WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 1, 175-192. Research Article ISSN 2277– 7105

HYDROMETHANOLIC LEAF EXTRACT OF *OCIMUM GRATISSIMUM* IMPROVES VISUO-SPATIAL LEARNING AND MEMORY IN MICE

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Article Received on
30 Oct. 2018,
Revised on 20 Nov.2018,
Accepted on 10 Dec. 2018
DOI: 10.20959/wjpr20191-13905

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ABSTRACT

This study evaluated the effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on visuo-spatial learning and memory in mice. Mice were randomly assigned into four groups of 6 mice each. Group 1 received distilled water normal control; group 2 received 10 mg/kg body weight of *Gingko biloba* (standard drug); while groups 3 and 4 received 200 and 400 mg/kg body weight of the extract, respectively, for 21 consecutive days. The acute oral toxicity (LD50) study and qualitative phytochemical screening were carried out. The body weight of mice was monitored and behavioural indices such as swim latencies, quadrant durations and annulus crossings (annulus acquisition and reversal frequency) were measured using Morris water maze. The result of LD50 of the extract was above 5000 mg/kg body weight of mice and qualitative phytochemical result indicates the presence of

flavonoids, alkaloids, glycosides and tannins. The results also showed a significant decrease ($p<0.001$) in body weight of the treated groups when compared with the control. Behavioural indices showed a significant decrease ($p<0.05$) in swim latency (visible platform day) and a significant increase in retention quadrant in mice treated with 400 mg/kg of extract when compared with the standard drug. This study shows that the extract may improve visuo-spatial learning and memory abilities and this beneficial health effect may be attributed to the phytochemicals in the extract.

KEYWORDS: Ocimum gratissimum; Gingko biloba; Morris water maze; behavioural; phytochemicals.

INTRODUCTION

The most common form of dementia that affects more than 35 million people worldwide is Alzheimer disease (AD) and it is estimated to reach 65.7 million by 2030. It is one of the most widely spread neurodegenerative disorders that causes progressive loss of memory, ability to understand and virtual depletion of all intellectual functions.[1] The low synaptic levels of acetylcholine (Ach) resulting from loss of cholinergic neurons has been majorly implicated to be responsible for cognitive decline and other neurodegenerative disorders.[2] AD is now known as the fourth leading cause of death in the elderly population (65 years of age and above) because of involvement of many biochemical pathways.[3] It is expected that AD will increase geometrically in no distant time as the growing population of people aged 65 years and above rises sharply.

According to World Health Organization (WHO), approximately 80% of the world populations live their life on herbal medicine, because it involves the use of extract derived from plants, which have shown potentials for development of new drugs.[4] Most developing countries such as Nigeria are involved in herbal prescription and natural remedy practices for the alleviation of various diseases; this is observed to be a means of compensation for various toxicity associated with orthodox medicine. One of the medicinal plants that is rich in folkloric claim in the treatment of diseases is *Ocimum gratissimum (O. gratissimum), Linn*, commonly called scent leaf, tea bush, fever plant or clove basil, is an herbaceous plant which belongs to family Labiatae.[5] In Nigeria, it is called *Efinrin* in Yoruba, *Daidoya* in Hausa, *Nchanwu* in Igbo, *Ntonng* in Calabar, *Aramogbo* in Edo.[6] Scent leaf as use in herbal medicine is prominent in the treatment of certain ailments and diseases which includes: upper respiratory tract infections, diarrhea, headache, conjuctunctivis, skin diseases,

pneumonia, tooth and gum disorder, fever, insomnia, skin and liver diseases and as mosquito repellants. The Igbo people in the Southeast Nigeria use it in baby's cord management after delivery; because they believe it keeps the baby's cord and wound surfaces sterile.[7] Previous phytochemical screening of the leaf extract of *O. gratissimum* (aqueous) reveals alkaloids, saponins, tannins, anthraquinone, flavonoids, steroids, terpenoids and glycosides.[8] Besides, *O. gratissimum* leaves showed the presence of essential oils such as eugenol, cineole, ocimol, citral, geraniol, thymol, linalool, tetratriacontane, gratimissin, gratimissic acid and β-caryophyllene. Eugenol represents its main physiologically active component and has been widely used in perfumery, flavour and pharmaceutical products, cosmetology and soap industries.[9] Other active components are ursolic acid, rosmarinic acid, lineoleic acid, oleanoic acid. The plant has a high content of phenols, carotene and vitamin C. Among the notable pharmacological effects of the leaf extract of *O. gratissimum* that have been reported are antidiarrheal,[10] wound healing,[11] anti-inflammatory,[12] antidiabetic,[13] antioxidant[8] and anticonvulsant.[14]

