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RESEARCH ARTICLE

Neurotrophic effects of turmeric on the memory of the mouse using the Morris water maze test

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ABSTRACT

Background: Turmeric is a yellow-orange spice found mainly in India and other parts of Asia and South America, and is widely used for its medicinal properties. Curcumin, the main part of the turmeric rhizome, may have properties that can improve the memory of mice in a step-through latency test. **Aims and Objectives:** In this study, turmeric was utilized to determine its effect on the memory of mice using the Morris water maze (MWM) to assess spatial memory of rodents. **Materials and Methods:** Determination of the effect of intake of turmeric powder in sterile olive oil was observed, as well as the effects of the different doses of administration (control, 5 mg/kg, 15 mg/kg, and 45 mg/kg) of turmeric based on the performance of mice in the MWM. Further investigation was done by analyzing the apoptotic count on the hippocampal area of the mouse brain. **Results:** No significant effects were detected on the analysis of both the pre-treatment and post-treatment data on the MWM test, which may imply that the observed downhill trend may be due to chance alone. On the other hand, the significant difference brought about by the treatments of 5 mg/kg, 15 mg/kg, and 45 mg/kg doses on the comparison against the control on the histological analysis by counting the apoptotic cells on the mouse hippocampus was observed. **Conclusion:** The effect of memory improvement of turmeric administration is, therefore, due to the preventive effect of the turmeric against oxidative stress and its ability to inhibit the apoptosis, or programmed cell death, of neurons. Thus, turmeric has the ability to protect brain cells from deterioration and maybe a potential neuroprotective agent if studied further.

KEY WORDS: Apoptosis; Curcumin; Hippocampus; Memory; Morris Water Maze; Turmeric

INTRODUCTION

Turmeric is a spice native to India and has been used for over 2,500 years. 1,7-bis(4-hydroxyl-3-methoxyphenol)-1,6-heptadiene-3,5-dione) or dipheruloyl-methane is the active component of the rhizome of turmeric.[1,2] Although native in India, turmeric is also produced in other parts of Asia and South America. Distinguished for its yellow-orange pigment contained in the curcuminoids in the rhizome, turmeric was first cultivated as a dye and was later on used as a cosmetic and as a food additive. Turmeric has been used in different aspects of people ives including as food additives, for cosmetic purposes and source of alternative medications.[3] Curcumin is used for the treatment of various medical conditions, including cystic fibrosis, hemorrhoids, gastric ulcer, colon cancer, breast cancer, atherosclerosis, liver diseases, arthritis, dementia, Parkinson's and Alzheimer's disease, and traumatic brain injury.[4,5] Moreover, it exhibits a remarkably wide range of pharmacological effects that may account for its diverse medicinal properties, including effects on neurotransmitters, trophic factors, protein kinases, and transcription factors.[6] In Ayurveda Indian System of

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Medicine, it is considered as "cleanser of the body" and is used as an anti-inflammatory agent to relieve pain and inflammation in the skin and muscles. Indians consume about an average of 80–200 mg of curcumin per day. The common therapeutic dose is 400–600 mg 3 times daily. Asians typically consume about 100 mg a day for over centuries. Oral administration of curcumin is atoxic at very high doses. Treatment of humans with 8000 mg in 3 months showed no side effects because curcumin has shown to be well-tolerated by human subjects with relatively few side effects when orally administered as it readily enters the brain in sufficient concentrations to impair intracellular processes in the lateral amygdala neurons, which are related to fear memory consolidation.[7,8] However, it was said that curcumin is readily conjugated in the intestine and liver to form curcumin glucuronides that cause its poor bioavailability. It has also been said that chronic use of curcumin can cause liver toxicity and is not recommended for persons with biliary tract obstruction because it stimulates bile secretion. It is also not recommended for individuals with gallstones, acute biliary colic, obstructive jaundice, liver disease, for heavy drinkers, and for those who take prescription medications that are heavily metabolized by liver.[8] Furthermore, curcumin is a very effective agent in brain disease models for it can cross the blood-brain barrier to enter brain tissue despite its poor bioavailability. The protective effect on memory deficit is mediated by prevention of oxidative stress.[9] Curcumin protects the brain mitochondria against various oxidative stress, such as peroxynitrite, a product of the reaction of nitric oxide with superoxide. Curcumin has also been shown to reduce the levels of amyloid and oxidized proteins and prevents memory deficits, and is thus beneficial to patients with Alzheimer's disease.[10] Moreover, curcumin also has a close interaction with the cholinergic system in memory retention process and improves memory retention process in rats.[11] A study revealed that curcumin improved learning and memory in mice. It investigated the neuroprotective effect of curcumin on the memory of mice with Alzheimer's disease in a step-through test. In the experiment, curcumin reduced the number of step-through errors and prolonged step-through latency of the mice.[12]

