Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.18203/2319-2003.ijbcp20151336

Research Article

Assessment of *Curcuma longa* linn. on learning and memory in rats

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Received: 06 September 2015
Accepted: 21 October 2015

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ABSTRACT

Background: There is an increase in elderly population and memory-related disorders. *Curcuma longa* linn. can induce cognitive improvement due to its antioxidant effects. This study evaluated the effect of chronic administration of *C. longa* linn. on learning and memory in rats.
Methods: Twenty four female Albino Wistar rats were randomized into control, standard, and test groups. The control group received 0.5% methyl cellulose; the standard group received piracetam and test group received *C. longa* orally for 24 days. Water maze and step-through passive avoidance test were used to evaluate the effect of *C. longa* linn. on learning and memory. Water maze training was done from day 23 to day 26. Assessment of spatial memory was done on day 27 and retention memory on day 29.
Results: The mean escape latency (EL) in control, standard, and test group was 42.48, 30.45, and 30.88 sec on day 23 which decreased to 11.08, 11.36, and 9.66 sec on day 26, respectively. The EL in all the three groups decreased from day 23 to day 26 ($p<0.01$). The time spent in the target quadrant was more in the test group (21.25 sec) compared to control (19.73 sec) and standard group (19.79 sec) ($p=0.768$). In passive avoidance test, test group showed a trend toward improvement in retention memory ($p=0.293$).
Conclusion: *C. longa* linn. may be useful in enhancing learning. Further dose ranging preclinical studies are required to evaluate the efficacy of curcumin on memory.

Keywords: *Curcuma longa*, Spatial memory, Step-through passive avoidance, Water maze

INTRODUCTION

Memory comprises registration (short-term memory), consolidation (long-term memory), and retrieval (process of recalling).[1] Short-term memory helps the individual to perform day to day tasks, allows recall and has limited capacity, whereas long-term memory helps in retention of facts, can store much larger quantities of information for potentially unlimited duration.[2] Loss of memory is normally seen with increase in age, and it is estimated that approximately 20% of people over the age of 70 years have mild cognitive impairment. More than 25 million people worldwide suffer from dementia and about two-thirds of these people live in developing countries.[3] The number of patients affected will double every 20 years and increase to 81.1 million by 2040. The number of affected cases increases by more than 300% cases in India, China, and their south Asian and western Pacific neighbors by 2040.[4] Treatment for memory loss depends on the underlying cause and severity of the condition. Commonly prescribed medications to prevent memory loss in neurodegenerative disorders include drugs which act by inhibiting cholinesterase enzyme thereby increasing the availability of acetylcholine or by antagonizing N-methyl-D-aspartate receptors in the brain

such as rivastigmine, galantamine, donepezil, tacrine, and memantine.[3,5] However, these drugs cannot be prescribed to all dementia patients because of modest benefit and potential adverse effects.[6] With increasing life expectancy, there is an increase in aging population and memory-related disorders. Currently, there is a need for searching newer, effective, and safer alternatives to existing synthetic drug for resolution of cognitive defects. Moreover, drugs that not only treat but also prevent diseases of older adults are needed in clinical practice.

In the traditional system of medicines, various plants and their isolated phytochemicals have been used for the treatment of various disorders related to learning and memory.[7] Hence, plant products have great potential as therapeutic options in Alzheimer's disease and dementia. *C. longa* linn. commonly known as "turmeric" belongs to the family *Zingiberaceae* and is widely known for its multiple actions, such as antioxidant, anti-inflammatory, cholesterol lowering, hemostatic, anti-amyloid, carminative, anthelmintic, appetizer, and diuretic.[8,9] Turmeric contains sesquiterpenoids called turmerones that are reported to be responsible for its activity. Piracetam, a derivative of the neurotransmitter gamma-aminobutyric acid, has a variety of physiological effects. At a neuronal level, it modulates neurotransmission in a range of transmitter systems (including cholinergic and glutamatergic), has neuroprotective and improves neuroplasticity. At a vascular level, it appears to reduce erythrocyte adhesion to vascular endothelium, hinder vasospasm, and facilitate microcirculation. Its efficacy is documented in cognitive disorders and dementia.[10] Oral administration of piracetam markedly improved the memory impairment, increased the amino acid content in hippocampus, and attenuated neuronal damage.[11] Central cholinergic pathways play a crucial role in learning, and memory processes and the degree of cholinergic neurodegeneration correlates positively with the severity of memory impairment.[12] Curcumin can induce cognitive improvement by enhancing the cholinergic system and due to its antioxidant activities.[13] In this study, we aim to evaluate the effect of chronic administration of *C. longa* linn. on learning and memory in rats.

METHODS

The experiments were carried out taking appropriate measures in accordance with the guidelines laid down by Committee for the Purpose of Control and Supervision of Experiments on Animals, India with regard to the care and use of animals for experimental procedures, and with approval from the Institute Animal Ethics Committee.

Animals

Twenty four inbred female, Albino Wistar rats, weighing 150-200 g were used. Wistar rats were housed in groups of 4 animals per cage, maintained on a standard pellet diet and water ad libitum.

