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RESEARCH ARTICLE

Effect of Hordeum vulgare, Moringa oleifera, and Vitex negundo extracts on the hippocampus and memory of Mus musculus

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ABSTRACT

Background: Herbal medicines such as *Hordeum vulgare*, *Moringa oleifera,* and *Vitex negundo* are widely recognized around the world as dietary supplements, remedy for ailments, and bodily sustenance. Moreover, studies have shown that intake these herbal medicines can have an effect in the memory retention in mice; however, no further studies have been conducted in comparing between barley, moringa, and lagundi and their optimal dosages on treatments for the effect of memory in mice. **Aims and Objectives:** The aim of this study is to comparatively assess the effects barley, moringa, and lagundi extracts on the histology of the hippocampus and its effect on memory using the Morris Water Maze test. **Materials and Methods:** The extracts were orally administered to the mice with three specific dosages (250 mg/kg, 500 mg/kg, and 750 mg/kg) of each herbal supplements, barley, moringa, and lagundi. The mice were subjected to a 2-day water maze test, and mice were sacrificed thereafter. Neurological effects such as hippocampal reduction and degeneration were analyzed histologically. **Results:** Results showed that there are no differences between the different treatments at all dosages with the control group; therefore, memory improvement or impairment was not exhibited during the maze test. In the histological analysis, however, it showed that barley did not show any effect on the histology of hippocampal neurons with the cells within the normal limit and did not undergo cell degeneration. Moringa and lagundi, on the other hand, resulted to neurotropic effects of impaired hippocampal neurons with lagundi causing more damage than moringa. **Conclusion:** There are no effects on memory between the three treatments administered to the mice. However, neuronal damage on the other may result from moringa and lagundi administration.

KEY WORDS: Barley; Hippocampus; Lagundi; Memory; Moringa

INTRODUCTION

Plants are excellent sources for the development of different kinds of drugs, and with the development of modern medicine, the use of plants in part or in whole became the foundation of herbal medicine. Herbal medicines have been widely utilized to as high as 87%[1] as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. This is due to a long list of advantages that include non-existence of side effects, few associated health risks, affordability, and most importantly, ability to fight symptoms of diseases such as obesity, malnutrition, coronary heart disease, and memory loss such as Alzheimer's disease.[2-4]

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The hippocampus is part of the brain located at the medial temporal lobe that is responsible for spatial memory and navigation.[5] It is also accountable for forming and reconstructing relational memory representations that underlie flexible cognition and social behavior[6] and is also involved in at least some aspects of declarative memory.[7] The hippocampus, as the brain's search engine, also allows a fast and efficient search among the deposited memories in the neocortex, which is a process essential for planning the future and generating creative ideas.[7-9] Most physiological studies on the hippocampus have been performed in rodents and gave rise to the spatial navigation theory.[10] However, when hippocampus is subjected to factors like aging, stress and diseases, it experiences degeneration and declined responses. In humans, the hippocampus tends to shrink as they grow old and there is a substantial loss of neurons as well.[11] Rats, however, do not exhibit cell loss but the synaptic connections are altered and the synapses in the dentate gyrus are lost as they grow old.[9,12] Stress greatly affects the hippocampus because it contains glucocorticoid receptors that are more vulnerable to long-term stress.[12,13] This long-term stress may result to hippocampal atrophy, which inhibits genesis of new neurons in the dentate gyrus. Studies have also shown that there are reductions in the size of the hippocampus as well as decline of synaptic activity between neurons in disorders such as epilepsy and schizophrenia.[14] Despite these factors, the hippocampus has the ability to repair itself, although slow in aged animals, but once it proceeds, it can go back to repairing itself with the same rate as young animals.[12] A study made by Anacker *et al.*[13] suggests that treatment with antidepressants could also increase in the neurogenesis of the hippocampus of an adult rat because antidepressants increase proliferation of hippocampal cells.