The rise in burden of mental health has remained a hidden problem with detrimental and dwindling socio-economic effects on the society. The treatment of these disorders is usually by a group of drugs known as nootropic agents such as piracetam, tacrine, Gingko biloba. However, most of these drugs are either with attendant side effects like headache, increase in body weight and GIT disturbances for those who can afford them or that they are out of reach for the larger population of the average Sub-Saharan African so this necessitated the need to find an alternative management for this disorder. Despite the numerous pharmacological researches on *O. gratissimum* as mentioned above, there appears paucity of report in the scientific literature on the effect of *O. gratissimum* on visuo-spatial learning and memory. Therefore, this study was aimed at assessing learning and memory in mice treated with hydromethanolic leaf extract of *O. gratissimum* (HMOG) using Morris water maze (MWM) learning paradigm. This is with a view to providing an alternative therapy in the management, control and treatment of mental disorders.

MATERIALS AND METHODS

Chemicals and drugs

All chemicals and drugs used in this investigation were of analytical grade and were obtained from Sigma, Saint Louis, USA. *Gingko biloba* was used as the reference learning and

memory improvement drug. In this study, *Gingko biloba* was administered orally to mice in a dose of 10 mg/kg suspended in distilled water.

Experimental animals

Twenty-four (24) male mice weighing between (25-30) g were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Nigeria. The animals were housed in cross ventilated room in cages at (22 ± 2.5°C) with 12 h dark/12 h light cycles and were fed with standard growers mash feeds (Pfizer Feeds LTD, Enugu, Nigeria) and tap water *ad libitum*. Animals were acclimatized for one week with free access to water, prior to experiment. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by National Institute of Health.[15] This study was approved by Animal Ethics Committee of the Faculty of Basic Medical Sciences, Ebonyi State University with reference number (EBSU/REC/MPC/1706/02/001).

Plant material and preparation of hydromethanolic extract (HMOG)

The fresh leaves of *Ocimum gratissimum* (Family: Labiatae) were collected from botanical garden of Ebonyi State University, Abakaliki, Nigeria, identified and authenticated by Mr. Nwankwo O.E of the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The method of[16] was used for the extraction. The leaves were air-dried and milled to fine powder with a domestic food processor (Compact kitchen grinding machine, Kenwood). A powdered dried leaf (186.4g) was weighed and soaked in 1L of hydromethanol (1:2) and shaken vigorously at interval for 72 hours in a dark room environment. After the extraction, the liquid phase was filtered through Whatman No. 4 filter paper (Whatman international Ltd; Maidstone, England) to obtain a pure filtrate (hydromethanol extract, *HMOG*). The filtered extract was concentrated in a rotary evaporator (BÜCHI, Vacuum Controller, V-800) at 40°C under a reduced pressure for 3 h. The concentrate was finally dried by exposing it to air and its percentage yield calculated. The extract was preserved for use throughout the study and reconstituted in sterile distilled water to give the required doses of 200 and 400 mg/kg b. wt., respectively after the acute toxicity study. The dosages were prepared fresh on the day of experiments prior to administration to the mice by oral dosing needles.

Preliminary Phytochemical Screening

Ocimum gratissimum leaf extract (HMOG) was subjected to qualitative phytochemical tests to identify the secondary metabolites; saponins, tannins, alkaloids, terpenoids, flavonoids, phenols, steroids, phytosterols, and glycosides using standard phytochemical methods as described by.[17,18,19]

Acute Oral Toxicity Study

The lethal dose (LD50) of the hydromethanolic leaf extract of *O. gratissimum* was determined by the method of[20,21] using thirteen (13) mice of both sexes. In the first phase, mice were divided into three groups of three (3) mice each and were treated with the hydromethanolic leaf extract of *O. gratissimum* at doses of 10, 100 and 1000 mg/kg body weight orally. They were observed for 24 hours for signs of toxicity. In the second phase, four mice were divided into four (4) groups of one mouse each and were also treated with the hydromethanolic leaf extract of *O. gratissimum* at doses of 1000, 1600, 2900 and 5000 mg/kg body weight (p.o). The median lethal dose (LD50) was calculated using the second phase.

Experimental Design

After the acclimatization period, the animals were randomised into 4 groups of 6 animals each. Normal mice in control group 1 received distilled water orally (1 ml daily) throughout the duration of the experiment. Mice in group 2 received 10 mg/kg body weight of the reference drug (*Gingko biloba*) while group 3 and 4mice received 200 and 400 mg/kg body weight of HMOG respectively for 21 consecutive days by single oral gavage daily and the body weight of all the mice closely monitored. At the end of the administration, visuo-spatial learning and memory was assessed in mice using Morris water maze (MWM).

