Ethanolic extract of Gongronema latifolium improves learning and memory in Swiss albino Mice

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Abstract

Learning and memory are vital attributes of human intelligence. These processes underlie the very nature of our self-awareness, planning and execution of task. The number of people living with dementia worldwide is currently estimated at 35.6 million. About 80% of the world’s population depend on herbal remedies to curb mental disorders. This study aimed to investigate the effects of administration of ethanolic leaves extract of Gongronema latifolium (GL) on learning and memory in mice. Thirty (30) adult male Swiss white mice were assigned into three groups of ten mice each. Group 1 served as the control. Groups 2 and 3 received 200mg/kg and 400mg/kg of GL extract respectively. All the animals were allowed food and water ad libitum. Learning and memory was assessed using the Morris water maze. Results showed that the swimming latencies during the acquisition and reversal trainings, and probe trial were significantly (p<0.001) decreased in the extract treated groups when compared with the control. The south-east duration was significantly (p<0.001) increased in the extract treated groups when compared with the control group. There was a corresponding decrease (p<0.001) in the south-east duration in the extract treated groups when compared to the control. In conclusion, extract of GL enhances visuo-spatial learning and cognitive memory. It could therefore be of therapeutically use in cases of memory loss or impairment.

Keywords: Gongronema latifolium, learning, memory, Morris water maze, mice.

INTRODUCTION

Learning is the process by which we acquire knowledge about the world. It is a change in behaviour or in potential behaviour that occurs as results of experience. Memory on the other hand is the ability to retain and retrieve information. Learning and memory are vital attributes of human intelligence. These processes underlie the very nature of our self-awareness, planning and execution of task. It is difficult to overstate the importance of learning and memory, but could be viewed in a situation of impairment or disorders as in dementia.

Dementia is one of the ages related mental problems and characteristic symptom of various neurodegenerative disorders including Alzheimer’s disease which is age related. The number of people living with dementia worldwide is currently estimated at 35.6 million. This number will double to 65.7 million in 2030 and 115.4 million in 2050. Dementia is debilitating in nature and due to which an enormous social and economic worry is placed on our society. Currently there is no proper cure for the disorder and much of the treatments available have been able to only delay the progression of the disease or provide symptomatic relief for a short time period. Therefore there is a need for a different approach to the treatment of these diseases.

In the quest to curb mental disorders, many Africans including Nigerians and in fact 80% of the World’s population depend on herbal remedies because of their affordability, efficacy and reduced side effect. One of such medicinal plants that has been used is Gongronema latifolium.

Gongronema latifolium (Asclepiadaceae) is a herbaceous climber with yellow flowers and stem that yields characteristic milky exudates. It is widespread in Tropical Africa and can be found from Senegal east to Chad south, and to Democratic Republic of Congo. It occurs in rainforest, deciduous, and secondary forest, and also in mangrove and disturbed roadside forests, from sea level up to 900m altitude.

Phytochemical analysis reveals that G. latifolium is nutritionally high in iron, zinc, vitamins, proteins and amino acids. The leaf extract contains alkaloids, saponins, tannins, flavonoids and glycosides. Its roots contain polyphenols in abundance, alkaloids, glycosides and reducing sugars.

The plant has anti-inflammatory property, antioxidant, antimicrobial, anti-diabetic, anti-ulcerogenic and anti-malaria activities.
In Nigeria, the plant is primarily used as spice and vegetable. It is traditionally used in the South-Eastern part of Nigeria for the management of many disease conditions to include diabetes, high blood pressure and dementia\textsuperscript{17}. Due to the purported use of \textit{G. latifolium} in the treatment of dementia by or traditional herbalist, it is the aim of this study to provide scientific prove to this claim by investigating the effect of \textit{G. latifolium} on learning and memory in mice using the Morris water maze test.

**MATERIALS AND METHODS**

**Experimental animals**

Adult male Swiss white mice (15g-33g) used for the study were purchased from the animal house of the department of Physiology, University of Calabar. They were kept in the animal house of the department of physiology, Abia State University, Uturu for two weeks for acclimatization before actual experimentation commenced. The temperature of the animal house was 26 ± 2.0\textdegree}C. The mice were exposed to natural day light cycles, and allowed access to rodent laboratory chow obtained from Vital Feeds Nig. Ltd., Lagos, Nigeria and clean drinking water \textit{ad libitum}. The research was approved by the ethical committee of the department of Physiology, Abia State University, Uturu.