There are no studies yet on the effectivity and safety of turmeric when given in animals when used as an alternative method to help in spatial learning and memory. Although there are studies already stating that there are no toxic effects,[3] there is a need to study whether there will be effects on the brain which may be detrimental to the animal. This study aimed to determine the effect of intake of turmeric powder before testing on the Morris Water Maze (MWM), a widely used model for studying learning and memory of mice, which assess spatial learning and memory, and the potentials of the different concentrations of turmeric to aid memory. The results of this study would pave way if turmeric would be a potential alternative to memory enhancement supplements. Its utility could help humans who would want to improve their memory and cognitive performance with the aid of an inexpensive and readily available solution. Furthermore, this study provides reliable data, which can be used for further studies, which aims to improve memory enhancing supplements and its increase in cost efficiency with the use of an inexpensive ingredient. This study also increases the feasibility of developing memory enhancement supplements that pose no detrimental side effects.

With the data on the effectiveness of turmeric on several conditions, it is imperative to look further into its neurological aspects and whether it can give synergistic effects when people take them as supplements. In lieu of the high demand for the "holy grail" cure or preventive drugs of certain neurological diseases such as dementia and Alzheimer's disease, turmeric was said to possess a potential to reverse effects and to increase cognitive function not only to people affected by the disease but also to those unaffected. Therefore, this study determined the effect of the administration of turmeric powder on the memory of mice using the MWM test. Specifically, the study aimed to compare the effects of different concentrations of turmeric powder on the memory of mice using the MWM and determine the effects of turmeric powder on the histological analysis of the mice brain.

MATERIALS AND METHODS

Procurement of Animals

A total of 28-week-old, weighing around 25–30 g male Institute of Cancer Research mouse were obtained from the food and drug administration (FDA), Alabang, Philippines. The sample size was determined based on the methods of Charan and Kantharia.[13] Animals were then placed in the animal house at De La Salle University and were housed in individual standard sized cages. Standard commercial rodent food (pellet form) and drinking water were provided. The cages were lined with autoclaved paddy husk and cleaned twice a week. Before the experiment, all mice were acclimatized for a period of 1 week to adapt to an environment with the temperature of 23°C and 55% humidity at a 12 h light: 12 h dark cycle. All succeeding experiments in animals were approved by the Institutional Animal Care and Use Committee of De La Salle University.

3 Days Maze Protocol

The MWM protocol consists of pre-training phase (day 1), training phase (day 2), and test phase (day 3). Random group assignment was done prior the mice exposed to the water maze test.

Pre-training Phase

The mice were allowed to swim freely on the pool as a familiarization session to acquire the procedural aspects of the

task.[14] This is compulsory for verifying that the mouse could swim and climb on the platform. The mouse was released facing the platform 1 cm above water level located two feet away, and the mouse was guided to it. The mouse must remain in the platform for 10 s before it is dried and caged.

Training Phase

Three trials were allotted for the mice during the training phase. The platform was placed on the center of one of the four imaginary quadrants: North, east, south, and west. The mice were released facing the wall of the pool. A maximum of 60 s was allowed for the mouse to locate the platform to prevent hypothermia in mice. During training day, the platform was submersed 2 cm under water. If the mouse failed to locate the platform within the given time, it was manually guided to it. In addition, if the mouse failed to find the platform, it was automatically scored with 60 s. The mouse must remain in the platform for 10 s before it is dried and caged. An intertrial interval of 120 s was allotted for the mouse to go back to the starting point in the pool.[15]

Test Day

Three trials were allotted for the mice during the test session, 24 h post-training day. 30 min was allotted for the treatment to take effect. The platform was retained from its position in the training phase. The mouse was timed on how long it will locate the platform. If the animal fails to locate the platform within 60 s, it was guided to it. In addition, if the mouse failed to find the platform, it was automatically scored with 60 s. The animal must remain on the platform for 10 s before placing it back to its cage. An intertrial interval of 120 s was allotted for the mouse to go back to the starting point in the pool.[15]

Histological Analysis

Brain extraction

Each mouse was euthanized by cervical dislocation. Subsequently, the brain was then extracted from each mouse and was placed in individual vials containing 10% buffered formalin.

Brain processing

The extracted brain specimens were submitted to the Philippine Kidney Disease Foundation Pathology Laboratory for brain processing and slide fixation.

Histological analysis

The processed brain specimens were analyzed by counting the normal cells and the cells that have undergone apoptosis.

