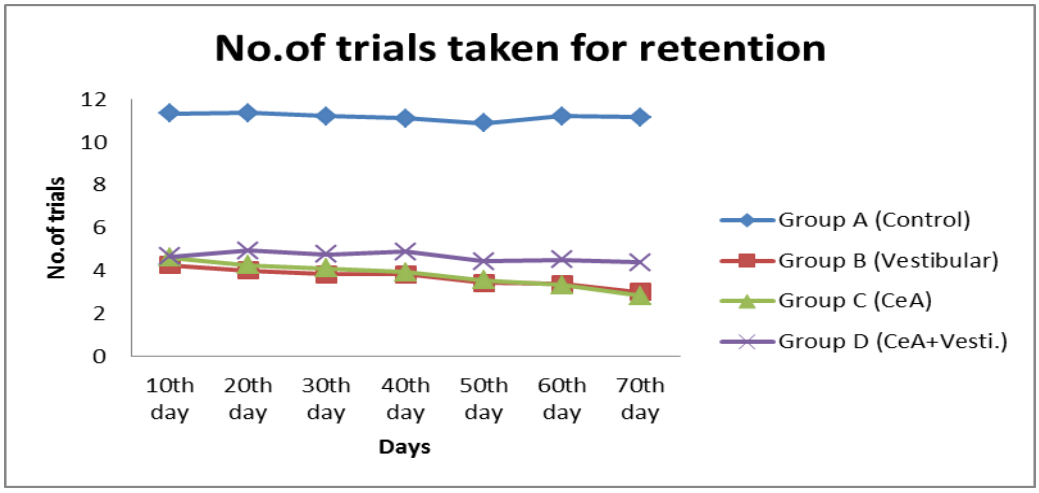


**Figure 2: No of trails in retention taken by different groups compare with control from 10$^{th}$ day to 70$^{th}$ day after acquisition in different groups. Data represented in Mean ± SD.**

**Neuromorphological Analysis**

**Dendritic Branching Points**

From the sholl analysis, it is clear that in every concentric circle the dendritic branching points significantly increased in all the treated groups when compared to Control (p value <0.01). In 0-20µm concentric circle, Group D (the rats treated with both Rotatory vestibular stimulation and *CeA*)shows a significant increase in branching points when compare with the other groups (p value <0.01). In the next

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concentric circle (20-40µm) Group B (Vestibular stimulated group) shows better result in number of dendritic branching points. In the next three consecutive circles, both Group B and D show equalency in number of dendritic branching points. Whereas in 100-120µm, *CeA*+Vestibular (Group D) shows a significant increase in dendritic branching points compare with the other groups (p value <0.01). There is a non significant increase in branching points between Group B and C. And it is concluded that both Rotatory vestibular stimulation alone and rotatory vestibular stimulation along with *CeA* enhances learning and memory via increasing the number of dendritic branching points in hippocampal pyramidal neurons. Results are shown in Fig.3.

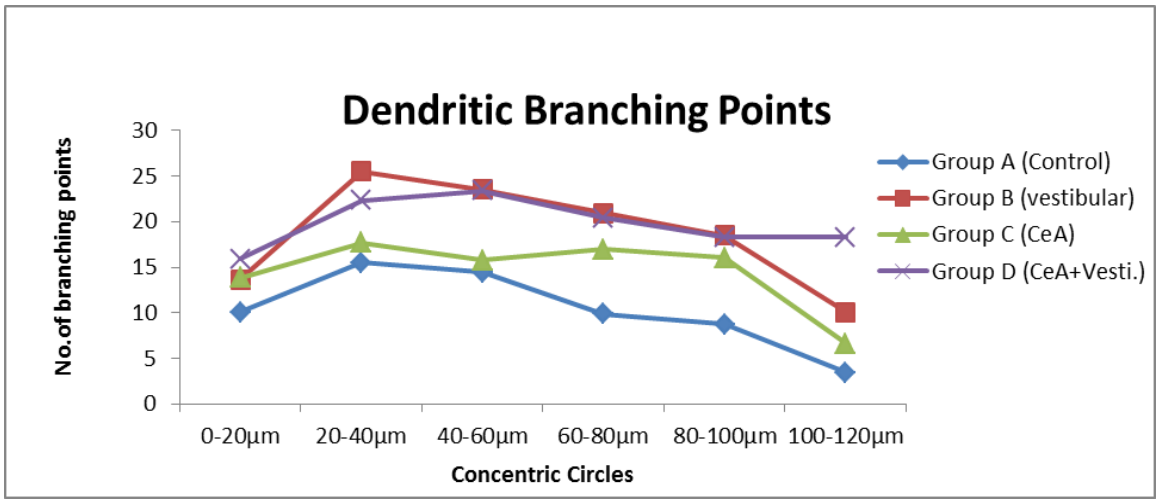


**Figure 3: No. of branching points of hippocampal pyramidal neurons of rats in different concentric circles. The values are shown in Mean+ SD.**