**Experimental Design**

Mice were weighed using a digital weighing balance. Their individual weights were between 17g and 33g. Identification of the animals was simply done using identification cards attached to each cage. The mice used for this study were thirty. There were grouped into three viz: the control group, the low dose group and the high dose group, each having ten mice.

**Preparation of ethanolic leaves extract of \textit{Gongronema latifolium}**

The leaves of the plant, \textit{Gongronema latifolium} were purchased in a local market in Calabar, Cross River State, Nigeria. The leaves were washed and dried under shade, then blended into coarse powder and stored in a cool dry place away from light until required for use. 400g of the grounded leaves was dissolved in 1250ml of ethanol (BDH Ltd Poole, England) and allowed to stay overnight. The mixture was then centrifuged and the supernatant collected. The supernatant was suction filtered, first, using Whatmann no. 1 filter paper, and then a second time using cellulose filter paper. The filtrate was evaporated to dryness at 30\textdegree}C using a vacuum rotary evaporator (Caframo VV2000, Ohio) and water bath (Caframo WB2000, Ohio). This extraction gave a percentage yield of about 4.3%. The dry ethanol extract was reconstituted to a stock of 500mg/ml in 0.9% saline from which various dose concentrations were obtained. Doses of 200mg/kg and 400mg/Kg representing the low dose and high dose respectively were obtained and administered on the mice at the rate of 0.1ml/10g body weight orally. The control mice were given normal saline via oral gavaging.

This dosage of administration was adopted from earlier published works\textsuperscript{14,15}.

**Assessment of learning and memory**

Assessment of the effect of ethanolic leaves extract of \textit{Gongronema latifolium} on visio-spatial learning was done using the Morris water maze. The Morris water maze is made of a circular plastic divided into four quadrants. Water was filled to a height of about 30cm. Non-toxic white tempura paint was added to make the water opaque. The diluted paint concentrate was poured into the pool, and water again added to the pool until the escape platform was submerged by 1cm. The water was left to sit overnight in order to achieve room temperature (25s + 1\textdegree}C).

The test consisted of three days of acquisition training and three days of reversal training (with the platform in the opposite quadrant) where the mice were trained to use extra-maze visual cues to locate an escape platform hidden just below the surface of the opaque water in one of the quadrants\textsuperscript{18}. On the seventh day, the platform was removed and the duration in each quadrant noted, to test for visuo-spatial learning and memory which is hippocampus dependent\textsuperscript{19}.

**Procedure**

Administration of the drug was done 5 minutes before experimentation. Mice were introduced to the pool with a plastic container at a predetermined start position (North, East, South or West) and allowed to locate the hidden escape platform in one of the quadrant (NE, NW, SE or SW) during first three days of acquisition. Mice were given four trials per day and allowed 5 minutes rest between trials. If mice did not locate the hidden platform within the stipulated 60 seconds trial period, there were guided to locate the platform and allowed to explore for about 10 seconds. The following three days consisted of reversal training with the hidden platform in the opposite quadrant, and each animal given four trials each day. In order to avoid repeating a particular sequence for introducing each mouse, and thereby eliminate stereotype, there was a chart for start position for each mouse in all the trial sessions. Day 7 of the experiment was the probe trial consisting of a single trial of 60 seconds without an escape platform.

Behaviours were scored based on visual assessment of the animals in the maze. The behaviours scored were swim latency, and quadrant duration\textsuperscript{20}. The mice were placed in cages with shredded paper towel bedding to make them dry easily. A heating lamp was also provided to prevent the animals from developing hypotherma.

**Data Analysis**

All data were analyzed using StatView 5.0.1 (SAS Institute) for PC or Mac, Microsoft office excel, 2007 version (Microsoft Inc.), and primer of Biostatistics (version 3.01), an MS Dos based statistical package (McGraw-Hill, Inc.). The analysis of variance (ANOVA) was used to test for variability within and among groups. Results were expressed as mean ± SEM (standard error of the mean) and probability level p<0.05 was accepted as significant.

**RESULTS**

**Swim latency during acquisition training (days 1-3)**

The swim latencies for the control, low dose and high dose groups during the acquisition training on the first day were 41.6 ± 0.87, 34.1 ± 0.80 and 28.1 ± 0.59 seconds respectively, on the second day, 35.3 ± 0.60, 30.5 ± 0.97, and 23.5 ± 1.07 seconds respectively were recorded, and on the third day, 31.5 ± 0.78, 27.7 ± 1.04 and 20.1 ± 0.71 seconds respectively were taken. The result showed a significantly (p<0.001) lower swim latencies in the high dose group compared with the control and low dose groups, it s also significantly (p<0.001) lower in the low dose group compared with control group, fig. 1.