Data analysis

The statistical tools used were paired *t*-test and one-way analysis of variance (ANOVA) for the determination of any significant differences in the results obtained from the experiment brought by the evaluation of the treatments. If a significant difference was detected, a *post hoc* test (Tukey-Kramer test) will be used to detect the differences among the groups. The level of significance used throughout the statistical analysis was set at 0.05. All analysis was performed using STATA v.12.

RESULTS

The results below are the four groups (control, 5 mg/kg, 15 mg/kg, and 45 mg/kg doses) tested for the effect of acute exposure of turmeric powder based on their performance in the MWM test [Figures 1-3] and the percentage of cellular apoptosis in the brain [Figures 4 and 5]. The rate of each group's performance in the training phase and test phase

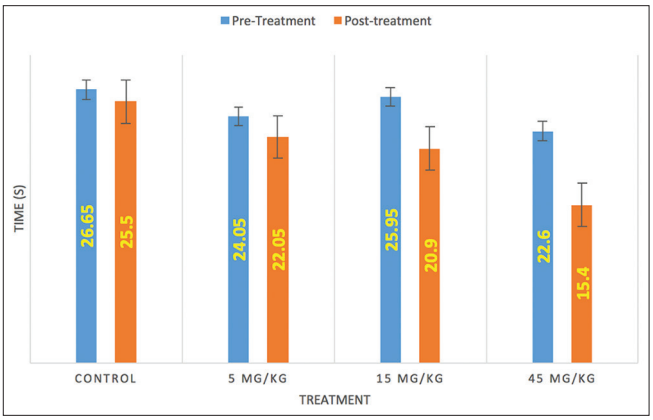

Figure 1: The average rates per group performance on the MWM test during the training phase (pre-treatment) and test phase (post-treatment). The mice were administered with their corresponding treatments 30 min prior their performance in the MWM test. Statistical analysis revealed both pre-treatment and post-treatment values do not differ. Similar letters (a) indicate that there are no significant differences in between groups

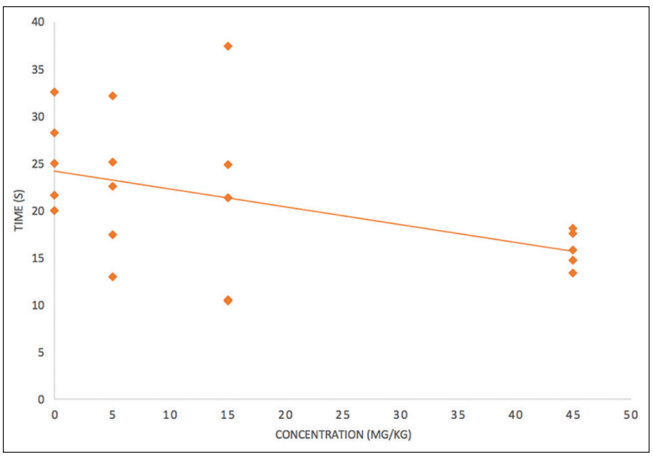

Figure 2: Downhill trend on the post-treatment administration. Concentrations at 0 mg/kg are the control group, containing 0.2 ml of olive oil, whereas the turmeric-treated concentrations are as follows: 5 mg/kg is the low dose group, 15 mg/kg is the middle dose group, and 45 mg/kg is the high dose group

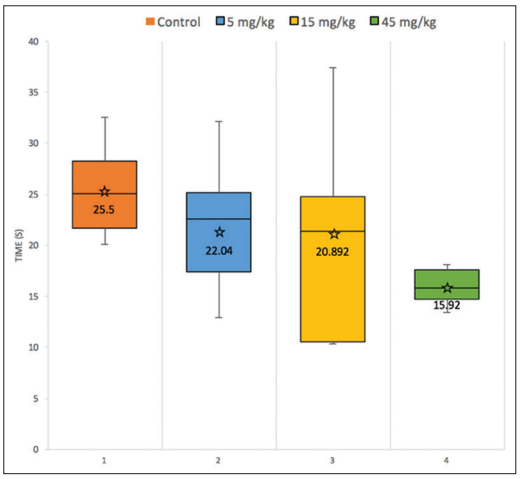

Figure 3: Box and whisker plots for four samples with sample size n = 5. The median of each of the four treatments were 25.05, 22.6, 21.35, and 15.8 (shown as solid lines in the boxes). There was no significant difference among these means when one-way analysis of variance was performed

