



Research Article

Effects of n-Hexane Extracts of *Ocimum gratissimum* and *Momordica charantia* Leaves on Learning and Memory in Scopolamine-induced Rat Model of Dementia

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ABSTRACT

Objective: Cholinesterase inhibitors such as donepezil are being used to improve memory in people with dementia, but adverse effects associated with the use of these drugs coupled with cost have limited their use, hence the need to explore options in medicinal plants for the treatment of dementia. The aim of this study was to investigate the memory restorative effects of n-hexane extracts of *Ocimum gratissimum* (OG) and *Momordica charantia* (MC) in scopolamine-induced dementia in Wistar rats.

Methods: Thirty male Wistar rats were randomly divided into six treatment groups as follows: Control, Scopolamine only (1 mg/kg b.w), Donepezil (2 mg/kg b.w) + scopolamine (1 mg/kg b.w), OG (400 mg/kg b.w) + scopolamine (1 mg/kg b.w), MC (400 mg/kg b.w) + scopolamine (1 mg/kg b.w) and co-administration of OG and MC (400 mg/kg b.w each) + scopolamine (1 mg/kg b.w). The animals were treated for 15 days. Morris water maze, light and dark box test were conducted to evaluate learning and memory. Malondialdehyde (MDA) level and Acetylcholinesterase (AChE) activities were also assessed using thiobarbituric acid procedure and Ellman method respectively.

Results: The result showed a significant reduction in escape latency, MDA level, AChE activity and a significant increase in the probing frequency in OG + scopolamine; MC + scopolamine and OG, MC + scopolamine treated groups when compared to the scopolamine only group ($p < 0.05$). There was no significant difference in the escape latency, MDA, AChE activity and probing frequency between the extracts treated groups and donepezil group.

Conclusion: The study concludes that n-hexane leaves extracts of OG and MC have the ability to restore memory impairment induced by scopolamine.

INTRODUCTION

Dementia is a neurodegenerative disorder wherein there is loss of memory and cognitive ability of sufficient severity to interfere with normal daily functioning of an affected individual as the disease progresses.[1] Its incidence is continually growing with an estimation of about 35.6 million people affected worldwide.[2] Due to these rapidly growth of prevalence, high expenditure cost, and unsatisfactory outcomes of therapeutic strategy, dementia has been recognized as one of the major medical and social challenges especially in developing countries.[3]

Recent findings show that these cognitive dysfunction in dementia occurs as a result of oxidative stress elevation in the brain,[4] hippocampal atrophy,[5] and the disturbances of neurotransmission with cholinergic hypofunction attributed to degeneration of cholinergic neurons projecting to the cortex and hippocampus, reduced choline acetyltransferase and choline uptake, reduction of acetylcholine (ACh) synthesis, increase acetylcholinesterase activities and depletion of brain acetylcholine receptors an essential hallmark.[6-7]

Therefore, the modulation of cholinergic function

becomes the approach to the treatment leading to the use of acetylcholine esterase (AChE) inhibitors such as tacrine, donepezil, and rivastigmine approved by U.S. Food and Drug Administration for the amelioration of dementia symptoms.[8-9] The adverse effects associated with these agents have limited their use.[10] Consequently research efforts have focused on finding other protective agents against dementia. Polyphenolic compounds in medicinal plants have been shown to promote learning, memory and intelligence and are currently being studied because of their potential antioxidative properties. This may provide an alternative treatment for dementia.[11]

According to the World Health Organization (WHO) about 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of these therapies involve the use of plant extracts and their active components. Hence WHO encourages, recommends and promotes traditional/herbal remedies in national health care programmes because of their availability at low cost, safety and faith people have in them.[12] Among these herbal remedies used for both medicinal and nutritional purposes are *Momordica charantia* and *Ocimum gratissimum*.