Assessment of learning and memory ability

Morris water maze (MWM)

Apparatus

Visuo-spatial learning and memory in all groups of mice were measured using the Morris Water Maze (MWM). MWM was developed by[22] for rats and[23] modified it for mice.

The MWM is circular pool filled with opaque water. Mice are trained to use extra-maze visual cues which include pictures and room furniture to find a hidden escape platform below the surface of the opaque water. The MWM was constructed out of a circular polypropylene pool that measures 110-cm in diameter and 20-cm in depth. The pool is filled to a depth of

14-cm (0.5-cm over the platform) with room temperature tap water, which is made opaque with the addition of non-toxic white chalk. The water is left to sit overnight in order to reach room temperature (22±1ºC). The pool is divided into four quadrants: Northeast, Northwest, Southeast and Southwest. These quadrants Boundaries are marked on the edges of the pool with masking tape and labelled: North, South, East and West. The escape platform of the maze was a plexiglas cylinder (13.75cm × 9 cm diameter), which has been filled with cement to weigh it down in the pool. The water level in the pool was adjusted 0.5-cm below for the visible platform tests, thereby making the escape platform visible or 0.5-cm above the white cylinder, causing a hidden escape platform during acquisition and reversal learning respectively.

The pool is located in a room measuring 5.2 × 2.4 where many posters are placed on the walls and the presence of furniture (tables and chairs) which help to provide visual cues needed. While testing, a white diffuse light was used to dimly light the room. Performance of animals as was observed in the MWM was recorded with a video camera hanged 2.1m above the pool to the ceiling on an IBM PC computer tracking system (Water maze, Actimetrics).

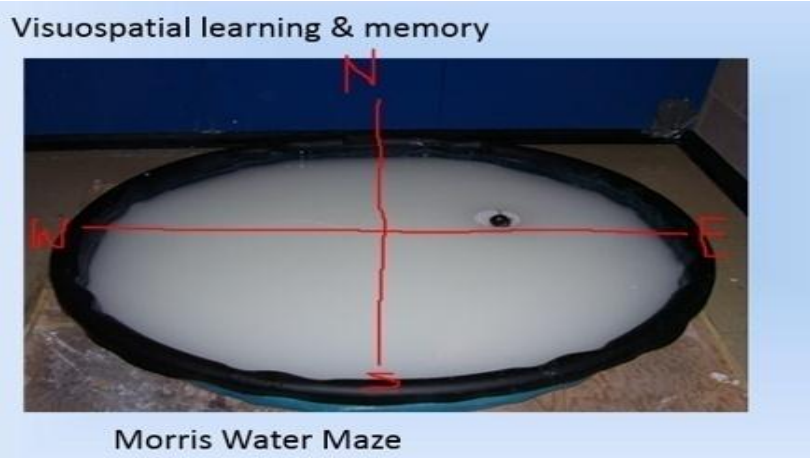

Figure 1: Morris Water Maze.[23]

Principle

In this study, testing in the water maze took 8 days as follows: day 1-3: Acquisition learning days, day 4-6: Reversal learning days, day 7: Probe trial and Day 8: Visible platform day.

The hidden platform (water is 0.5-cm above the platform) was used for acquisition and reversal training. The hidden platform during reversal was moved to the opposite side of the maze. There was no escape platform during the probe trial so that visuo-spatial memory can

be assessed. The visible top was added to the platform and moved to another quadrant of the pool during the visible- platform day. The basic visual ability and motivation to locate the platform was assessed. The mouse was removed from its home cage and placed in a clean holding cage without woodchip bedding on each day. A Paper towel that was torn into pieces was placed in the bottom of the holding cages to allow for easy and quick drying of the mouse. After it was wet (paper towel), it was changed. Mice are run in squads of 4-6 with 5-minuites between each trial (inter-trial interval) for each mouse. Short inter-trial intervals (ITIs) was discourage as it is capable of producing memory deficits in mice due to hypothermia.[24]

Procedure

Acquisition training (day 1-3)

