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Research article

Impact of rotatory vestibular stimulation and *Myristica fragrans* on spatial learning and memory in wistar albino rats

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Abstract

The altered dendritic length and de novo formation of new dendritic branches in cholinoceptive cells are responsible for learning and long term memory storage, a process enabled by the central cholinergic pathways. Living in an enriched environment and also physical activities may stimulate hippocampal neurogenesis in adult mice. Acetyl cholinesterase an enzyme that inactivates the neurotransmitter acetylcholine and the present study was undertaken to find out the enhancement of cognition in rats with Rotatory Vestibular Stimulation (RVS), and treatment with *Myristica fragrans* via estimating the dendritic arborization and acetylcholinesterase activity. A total of 72 adult male Wistar albino rats were randomly assigned into four groups. For Group A neither Vestibular Stimulation, nor *Myristica fragrans*was administered, Group B, 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 treated with 2mg/kg of *Myristica fragrans*for 30days, and Group D, treated with 2mg/kg of *Myristica fragrans* followed by 5 minute of Rotatory Vestibular Stimulation for 30days in a rotatory vestibular apparatus at a rate of 50 rpm in clock wise direction for 30days. It has been concluded that*Myristica fragrans* in combination of Rotatory Vestibular Stimulation provides significant improvement in cognition than vestibular stimulation and *Myristica fragrans* alone. From the analysis it is clear that learning and memory has improved via altering the dendritic arborization and level of AChE Hence we recommend further detailed study on combination of rotatory vestibular stimulation and *Myristica fragrans*to explore the exactmechanism of actionunderlying in this behavioral change and the therapeutic validity for treatment of cognitive disorders.

Key words: *Myristica fragrans, Hippocampus, Learning and Memory, Rotatory Vestibular Stimulation*

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1. Introduction

In the developing countries most of the people rely on phytomedicine for primary health care for man and livestock, as herbal products contain complicated

mixtures of organic chemicals, such as, fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes etc and the therapeutic value of a plant from the synergistic effects of the various components of the plants in contrast to the individual chemicals of conventional medicines isolated in laboratories by the pharmacologists and it is believed that the traditional medicines are most effective with less or no side effects [1]. Though brain possesses a large potential oxidative capacity, it has a limited ability to counteract oxidative stress, which leads to neuronal cell injury in various pathological states of the brain including neurodegenerative disorders. Brain consumes about 20% of the oxygen available through respiration, and became of its high oxygen demand the brain is the most susceptible organ to oxidative damage [2]. Antioxidants are very effective in defense against free radicals. This defense system can be activated or modulated by nutritional antioxidants such as polyphenols, flavonids, terpenoids, and fatty acids, where as plant derived alternative antioxidants (AOX) are effective in controlling the effects of oxidative damage [3-4]. Phytochemical based antioxidants are neuroprotective by reducing or reversing cellular damage and also slow down the progression of neuronal cell loss. The long-term memory storage mainly occurs in dendritic arbors of the cholinoceptive cells of the cerebral cortex, hippocampusand amygdala [5]. The dendrites of cholinoceptive cells, which prominently involved in memory, undergo restructuring during memory formation [6].

Myristica fragrans (nutmeg) is a well known spice belongs to the family Myristiacea and the nutmeg oil in recommended and restricted amount stimulates the brain and relieves stress, stimulates the mental activity, improves concentration power, and also increases better blood circulation in brain as it consists of cellular and solid matter, fixed oils, volatile oils and phenolic compounds which possess the antioxidant properties [7]. The effects of nutmeg on the CNS is variable and reflect anticholinergic and CNS excitatory effect and the comparative brain cholinesterase inhibiting activity of *Myristica fragrans* in the central cholinergic pathways play a prominent role in the learning and memory processes [8].