**Dendritic Intersections:** From the sholl analysis, it is clear that in every concentric circle the dendritic intersections significantly increased in all the treated groups when compared to Control group (p value<0.01). In 20µm concentric circle, in Group D (the rats treated with both Rotatory vestibular stimulation and *CeA)* shows an increase in dendritic intersection when compare with the other groups. In the next two concentric circle (40µm, 60µm), both the vestibular stimulated group (Group B) and vestibular stimulation along with *CeA* (Group D) shows more similarity in number of dendritic intersection. In 80µm, the number of dendritic intersection increased in Group D, Group C and Group B respectively. Whereas in 100µm, *CeA*+Vestibular (Group D) shows increased dendritic intersection, though there is no significant difference with Group B and C. And in 120µm concentric circle, *CeA*+vestibular group (GroupD) shows more increase in dendritic intersection. It is concluded that both rotatory vestibular stimulation and also rotatory vestibular stimulation along with *CeA* improves learning and memory via increasing the number of dendritic branching points in hippocampal pyramidal neurons, and than *CeA* alone. Results shown in Fig. 4.

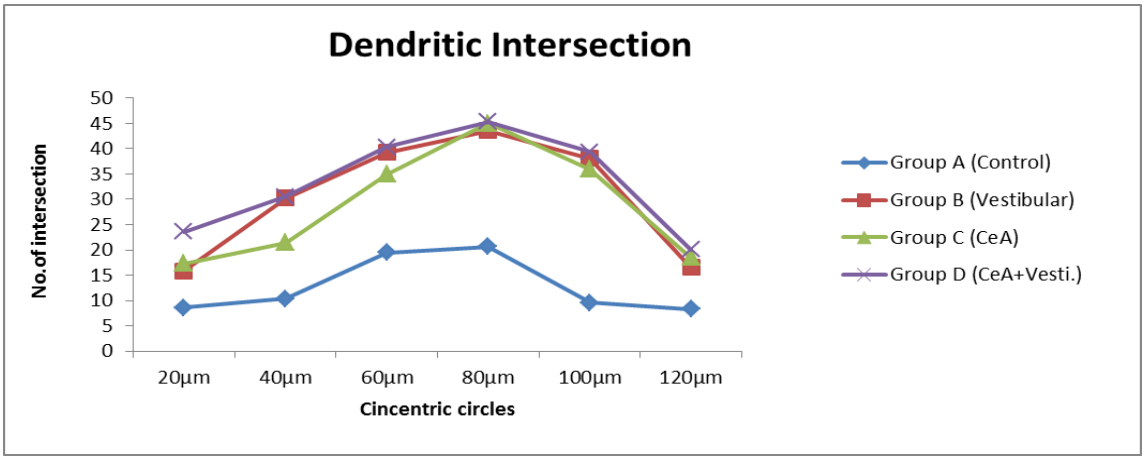


**Figure 4: Number of Intersections of hippocampal pyramidal neurons of rats in different concentric circles. The values are shown in Mean+ SD.**

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**Acetylcholinesterase level:**

From the analysis it is clear that, level of Acetylcholine esterase decreased significantly in Group D (*CeA*+Vestibular), (p value < 0.01),Group B and Group C respectively when compare with the control group and in turn it enhances learning and memory via facilitating the synaptic transmission through altering the level of Acetylcholine, the neurotransmitter. In addition, the action of rotatory vestibular stimulation and the administration of *CeA* alone also improve learning and memory through the same mechanism. Results are shown in Fig. 5.