During acquisition training, the platform was placed in the center of the Northwest quadrant (NW). Each mouse receives 4 trials per day and a maximum of 60-seconds was given to each mouse per trial to locate the escape platform. A Latin square design was used to pre-determine the starting positions of the mouse so as to avoid the repetition starting location sequences on back-to-back test days. The West, North, East, and South boundary of the quadrant were the possible start positions. Each mouse is removed from its holding cage using a small, clean 500-mL plastic container to minimize handling stress on each trial. At the appropriate start position, the animal was then place into the water. The mouse is then permitted to explore the pool and to search for the hidden escape platform for 60-sec. when the animal locates the platform, the timer was stopped (manually) and the mouse is allowed to stay on the platform. The mouse was allowed to view the extra-maze environment for 10-sec once on the platform, and thereafter, it was removed with the plastic container and returned to its appropriate holding cage. The mouse was only removed after it is on the platform for it to associates escape with the platform. Where the mouse failed to locate the platform after the allotted time, it was then guided unto the platform using the plastic container. The same procedures were followed for the rest of the mice. During acquisition training, each animal completes 4 trials per day over 3 days, making a total of 12 trials from different 4 start locations.

Reversal training (day 4-6)

Day 4 begins the reversal training. It was started by moving the platform in the acquisition phase to the opposite quadrant (SE). The same procedures as described above were followed.

In this phase, each animal also completes 4 trials per day over 3 days, making a total of 12 trials from different 4 start locations.

Probe trial - day 7

Visuo-spatial memory was assessed on probe trial day (day 7). This involves no escape platform. At four possible different start positions, each mouse was placed in the pool and allowed to explore the pool for 60-sec, the time spent in each quadrant of the maze were recorded. The mouse was removed by using the container and put in its holding cage to dry before being returned to its home cage at the lapse of the allotted time.

Visible platform – day 8

The visible platform task was conducted on day 8. The location of the visible platform was change, specifically placed within the Northeast quadrant (NE) of the pool. The same procedures as in acquisition and reversal training were carried out and mice complete 4 trials. This was done for observation of any visual impairment in the mice used for the study.

The behaviors scored in the MWM are swim latency (SL), quadrant durations (SE, NE, NW and SW) and annulus crossings frequency (annulus acquisition and annulus reversal frequency).

Statistical Analysis

The data were expressed as mean ± SEM (n=6). Differences between group means were estimated using a one-way ANOVA followed by Tukey test, using SPSS version 20.0 for Windows (SPSS Inc., Chicago IL, USA). Results were considered statistically significant at $p<0.05$.

RESULTS

Extract yield

The hydromethanolic extraction of 184.6g of *O. gratissimum* leaf powder yielded 8.4% (w/w) dark brown semisolid extract with a pleasant scent smell and pasty consistency.

Qualitative Phytochemical Screening

Phytochemical analysis of HMOG qualitatively revealed the presence of tannins, flavonoids, glycosides, alkaloids, steroids, terpenoids and saponins as shown in Table 1.

Table 1: Qualitative phytochemical analysis of *O. gratissimum* leaf extract.

Secondary metabolites	HMOG
Phytosterols	-
Tannins	+
Flavonoids	+
Glycosides	+
Alkaloids	+
Steroids	+
Terpenoids	+
Phenols	-
Saponins	+

Key: + present; - absent

Acute Toxicity Test

No mortality was recorded and neither was there any visible drug-induced sign of toxicity even with the highest dose (5000 mg/kg) in the treated mice. The behavioral changes observed were weight loss and increased in bodily activities in the extract treated animals when compared to normal control animals. Thus, the LD50 was determined to be above 5000 mg/kg body weight.