Environmental enrichment effects changes the ability to acquire motor skill. Acrobatic Skill learning in the rat leads to increased synapse numbers [9]. Various stimuli like visual, auditory, olfactory, and tactile produced an increased level of Ach in the hippocampus [10]. There is a close relationship between stimulation and cholinergic response, that in a paired tone and light stimulus significantly increased Ach release in the frontal cortex and hippocampus, when it was presented for the first time and no further increase in Ach release and behavioral response occurred if the tone and noise stimuli were presented repeatedly over an 8-day period leading to habituation development [11]. The vestibular system is a contributor to our balance system and our sense of spatial orientation is the sensory system that provides the dominant input about movement and equilibrioception and the vestibular sense provides information related to head position and movement, and is very important for the development of balance and co-ordination of our body and attention and various studies on spatial navigation suggest that there is a link between spatial memory and vestibular

function [12]. The acetylcholinesterase is a major component of the central and peripheral nervous system and its main function is to terminate synaptic transmission by hydrolyzing the neurotransmitter acetylcholine [13]. Vestibular Stimulation induces acetylcholine release in the hippocampus and it is known to facilitate long term potentiation (LTP) in the hippocampus, via the activation of septohippocampal cholinergic neurons and also via inhibiting AChE activity [14].

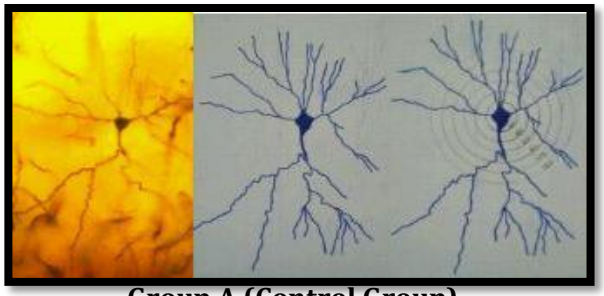
Acetylcholinesterase, the enzyme that hydrolyzes the neurotransmitter acetylcholine plays significant role in the pathology of Alzheimer's disease [15- 16]. Enhancement of acetylcholine level in the brain using AChE inhibitors is the most effective approach in the treatment of cognitive disorders [17]. AChE activity can be inhibited by various compounds. Toxins and drugs are two main types of inhibitors, which inhibits AChE activity [18]. Memory is the ability of an individual to record sensory stimuli, events and information and to retain them over short or long periods of time and recall the same at the time when needed. Age, stress, emotions are conditions which may lead to memory loss, amnesia, anxiety, high blood pressure, dementia and Alzheimer's disease. Antioxidants reduce oxidative stress and there by facilitating neuro transmission with anticholinesterases. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive function. This serves as a rationale for the use of acetyl cholinesterase inhibitors for the symptomatic treatment of cognitive disorders as well as improvement of learning and memory. Repeated exposure to enriched environments has been shown to increase the density of spines and dendritic complexity in certain brain structures. The increased numbers of dendritic branching points and intersections of hippocampal neurons may have profound effects on behavior because of the additional dendrites that are available on these neurons for the formation of new synapses. The increase in dendritic arborization increases the number of possible synaptic connections with the neurons. This may be the neural basis for the improved learning and memory. The study aims to evaluate the neuromorphological changes and acetylcholinesterase inhibitory ability of both rotatory vestibular stimulation and extracts of *Myristica fragrans* in rats that leads to the enhancement of cognition.

2. Materials and Methods

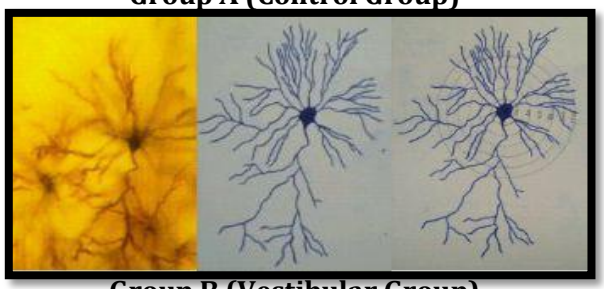
Animals

A total of 72 adult male Wistar albino rats were used for the study. 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.

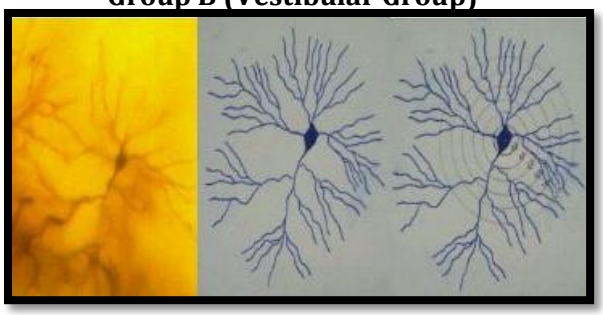
Group A: (n=18) Control group (CG) - Neither Vestibular Stimulation, nor Nutmeg was administered. Group B: (n=18) (RVSG) - Rotatory Vestibular Stimulation was given for 5 minutes in a Rotatory Vestibular apparatus at a rate of 50 revolutions per minute for 30 days. Group C: (n=18) (Nutmeg Group) - Treated with 2mg/kg of *Myristica fragrans* for 30days. Group D: (n=18) (Nutmeg+ RVS) Treated with 2mg/kg of *Myristica fragrans* followed by 5 minute of Rotatory Vestibular Stimulation at a rate of 50 rpm for 30 days.



