The cognitive-enhancing activity of hydromethanolic leaf extract of Ocimum gratissimum in mice

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Abstract
Cognitive effect of hydromethanolic leaf extract of O. gratissimum (HMOG) was evaluated in mice. Twenty four male mice weighing between 25-30 g were randomly assigned into four groups of 6 mice each. Group 1 received distilled water and served as control, group 2, 3 and 4 received 10 mg/kg body weight (b.wt) of Ginkgo biloba (standard drug), 200 and 400 mg/kg b.wt. of HMOG respectively for 21 consecutive days. Data were analyzed using one way and student`s t-test, where \( p < 0.05 \) was considered significant. Qualitative phytochemical screening of HMOG done indicated the presence of flavonoids, alkaloids, glycosides and tannins. The cognitive indices: latency to alternate and percentage spontaneous alternation (\( \% \) SA) showed a significant decrease (\( p < 0.001 \)) respectively when compared with the control and the standard drug group. This study concluded that HMOG improved cognition in mice which may be attributed to the phytochemicals in the extract.

Keywords: Cognitive, O. gratissimum, Ginkgo biloba, T-Maze. Phytochemicals

1. Introduction
Memory is the most important function of the brain; a complex process which involves acquisition of information from the surroundings and consolidation of the acquired information and then retrieving it for future use. Central cholinergic system plays a major role in the learning and memory process through various neuronal pathways and neurotransmitters. Presence of acetylcholine within the neocortex is sufficient to ameliorate learning deficits and restore memory \([1]\). Deficits occurring in these pathways may result in occurrence of various cognitive disorders like amnesia and dementia. Alzheimer`s disease (AD) is one of the most common causes of impaired cognitive functions. The prevalence of AD increases with the age (65 years) from 2% to 30-45% in those over 85 years \([2]\), AD and stroke together rank as the third most common causes of death \([3]\). The incidence of AD for those age 65 years and above was 3.24 per 1000 individual in a year \([2]\). Besides reduced cholinergic activity, oxidative stress is also one of the major causes for memory loss in AD. Hence, agents which act by reducing oxidative stress and increased cholinergic activity are found to be useful in treating memory impairments \([4]\). These neurodegenerative diseases are usually treated with ACHE inhibitors and muscarinic receptors agonists which increases cholinergic neurotransmission causing an improvement in cognitive deficits in AD \([5]\).

Extensive research is going on in different plants all around the world as plants extracts have a relatively higher therapeutic window, lesser side effects and are economical. Plants extracts may also provide a source of new compound as many synthetic drugs originated from herbal sources. *Ocimum gratissimum* (*O. gratissimum*) Linn, commonly called scent leaf, tea bush, fever plant or clove basil, is an herbaceous plant which belongs to the family labiatae \([6]\). In Nigeria, it is called *Efinrin* in Yoruba, *Daidoya* in Hausa, *Nchanwu* in Igbo, *Ntonng* in Calabar, *Aramogbo* in Edo \([7]\). 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, conjunctivitis, skin diseases, pneumonia, tooth and gum disorder, fever, insomnia, skin and liver diseases and as mosquito repellants \([8]\). The Igbo people in the South-East Nigeria use it in Baby's cord management after delivery, because they believe it keeps the baby`s cord and wound surfaces sterile \([6]\). Previous phytochemical screening of the leaf extract of *O. gratissimum* (aqueous) reveals alkaloids, saponins, tannins, anthraquinone, flavonoids, steroids, terpenoids and glycosides \([9,10]\). Besides, *O. gratissimum* leaves showed the presence of essential oils such as eugenol, cineole, ocimol, citral, geraniol, thymol, linalool, tetratriacontane, gratimissin, gratimissic acid and \( \beta \)-caryophyllene \([11]\).
Eugenol represents its main physiologically active component and has been widely used in perfumery, flavour and pharmaceutical products, cosmetology and soap industries [12-13]. Other active components are ursolic acid, rosmarinic acid, lineoleic acid, oleic acid, and linoleic acid [14]. The plant has a high content of phenols, carotene and vitamin C [14]. Among the notable pharmacological effects of the leaf extract of O. gratissimum that have been reported are antidiarrheal [15], wound healing [16], anti-inflammatory [17], antiangiogenic [18], antioxidant [19] and anticonvulsant [19]. However, no studies were conducted to explore the effect of hydromethanolic leaf extract of Ocimum gratissimum (HMOG) extract in male mice against cognition in-vivo, hence this study.

2. Materials and Methods
2.1 Chemicals and drugs
All chemicals and drugs used in this investigation were of analytical grade and were obtained from Sigma, Saint Louis, USA. Ginkgo biloba was used as the reference drug and memory improvement drug. In this study, Ginkgo biloba was administered orally to mice in a dose of 10 mg/kg suspended in distilled water.

2.2 Experimental animals
This study was carried out using 24 male mice weighing between (25-30) g. The animals were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Nigeria. The animals were housed in a cross ventilated room in cages at (22 ± 2.5 °C) with 12 h dark/12 h light cycles and were fed with standard grower 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 [20]. This study was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences, Ebonyi State University with reference number (EBSU/REC/MPC/1706/02/001).

2.3 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 [21] (2015) 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 [21]. 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 (BUCHI, 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.

2.4 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, phytoestrogens and glycosides using standard phytochemical methods as described by [22-24].

2.5 Acute Oral Toxicity Study
The lethal dose (LD₅₀) of the hydromethanolic leaf extract of O. gratissimum was determined by the method of [25] and 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 1000mg/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 5000mg/kg body weight (p.o.). The median lethal dose (LD50) was calculated using the second phase.