Ocimum gratissimum (Lamiaceae) is a perennial plant native to Africa, Southern Asia. It is known by different names such as sweet basil in English, *Efirin* in Yoruba, *Daidoya ta gida* in Hausa, *Nehonwu* in Igbo and *Tanmotswangiwawagi* in Nupe.[13] The plant has been claimed to be used as an analgesic, it is used to treat upper respiratory infections, fever and headache. [14,15] Other uses are as a stomachic, expectorant, anthelmintic, laxative, also in curing angina and madness,[16,17] and in central nervous system diseases.[18]

Phytochemical screening of the leaf extract of *Ocimum gratissimum* (OG) had shown the plant to contain Alkaloids, Saponins, Tannins, Anthraquinone, Flavonoids, Steroids, Terpenoids and Cardiac glycosides.[19-21] These phytochemicals have been reported to have various protective and therapeutic effects essentially to prevent diseases and maintain a state of well-being by scavenging free radicals produced within the body.[22]

Momordica charantia belongs to *Cucubitaceae* family. It is widely grown in Africa, Asia and Caribbean for its fruit. It is known by different names such as bitter melon in English, *Ejirin* in Yoruba, *Alo-ose* among the Igbos and *kakayi* by the Hausa speaking tribe of Nigeria.[23] It is used as a remedy for different ailments like stomach ache,[24] diabetes, ulcer, gout respiratory disease, also used as an antioxidant.[25]

Phytochemical screening of the leaves showed presence of biologically active chemicals such as essential oil, Flavonoids, Phenolic compounds, Glycosides, Triterpenes, Alkaloids, Tannins, Saponins, Reducing compounds and Steroids which are antioxidant molecules contributing to its medicinal value.[26,27] It is also rich in nutrients such as essential amino acids, folic acid, minerals like potassium, calcium and zinc, and vitamins C and A which add to the nutrient and energy requirement of man when the plant is taken for curative purposes in disease conditions.[26]

As antioxidants have been reported to prevent oxidative damage caused by free radicals in oxidative stress which is related to the pathogenesis of various disorders including dementia;[28] and antioxidant property of medicinal plants have shown beneficial therapeutic potential in reducing oxidative stress,[29] therefore, the present study was designed to investigate memory restorative effects of n-hexane leaf extract of *Ocimum gratissimum* and *Momordica charantia* in scopolamine-induced rat model of dementia, by determining their effects on learning and memory, lipid peroxidation and acetylcholine esterase activity.

MATERIALS AND METHODS

Experimental Animals

Male albino rats (*Rattus norvegicus*) of Wistar strain with initial weight between 100–120 g were used in the present study. The animals were fed on rat pellets (Ogooluwa Feeds, Ilorin, Nigeria) and water *ad libitum*. The animals were kept under standard laboratory conditions (under the prevailing light and dark photoperiod) and acclimatized for two weeks before behavioural studies.

Drugs and reagents

Dimethyl sulphoxide (DMSO) was a product of Sigma-Aldrich CHEME GmbH, Steinheim, Germany and purchased from Integrated SUNAF ventures, Ilorin.

Scopolamine was a product of Hubei Tianyao Pharmaceutical Co. Ltd. Hubei, China and Donepezil was a product of Torrent Pharma (UK) Ltd, United Kingdom and were purchased from Aromokeye Pharmacy, Ilorin, Kwara state. All chemicals/drugs used were of analytical grade and drug solutions were freshly prepared before use.

Preparation of *Ocimum gratissimum* and *Momordica charantia* extract

Fresh leaves of *Ocimum gratissimum* and *Momordica charantia* were purchased from Oro town, Kwara state, Nigeria and authenticated at the Plant Biology Department, University of Ilorin, Kwara State, Nigeria. The fresh leaves were air dried at room temperature for about two weeks until fully dried. The dried leaves were pulverized using an electric blender. A known weight of the powdered *Ocimum gratissimum* (283.4 g) and *Momordica charantia* (300 g) was extracted each in 4 litres of n-hexane solvent for 72 hours. The filtrates were dried using rotatory evaporator and then water bath which yielded 32.5g of *Ocimum gratissimum* and 33.6 g of *Momordica charantia*. About 2 g of each extract was dissolved in 25 ml of DMSO to obtained required dose of 400mg/ kg body weight. The dried powders were stored in separate airtight containers till use.