**Swim latencies during reversal training (days 4-6)**

The swim latencies between the control group, low dose and high dose groups during the reversal training was 41.4 ± 0.87, 34.1 ± 0.80 and 28.1 ± 0.59 seconds respectively, on the second day, 35.3 ± 0.60, 30.5 ± 0.97, and 23.5 ± 1.07 seconds respectively were recorded, and on the third day, 31.5 ± 0.78, 27.7 ± 1.04 and 20.1 ± 0.71 seconds respectively were taken. The result showed a significantly (p<0.001) lower swim latencies in the high dose group compared with the control and low dose groups, it s also significantly (p<0.001) lower in the low dose group compared with control group, fig. 1.
0.95, 32.9 ± 1.23 and 26.9 ± 0.60 respectively for day one, 37.7 ± 0.78, 29.1 ± 1.25 and 20.3 ± 0.63 for day two, and 32.2 ± 1.19, 25.0 ± 1.36, 16.2 ± 0.65 for day three. The result shows a significant (p<0.001) lower swim latencies during the reversal training in the extract treated groups compared to the control. This is shown in fig. 2.

Comparison of duration in the south-east and south-west quadrants during probe trial

The comparison of duration in the south-east quadrant during probe trial (recorded on day 7) in the control, low dose and high dose groups was 16.5 ± 0.65, 25.3 ± 0.84 and 30.3 ± 0.60 respectively. The result showed a significant (p<0.001) increased duration in the south-east quadrant in the test groups compared with the control group. The high dose group also showed a significant (p<0.001) increase in the south-east quadrant duration compared with the low dose group. This is shown in fig. 3.

The comparison of duration in the south-west quadrant during probe trial in the control, low dose and high dose groups was 91 ± 0.43, 10.5 ± 0.54, and 7.2 ± 0.29 respectively. The result showed a significant (p<0.05) decreased quadrant duration in the extract treated groups compared with the control. It was in turn significantly (p<0.05) lower in the high dose group compared with the low dose group. This is shown in fig. 3.

Comparison of swim latency during the visible platform task

The swim latency during the visible platform task recorded on day 8 for the control, low dose, and high dose groups were 9.3 ± 0.42, 7.2 ± 0.5 and 5.2 ± 0.29 respectively. The swim latency in the extract treated groups was significantly (p<0.001) decreased compared with the control. The high dose group showed a decreased (p<0.001) latency compared with the low dose. This is shown in fig. 4.
Fig. 2: Swim latency during reversal training of the Morris water maze in control and test groups. Values are expressed as mean ±SEM.

*** = p<0.001 vs control,
c = p<0.001 vs low dose.

Fig. 3: Swim latency in the south east and south west quadrants during probe trial of the Morris water maze in the different experimental groups. Values are expressed as mean ±SEM.

* = p<0.05, ** = p<0.01, *** = p<0.001 vs control,
a = p<0.05, c = p<0.001 vs low dose.
DISCUSSION

This study was aimed at studying the effect of acute administration of ethanolic leaves extract of *Gongronema latifolium* on learning and memory in Swiss white mice. Learning and memory in this study was assessed using the Morris water maze. The Morris water maze is employed as a test for spatial learning in rodents. The hidden platform version of the Morris water maze test is a test of spatial memory which is sensitive to hippocampal damage, while the visible platform of the maze is a non-hippocampal task which is disrupted by dorsal striatum lesions. In this study, the swimming latencies during acquisition and reversal training were dose dependently decreased significantly in the extract treated groups. This is an index for better learning abilities exhibited by mice treated with extract of *Gongronema latifolium*. The South-East and South-West quadrants duration test showed that extract treated mice spent much time in the South-East quadrant, and less time in the South-West quadrant. This is an index for better memory exhibited by the extract treated groups as earlier reported. The visible platform task showed a dose-dependent decrease in swimming latency among the extract treated groups compared to the control group. This is an index for visio-spatial learning. Thus, *Gongronema latifolium* enhances visio-spatial learning, and improves memory.

In conclusion, the ethanolic leaves extract of *Gongronema latifolium* improved visio-spatial learning and memory in mice. This ability of the extract to enhance vision-spatial leaning and cognitive memory could be of therapeutic use in the management of memory loss or impairment.

REFERENCES