2.6 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 4 mice received 200 and 400 mg/kg body weight of HMOG respectively for 21 consecutive days by single oral gavage daily. At the end of the administration, cognitive memory was observed in mice using spontaneous alternation test (T-Maze).

2.7 Assessment of Cognitive functions
Cognitive ability in all the groups of mice was carried out using T-Maze according to the method described by [27]. T-Maze test for cognitive ability in animals, also known as spontaneous alternation behavior (SAB). Rodents have the natural tendency in a T-maze to alternate their choice of goal arm [27], by making use of their ‘working memory’. This implies that each response varies according to what was previously done [28]. Alternation reflects the motivation of the animal to explore its environment and locate the presence of resources such as food, water, mates or shelter. Animals do not need to be deprived of such resources to show alternation behavior; because it is an innate response, which comes about as survival instinct [27]. Spontaneous alternation behaviour (SAB) comprises the tendency for rats, mice and other animals to alternate their (conventionally) non reinforced choices of T- or Y-maze arms on successive opportunities. Research has shown that an animal must remember which arm it had entered on a previous occasion to enable it to alternate its choice on a following trial [29]. The T-maze is an elevated or enclosed apparatus in the form of a T placed horizontally. Animals were started from the base of the T and allowed to choose one of the goal arms abutting the other end of the stem. If two trials are given in quick succession, on the second trial the rodent tends to choose the arm not visited before, reflecting memory of the first choice. Each trial is completed in less than 2 minutes. The maze was set so that the central partition was in place and
all guillotine doors were raised. Then the animal was placed in the start area and allowed to choose a goal arm. It was then confined in the chosen arm by quietly sliding the door down. After 30s, the central partition was removed and the animal removed, the guillotine door of the sample arm was then raised and the animal replaced in the start area facing away from the goal arms and allowed to choose between the open goal arms. Each trial took one minute. Behaviours that were scored in mice on the T-maze are latency to alternate (LTA) and percentage spontaneous alternation (%SA).

2.8 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.

3. Results
3.1 Extract Yield
The hydromethanolic extraction of 184.6 g of O. gratissimum leaf powder yielded 8.4% (w/w) dark brown semi-solid extract with a pleasant scent smell and pasty consistency.

3.2 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>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>HMOG</th>
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<tbody>
<tr>
<td>Phytosterols</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
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<tr>
<td>Glycosides</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
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<td>Steroids</td>
<td>+</td>
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<td>Terpenoids</td>
<td>+</td>
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<tr>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
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</tbody>
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Key: + present; - absent

3.3 Result of 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 LD<sub>50</sub> was determined to be above 5000 mg/kg b.wt.

Fig 1: Effect of HMOG on Latency to Alternate (LTA). Values were expressed as mean ± SEM (n = 6 in each group), ** Significant at p< 0.001 against control, * Significant at p< 0.05 against control, a Significant at p< 0.05 against Ginkgo biloba group.

Fig 2: Effect of HMOG on Percentage Spontaneous alternation (%SA). 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 Ginkgo biloba group and b Significant at p< 0.05 against HMOG (200mg/kg).
4. Discussion
The use of medicinal plants in the treatment of ailments in both the developed and developing countries is on the increase. Researchers have authenticated the therapeutic efficacy of most of these herbs [30-31]. In this study, the cognitive effect of hydromethanolic leaf extract of Ocimum gratissimum in mice was investigated using spontaneous alternation test (T-Maze) as behavioural paradigm. 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 [30] and [8], [32] reported that substances with LD₅₀ value above 5000/kg body weight could be classified as substances with low toxicity.

The decrease in LTA (figure 1) and an increase in % SA (figure 2) showed that the mice have good cognitive ability as examined on the T-maze. Hence, increase in learning and memory. This test relies on an innate ability of rodents to explore their natural environment [53, 34] reported that shortened latency to alternate has been shown to be related with improved memory processes. The decrease in LTA and an increase in % SA as observed in this study is in agreement with the work done by [33] (2009) who revealed that the seed extracts of Ziziphus mauritiana (L.) have effect on cognitive memory of rats in a Y-maze test by increasing the % SA in rats.

HMOG was shown to contain phytochemicals such as alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and glycosides as shown in table 1. These phytochemical constituents present in this extract are in agreement with the work done by [10]. Plants constituents have been reported to be responsible for a host of pharmacological actions, most notably antioxidants effect [9-10]. The flavonoids and glycosides present in this extract (HMOG) had earlier been reported by [10] to be rich in antioxidant properties. For example, the antioxidant effect of flavonoids is by scavenging the free radicals and reactive oxygen species and glycosides is by its anti-inflammatory responses [10]. Studies by [35-36] 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 increase blood-brain circulation, increased development of neurons (neurogenesis), leading to improved neuroplasticity; improvement in the brain immune system against brain neurons degenerating diseases causing agents [36]. Thus, the learning and memory improvement effect of this extract (HMOG) may be attributed to its antioxidant phytochemical constituents.

In this study, Gingko biloba (AChE inhibitors class), was the standard drug of choice that improved 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, that may have come about in their (HMOG and Gingko biloba) similarities in phyto-chemicals constituents [37-38]

Studies have shown that some plants or plant products such as Melissa officinalis [39-40], Ginseng [41-42] and Morinda citrifolia [43-44] could exert Gingko biloba like effect [38] reported that Gingko biloba extract promotes proliferation of endogenous neural stem cells, which might be a reason it improves memory and cognitive impairments. Therefore, there is a 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.

5. Conclusion
This study has shown that hydromethanolic extract of O. gratissimum leaf enhanced cognition in mice particularly at 400 mg/kg body weight of the extract administered.

6. References


