Efficacy of long term administration of curcuma longa linn. on learning and retention memory in albino mice

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ABSTRACT

There is an increase in non-degenerative memory-related disorders in elderly people. Curcuma Longa linn. can induce cognitive improvement by reducing oxidative stress and antioxidant property. This study evaluated the effect of chronic administration of Curcuma Longa linn. on learning and memory in mice. Twenty four male swiss albino mice aged 6 to 8 weeks, weighing 25-30 g were randomized into control, standard, and test groups. Control group received 0.5% Carboxymethylcellulose; the standard group received piracetam and test group received Curcuma Longa linn. orally for 42 days (6 weeks). Water maze test and step-through passive avoidance test were used to evaluate the effect of Curcuma Longa linn. on learning and memory. 1 week of water maze training done (day 40 to 46). On day 47, spatial memory assessment was done. On day 49 retention memory assessment was done. In control, standard, and test groups, the mean escape latency (EL) was observed to be 42.7, 30.7, and 31.3 seconds on day 43 which decreased to 11.3, 11.6, and 9.8 seconds on 46th day, respectively. The EL in all the three groups decreased from day 43 to Day 46 (p<0.01). The time spent in the northeast target quadrant was more in the test group (21.6 seconds) compared to control (19.9 seconds) and standard group (19.6 seconds) (p=0.768). Test group showed a trend towards improvement in retention memory (p=0.293) in passive avoidance test. In conclusion, Curcuma Longa linn. demonstrated that long term administration may be useful in enhancing the learning in albino mice but there was no improvement in retention of memory in test group mice as compared to the standard and controlled animals.

INTRODUCTION

As age increases, there is a high risk of memory loss, cognitive impairment. It is estimated that approximately 20 to 30 % of people over the age of 70 years have mild to moderate cognitive impairment. More than 30 million people suffer from dementia globally and approximately two-thirds of those people live in developing countries. (Qiu et al., 2009)

Lifestyle changes like adequate sleep, healthy diet, regular exercises forms an important part of management. Medications like tacrine, rivastigmine, galantamine, donepezil, and memantine act by inhibiting enzyme cholinesterase thereby increasing the availability of acetylcholine or by antag-
onizing NMDA receptors in brain. (Tibor, 2009) Treatment for cognitive impairment depends on the underlying cause and severity of the condition. Commonly prescribed medications to prevent memory loss in neurodegenerative disorders and non-neurodegenerative disorder patients have limited role and outcome is not satisfying.

Memory comprises of registration (short term memory), consolidation (long term memory) and retrieval (process of recalling). Cholinergic pathways in CNS have important role in learning and memory. The amount of cholinergic neurodegeneration correlates positively with severity of memory impairment. (Singh and Parle, 2003) Curcumin commonly known as ‘turmeric’ can stimulate cognitive functions by enhancing the cholinergic system, reducing oxidative stress (Pan et al., 2009) and with its antioxidant properties. (Vohora et al., 2000)

Curcuma longa linn., from family Zingiberaceae (Sharma et al., 2000) is widely known for its multiple actions, such as anti-inflammatory, carminative, anthelminthic, appetizer & diuretic. (Lantz et al., 2005) They contains sesquiterpenoids called turmerones that are reported to be responsible for its activity.

Piracetam, a derivative of the neurotransmitter gamma-aminobutyric acid (GABA), modulates neurotransmission (including cholinergic and glutamatergic), has neuroprotective and anticonvulsant properties, and improves neuroplasticity. It helps in reducing erythrocyte adherence to vascular endothelium, prevents vasospasm, and improves microcirculation. Its found to be useful in cortical myoclonus, dyslexia, and sickle cell anemia. (Winblad, 2006) Oral administration of piracetam helps in increasing the amino acid content in hippocampus, and reduces the neuronal damage. (He et al., 2008)