Animal grouping and administration

Groups	Dosage administered to each group
Control	Vehicle (0.5 ml DMSO) oral
Scopolamine treated	Scopolamine (1 mg/kg b.w) ip.
Donepezil treated	Donepezil (2mg/kg) oral+ Scopolamine (1 mg/kg) intraperitoneally (ip).
<i>Ocimum gratissimum</i> scopolamine group	<i>Ocimum gratissimum</i> (400mg/kg b.w) oral + Scopolamine (1 mg/kg) ip.
<i>Momordica charantia</i> scopolamine group	<i>Momordica charantia</i> (400mg/kg b.w) oral + Scopolamine (1mg/kg) ip.
<i>Ocimum gratissimum</i> and <i>Momordica charantia</i> scopolamine group	<i>Ocimum gratissimum</i> and <i>Momordica charantia</i> (400mg/kg b.w) oral + Scopolamine (1mg/kg) ip.

Behavioural Tests

Morris Water-Maze Test

Spatial memory was evaluated using the Morris water maze. The maze is made up of an open circular pool of about 200cm in diameter and 70cm deep filled with water up to about 60cm of the pool. A hidden platform with a top surface of about 15cm, maintained at the same position throughout the experiment was submerged at about 1.5cm below the water surface. The platform is camouflaged by adding milk to make the water opaque to create a nearly invisible platform-to-background colour match. First, animals were trained to locate the platform. During acquisition, trial escape latency time (ELT), time measure to locate the hidden platform, was noted as an index of learning which was recorded with the aid of a video system. Each animal was subjected to the four acquisition trials per day for 5 consecutive days before the administration. On the last day of administration (15th day), the animals were re-exposed to the maze (to test for their spatial and long-term memory functions), a video camera was placed above the centre of the pool to capture images of the swimming animal, for measures of the escape latency.[30]

The time spent by the animal in locating the hidden platform (escape latency) was noted as an index of learning.

Light and dark box

The light and dark box, an apparatus normally use for mood assessment was modified to evaluate spatial memory. The tool consists of two compartments, the light and dark section. The maze derived its name from the fact that one of the compartments is painted white (light section) while the other is painted black (dark section). The light and dark box used was 50cm x 30cm x 100cm in size and the two sections were linked with an opening of 8cm². Before administration, the rats were trained three trials a day (5 minutes per trial) for three consecutive days. At the end of training, only animals that developed memory of an opening route between the light and dark compartments were used for the experiment. To establish formation of memory in rats, the opening route was blocked from the dark compartment using black plank. Animals were then reintroduced and allowed to move freely, increased probing of the blocked-exit showed memory formation. One (1) hour after the last treatments on day 15, the rats were re-introduced to the modified (opening route-blocked) light and dark box and were allowed to freely explore the maze for 5 minutes. This was recorded with an overhead camera (Logtech Webcam, 5MP) and videos from the studies were later watched to extract data. The number of time (frequency) each rat probed the blocked-aperture was documented as the index for intact/enhanced spatial memory.

Sample Collections

On the 15th day of administration, after behavioral assessments, the rats were anaesthetized with intraperitoneal injection of ketamine (0.5 ml), after which the animal brain tissues were isolated weighed and homogenized in 0.1 M phosphate buffer solution (pH 7.4) (the weight of the brain tissue determines the volume of phosphate buffer solution used in homogenation, that is, brain weight multiplied by 4). The homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant were separated and used for biochemical analysis

Biochemical Analysis

Acetylcholine esterase (AChE) Estimation

The cholinergic marker, acetylcholinesterase, was estimated in the whole brain using Acetylcholinesterase Activity Assay kit (Elabscience, China). The assay kit is an optimized version of the Ellman method in which thiocholine, produced by AChE, reacts with 5, 5-dithiobis (2-nitrobenzoic acid) to form a colorimetric (412 nm) product, proportional to the AChE activity present. This homogenate was incubated for 5 min with 2.7mL of phosphate buffer and 0.1ml of Ellman's reagent (which is 5, 5-dithiobis 2-nitrobenzoate, DTNB). Then, 0.1ml of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412nm.[31-33]

Malondialdehyde (MDA) level estimation

Malondialdehyde (MDA), marker of oxidative stress was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS). 0.5ml of distilled water was added with 1 ml of 10% trichloroacetic acid and was added with 0.5ml of brain tissue homogenate.