Study drug and dosage

The control group received 0.5% carboxymethyl cellulose (CMC). The standard group received Piracetam, 2000 mg/kg given orally. Aqueous extract of *C. longa* linn. (0.015%) suspended in 2 ml of 0.5% CMC was used as test drug. This suspension was administered orally every day for 23 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 *C. longa* linn. on learning and memory in rats.

Water maze apparatus

A modified version of Morris's water maze of 120 cm diameter and 60 cm height, filled with opaque water just 1 cm above the hidden movable plexiglass platform was used.

STPAT

STPAT apparatus was divided into bright (29 cm height × 29 cm width × 26 cm length) and dark compartments (29 cm height × 10 cm width × 30 cm length) by a wall with a guillotine door. The bright compartment was illuminated by a fluorescent light (8 W) while the dark compartment was not illuminated. Floor of the dark compartment was made with iron grid and was designed to deliver an electrical foot shock of 0.6 mA through the iron grid.

Study procedure

Twenty four rats were randomized into control, standard, and test groups consisting of eight rats each based on the random numbers obtained by using graph pad software. The animals were housed in groups of four per cage with paddy husk as bedding. Animals were acclimatized at room temperature with 12:12 hrs light and dark cycle for 5 days prior to the experiment.[14] Lights were switched on at 18.00 hrs and switched off at 6 hrs. The animals had free access to pellet food and water ad libitum. The same conditions were maintained throughout the experiment. Control group received 0.5% methyl cellulose, 2 ml/kg body weight; standard group received piracetam, 2000 mg/kg; and test group received 0.015% of *C. longa* reconstituted in 0.5% methyl cellulose.[15] All the drugs were administered once a day, orally, at 10.30 hrs for 23 days. A single person handled the animals throughout the experiment.

Water maze test

The animals were trained in a water maze, with opaque water at or above 25°C±2°C for 4 days starting from the 23rd day.

Visual cues in the form of objects or geometrical shapes of different colors were placed in the external environment of the maze, to aid learning. Each animal was allowed to swim and trace the hidden platform for 3 trials every day. Each trial lasted for 1 min. The position of the platform was kept constant in the northeast quadrant of the maze while the rats were introduced into the maze in different quadrants randomly. We terminated the trial when the rat reached the platform, or after 60 sec from the time of introduction into the maze. If the rat did not find the platform within this time, it was placed on the platform for 15 sec before the next trial is initiated. We assessed learning by comparing the escape latency (EL) (time to find the platform) during the training period. On the 27th day, the animals were tested for memory retention by a probe trial conducted 24 hrs after the previous training by removal of the platform. The time spent in the northeast quadrant during the 60 sec trial period was calculated (Figure 1).

STPAT test

Rats were placed in the bright compartment and allowed to explore for 30 sec, at which point the guillotine door was raised to allow the rats to enter the dark compartment. When the rats enter the dark compartment, the guillotine door was closed, and an electrical shock (0.6 mA) to the foot was delivered for 3 sec. The animal was then quickly removed (within 10 sec) from the apparatus and returned back to its home cage. Training sessions were conducted twice during the light phase (1:00-4:00 pm) of the 12 hrs day/night cycle. The second session was carried out immediately after the first session. Training sessions was on the 28th day and the retention test after 24 hrs of training sessions. The latency to enter the dark compartment was recorded for up to 300 sec on day 2 (Figure 1).

Assessment of learning and memory of test drug

- Spatial learning in water maze was assessed by comparing improvement in the mean EL of each experimental group over 4 days. Memory is assessed by comparing the duration of stay (sec) in target quadrant in search of hidden platform between groups. A significant increase in the duration of stay in target quadrant on the 27th day compared between the groups was considered as an improvement of memory in rats.
- Acquisition and retention memory were assessed by passive avoidance test. Increase in transition time to enter the dark compartment on the 29th 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 were made. Between group comparisons (time spent in target quadrant, transition time to enter dark compartment) carried out by analysis of variance (ANOVA). Repeated measures ANOVA was used to compare within the groups for improvement in memory from day 23 to day 26.

RESULTS

The mean±standard deviation EL in the control group was 42.48 and 11.08 sec on day 23 and 26. The mean EL in standard and test group was 30.45 and 30.88 sec on day 23 which decreased to 11.36 and 9.66 on day 26, respectively. The EL in all the three groups decreased significantly from day 23 to day 26 ($p<0.01$) (Table 1).

The mean time spent in the northeast quadrant of the water maze on the test day (day 27) was 19.73, 19.79, and 21.25 sec in the control, standard, and test groups, respectively, which were not statistically significant. There is a trend toward increased time spent in the target quadrant in the test group rats (Table 2).