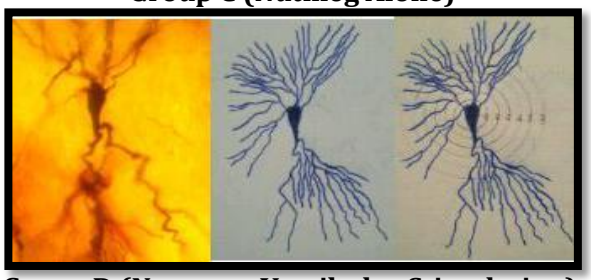
Group A (Control Group)



Group B (Vestibular Group)



Group C (Nutmeg Alone)



Group D (Nutmeg+ Vestibular Stimulation)

Microphotograph and Camera lucida tracing of hippocampal pyramidal neurons in different groups of rats

Drug

Nutmeg in ground form (whole nutmeg) were dried and minced with distilled water (2ml/gm) and used for oral administration to rats.

Apparatus used for the study

Radial Arm Maze

The behavioral experiments included in the study were the Radial Arm Maze Task. The details of the procedure and apparatus used are same as described in the previous papers from our research centre [19]. However, in the present study instead of score and error the number of trials taken for attaining the correct entries was recorded for the analysis of acquisition and retention.

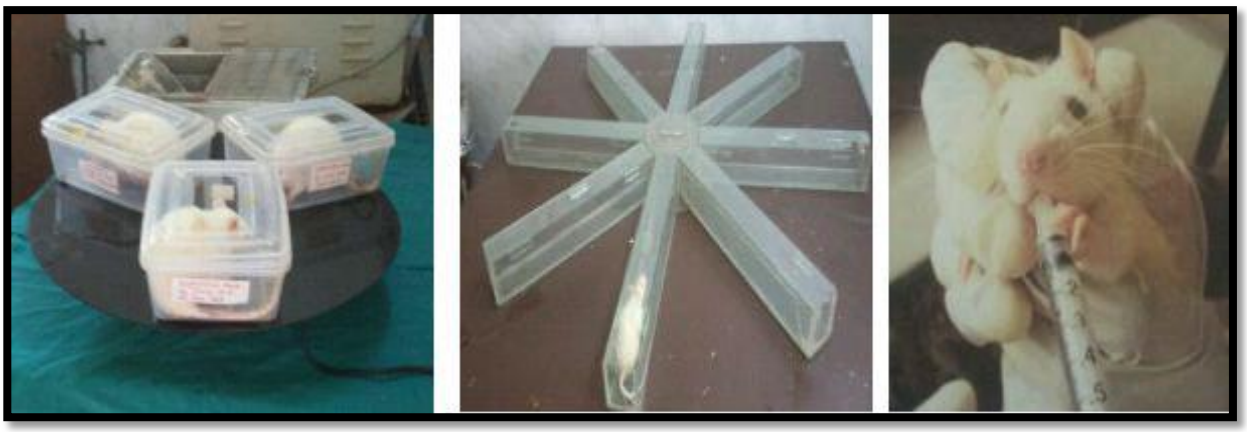
Rotatory Vestibular Stimulation Instrument

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 in Radial Arm Maze after 30days of rotatory vestibular stimulation and Nutmeg administration. 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 10 minutes if all the eight arms were not visited. Six trials per day was given with an inter trial interval of 1 hour. 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 *Nutmeg* 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 *Nutmeg* administration for 30 days continuously before the behavioral task and also 15 minutes before each acquisition and retention test.



Rotatory Vestibular Apparatus **Behavior Study in RAM** **Nutmeg Administration**

Neuromorphological analysis of Pyramidal Neurons for Dendritic Quantification:

From each group six rats were sacrificed immediately 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 there after decapitated and the brain was shelled out, 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 120µ m 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 method [20].