This was centrifuged at 3000 rpm for 10 min. To the mixture, 0.1ml of thiobarbituric acid (0.375%) was added. Total solution was placed in water bath at 80°C for 40 min and cooled at room temperature. Absorbance was read at 532nm.[34]

Statistical Analysis

The results were expressed as mean $\pm$ standard error of mean (SEM). Statistical significance was done using one-way analysis of variance (ANOVA) and then subjected to post-hoc Newman-Keul test using Graph pad prism version 5. Values were considered statistically significant at $p < 0.05$.

RESULTS

Effect of n-hexane leaves extract of *Ocimum gratissimum* and *Momordica charantia* on learning and memory in Morris water maze test is shown in Figure 1. The result shows that: There is a significant increase in the escape latency of rats treated with scopolamine only when compared with control. Administration of the n-hexane leaves extracts of either *Ocimum gratissimum*, *Momordica charantia* or combination of the two extracts causes a significant decrease in the escape latency ($p < 0.05$) when compare with scopolamine only group (demented group). However, there is no significant difference in the escape latency of the animal treated with donepezil and the n-hexane leaf extracts of either *Ocimum gratissimum*, *Momordica charantia* or combination of *Ocimum gratissimum* and *Momordica charantia*.

Figure 2 shows the result of the light and dark box test. There is a significant decrease in the probing frequency (number of times the rats visited the opening route) in scopolamine group compared with control. Conversely, treatment with n-hexane leaf extracts of either *Ocimum gratissimum*, *Momordica charantia* or combination of *Ocimum gratissimum* and *Momordica charantia* causes an increase in the probing frequency significantly ($p < 0.05$) when compare with scopolamine only group (demented rats). However, there is no significant difference in the probing frequency between the animals treated with donepezil (reference drug) and the three intervention (n-hexane leaf extracts of *Ocimum gratissimum* and *Momordica charantia*) groups.

Acetylcholine esterase (AChE) activity in the brain of scopolamine only treated group was significantly increased when compared with the control. Administration of the n-hexane leaf extracts of either *Ocimum gratissimum*, *Momordica charantia* or combination of *Ocimum gratissimum* and *Momordica charantia* causes a significant decrease in Acetylcholine esterase (AChE) activity similar to donepezil group when compared with scopolamine only treated group. Likewise, the activity of AChE was not significantly altered across all the intervention groups (Figure 3).

Figure 4 shows the effect of n-hexane leaves extract of *Ocimum gratissimum* and *Momordica charantia* on malondialdehyde (MDA) level. The result shows a significant increase in the MDA level in scopolamine only group when compared with the control. Treatment with n-hexane leaf extracts of either *Ocimum gratissimum*, *Momordica charantia* or combination of *Ocimum gratissimum* and *Momordica charantia* causes a significant decrease in the MDA level when compare with scopolamine only group ($p <$

0.05). Similarly, there is no significant difference in MDA level between the donepezil (reference drug) and all the extract treated groups. Across the three extract treated groups, OG treated groups had the lowest MDA level.

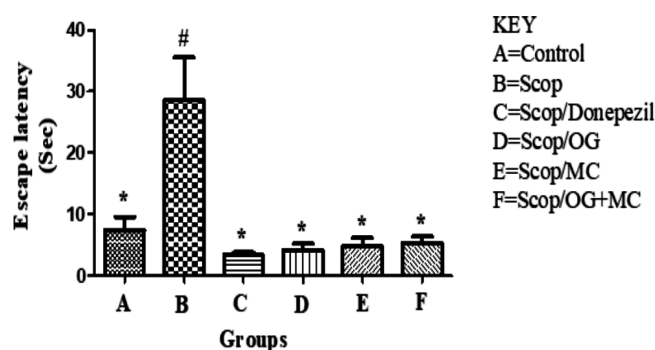


Figure 1: Effect of n-hexane leaves extracts of *Ocimum gratissimum* and *Momordica charantia* on learning and memory in Morris water maze test. Values are expressed as mean $\pm$ SEM of 5 rats per group.