In the SPAT test, test groups rats stayed more in the dark compartment (264.48 sec) compared to control

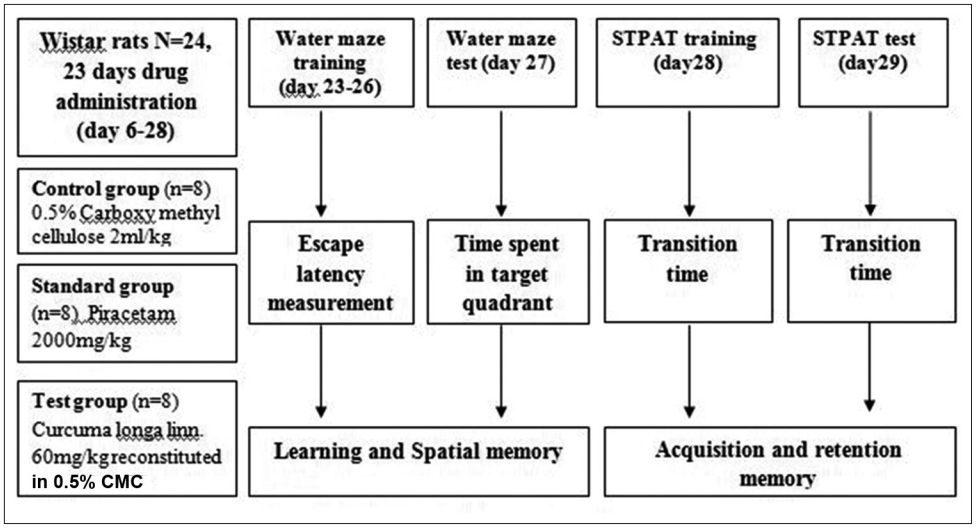

Figure 1: Scheme of the experiment.

Table 1: EL on day 23 to day 26.

Groups	Day 23	Day 24	Day 25	Day 26	p	F	df
Control group	42.48±4.3	16.47±2.2	11.94±2.3	11.08±2.2	0.001	24.54	3
Standard group	30.45±2.8	13.45±1.6	9.32±2.9	11.36±4.1	0.010	13.14	3
Test group	30.88±4.2	20.81±3.4	15.65±3.5	9.66±1.6	0.001	10.70	3

Data expressed as mean (±SEM); time in sec (s), EL: Escape latency, SEM: Standard error of mean

Table 2: Time spent in NEQ between the groups on day 27.

Groups	Control group	Standard group	Test group	p	F	df
Time spent in NEQ	19.73±1.3	19.79±2.4	21.25±1.5	0.768	0.28	2

Data expressed as mean (±SEM); time in sec (s), SEM: Standard error of mean, NEQ: North East quadrant

Table 3: Transition time in SPAT test between the groups on day 28 and day 29.

Groups	Control group	Standard group	Test group	p	F	df
Transition time (training day)	94.17±32.4	155.82±24.2	179.87±15.2	0.040	3.83	2
Transition time (test day)	156.94±54.2	252.83±47.2	264.48±35.5	0.293	1.69	2

Data expressed as mean (±SEM); time in sec (s), SEM: Standard error of mean

(156.94 sec) and standard group rats (252.83 sec) (p=0.293) (Table 3).

DISCUSSION

In this study, we demonstrated the significant reduction in the EL within groups from day 23 to day 26. Decrease in EL within the groups indicates learning during the training schedule. In probe test and STPAT, curcumin treated rats showed a trend toward improved spatial learning and retention memory compared to standard and control group rats. In a study by Pan et al.,[13] curcumin administration for 45 days improved the memory ability of Alzheimer's disease mice as indicated by prolonged step-through latency ($p<0.05$).[3] Similar results were seen in Pyrzanowska et al., study[14] which showed effects of chronic pre-treatment (60 days) with *C. longa* in doses 10 and 50 mg/kg/day on learning and spatial memory in male Wistar rats using Morris water maze paradigm. There was a significant decrease in EL over four days of training and in probe trial test group rats spent more time in the target quadrant than the control group.[14] The exact neurobiological mechanism by which curcuma mediates its beneficial effects on cognitive deficit is not clearly known. Possible mechanisms to explain its beneficial effects include enhancing the cholinergic system, antioxidant activity, inhibition of beta-amyloid formation, reversal of hippocampal glutamate induced excitotoxicity and neurodegeneration.[13,14]

The lack of a significant improvement in retention memory in curcuma treated rats in our study could be due to the relatively short duration of drug administration as compared to previous studies where test drugs were administered for at least 6-8 weeks. It is difficult to generalize the results of the present study to the clinical setting because healthy laboratory animals are biologically different from the dysfunctional human patient. We also used a single dose level to assess the efficacy of *C. longa* linn. in improving memory and learning. Therefore, further studies are required, wherein a range of doses administered for longer periods of time and multiple paradigms used to assess the efficacy of curcuma in improving memory. In conclusion, this study demonstrated that *C. longa* linn. may be useful in enhancing learning. Further dose ranging preclinical studies are required to evaluate the efficacy of curcumin on memory.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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Cite this article as: Lohit K, Anthony N, Pandit AA, George J, Jose M, Rao M, Xavier D. Assessment of *Curcuma longa* linn. on learning and memory in rats. Int J Basic Clin Pharmacol 2015;4:1086-90.