Ethical approval

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

3. Results

Data was analyzed by SPSS 20.0 by one way Anova and Bonferonni Post Hoc Test. The level of significance was fixed at 5% ($p<0.05$) and at 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<0.01) when compared with Group A. From the result it is observed that the number of trials taken for acquisition in Group D decreased far better than Group B and C (p value<0.01).There is a non-significant difference between Group B and Group C in no. of trials taken for acquisition. From the above observation it is clear that rats treated with Rotatory vestibular stimulation followed by the administration of nutmeg is effective in learning. Results shown in Figure 1.

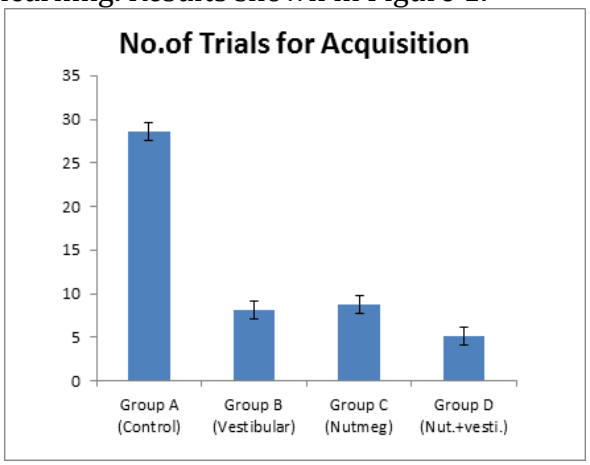


Figure 1. Mean number of trials for acquisition of different groups of rats - Control (Group A), Vestibular Group (Group B), Nutmeg treated Group (Group C) and Nutmeg+ Vestibular (Group D) of rats in learning.

Retention:

Memory on the 10th day after acquisition: When compared to Control (Group A), the Vestibular (Group B), Nutmeg (Group C), and Nutmeg+ Vestibular (Group D) are shows a significant decrease in the number of trials (p-value <0.01). However, there is no significant difference between Group B, C and D, a better performance in memory was shown by Group B.

Memory on the 20th day after acquisition: When compared to Group A, the other groups, B, C and D are shows significant decrease in number of trials (p value <0.01). However in Group B, there is a significant reduction in number of trials when compare with Group C (p value <0.05). And there is no significant

difference between Group B and D, as well as Group C and D. Here also Group B shows improvement in memory.

Memory on the 30th day after acquisition: When compared to Group A, the other groups, B, C and D are shows significant decrease in number of trials (p value <0.01). However Group B, there is non-significant decrease in number of trials when compare with Group C and Group D. Similarly, there is a non-significant difference in no. of trials between Group C and D.

Memory on the 40th day after acquisition: When compared to Group A, in the other groups, B, C and D, observed a significant decrease in number of trials (p value <0.01). However Group B, there is non-significant decrease in number of trials when compare with Group C and Group D. Similarly, there is a non-significant difference in no. of trials between Group C and D.

Memory on the 50th day after acquisition: When compared to Group A, the other groups, B, C and D showed a significant decrease in number of trials (p value <0.01). However Group B, there is non-significant decrease in number of trials when compare with Group C and Group D. Similarly, there is a non-significant difference in no. of trials between Group C and D.

Memory on the 60th day after acquisition: When compared to Group A, the other groups, B, C and D showed a significant decrease in number of trials (p value <0.01). However Group B, there is non-significant decrease in number of trials when compare with Group C and Group D. Similarly, there is a non-significant difference in no. of trials between Group C and D.

Memory on the 70th day after acquisition: When compared to Group A, the other groups, B, C and D showed a significant decrease (p value <0.01). However in Group B, there is a significant reduction in number of trials when compare with Group C (p value <0.05). And there is no significant difference between Group B and D, as well as Group C and D. Here also Group B shows improvement in memory.

From the analysis, it is observed that, all the treated Groups (B, C and D) shows significant decrease in number of trials taken for retention when compared with the Control (Group A), (p value <0.01) and Group B shows a better retention capacity throughout the period of retention, indicates that rotatory vestibular stimulation alone is enough to enhance learning and memory. Results are shown in Figure 2.