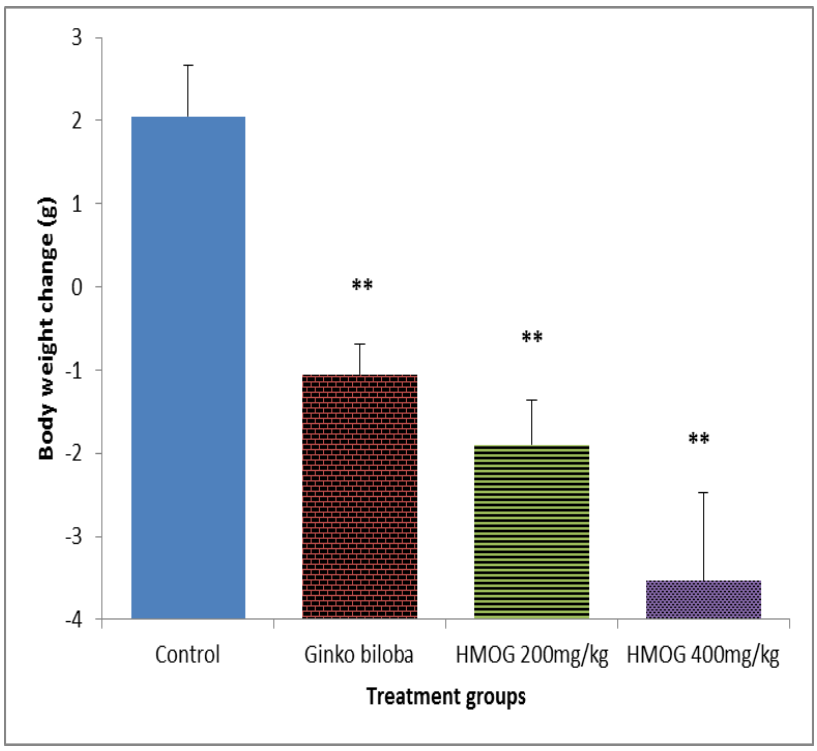

Figure 2: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on body weight. Values were expressed as mean ± SEM (n = 6 in each group), ** Significant at p<0.001 against control.

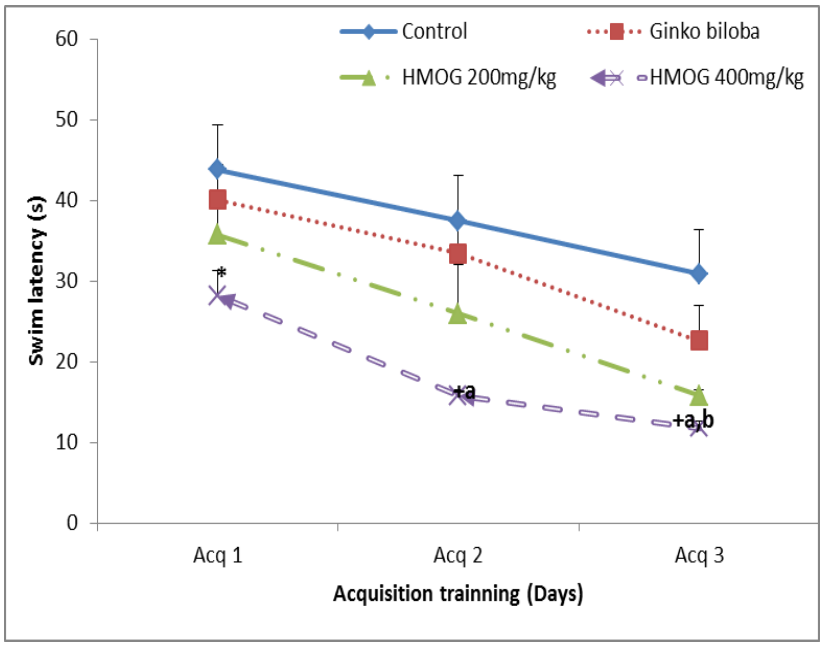

Figure 3: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on swim latencies during acquisition learning in MWM. Values were expressed as mean ± SEM (n = 6 in each group), * significant at p<0.05 against control, [+] significant at p<0.01 against control, [a] significant at p<0.05 against *Gingko biloba* group and [b] significant at p<0.05 against HMOG (200mg/kg).

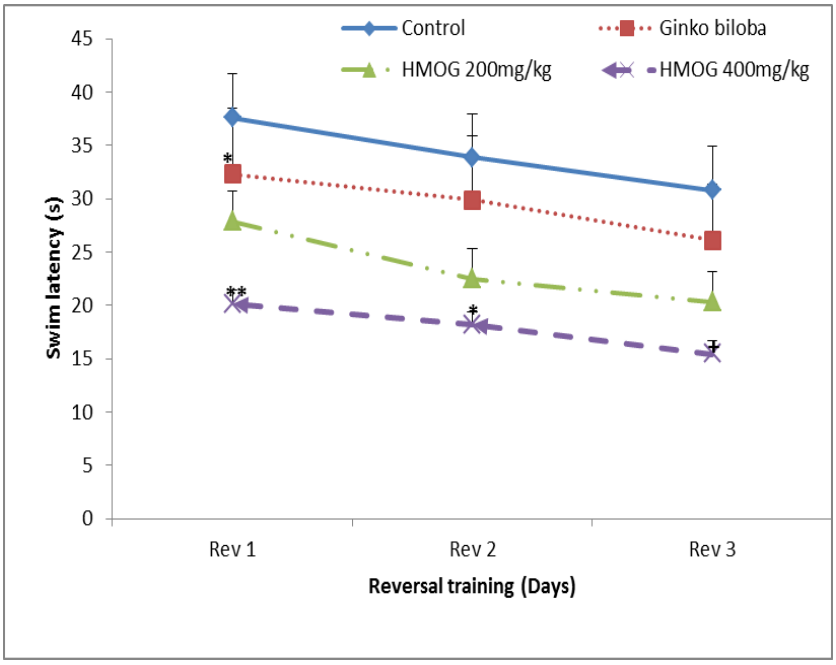

Figure 4: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on swim latencies during Reversal learning in MWM. Values were expressed as mean ± SEM (n = 6 in each group), * significant at p<0.05 against control, [+] significant at p<0.01 against control, ** significant at p<0.001 against control.