Memory loss is a condition that affects a large number of people in a population and is classified as a problematic health issue, especially for the elderly.[9,15,16] Most people have chosen to rely on herbal supplements in trying to prevent the effects of memory loss for the betterment of one's health.[17,18] Commercialized herbal supplements contain plant extracts that treat diseases and promote health, which includes *Hordeum vulgare*,[19,20] *Moringa oleifera,*[21-23] and *Vitex negundo*.[1,24]

H. vulgare L., more commonly known as barley, has historically been used for malt and livestock feed. Recently, however, there has been growing interest in the potential use of barley in human foods largely due to its high content of fiber, β-glucan, and antioxidants.[25] Antioxidants are crucial in maintaining the health of tissues and organs because of their ability to slow tissue damage by preventing the formation of free radicals, scavenging them, or by promoting their decomposition.[26] A recent study showed that administration of young green barley leaves is a good source of potent anti-oxidants, as well as exhibiting hypolipidemic, antidiabetic, and antiulcer effects. Furthermore, it has been shown to have antidepressive and anti-stress properties.[27] Barley contains Vitamin C and E, calcium, iron, all essential amino acids, flavonoids, Vitamin B12, and a number of enzymes and minerals.[28,29]

M. oleifera, more commonly known as Malunggay, is a widely cultivated species of the family *Moringaceae* common in the tropics and subtropics.[21,23,30] *M. oleifera* is a good source of protein, vitamins, b-carotene, amino acids, and various phenolics and has compelling water purifying powers and high nutritional value.[21] Recent studies show that *M. oleifera* has the potential to enhance cognitive and spatial memory as well as a neuroprotectant in the brain[23] by altering the monoamine oxidase levels of the brain.[31] It also enhances learning and memory and increased anxiogenic effect[22] and helps in maintaining the integrity of the cell morphology of the hippocampus.[30]

V. negundo, locally known as lagundi, is one of the ten herbal medicines endorsed by the Philippine Department of Health as an effective herbal medicine with proven therapeutic value.[32] Its roots, leaves, flowers, and fruit have multiple medicinal uses and biological activities such as analgesic, anti-inflammatory, antirheumatic, diuretic, anticonvulsant, antioxidant, insecticidal, and pesticidal activities among others.[33,34] A report in India showed that animals treated with *V. negundo* decrease the phenomenon of scopolamine-induced amnesia through its antioxidant effect and decrease acetylcholinesterase activity.[35] *V. negundo* can also trigger a reduction in oxidative stress mainly by reducing lipid peroxidation.[33]

Conducting further studies to analyze the effects of *H. vulgare*, *M. oleifera,* and *V. negundo* concerns not only of their effects on the hippocampus but also their influence on memory. Specifically, the objectives of the study were to analyze the effects on the rate of finding the platform in the water maze test and to observe qualitatively its effect on the hippocampus of the mice.

MATERIALS AND METHODS

Procurement of Animals and Maintenance

Thirty 10–16-week-old male Institute of Cancer Research mice were purchased from the Food and Drug Association in Muntinlupa city and were housed individually at room temperature (27°C ± 2°C) having 58% humidity with controlled 12:12 h of light/dark cycle. The mice were acclimatized in laboratory conditions for at least 7 days. The study was conducted in the animal house in De La Salle University where the temperature is controlled by the air conditioner; with proper ventilation using an exhaust fan, and a dim and quiet environment was conserved. All cages were cleaned and sanitized weekly and bedded with husk which was autoclaved. The plates and water bottles were cleaned and dried twice a week. Food and water were given *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of De La Salle University following the standard guidelines for animal care as recommended by the Philippine Association of Laboratory Animal Science and the Department of Agriculture (DA) Bureau of Animal Industry (BAI). The experiment from extraction to administration of samples and extrapolation of results was performed from September 2015 to December 2016.

Treatment Preparation and Administration

Herbal extracts (barley, moringa, and lagundi) were purchased from local markets, were dissolved in 0.2 ml of distilled water based on the amount to be given to the mouse, and were administered through oral gavage for 28 days (Table 1).

