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ORAL ADMINISTRATION OF NUTMEG ON MEMORY BOOSTING AND REGAINING IN WISTAR ALBINO RATS

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Background: This study provides further evidence for improvement of memory by oral consumption of nutmeg. The present study was undertaken with an objective to study the effects of oral administration of nutmeg on memory boosting and regaining. **Methods:** Here we investigate the influence of oral intake of nutmeg on behavioral task performance by using T-maze and radial arm maze and physiological measures relative to a milk control group. **Results:** We have observed significant memory boosting and memory regaining effects of nutmeg when administered orally. This effect may be due to facilitation of acetylcholine activity by decreasing acetylcholinesterase activity of nutmeg. Hence we recommend further research in this area by investigating compound metabolism to optimize quantification of memory performance following nutmeg consumption.

Key words: Memory boosting, Memory retention, Nutmeg consumption

INTRODUCTION

The ability to alter behaviour on the basis of experience is called learning.[1] Memory is the retention and storage of the information.[2] Several areas of the brain contribute to memory. There is something to be said for Lashlel's hypothesis that memory is not a location specific process. He found that memory loss was proportional to the amount of cerebral cortex ablated rather than the area from which it was ablated.[3] There are many mazes that have been used to test hippocampal function. The Radial Arm Maze (RAM), T-maze and water maze are perhaps the most used among them.

Medicinal plants improves memory by their acetylcholine esterase inhibitory activity.[5] Nutmeg, now a common household spice, comes from the tree *Myristica fragrans*, which originates from the Indonesian Banda Islands (also known as the Spice Islands). The Nutmeg plant, *Myristica fragrans* Houtt, is a member of the small primitive family called Myristicaceae, taxonomically placed between the Annonaceae and Lauraceae.[8] The name nutmeg comes from Latin, *nux muscat*, meaning musky nut. Myristicin found in nutmeg has been shown to inhibit an enzyme in the brain that contributes to Alzheimer's disease and is used to improve memory.[4]

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Nutmeg is a reasonably non-toxic alternative. The drugs currently prescribed for Alzheimer's disease. Nutmeg has strong antibacterial properties. It is effective in killing a number of cavity-causing bacteria in the mouth.[6] Oral administration of nutmeg may have some deleterious effects on the kidneys of adult Wistar rats at higher doses.[7] Nutmeg, the dried seed kernel of *Myristica fragrans*, MF (Family: Myristicaceae) possesses antifungal, hepatoprotective and antioxidant properties.[16]

The present study was undertaken with an objective to study the effects of oral administration of nutmeg commonly used as spice in various dishes, as components of teas and soft drinks or mixed in milk and alcohol on memory boosting and regaining.

Materials and Methods

Subjects

A total of 24 male and female wistar albino rats were used for this study. They were housed in groups, in propylene cages in an acclimatized (25-27^{o}C) room and were maintained on a 12hr light / dark cycle. Food and water was given ad libitum until they aged 30 days at the beginning of the experiment. They were randomly assigned into control and Nutmeg groups with 12 rats in each group. Nutmeg was administered to Nutmeg group and milk without Nutmeg was given to control group.

Materials

T-maze

The T-maze is made of wood with smooth polished surface. It consists of a stem (35 x 12 cm), a choice area (12 x 12 cm) and two arms (35 x 12 cm); at the end of each arm contain a food well. The sidewalls are 40 cm high. The choice area is separated from the arms by a sliding door.

Radial arm maze

Radial arm maze is made of Plexiglass; consist of eight equally spaced arms radiating from an octagonal central platform. Each arm was having a length of 56.2cm, width of 7.9 cm and height of 10 cm. The entire maze is elevated 80 m above the floor for easy locating of spatial cues by rats.

Nutmeg extract

Nutmeg extract is purchased from Kancor ingredients limited, Kancor road, Angamaly, Kerala, India.