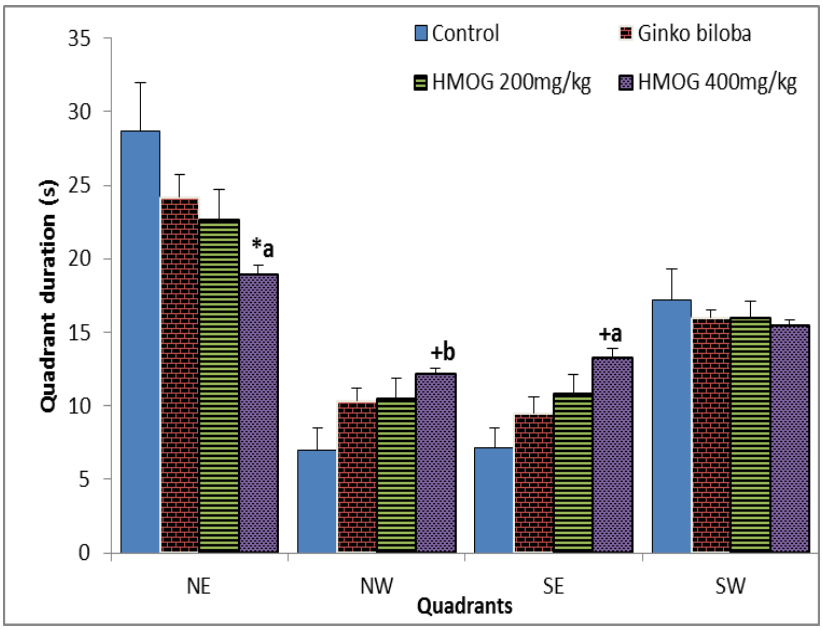

Figure 5: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on quadrant duration. Values were expressed as mean ± SEM (n = 6 in each group). * significant at p<0.05 against control, + significant at p<0.01 against control, a significant at p<0.05 against *Gingko biloba* group and b significant at p<0.05 against HMOG (200mg/kg).

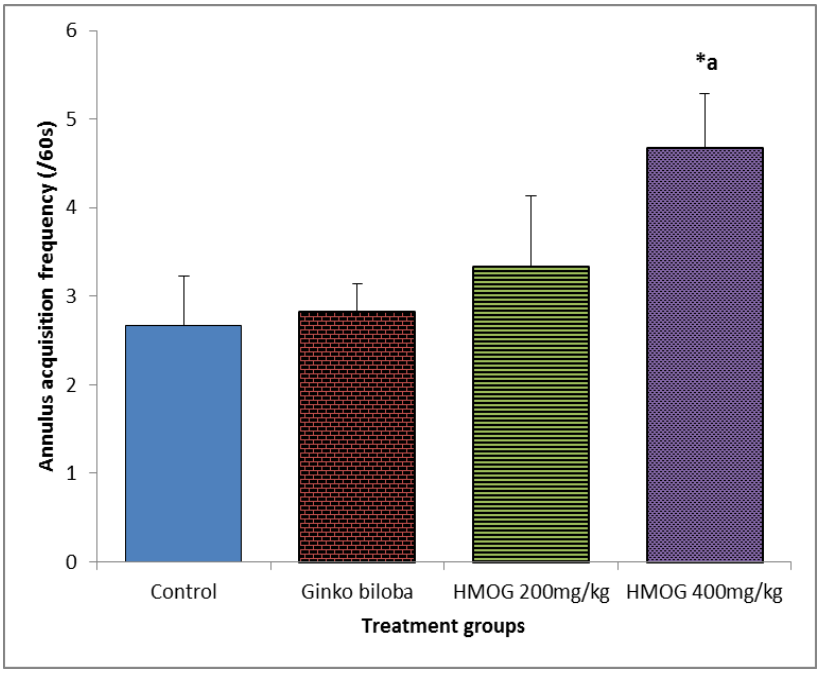

Figure 6: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on Annulus acquisition frequency. Values were expressed as mean ± SEM (n = 6 in each group).* Significant at p<0.05 against control and a significant at p<0.05 against *Gingko biloba* group.