Scop = scopolamine, OG = *Ocimum gratissimum*, MC= *Momordica charantia*.

* $p < 0.05$ Vs A; * $p < 0.05$ Vs B

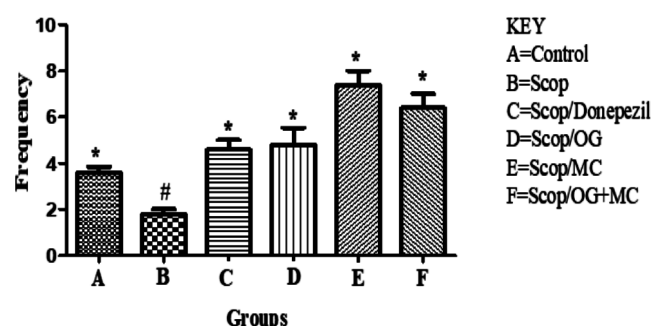


Figure 2: Effect of n-hexane leaves extracts of *Ocimum gratissimum*, and *Momordica charantia* on learning in light and dark box. Values are expressed as mean $\pm$ SEM of 5 rats per group.

Scop = scopolamine, OG = *Ocimum gratissimum*, MC= *Momordica charantia*.

* $p < 0.05$ Vs A; * $p < 0.05$ Vs B

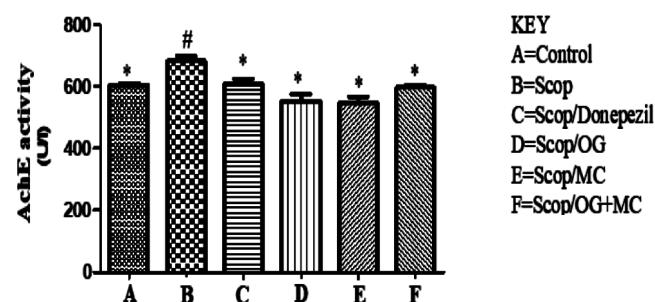


Figure 3: Effect of n-hexane leaves extract of *Ocimum Gratissimum* and *Momordica charantia* on acetylcholine esterase (AchE) activity. Values are expressed as mean $\pm$ SEM of 5 rats per group.

Scop = scopolamine, OG = *Ocimum gratissimum*, MC= *Momordica charantia*.

* $p < 0.05$ Vs A; * $p < 0.05$ Vs B

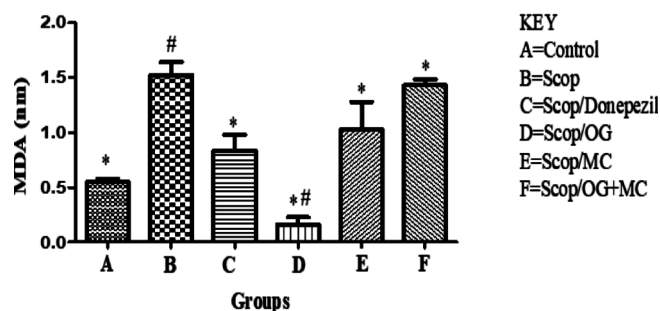


Figure 4: Effect of n-hexane leaves extract of *Ocimum gratissimum* and *Momordica charantia* on plasma malondialdehyde (MDA) level. Values are expressed as mean $\pm$ SEM of 5 rats per group.

Scop = scopolamine, OG = *Ocimum gratissimum*, MC= *Momordica charantia*.

* $p < 0.05$ Vs A; * $p < 0.05$ Vs B

DISCUSSION

Medicinal plants have been used for centuries before the advent of orthodox medicine and their medicinal values have been attributed to various phytochemicals component, which produce definite pharmacological actions on the human body. These phytochemicals which are naturally-occurring and biologically-active plant compounds have been reported to have potential diseases inhibiting capabilities believed to be effective in combating or preventing many diseases due to their antioxidant effect.[21]