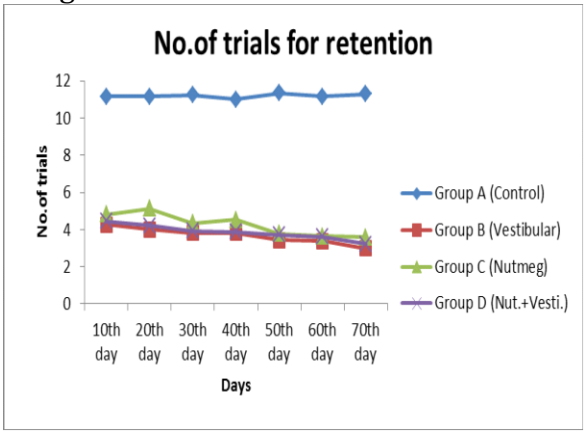


Figure 2. No. of trails taken for retention in different groups of rats.

Neuromorphological Analysis

Dendritic Branching Points

In 0-20µm concentric circle: When compared to Control (Group A), the Vestibular (Group B), Nutmeg (Group C), and Nutmeg+Vestibular (Group D) the

dendritic branching points are significantly increased (p value <0.01). There is no significant difference in dendritic branching points between Group B and C, but it shows a significant increase in Group D when compared with Group B (p value <0.01) , as well as in Group D when compared with Group C (p value <0.05).

In 20-40µm concentric circle: When compared to Group A, the groups B, C and D shows a significant increase in dendritic branching points (p value <0.01). There is a significant increase in dendritic branching points in Group B than Group C (p value <0.01), while no significant increase in branching points is shown in Group B than Group D. And there is a significant increase in dendritic branching points in Group D than Group C (p value <0.01).

In 40-60µm concentric circle: When compared to Group A, the groups D, B and C showed significantly increased no. of branching points respectively (p value <0.01). There is significant increase in dendritic branching points in Group B when compared with Group C and D (p value <0.01). Dendritic branching points are significantly increased in Group D when compared with Group C (p value <0.01).

In 60-80µm concentric circle: When compared to Group A, the dendritic branching points in groups D, B, and C are significantly increased respectively (p value <0.01). Though there is a non-significant increase in dendritic branching points in Group B than Group C, it shows a significant increase in dendritic branching points in Group D than Group B(p value <0.01). However there is a significant increase in the no. of dendritic branching points in Group D when compare with Group C (p value <0.01).

In 80-100µm concentric circle: When compared to Group A, the groups D, B, and C shows a significantly increased no. of dendritic branching points respectively (p value <0.01). It shows a non-significant increase in branching points in Group D when compare with Group B and C. It shows a significant increase in dendritic branching points in Group D than Group C (p value <0.05).

In 100-120µm concentric circle: When compared to Group A, the groups D, B, and C shows a significant increase in the dendritic branching points respectively (p value <0.01). It shows a significant increase in dendritic branching points in Group Dwhen compared with Group B and C (p value <0.01). And also a significant increase in branching points is shown by Group D when compare with Group C (p value <0.01).

From the Sholl analysis it is clear that in concentric circles, 0-20µ m and in 40-60µ m onwards Group D shows a better increase in Dendritic Branching points when compare with other treated groups, and it may indicate that Rotatory Vestibular Stimulation along with Nutmeg administration plays a significant role in increasing the dendritic branching points in hippocampal pyramidal neuron while Rotatory vestibular stimulation alone is enough to increase the dendritic branching points in hippocampal pyramidal neurons. Results are shown in Figure 3.

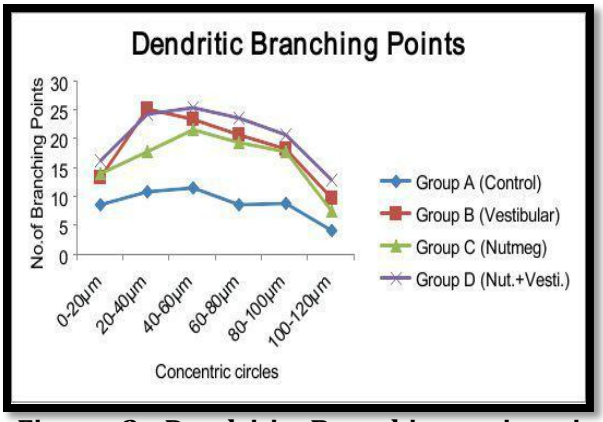


Figure 3. Dendritic Branching points in hippocampal pyramidal neurons of different groups of rats.