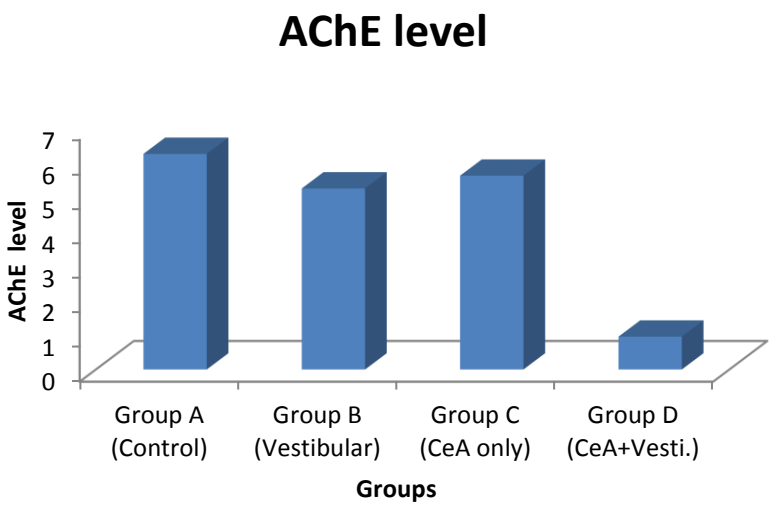
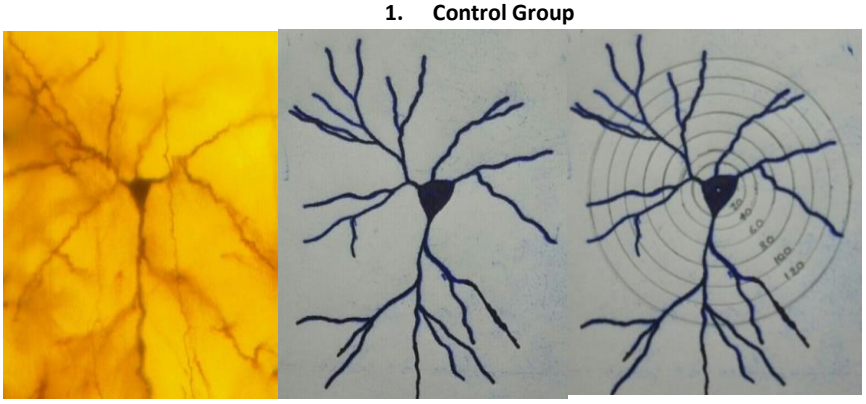