2-Day Water Maze Protocol

A 2-day water maze protocol by Hodges[36] as modified by Villareal *et al.*[37] was performed to assess the effect of the extracts on memory. Specifically, the Morris water maze is used in the field of behavioral neuroscience in understanding the psychological processes and neural mechanisms of spatial learning and memory, as it is used to tap into the cognitive functions of the brain.[38] This protocol has the advantage of adapting quickly without pre-training or restriction of food and water. The water maze pool is 1.8 m in diameter and 1.5 feet in height. The water was maintained at room temperature with visual cues were be placed at the north, south, east, and west of the pool acting as landmarks to help the mice in locating the hidden platform. The hidden platform measured 10 cm in diameter and 6 inches in height. The platform is made of glass, to prevent visual aid for the mice in easily locating the platform. The measurement between the depth of the water and the height of the platform is approximately 1 cm.

The protocol spanned 2 days consisting of a training phase (day 1) and a test phase (day 2) conducted twice per week, with a 3-day rest period to prevent further stress to the mice. The timetable for the experiment was done for 28 days. The rate (cm/s) was computed for the testing phase and was subsequently analyzed for differences among different groups.

Training phase (day 1)

The platform was placed at a random point within the pool. Landmarks were labeled around the pool as the release points (north, south, east, and west) and the distance between these release points and the platform was recorded. Before the release of the mouse into the pool to start the experiment, they were facing the wall of the pool to prevent visual bias of the mouse.

The allotted time to locate the platform is 60 s and failure to do so will yield a 60-s result. If they failed to reach it within the allotted time, they will be guided to the platform and remain there for 15 s before it is brought back to its cage. This helps the mice know that the platform is a safe area within the water maze. Two trials per mice with a rest period of 30 s during the trial phase.

Test phase (day 2)

The platform remained at the same point of the training phase. The testing phase procedure followed the training phase, except only one trial, was conducted. If the mouse fails to locate the platform, it will be guided to it and remain there for 15 s.

Histological Analysis

After 28 days of experiment, the mice were euthanized by cervical dislocation and the brain was extracted and placed in a vial containing 10% buffered formalin. Samples were then brought to the BAI, DA, Quezon city, for brain processing and routine hematoxylin and eosin slide preparation of the hippocampus. Morphology and reduced hippocampal neurons of the brain were qualitatively observed with the histopathologist blinded to the treatment.

Statistical Analysis

The differences in the mean rate in the water maze were analyzed using one-way analysis of variance, and significant differences were analyzed using Tukey's test at $P < 0.05$. All statistical analysis was performed using STATA v. 12.

RESULTS

Analysis of the Rate of Locating the Platform in the Water Maze Test

The testing phase trials were averaged to determine which treatment resulted to the mice having memory improvement or memory impairment based on the rate of each mouse finding the hidden platform. Statistical analysis shows that there are no significant differences between the different groups ($P = 0.7786$). This shows that no memory improvement or memory impairment resulted from administration of the herbal extracts. However, it can be seen by observing the graph [Figure 1] that the group treated with barley at 750 mg/kg

Table 1: Treatment given to the different groups for a duration of 28 days

Groups	Dose[1]	Reference
Control	0.2 ml distilled water (mg/kg)	
Barley		
Low	250	Yamaura *et al.*[27]
Mid	500	
High	750	
Moringa		
Low	250	Roy[30]
Mid	500	
High	750	
Lagundi		
Low	250	Kanwal *et al.*[35]
Mid	500	
High	750	