Pharmacological drug administration

Buscopan® tablets manufactured by Cadila Healthcare limited, is used in the present study. Each Buscopan tablet contained Hyoscine (scopolamine) Butylbromide I P 10 mg and excipients (q. s.). The tablets were powdered and mixed with 50ml sterile 0.9% w/v normal saline. It was administered to the rats as intraperitoneal injection at a dose of 1 mg / Kg.[9]

Scopolamine was injected at a dose of 1 mg / Kg body weight of rat, only during the phases where it was assigned. In those groups were the drug is administered, SC was injected 30 minutes before beginning the behavioral trials, every day, either during acquisition or retention depending on the group.[10, 11, 12, 13]

Polybion

B-complex vitamin manufactured by Merck, Germany. Thiamine mononitrate 10 mg, pyridoxine hydrochloride 3 mg, cyanocobalamin 15 mcg, riboflavin 10 mg, nicotinamide 100 mg, calcium pantothenate 50 mg, ascorbic acid 150 mg, folic acid 1.5 mg, biotin 100 mcg.

Experimental design

The rats in the nutmeg group were given 1-2 mg/kg body weight of nutmeg extract orally for 30 days continuously. The control rats were given equal quantity of milk for 30 days without nutmeg extract. All the rats were fed with pellets and water mixed with B complex tonic liberally in these 30 days. After 30 days, the rats were starved for 48 hours and after 48hours the behavioural task is performed on T-maze and radial arm-maze for acquisition. This task is continued till we recorded full score without any error. Now ten days gap was given for the retention of the task. In these ten days only pellets and water mixed with B complex tonic was given to both the groups. On eleventh day behavioral task is performed on T-maze and radial arm-maze and number of trials required to get full score is recorded in both the groups to test memory boosting effect of nutmeg. From the next day we have started administration of scopolamine intraperitoneally to both the groups to cause partial amnesia. This procedure continued for 9 days. Scopolamine administration was done at 10 am daily. Only water mixed with B complex tonic is given to both the groups during this 9 days. From tenth day administration of scopolamine is stopped and nutmeg is administered to nutmeg group where milk without nutmeg is given to the control group. This procedure continued for 30 days and food and water mixed with B complex was given to both the groups during these 30 days. On 31st day behavioral task is performed on T-maze and radial arm maze in both the groups for acquisition and number of trails required to get the full score is recorded. Now ten days gap is given where only food and water mixed with B complex is given to the rats in both the groups. On eleventh day behavioral tasks were performed on both the mazes to test the retention in both the groups and number of trails required to get the full score is recorded. The memory score was calculated by taking the difference between the number of trials required for acquisition test and number of trials for retention test.

The body weight was maintained at 85% of the original body weight, throughout experiment. Behavioral experiments were conducted in the same room with the same allocentric cues, such as doors, windows.

T-maze task

This was analogous to non-matching to sample task, where the rat was rewarded only if the current choice doesn't match the previous one. As reward is used it can also be considered as a learned alternation procedure. In the orientation phase, the starved rats were allowed to spend 10 minutes / day for three days in the T-maze and trained to collect food pellet from the food wells.

During the acquisition test, all the rats were given six trials / day with an inter trial interval of one hour. Each trial consists of four sample and choice run. In the sample run, the rat was placed at the start end of the T-maze stem.

Allowed to move towards one arm and collect the food pellet, while keeping the sliding door of other arm closed. In the choice run, the rat was placed at the start end of stem and both arms were kept open.

If the rat visits the same arm as that of sample run, it was recorded as error and the rat was not rewarded with food. Instead, if the rat visits the alternate arm, it was recorded as correct score and the rat was allowed to eat food pellet (reward) in the food well. There was an interval of 30s between each run. Score was given for alternate selection of

arm during choice run and a maximum score of '4' can be obtained per trial.

Radial arm maze task

The rats was placed in the centre of the maze and allowed to freely explore the maze for 15 minutes on the first day. The rats were required to take the food pellets from each arm without making a re-entry into the arm already visited.

The trial was terminated when the animal takes the food reward from all the eight arms or after 10 minutes if all the eight arms were not visited. Correct sore was give when the visits an arm and collects the food reward, and a maximum score of '8' can be attained per trial. When a rat reenters an already visited arm it was taken as a working memory error.