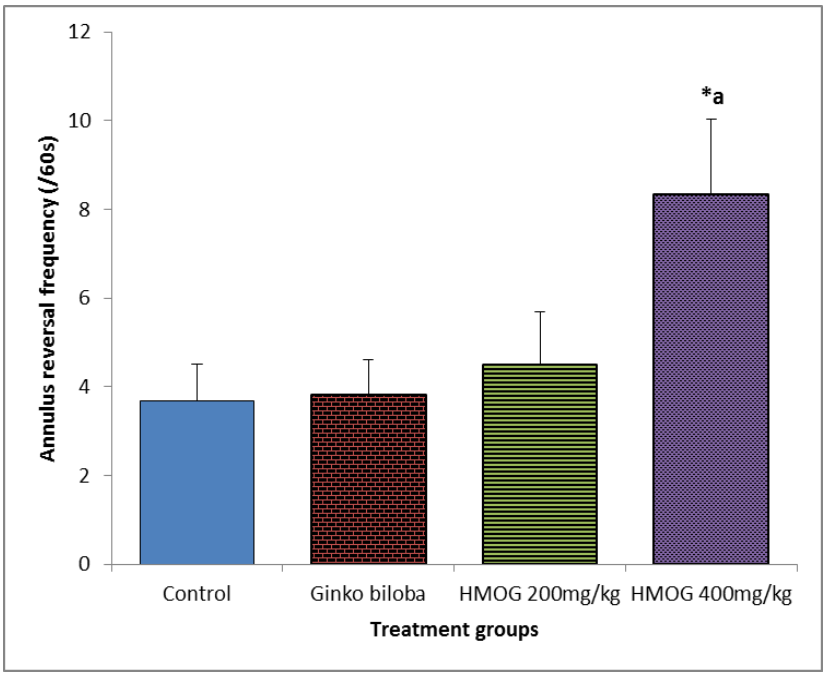

Figure 7: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on Annulus reversal frequency. Values were expressed as mean ± SEM (n = 6 in each group). * Significant at p<0.05 against control and [a] significant at p<0.05 against *Gingko biloba* group.

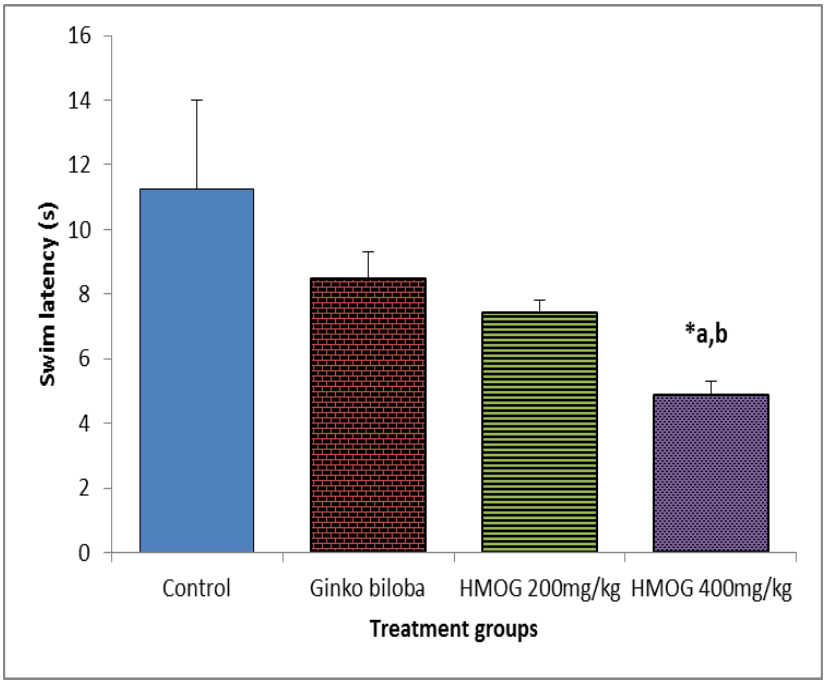

Figure 8: Effect of hydromethanolic leaf extract of *Ocimum gratissimum* (HMOG) on swim latencies (visible platform day). Values were expressed as mean ± SEM (n = 6 in each group),* significant at p<0.05 against control, [a] significant at p<0.05 against *Gingko biloba* group and [b] significant at p<0.05 against HMOG (200mg/kg).

DISCUSSION

The present study was carried out to assess visuo-spatial learning and memory in mice treated with HMOG. These behavioural outputs were examined by Morris water maze (MWM).

The results of the acute toxicity of this study revealed no adverse effects on the treated animals after observation. This is an indication that the extract has a wide margin of protection and thus administration as done in folk medicine may not have any immediate adverse effects as advanced by.[7] [25] reported that substances with LD_{50} value above 5000/kg body weight could be classified as substances with low toxicity.