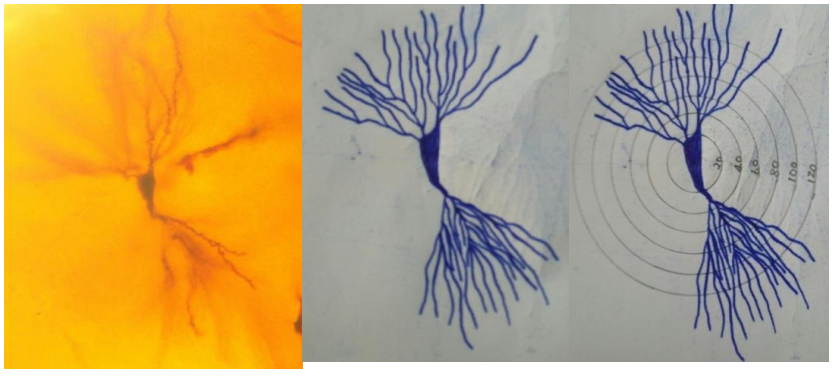

**Figure 5: AChE levels in different groups of rats. The values are shown in Mean+ SD**

**Microphotographs and Camera Lucida tracings of Hippocampal Pyramidal neurons from different groups of rats.**

**1. Control Group**



**2.Rotatory Vestibular Stimulated Group**



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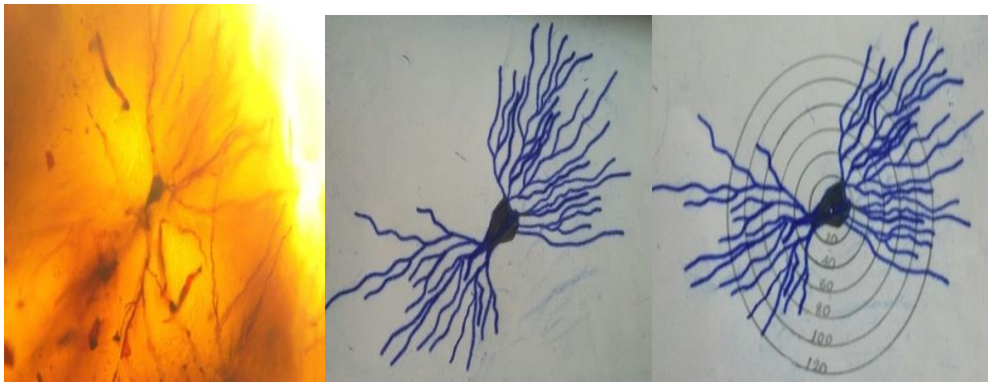
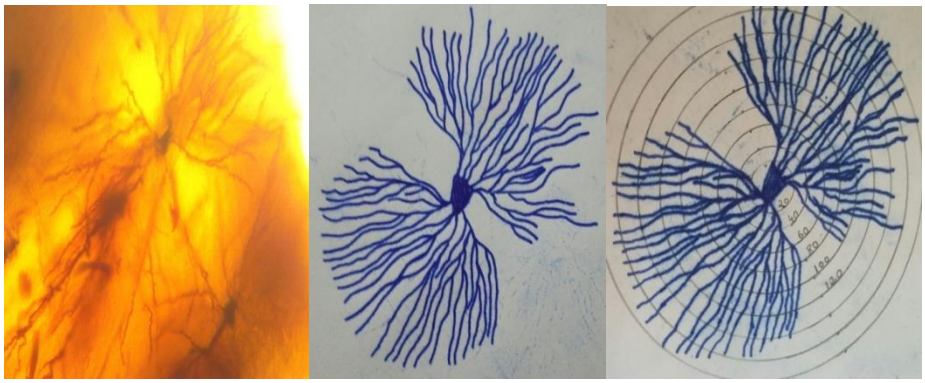
**3. *CeA* Group**



**4. *CeA*+Vestibular Group**



**Rotatory Vestibular Apparatus**

**Behavioral analysis in Radial Arm Maze**

## DISCUSSION

The long-term memory storage is attributed to the dendritic arbors, of the cholinoceptive cells of the cerebral cortex, hippocampus and amygdala. The high degree of plasticity is demonstrated by cholinergic terminals. Alternation in these afferent terminals affects the release of acetylcholine and also the sensory processing as well as memory retrieval. The permanent long-term memory is encoded by a set of new dendritic branches. New neurons provide new sparse codes for encoding new information, and then, the older memories are preserved, as they are represented by older neurons, and this facilitates the formation of new memories while avoiding catastrophic interference, saving older memories. The history of vestibular research dates back atleast 400 years [14]. Vestibular stimulation is a very effective and reliable approach for treating attention deficit or hyperactivity disorder particularly when combined with other training [15]. Vestibular system is intrinsically highly convergent with other sensory and motor signals which interact with various cognitive processes like spatial navigation [16], attention, memory [17], and social cognition [18]. There are many standardized vestibular stimulation equipments like, caloric, galvanic and vestibular evoked myogenic