The drugs currently used in the management of memory disorders are not adequate and effective. Role of these drugs in preventing memory loss in non-neurodegenerative disorder patients is not clear. Hence, newer, effective and safer medicines are required to treat memory disorders. Synthetic drugs have more adverse effects on long term consumption with unknown efficacy. Plant products have better patient acceptability and are safer compared to the other available medicines. (Janus and Welzl, 2010; Abdel-Salam et al., 2016) Therefore in this study, we try to evaluate the effect of this preparation on vital organs have shown a good safety profile from previ-ous literatures. (Pyrzansowska et al., 2010; Dawson et al., 2018)

**Study materials and methodology**

The experiments was done taking appropriate measures in accordance with regards to the care and use of animals for experimental procedures from Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA). Institutional Animal Ethics Committee. (Reg. No: 142/PO/ReBi/S/99/CPCSEA - 11/05/2017). Mahadevappa Rampure Medical College, Kalaburagi approved the conduct of the study.

**Animals**

Twenty four inbred swiss albino mice, aged 6 to 8 weeks, weighing 25-30 g were used. Animals were procured from Central Animal House, Mahadevappa Rampure Medical College, Kalaburagi. The control group received 0.5% carboxymethyl cellulose (CMC) 2 ml/kg body weight. The standard group received Piracetam, 200 mg/kg given orally 60 minutes before retention. Aqueous extract of C. Longa linn. 20 mg/kg suspended in 0.5 ml of 0.5% CMC was used as test drug. The dose was choosen based on the maximum volume possible to administer to the animal by oral pathway. This suspension was administered orally every day for 42 days. Test drug was provided in powder form by Natural Remedies Pvt. Ltd., Bengaluru.

**Tests for learning and memory**

Water maze and step-through passive avoidance test (STPAT) were used to evaluate the effect of Curcuma Longa linn. on learning and memory in mice.

**Study procedure**

Twenty four Albino mice in groups of eight each, were randomized into control, standard, and test groups using random numbers obtained by graph pad software. Per cage 8 animals were housed with food and water ad libitum. They were maintained at 23 ± 2°C with relative humidity of 50 – 60% under a 12:12 h light: dark cycle for proper acclimatization 5 days prior to start of study. Mice were matched for body weight (25 to 30 g) prior to performing the study. Lights were switched on at 18.00 hours and switched off at 06:00 hours. The same conditions were maintained throughout the experiment. All the drugs were administered on same timings at 10.30 hours once a day, orally, for 42 days. Animal handling was done by single person throughout the experiment. (Table 1)

**Water maze apparatus and Test**

A Morris’s water maze of 60 cm diameter and 30 cm height, filled with opaque water just 1 cm above the
hidden movable plexiglass platform was used. The animals were trained in a water maze, with opaque water at or above 23°C ± 2 °C for 4 days starting from the 43rd day. Visual cues like objects or geometrical shapes having different colors were placed in the external wall of the maze, to aid learning. Each animal had three trials (1 minute each) everyday and were allowed to swim and trace the hidden platform. The position of the platform was kept constant in the North-East Quadrant (NEQ) of the maze while the mice were introduced into the maze from different quadrants randomly. The training trial was terminated when the mice reached the platform, or after 1 minute from the time of introduction into the water maze. If the mice did not find the platform within 1 minute, it was placed on the platform for 15 seconds before the next trial was initiated.

Learning was assessed by comparing the escape latency (EL) (time taken to reach the platform) during the training period. Memory retention was tested by a probe trial conducted 24 hours after the previous training by removal of the platform on 47th day. Time spent in the northeast quadrant (target quadrant) during the 1 minute or 60 seconds trial period was recorded.

**STPAT**

STPAT apparatus was divided into bright (29 × 29 × 26) and dark compartments (29 × 10 × 30) (H x W x L in cm) by a wall having a guillotine door. The bright compartment was illuminated by 8W fluorescent light and dark compartment was not illuminated. Floor of the dark compartment had iron grid and was designed to deliver an electrical shock of 0.6 mA to foot through the iron grid.

Mice was placed in the bright compartment and
allowed to explore for 30 seconds, at which point the guillotine door was raised above to allow the mice to enter the dark compartment. Once mice entered the dark compartment, the guillotine door was closed, and an electrical shock (0.6 mA) was delivered to foot for 3 seconds. Within 10 seconds mice was removed from the apparatus and returned back to its home-cage. Training sessions were conducted 48th day two times in succession during the light phase of the 12:12 hours of day/night cycle. The retention test was conducted on 49th day 24 hours after the training sessions. The latency to enter the dark compartment was recorded for up to 300 seconds on 49th day.