[1]All extracts were dissolved at the appropriate dose in 0.2 ml distilled water

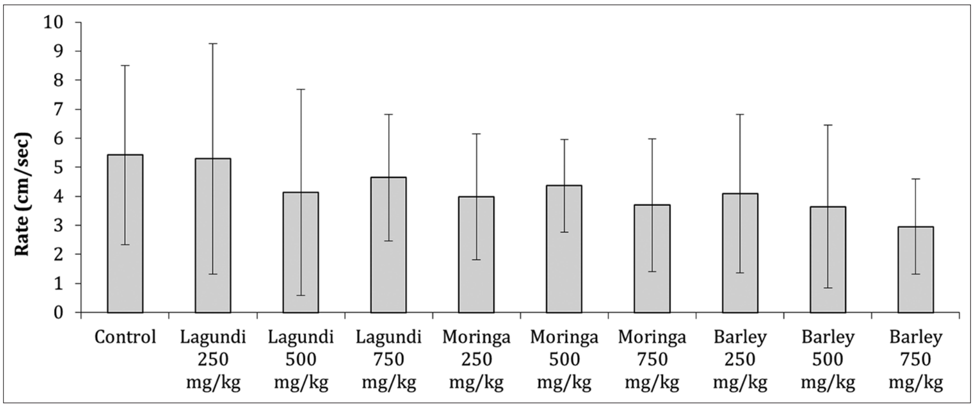

Figure 1: Bar graph showing the rate (cm/s) for different groups in finding the hidden platform in the water maze test

had the shortest time in finding the platform followed by moringa given at the same dose. Furthermore, it can be seen that for the groups treated with barley, there is a decreasing rate from the lowest dose to the highest dose when compared with the two treated groups that showed variability in the rate at different doses. This may mean that administration of barley may be dose dependent, whereas the two treatments may have been affected by other inherent factors in the mice not tested in the current experiment.

Histological Analysis of Hippocampal Neurons

Based on Figure 2, barley and the control grow showed similar histological results with the neurons having a cone-shaped morphology, distinct nuclear membranes, uniform chromatin, and normal limit of space surrounding the cells and capillaries with dense stroma. Moreover, they both exhibit few shrunken pyramidal cells, which are seen to have condensed and deeply stained nuclear chromatin. The only difference between the groups treated with barley with that of the control group is that there is the presence of chromatolysis in the hippocampus of the previous group.

The group treated with moringa given at 250 mg/kg showed the same microscopic findings with that of the control; however, those administered with 500 mg/kg and 750 mg/kg showed the presence of reduction of hippocampal neurons, distortion and degenerative changes in neurons, and mild chromatolysis.

Lagundi-treated mice at 250 mg/kg showed similar results as well with the control group but show the presence of swollen neurons and glial cells. Animal treated with 500 mg/kg shows reduction of normal hippocampal neurons while those at 750 mg/kg showed additional condensed and deeply stained nuclear chromatin.

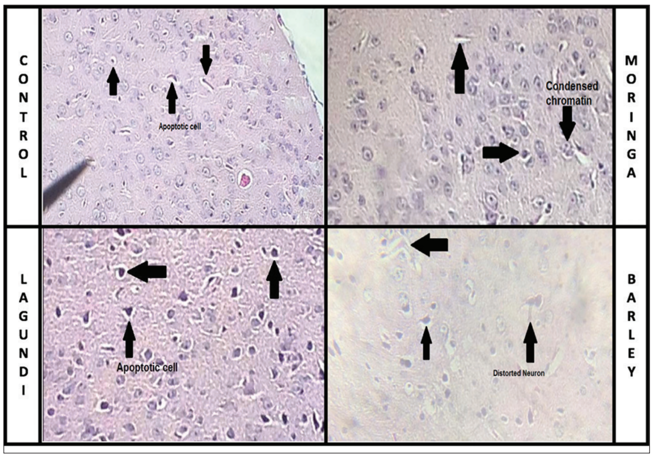

Figure 2: Microscopic findings of the hippocampus of the Mus musculus in ×400 magnification