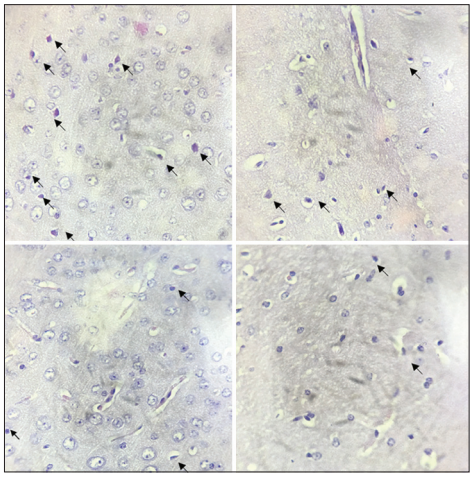

Figure 4: Representative histological analysis photos from control (upper left), 5 mg/kg (upper right), 15 mg/kg (lower left), and 45 mg/kg (lower right) groups. The figures show the neuronal apoptosis on the hippocampal area of the mouse brain. The arrows represent the neurons that have undergone apoptosis. Each photo is viewed under HPO (×400)

on the water maze was recorded. The percentage of cellular apoptosis on the brain was then recorded after the water maze test to determine the effect of exposure of turmeric.

To establish the homogeneity of values before treatment administration; the pre-treatment data were subjected to one-way ANOVA. Results show that there were no significant differences in between pre-treatment groups and that all pre-treatment values are similar. Confirming that the pre-treatment values are not different, the similar analysis was done to the post-treatment values to detect the effect of the administration of turmeric treatment [Figure 1]. The post-treatment values of the treatments (control, 5 mg/kg, 15 mg/kg, and 45 mg/kg) were all subjected to one-way ANOVA. Statistics show that there were also no significant differences in the performance of the mice in the MWM test among the four treatments.

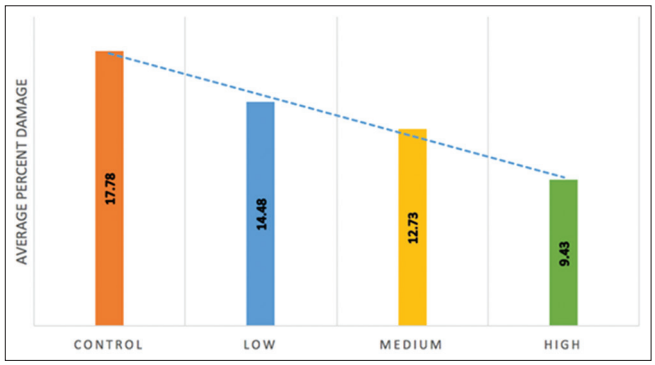

Figure 5: The average percentage damage of each treatment was collected and subjected to the *t*-test. Results show that each treatment has a significant difference against the control. Furthermore, there is a downhill trend, indicating that with higher doses of turmeric results to fewer apoptosis events. Different assignment of letters indicates significant differences in the values of the four groups

Nevertheless, the pre-treatment and post-treatment difference (not shown in graph) among the groups were 1.15 (control), 2 (5 mg/kg), 5.05 (15 mg/kg), and 7.2 (45 mg/kg). Thus, the graph reveals a downhill trend on the post-treatment administration, which may suggest that with higher doses, the mice were able to locate the platform at a lesser time [Figure 2]. Figure 3 shows that the mean of each of the groups are 25.5 (control), 22.04 (5 mg/kg), 20.892 (15 mg/kg), and 15.92 (45 mg/kg). Although results reveal a downhill trend on the mean of the samples, there was no statistical significance, and the downhill trend may be due to chance alone.

The assessment and scoring of the apoptotic damage were done with the following criteria: [16] (1) Cell shrinkage, (2) condensation of the cytoplasm, (3) deep eosinophilia of the cytoplasm, and (4) irregularly-shaping of the nucleus. The number of cellular apoptosis in the hippocampus was recorded to determine and compare the effect of the three different treatments (5 mg/kg, 15 mg/kg, and 45 mg/kg). The averages of the apoptotic index of each mouse of each treatment were taken and subjected to means comparison against the control. Results show that with increasing dose of turmeric, there is a prominent decrease in cellular damage or apoptosis. Furthermore, there are significant statistical differences when the three treatments were subjected to *t*-test against the control [Figures 4 and 5]. In addition, *t*-test of the treatment groups shows that there are significant differences in all treatments. With the high dose (45 mg/kg) having the least number of apoptotic cells, it is, therefore, the most effective dosage of all three treatments.