HMOG was shown to contain phytochemicals such as alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and glycosides. These phytochemical constituents present in this extract are in agreement with the work done by.[8] Plants constituents have been reported to be responsible for a host of pharmacological actions, most notably antioxidants effect. The flavonoids and glycosides present in this extract (HMOG) had earlier been reported by[8] to be rich in antioxidant property. For example, the antioxidant effect of flavonoids is by scavenging the free radicals and reactive oxygen species and glycosides are by its anti-inflammatory responses. Studies by[26,27] have established that antioxidant ways of improving learning and memory are attenuation of apoptosis, the inhibition of membrane lipid peroxidation, anti-inflammatory effects and the direct inhibition of amyloid-β aggregation. All these will predispose to increased blood-brain circulation, increased development of neurons (neurogenesis), leading to improve neuroplasticity; improvement in the brain immune system against brain neurons degenerating disease-causing agents. Thus, learning and memory improvement effect of this extract (HMOG) may be attributed to its antioxidant phytochemicals constituents. There was a reduction in body weight of the treated mice (400 mg/kg) in this study. This is in agreement with previous study by.[28] Increased body weight has been reported to be associated with learning and memory impairment as a result of sedentary life style observed in those suffering from various neurodegenerative disorders.[29] This reduction in body weight as a result of administration of the extract is an improvement when compared with the nootropic drugs that are usually associated with increased body weight.[30] It is therefore deduced that the extract may be useful in body weight gain control.

In this study, the significant decrease in swim latencies during reversal learning and visible platform day and an increase in retention quadrant and annulus reversal frequency as seen with the MWM showed that there is better learning and memory and no visual impairments.

These findings indicate that the extract could enhance memory and may be a potent neuroprotectant in mice. Therefore, visuo-spatial memory was well developed by the MWM. This decrease in the time to find the hidden platform in this study is in agreement with the work done by,[31] which assessed learning and memory in mice treated with *Bramhi ghrita* and it was discovered that the mice had good visuo-spatial memory as a result of their reduction swim latencies. This result suggests that the brain areas involved in this function such as cerebral cortex, neocortex and hippocampus[32] are well affected by the treatments.

Hippocampus enhanced learning and memory by ensuring rapid neurogenesis.[33] The phytochemicals attributed to antidepressants like tannins and antioxidants activities like flavonoids and glycosides have been reported to stimulate neurogenesis in adult rodent brains.[34] The reported phytochemicals that were responsible for improved neurogenesis were also present in this extract (HMOG). The results suggest that the extract may enhance neurogenesis through these constituents.

In this study, Gingko biloba (AChE inhibitors class), was the standard drug of choice that improves learning and memory. The findings of this study showed that Gingko biloba effect is comparable to the extract-treated groups suggesting a possible inter-relationship in their mechanism of action, which could be as a result of similarities in their (HMOG and *Gingko biliba*) phytochemical constituents.[8,35]

Studies have shown that some plants or plant products such as *Melissa officinalis*[36] *Ginseng*[37] and *Morinda citrifolia*[38] could exert *Gingko biloba* like effect.[30] reported that *Ginkgo biloba* extract promotes proliferation of endogenous neural stem cells, which might be a reason it improves memory and cognitive impairments. Therefore, there is possibility that the extract (HMOG) may be exhibiting *Gingko biloba*-like effects as it relates to learning and memory improvement. The behavioral effect of HMOG may be likened to have occurred by AChE inhibiting mechanism which will lead to an increase in the level of acetylcholine in the brain thereby, facilitating neurotransmission in the brain systems.

The findings of this study suggests the possible involvement of phytochemical constituents particularly those of antioxidants and anti-depressants class to be responsible for the observed learning and memory improvement activity of the extract (HMOG). The extract at high dose (400 mg/kg), showed a better learning and memory improvement potential over *Gingko biloba*. This reveals that the effects of the extract were dose-dependent.

CONCLUSION

From the foregoing therefore, it is quite obvious that the dosage dependent and statistically significant decrease in swim latency and an increase in retention quadrant and annulus reversal frequency indicate that the hydromethanolic leaf extract of *Ocimum gratissimum*'s has capacity to improve visuo-spatial learning and memory. Thus, visuo-spatial learning and memory was well improved in mice, which may be associated with the phytochemicals present in the extract, and this may provide a new therapeutics option for memory loss.

ACKNOWLEDGEMENT

Authors are thankful to the entire staff of department of Physiology of Ebonyi State University, Abakaliki, Ebonyi State for extending their kind help during the experimentation.

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