DISCUSSION

Normal sized pyramidal cells are characterized to have an extensive branching of axons and dendrites.[39] Having that said, pyramidal cells that have shrunken along with loss of its extensive branching may affect the hippocampal activity of the *Mus musculus* negatively as these pyramidal-shaped cells play a key role in cognitive functions such as understanding, perception, remembering, and thinking.[9] Reduction of normal hippocampal neurons has been attributed to stress[40] and this affects the transfer of information among neurons in different areas of the brain as well as neuron-glia communication.[41] This affects that the memory processes of the hippocampus have been found to lead to neurodegenerative diseases, such as Alzheimer's disease in CA1 and CA3 hippocampal areas of the brain.[9] Hippocampal memory processes are affected as glial cells are known to be "guardians" of one's memory and learning processes.[11] Swelling causes pressure on the nerve, which results to damaged nerve cells that affects the signals that are transmitted back and forth to the brain through the nerve.[42] When affected negatively due to the presence of swollen glial cells, brain function will be far from optimal. This results to brain functions, such as memory and learning, being affected negatively.[11,43]

Casticin has been extracted in the leaves of *V. negundo* and has been shown to induce human glioma cell death through apoptosis and mitotic arrest.[44] This may be the reason to the increased apoptotic cells as well as the swollen appearance of glial cells in the mice treated with lagundi. This negative impact on the administration of lagundi is different from other studies that show that there are anti-oxidant effects as well as decreasing acetylcholinesterase activities.[35] These may indicate that there is variability in the effects of lagundi in the brain, and further studies warrant to deduce the exact mechanism of actions. *M. oleifera* contains essential vitamins and minerals such as Vitamins A and D, potassium, and calcium.[21] However, it lacks the B vitamins that are usually needed for memory improvement, which may be the reason for the negative neurotropic effect of moringa. Another feasible reason for the reduction of the hippocampal cells is the increased intake of Vitamin A as it has been shown to increase intracranial pressure, which may result to decreased mental abilities. This is contrast to studies showing that moringa has the potential to enhance cognitive and spatial memories.[23] Both the effects shown by barley and moringa may warrant that the effects are dose dependent with increasing doses not suitable for treatment and administration to enhance memory. Barley has been shown to contain Vitamins B12 and E, which has been attributed to improving memory loss.[45] This may be the reason for the decreased rate in finding the platform as well as the less damaged hippocampal neurons when compared with the groups treated with lagundi and moringa. Vitamin E exhibits good evidence in treating cognitive decline or dementia. Furthermore, there are evidence that shows that supplementation with a high dose of Vitamin E at 2000 IU per day may be beneficial for patients with moderate Alzheimer's disease.[46] Furthermore, Vitamin B12 in conjunction with folic acid supplementation has been shown to improve gross motor and problem-solving skills as well as beneficial effects in neurodevelopment of the brain.[45] Performance enhancers have been formulated to contain Vitamin B-complex and C, along with calcium, magnesium, and zinc, due to the known biochemical and physiological roles and contributions of the different components in supporting several aspects of both mental and physical performance.[22,47]

It has been shown that there are no differences in the treatment groups with the control when used as a memory enhancer using the Morris water maze test. This is different with some studies that showed that the different treatments were able to enhance memory, and as such, further studies using different sources may be warranted. However, there are significant results on the effects of the different treatments on the histology of the hippocampus, which shows that barley may be a safer choice when taking in as supplement when compared with lagundi and moringa.

CONCLUSION

The herbal extracts moringa (*M. oleifera*) and lagundi (*V. negundo*) exhibited impairment in the hippocampal neurons with lagundi causing more damage than moringa. Barley (*H. vulgare*), although showed some natural apoptosis, is similar to that of the control group of mice, having cells within the normal limit, and did not undergo any neurological degeneration. While it is established that the herbal extracts posed changes in the neurons of the mice, the present findings also showed that during the water maze test, the extracts did not have any effect on the memory performance of the *M. musculus*; the trials did not have a constant pattern of improvement or impairment, and statistical analysis confirmed that there was no significant difference with the control group and the treatment groups ($P > 0.05$). In summary, *M. oleifera* and *V. negundo* extracts showed greater neurological degeneration and apoptosis on the hippocampus, while no significant effects are shown when testing the memory of the *M. musculus* using a water test maze.

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