Several drug induced dementia models have been used to search for and evaluate the effects and therapeutic potentials of novel drug candidates in dementia; these models include colchicines, scopolamine, okadaic acid and trimethyltin.[35] Scopolamine model used in this study has been reported to antagonize acetylcholine activities, increase brain oxidative stress status and impair learning and memory functions similar to memory deficit observed in dementia.[36-38] Furthermore, the use of acetylcholinesterase (AChE) inhibitors such as Tacrine, Donepezil, and Rivastigmine has been approved for the treatment of dementia symptoms.[8,39] Donepezil used in the present study as a reference drug is a known acetylcholine esterase inhibitor which increases acetylcholine concentration and up-regulate brain cholinergic receptors.[40]

In this study, the significant decrease in the escape latency recorded in the group treated with n-hexane leaves extracts of *Ocimum gratissimum* and *Momordica charantia* shows memory restorative effect of the extracts. This agrees with the reports of Roja *et al.*,[41] although these authors used the ethanolic fraction of *Momordica charantia*. This recorded memory restorative effect may be due to the active metabolites such as Saponins, Tannins, Glycosides, Flavonoids and Steroids which have been reported to be present in these plants.[21,26,27,42] These constituents have been reported to delay progress of neurodegenerative disease (dementia) due to their antioxidant properties.[43]

Antioxidant properties of these secondary metabolites may be attributed to their ability to protect against oxidative stress-induced brain damage by scopolamine as evidenced in this study by decreased MDA level in all the three extract intervention groups. Thus, the memory restorative effect of

these extracts may be due to the antioxidant effect of these constituents either alone or in synergy with one another. Similarly, the presently observed increase in probing frequency in the three intervention groups (n-hexane leaf extracts of OG, MC, OG and MC) in a manner similar to the reference drug (Donepezil) further suggest that the extracts (OG and MC) has memory restorative effect. The decreased probing frequency in the extracts treated groups might be due to their phytochemical constituents which has been reported earlier.[21,26]

Scopolamine has been reported to elevate acetylcholinesterase (AChE) levels in the cortex and hippocampus,[44] resulting in cholinergic transmission blockage and cognitive impairment. The data obtained from this study showed increased acetylcholinesterase activity in scopolamine only group which was ameliorated upon intervention by the n-hexane extracts of OG and MC. This ameliorative effects of n-hexane leaf extracts of OG and MC suggests that these extracts act by inhibiting the production and action of acetylcholine esterase activity, thereby enhance cholinergic transmission which restore memory deficit as evidenced by the decreased escape latency (in Morris water maze) and increased probing frequency (number of visit to the opening route) by all the three extract intervention groups. Elevated level of MDA recorded in the scopolamine rats agree with the study that documented that the impairment of memory in scopolamine induced animal model is associated with altered status of brain oxidative stress.[41] However, the significant decrease in the MDA level of groups treated with n-hexane leaf extracts of *Momordica charantia* and *Ocimum gratissimum* may be attributed to their antioxidant properties, the antioxidants (plant metabolites) common to these plants include Flavonoids, Tannins, Saponins and Vitamins. These plant metabolites protect brain cells from oxidative stress; thereby reduce brain damage and improved neuronal function thus ameliorating oxidative stress-mediated action of scopolamine. Also, epidemiological and laboratory findings reveal that foods containing antioxidants delay progress of neurodegenerative disease such as dementia probably due to prevention or neutralization of detrimental effects of free radicals.[45] All these components present in the leaves have been shown to reduce oxidative stress through antioxidant mechanism by scavenging free radicals, inhibiting the lipid peroxidation and thus suggesting that the n-hexane extract of *Ocimum gratissimum* and *Momordica charantia* may help to restore memory impairment caused by scopolamine, hence dementia.

CONCLUSION

This study concludes that the memory restorative effect of n-hexane leaves extracts of *Momordica charantia* and *Ocimum gratissimum* might occur via the decreased oxidative stress and the enhanced cholinergic function. Therefore, the memory-restorative activity of n-hexane leaves extracts of *Momordica charantia* and *Ocimum gratissimum* may be attributed to the antioxidant, anti-AChE properties. In the light of the above, it may be worthwhile to explore the potential of these plants in the management of cognitive dysfunction and the development of novel therapies for dementia.

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