Assessment of learning and memory of test drug
Spatial learning in water maze was assessed by comparing the improvement in mean EL of mice in each experimental group over 4 days. (Table 1)

Memory is assessed by comparing the duration of stay in target quadrant in search of hidden platform between the groups. A significant increase in the duration of stay in target quadrant on the 47th day compared between the groups was considered as an improvement of memory in mice.

Acquisition and retention memory were assessed by passive avoidance test. Increase in transition time to enter the dark compartment on the 49th day between groups was considered as evidence of improvement in retention memory.

Statistical analysis
For assessing improvement in learning and memory, within the group and intergroup comparisons will be made. Between group comparisons (time spent in target quadrant, transition time to enter dark compartment) were carried out by ANOVA. Repeated measures ANOVA was used to compare within groups for improvement in memory (day 43 to 46).

RESULTS
The EL in all the three groups decreased significantly from day 43 to day 46 (p<0.01). (Table 2). The EL in the control group was 42.7 and 11.3 seconds on day 43 and 46 (mean ± SD). The EL in standard was 30.7 and 11.6 Seconds. The EL in test group was 31.3 and 9.8 seconds.

The mean time spent by control, standard, and test groups mice in the northeast quadrant in water maze test on day 47 (test day) was 19.9, 19.6, and 21.6 seconds, respectively. There was no statistically significant difference among three groups, but the study observed that there was a trend toward increase in time spent by mice receiving piracetam in the north east target quadrant (Table 3).

Similarly, on day 49, in the SPAT test, test groups mice stayed more in the dark compartment compared to control and standard group mice but it was not significant among three groups (p=0.293) (Table 4).

DISCUSSION
Our study demonstrated significant reduction in the EL within groups from 43rd day to 46th day. Decrease in EL which was observed within the groups indicates learning during the training schedule. In probe test and STPAT, curcumin treated mice showed a trend toward improved spatial learning and retention memory compared to standard and control group mice. In a similar study (Pan et al., 2008) were curcumin was administered to Alzheimer’s disease mice for 45 days, showed improvement in memory indicated by prolonged step-through latency (p<0.05). Effects of chronic pre-treatment with Curcuma Longa linn. for 60 days in different doses of 10 and 50 mg/kg/day on learning and spatial memory using Morris water maze paradigm also showed similar results (Pyrzanowska et al., 2010). Our study observed significant decrease in EL over four days of training and in probe trial, test group mice spent more time in the target quadrant than the control group. Appropriate mechanism of action to explain the beneficial effects of curcumin include enhancing the central cholinergic system activity, enhanced antioxidant property, (Pan et al., 2008; Janus and Welzl, 2010) reduced beta-amyloid formation, and reversal of glutamate induced excitotoxicity in hippocampus and prevention of neurodegeneration (Dawson et al., 2018). However the exact neurobiological mechanism by which curcumin mediates its beneficial effects on cognitive deficit is not clearly known.

Our study noted the lack of a significant improvement in retention memory in test group mice which could be due to the relatively low drug dosage given and single dose used as compared to other studies to assess the efficacy of curcumin in improving memory and learning. It is also difficult to generalize the results of the present animal experiment to the human clinical settings, as laboratory animals which are healthy and living in controlled environment are biologically different from the dysfunctional human patient. Further dose ranging studies are required where multiple doses of Curcuma Longa linn. are administered for longer periods of time and multiple paradigms are utilized to assess the efficacy of curcuma in improving memory.
CONCLUSIONS

In conclusion, Curcuma Longa linn. demonstrated that long term administration may be useful in enhancing the learning in albino mice but there was no improvement in retention of memory in test group mice as compared to the standard and control group animals.

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Ethical approval

The study was approved by the Institutional Animal Ethics Committee, HKE Society’s Mahadevappa Rampure Medical College, Kalaburagi. (Ref.No: HKES/MRMCK/IAEC/180203) dated 02-02-2018.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

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