Dendritic Intersections:

In 20µm concentric circle: When compared to Control (Group A), the no. of dendritic intersections increased in Group D, C, and B respectively (p value <0.01). There is significant increase in the no. of dendritic intersections in Group D and in Group C than Group B (p value <0.01), as well as significant increase in the no. of intersections showed by Group D when compared with Group C, (p value <0.01).

In 40µm concentric circle: When compared to Control (Group A), the Group D, B, and C showed a significant increase in the no. of dendritic intersections (p value <0.01). There is significant increase in the no. of dendritic intersections between Group C than Group B (p value 0<0.01) but no significant difference between Group B and D. It shows a significant increase in the no. of dendritic intersections in Group D when compared with Group C (p value <0.01).

In 60µm concentric circle: When compared to Control (Group A), Group D, B, and C showed a significant increase in the no. of dendritic intersections respectively (p value <0.01). It shows a non-significant increase in the no. of dendritic intersections between Group D, B and C respectively.

In 80µm concentric circle: When compared to Control (Group A), it shows a significant increase in the no. of dendritic intersections in Group D, C, and B respectively (p value <0.01). There is a significant increase in dendritic intersections in Group D and C, than Group B respectively (p value <0.01). and the no. of dendritic intersection is almost similar in Group C and Group D.

In 100µm concentric circle: When compared to Control (Group A), Group D, C and B shows a significant increase in dendritic intersections (p value <0.01). There is no significant difference between Group B and C, and D in case of dendritic intersections.

In 120µm concentric circle: When compared to Control (Group A), in Group D, C, and B the dendritic intersections were significantly increased (p value <0.01). There is a significant increase in dendritic intersections in Group D and Group C when compared with Group B respectively (p value 0.01).There is a significant increase in dendritic intersections in Group D than Group C (p value <0.01).

From the analysis it is clear that except in two concentric circles (40µ m, 60µ m) the no. of dendritic intersection increased in Group D and Group C respectively and it may be concluded that Rotatory vestibular stimulation along with nutmeg is best for increasing in the no. of dendritic intersections and nutmeg alone is also good for increasing the same in hippocampal pyramidal neurons of rats. Results shown in Figure 4.

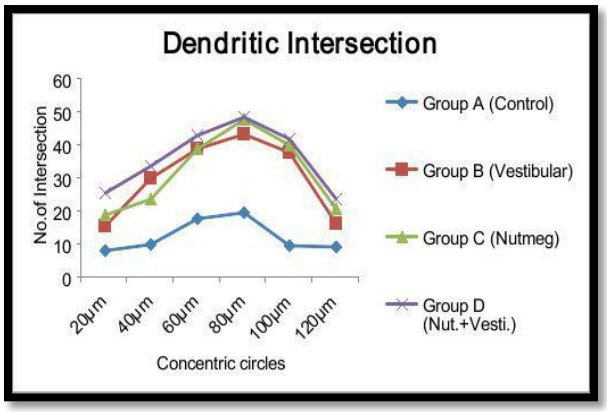


Figure 4. Dendritic Intersections of hippocampal pyramidal neurons in different groups of rats.

Biochemical Analysis

Acetylcholine esterase level

When compared to Control (Group A), the level of hippocampal AChE significantly decreased in Nutmeg+Vestibular (Group D), Vestibular (Group B), Nutmeg group (Group C) respectively (p value<0.01). There is no significant difference between Group B and C, but it shows a significant decrease in AChE level in Group D when compared with Group B and Group C (p value < 0.01). From the result, it is clear that Group D enhances learning via inhibiting the level of AChE than other groups. Results shown in Figure 5.