DISCUSSION

In a similar study conducted by Sarlak *et al*.,[11] all three treatments also failed to show any statistical effects on the step-through latency when the data were subjected to one-way ANOVA. The curcumin of turmeric has played a role in the memory of mice by its antioxidative properties, blocking apoptosis. According to the preceding study, curcumin has

a close interaction with the cholinergic system, thus, it has the ability to improve memory retention process in rodents. In other studies, curcumin has also proven to improve the performance of ovariectomized rats in the MWM and concluded that curcumin can improve cognitive performance and prevent memory impairment induced by ovariectomy due to the sudden rapid decline in the hormone estrogen.[17] In another study by Noorafshan *et al*.,[18] curcumin prevented sulfite-induced learning and memory changes in rats. Moreover, curcumin treatment significantly improved colchicine-induced cognitive impairment in mice by decreasing lipid peroxidation.[9] This statement strengthened the claim of Ataie *et al*.,[19] who studied the effects of curcumin in the lipid peroxidation of the brain of which there is increased latency in passive avoidance tests.

The data above agrees with the study of Ataie *et al*.,[19] where the turmeric treatments at low, middle, and high doses (5 mg/kg, 15 mg/kg, and 45 mg/kg, respectively) have inhibited lipid peroxidation significantly. He further stated that curcumin at the dose of 45 mg/kg has inhibited apoptosis in the hippocampus most efficiently; thus, curcumin has dose-related antioxidant effects on the whole brain tissue. Turmeric's ability to inhibit apoptosis is due to curcumin's ability to reduce p53 gene expression, along with the induction of hsp-70 gene, protecting cells from caspase-3 activity and oligonucleosomal DNA fragmentation.[4] Curcumin's preventative and protective roles on learning and memory is related to apoptotic effect, as it impairs the consolidation of memory and reverses cognitive alterations, by inhibiting programmed cell death.[8,10] Accordingly, Nam *et al*.[9] reported that the protective effect of curcumin is mediated by the prevention of oxidative stress leading to apoptosis. Curcumin as an antioxidant primarily protects biomembranes against peroxidative damage, a free-radical-mediated chain reaction, damaging cell membranes. The inhibition of peroxidation of curcumin is done by scavenging the reactive free radicals. Curcumin is a unique antioxidant; its distinctiveness is attributed by the presence of a variety of functional groups: β-Diketo groups, carbon-carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxyl substituents, rather than just having either a phenolic functional group or a β-Diketone group, like most other antioxidants.[20] Curcumin is also considered a superb H-atom donor by donating hydrogen atoms from the central methylenic group in acidic and neutral aqueous, as well as acetonitrile solutions.[21] Furthermore, Barclay *et al*.[22] stated that curcumin is a classical phenolic chain-breaking antioxidant, being able to donate H-atoms from the phenolic groups as well. It was also claimed that the phenolic group is essential for the free radical scavenging activity, strengthened by the presence of methylenic group.[23] Curcumin can also degrade into trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, diferuloylmethane, and vanillin at basic pH in a span of only 30 min. Ferulic acid and vanillin are stable and potent antioxidants, among others.[20]

This study showed that turmeric treatment may inhibit apoptosis or at least decrease its rate, and at increasing doses of turmeric administration, a downhill trend was observed on the apoptotic cell count. However, this study failed to show any significance on the different treatments on the performance of mice on the MWM test, which may be due to different errors committed in the experiment such as environmental bias (maze), mice variability, and personal bias (scoring of apoptotic cells). Although the inhibition of apoptosis did not wholly affect the performance of mice in MWM, which may indicate that there are multiple mechanisms of memory retention, not just the prevention of oxidative stress. Furthermore, Ataie *et al*.[19] claimed that curcumin by itself did not affect the learning and memory but improved these functions which were impaired, after observing in a similar study that a higher dose (45 mg/kg) of curcumin did not have an increased lipid peroxidation than that of the lower dose (5 mg/kg).

It may be inferred based on the data analyzed and literature used, turmeric may then have neuroprotective effects particularly on the brain and cell damage; furthermore, this effect is brought into on its possible role as an antioxidant.

CONCLUSION

The study was conducted to determine the neurotrophic effects of turmeric on the memory of mice. Turmeric extract has been proven by the US FDA to be safe in animal studies and declared curcumin as generally regarded as safe. Thus, turmeric may have a protective effect on the memory by preventing programmed cell death or apoptosis and in turn, may be a potential neuroprotective agent in the prevention of oxidative stress. Curcumin as an antioxidant was thought to efficiently donate hydrogen atoms contain different functional groups necessary for scavenging free radicals and degrade into even smaller yet powerful antioxidants. Moreover, more studies must be conducted to determine its biochemical pathway, synthesis, as well as its adverse effects on the memory.

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