potential devices. But these techniques stimulate the vestibular organ in an unnatural and non physiological way. Bhatara et al, in 1981 conducted an exploratory cross over study in 4 to 14 years aged children with hyper kinetic attention deficit, using rotator stimulation of the semicircular canals using swivel chair twice weekly for 4 weeks by rapid acceleration of 33 rpm, and found significant improvement in their attention span [19].Here in this experiment we used rotational vestibular stimulation as the horizontal semicircular canals activated by rotation. Custom- built rotatory chairs stimulate especially the horizontal semicircular canals when the participant is in upright sitting position, with the center of the head passing through the chair rotation axis, and the vertical semicircular canals may also be stimulated. Vestibular stimulation exerts certain influence on various processes of learning and memory through otolithic and visual stimulation [20]. Performance in Radial Arm Maze shows a dose dependence increase in learning enhancement in *C. asiatica* treated rats. *C. asiatica* possess many medicinal properties, memory improving effects in normal rats [21, 22] .The observed decrease in the number of trials taken for acquisition and retention performance in radial arm maze of the *CeA* treated rats compared to the control, indicates increase learning and memory efficiency of the treated rats. This result of the present study is supported by the early finding that treatment with fresh leaf extract of *Centella asiatica* (2ml/kg) improved learning and memory in neonatal mice [21]. The predominant integrative centre involved in the regulation of exploratory activities and incorporation of spatial information is the Hippocampus of the brain [23]. The learning process and the dendritic morphology of amygdaloid and hippocampal neurons are closely associated. Increase in spine density and dendritic complexity in various brain structures may closely associated with the exposure to enriched environment via making more synapses [24]. And as a result new spines appear on post-synaptic dendrites, after the formation of long-lasting functional enhancement of synapses in the hippocampal area, whereas in control regions on the same dendrites no significant spine growth occurs. Thus the present study revealed that dendritic structural reorganization may also be the key feature in learning and memory. The additional dendrites on the neurons resulted in the formation of new synapses that may facilitate more rapid and effective conduction of impulse is one of the reasons for the enhanced learning and memory [25]. The facilitation of acquisition and performance in the spatial learning task is associated with an increase in the dendritic arborization and synapses in the hippocampal pyramidal neurons [9]. This suggests that dendrites are the major determinants of neuron integration and processing of incoming information and thence play a vital role in the functional properties of neuronal circuits and the functional organizations of various brain regions are dynamic and can change in response to experimental manipulations, which may results cause changes in synaptic function, neuronal membrane properties and axonal trajectories [3]. The neuroprotective effect and multiple active fractions of *Centella asiatica* accelerates nerve regeneration and also increased neuronal arborization and thereby accelerating repair of damaged neurons [26].

In the present study *C. asiatica* administration significantly increased the dendritic arborization in hippocampal pyramidal neurons. The increased dendritic arborization inturn increases the number of synaptic connections with the neurons which may be the neural basis for the better learning and memory in treated rats, as reflected in the Radial Arm Maze performance. Although the exact mechanism of this nootropic effect is not understood, the involvement of stimulation of neurosecretory activity of the cholinergic neurons is very clear. Vestibular stimulation alters the level of acetylcholine release in the hippocampus to facilitate the long term potentiation in the hippocampus and this may depends on the activation of septohippocampal cholinergic neurons via providing single stimulation train (100 pulses at 200 Hz) during passive whole-body rotation or during awake immobility and it was proved that LTP singnificantly enhanced during rotation than induced during immobility [27]. Caloric vestibular stimulation enhances hippocampal Long Term Potentiation by stimulating acetylcholine secretion from septohippocampal cells [28]. Though the neurotransmitters not only facilitates the transmission of brain impulses from one neuron to another throughout the body, but it also bridge the synaptic gaps and thereby make it easier for the brain to communicate to the rest of the body, the overabundance or deficiency of any neurotransmitter leads to various health problems. An overabundance of a particular biochemical can flood the synaptic cleft and a deficiency will interrupt the brain signal getting to the part of the body that needs information. The neurotransmitter acetylcholine level in the hippocampus increase during learning and during performance of a learned spatial memory task and the better performance in the radial arm maze is positively correlated with the increased Ach level in the hippocampus [29]. In developing rats choline supplementation increases the efficiency in cognitive functions by altering the hippocampal long term potentiation [30]. Oral administration of *C.asaitica* increases the ACh level in the brain and the acquisition of behavior is strongly related to the up-regulation of the neurotransmitter ACh level. AChE is the enzyme specific for acetylcholine and is inhibited by high acetylcholine concentrations. The inhibition of this enzyme results in increase in local acetylcholine concentrations, and this mechanisms forms the basis of

pharmacologic, therapeutic and toxicologic properties of drugs that inhibit AChE. Acetylcholinesterase is the enzyme with very high catalysis rate, is synthesized in the neuronal cell body and distributed throughout the neuron by axoplasmic transport. Cholinergic neurotransmission is altered by effective drugs that inhibit breakdown of acetylcholine as AChE hydrolyses the Acetylcholine to terminate synaptic transmission [31]. The experimental manipulations used in the present study also may alter the activity of neurotransmitters involved in various cognitive functions, like learning and memory.

## CONCLUSION

We conclude that Rotatory vestibular stimulation in combination of *Centella asiatica* provides significant improvement in cognition than vestibular stimulation and *Centella asciatica* alone. Hence we recommend further detailed study on combination of Rotatory vestibular stimulation and *Centella asiatica* to explore the mechanism of action of the interventions and therapeutic validity to recommend this intervention for treatment of cognitive disorders.

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