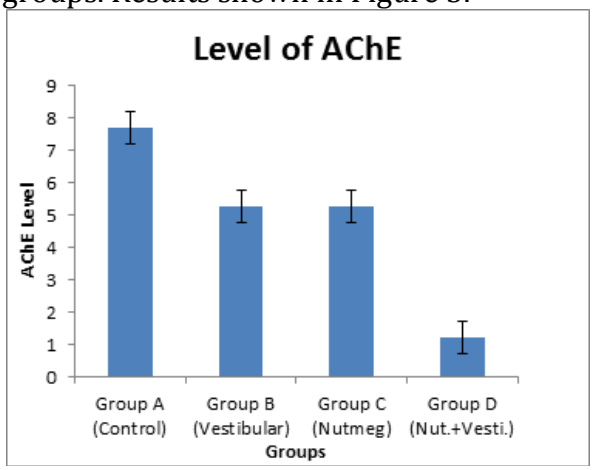


Figure 5. Level of Hippocampal AChE in different groups of rats

Discussion

An increase in the dendritic arborization and synapses in the hippocampal pyramidal neurons results in the facilitation of cognition and performance in the spatial learning tasks. In the present study also, rotatory vestibular stimulation followed by the administration of nutmeg extract significantly increased dendritic arborization of the hippocampal pyramidal neurons in rats. The increase the dendritic arborization may also increases the number of synaptic connections with the neurons and this can be considered as the neural basis for the improved cognitive functions in treated rats. Oral intubation with aqueous extract of *Clitoria ternatea* during the developmental stages improved learning and memory, due to the significant increase in dendritic arborization in hippocampal neurons [21]. The differences in the dendritic arborization between the control and test group (patients) are quantitative and the analysis of basal dendrites in senile dementia, showed an increase in total number of intersections for basal dendrites of pyramidal cells in the control than in the patients with senile dementia. The patients showed a significantly lower number of total intersections than the controls. The dendritic density was maximum at the 60um sphere in both the patients and in controls [22]. The neuromorphological analysis shows that synaptic plasticity involves changes in the intrinsic excitability affecting the firing of action potential and also the changes occurs in individual dendritic branches of the pyramidal neurons. [23-24]. Treatment with *C. asiatica* extract during the developmental stages can produce long lasting beneficial effects on the mouse brain, enhancing cognitive

functions through their effect on the cholinergic system and also by influencing the neuronal morphology [25]. *CeA* Fresh leaf juice treatment in rats increased the dendritic arborization and these dendritic modifications brought about by various factors improved learning and memory [26]. Our study also indicates that the dendritic arborization between the control and test group is quantitative and showed a total number of dendritic intersection of pyramidal cells in control group was lower than in test groups vestibular stimulated, and nutmeg treated group.

The neurotransmitters like acetylcholine, dopamine, serotonin, and norepineprine, act on specialized metabotropic receptors in the pyramidal neurons as well as other neurons leading to the activation of impulse / signal transduction pathways, which results in the modulation of intrinsic excitability and also synaptic function and there is a correlation between increased acetylcholine and improved spatial memory task as well as improvement of radial arm maze performance [27]. Ronsted et al, 2008 mentioned the co-relation of plan alkaloids and AChE inhibition and explains that all species of Narcissus inhibits AChE [28]. Lopez et al, 2002 have made the similar attempt to prove the AChE inhibitory effect of various plant origins, and investigated 26 species of Narcissus and found many fold variation in AChE inhibitory activity by different Narcissus species [29]. The essential oils of plant extract like *Pinus nigra* are also potent AChE inhibitor, reported by Bonesi et al, 2010 [30]. Acetylcholinesterase inhibition in aqueous extract of stem bark of *F. racimosa* was proved by Ahmed and Urooj [31]. According to Ahirwar S[32], the AChE inhibitory activity of plant extracts is a type of competitive inhibition, that, the plant extract might compete with ATCI for binding at the substrate binding site of AChE. So all these studies and the present study also suggests that the plant extracts may be a new potential resource of natural anticholinesterases compounds as a herbal alternative for Alzheimer's disease treatment as well as for cognition enhancement because the cholinesterase from plant origin may produce a substantial, long-term acetylcholine-esterase inhibition in brain, produce steady state long-term increases of Ach levels and also have no severe side effects, at therapeutical doses. Such requirements have not been met by the standard cholinesterase inhibitors till date. Therefore, it seems to be important to develop new cholinesterase inhibitors with fewer or no side effects and with greater therapeutic effects. [33]. The memory enhancing property of *Ferula asafoetida* is attributed to acetylcholinesterase inhibiting and anti-oxidant properties explained by Vijayalakshmi et al, 2012 [34] in a study conducted on Snail nervous system. The enhancement of memory by *F. foetida* in rats may be due to the facilitation of cholinergic transmission due to inhibition of AChE in rat brain, and partly due to its boosting effect on endogenous anti-oxidant system. This can be taken as indirect evidence of its ability to cross the blood brain barrier and also due to the active principles of ferulic acid and umbelliferone present in *F. asefoetida* [35]. According to Kim, 2009 [36], and Parle M, et al. 2007 [37], *Myristica fragrance* extract enhanced learning and retention capacities of both young and aged mice and the observed memory enhancing effect may be attributed to the antioxidant, anti-inflammatory or perhaps

procholinergic activity. A dose dependent AChE inhibition was also reported due to carbazole alkaloid, mahanimbinc isolated by *Murraya koenigi* [38].