Data analysis

The analysis of data was done by Spss 20.0. The Independent-Samples t Test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment (or lack of treatment) and not to other factors.

Ethical approval

The study protocol was approved by Institutional Ethics Committee of Little Flower Medical Research Centre, Angamaly.

RESULTS

The mean number of trials of acquisition in control group and in nutmeg group is presented in Table 1.

Table 1
Mean number of trials of acquisition and retention in control and nutmeg groups (Radial arm maze-memory boosting)

Parameter	Control	Nutmeg	*p* value
Acquisition	27.83±2.71	16±2.90	<.001
Retention	22.67±2.42	9.67±2.73	<.001

The mean number of trials of acquisition in control group is 27.83±2.71 and in nutmeg group is 16±2.90, which indicates that nutmeg group is having more memory boosting effect than control group. This is statistically significant ($p < 0.001$). The mean retention of control group is 22.67±2.42 and in nutmeg group is 9.67±2.73, which indicates that nutmeg group is having more memory boosting effect than control group. This is statistically significant ($p < 0.001$).

Mean number of trials of acquisition and retention in control and nutmeg groups (Radial arm maze-memory regaining) was listed in Table 2.

Table 2:
Mean number of trials of acquisition and retention in control and nutmeg groups (Radial arm maze-Memory regaining)

Parameter	Control	Memory regain Nutmeg	*p* value
Acquisition	25.00±5.22	45.00±3.16	<.001
Retention	17.67±4.08	27.33±3.08	0.001

The number of mean trials of acquisition in control group is 25.00±5.22 and in nutmeg group is 45.00±3.16, which indicates that nutmeg group is having memory regaining effect. This is statistically significant ($p < 0.001$). The mean retention of control group is 17.67±4.08 and in nutmeg group is27.33±3.08, which indicates that nutmeg group is having memory regaining effect. This is statistically significant ($p < 0.001$).

Mean number of trials of acquisition and retention in control and nutmeg groups (T maze-memory boosting) is listed in Table 3.

Table 3
Mean number of trials of acquisition and retention in control and nutmeg groups (T maze-memory boosting)

Parameter	Control	Nutmeg	*p* value
Acquisition	15.83±2.64	11.67±2.07	0.012
Retention	13.00±2.37	8.50±1.52	0.003

The number of mean trials acquisition in control group is15.83±2.64 and in nutmeg group is 11.67±2.07, which indicates that nutmeg group is having more memory boosting effect than control group. This is statistically significant ($p < 0.012$). The mean retention of control group is 13.00±2.37 and in nutmeg group is 8.50±1.52, which indicates that nutmeg group is having more memory boosting effect than control group. This is statistically significant ($p < 0.003$).

Mean number of trials of acquisition and retention in control and nutmeg (T maze-memory regaining) is listed in Table 4.

Table 4
Mean number of trials of acquisition and retention in control and nutmeg (T maze-Memory regaining)

Parameter	Control	Memory regain Nutmeg	*p* value
Acquisition	34.33±3.01	32.67±3.50	0.398
Retention	25.83±2.79	18.50±2.95	0.001

The number of mean trials of acquisition in control group is 34.33±3.01and in nutmeg is 32.67±3.50, which indicates that turmeric group is having memory regaining effect. This is not statistically significant. The mean retention of control group is 25.83±2.79 and in nutmeg group is 18.50±2.95, which indicates that turmeric Acquisition group is having memory regaining effect. This is statistically significant ($p < 0.001$).

DISCUSSION

It was reported that M. fragrans significantly decreased acetylcholinesterase activity as compared with their respective vehicle-treated control groups. Acetylcholinesterase is an enzyme that inactivates acetylcholine and the central cholinergic pathways play a prominent role in the learning and memory processes.[14,15] We agree with these studies as we have observed significant memory boosting and memory regaining effects of nutmeg consumption.

CONCLUSION

We conclude that oral administration of nutmeg is having memory boosting and memory regaining effects in rats. Hence we recommend further research in this area by investigating compound metabolism to optimize quantification of memory performance following nutmeg consumption

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