Dendrites are considered as the chief determinants of integration and information processing of neurons and hence dendrites play a vital role in the functional properties of neuronal circuits. The functional architecture of the different parts of the brain is dynamic and can change in response to various experimental manipulations. After the formation of long-lasting functional enhancement of synapses in the hippocampal area, new spines appear on post-synaptic dendrites, whereas in control regions on the same dendrites no significant spine growth occurs and thus the dendritic structural reorganization is the key feature in learning and memory [39]. The vestibular stimulation and herbal extracts used in this study may play a role in these manipulations and lead to dendritic alterations. The experimental manipulations used in the present study may stimulate the release of neuromodulators, which alter the activity of neurotransmitters involved in various cognitive functions, including learning and memory. Our present study showed that the controlled vestibular stimulation and administration of plant extract (Nutmeg) in rats improved spatial learning performance and enhanced memory retention.

Vestibular nuclei and hippocampus have anatomical connections, and vestibular lesion leads to hippocampal atrophy [40]. The hippocampal long-term potentiation is enhanced by caloric vestibular stimulation by stimulating acetylcholine secretion from septohippocampal cells [41]. In normal rats after modification with interspersing rotational stimulation for 20 days with ten full revolutions showed improvement in spatial discrimination task using cross maze, where as in fornix-lesioned rats showed no significant improvement. This suggests that adaptation to vestibular system stimulation is required for the improvement of cognition even in normal rats and such processes were disrupted in lesioned rats [42].

Connections of the semicircular canal are primarily associated with brain acetylcholine and the vestibular input enters directly to the cerebellum without crossing a synapse, while most of the other sensory inputs relay through synapses in thalamus or brain stem [43]. An exploratory cross over study in 4 to 14 years aged children using rotatory stimulation of the semicircular canal using swivel chair twice weekly for 4 weeks by rapid acceleration of 33 rpm, and found significant improvement in children with hyperkinetic reaction [44]. Another study also showed a significant improvement in behavior of the children with attention deficit disorder with hyperactivity by providing them rotatory motion, which in turns leads to the stimulation of the semicircular canal. [45]. Ferrare *et al.*, 1999 [46] found encouraging results in children with learning disorders when subjected them to otolith stimulation in a Comprehensive Motion Apparatus(CMA) for 30 minutes 3 times per week for 12 weeks. In another study conducted by Arnold et al, 2008[47], in CMA, rotatory vestibular stimulation for both semicircular and otolith system was given in a recycling chair in which the subjects were rotated with programmed gradual acceleration to 4 rpm accompanied by rocking and tilting, shows improvement in behavior and cognition in ADHD children. In our study also it is clear from the less

no. of trails taken by the treated rats for acquisition and retention tasks than the control group of rats, that cognition is improved by rotatory vestibular stimulation and this may be due to low Acetylcholinesterase level and increased dendritic arborization.

Memory can be enhanced by a modest increase in circulating glucose levels. The mechanism underlying glucose effects of memory may be an increase in acetylcholine (Ach) release [48] and in the present study, also glucose level slightly increased in treated rats, but it is not statistically significant.

The present study was done on the basis of the previous study conducted in our laboratory using rotatory vestibular stimulation and *Centella asiatica* [49].

Conclusion

It can be concluded that Rotatory Vestibular Stimulation along with nutmeg extract improves learning and memory, as it induces structural changes in the hippocampal pyramidal neurons *viz*, increasing the dendritic arborization and also it is suggested that both combination of rotatory vestibular stimulation and nutmeg enhances learning and memory by reducing the level of acetylcholine hydrolyzing enzyme, AChE. In net effect the combined action of Rotatory Vestibular Stimulation and Nutmeg significantly enhancing learning while either rotatory vestibular stimulation or nutmeg alone is sufficient enough to enhance the retention. Further study is required to get even more detailed information on it.

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