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Running Title: Memory Restorative Role of Medicinal Plants

***MARYAM T. AYINLA [1], VICTOR B. OWOYELE [1], WAHAB I. ABDULMAJEED [1] & AMINU IMAM [2]**

1. Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.
2. Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

*Corresponding author: gazmark@unilorin.edu.ng.

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4. Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

*Corresponding author: gazmark@unilorin.edu.ng.

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ABSTRACT:
This study aimed to explore the effect of *Momordical charantia* (MC), *Ocimum gratissimum* (OG) alone or their synergistic action on memory impairment induced either by scopolamine treatment or high fat diet in rats. Using Morris water maze (MWM) test, we show that treatment of adult rats with scopolamine or high fat diet (HFD) caused a significant impairment in spatial memory. Specifically, the time to locate the hidden platform in MWM (a memory index) was significantly higher in scopolamine- and HFD- treated rats. However, treatment with MC or OG prevents the cognitive impairment induced by the two animal models of Alzhemier's disease (AD). To understand the mechanisms of actions of MC and OG, we examined the brain Acetylcholinesterase (AchE), Malondialdehyde (MDA) and Glutathione (GSH) activities in the brain as well as plasma cholesterol level. We found that scopolamine or HFD treatment significantly increased their activities in the brain and increase plasma cholesterol level in HFD-treated rats. Treatment with MC or OG restores these activities. In conclusion, our findings indicate that the treatment with MC/OG alone could improve the memory function in animal model of AD by regulating the brain cholinergic system and oxidative stress as well as plasma cholesterol level.

Keywords: *Momordica charantia*, *Ocimum gratissimum*, Alzheimer's disease, scopolamine, high fat diet.
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INTRODUCTION:

Dementia is a form of neurodegenerative disorder that is characterized by decline in the ability to think and remember events which negatively affects the person's daily functioning [1]. Apart from cognitive deficit, other symptoms of dementia are decreased motivation, emotional problem, language difficulties among others [2]. Increase in age is most important risk factor but it is not part of the normal aging process [3]. However, research has revealed a strong correlation between unhealthy life styles (obesity, physical inactivity, intake of alcohol, smoking diabetes) and early onset of dementia [4,5]. The most common type of dementia is Alzheimer's disease (AD) that account for 60 to 70% of cases [3]. Other types are vascular dementia (25% of cases), Lewy bodies (15% of cases) [2]. Less common types are Parkinson disease dementia, mix dementia among others [6,7].

Globally, over 54 million people have been reported to have dementia, 60% of these live in low and middle income countries, with an annual 10 million new cases [8]. It has been projected that more than 75 million people will have dementia globally by the end of year 2030 and this figure will rise to 139 million by 2050. This significant increase is being attributable to rise in the number of people with dementia living in low and middle income countries [9].

Dementia places a great burden on the families and caregivers physically, socially, psychologically and financially. World-wide, the cost of caring for people with dementia as at 2019 was over 1.1 trillion US dollars which is expected to double by 2030 [8]. Dementia is currently being managed by medications to treat its cognitive problem, some of which are acetylcholine esterase inhibitors (Donepezil or Galantamine). However, the use of these drugs in mild cognitive impairment has not shown a significant benefit. In addition, most often these drugs are associated with side effects like bradycardia, decrease appetite and others. Therefore, there is need to explore options in traditional medicine system. Similarly, the World Health Organization (WHO) encourages scientific research into indigenous herbal medicine [10]. About 80% of the world population depends mainly on medicinal plants for their primary health care [11] and the remaining 20% in developed countries like Europe still make use of products derived from plants for their health care system; for instance plant derived alkaloid (galantamine) gotten from Galanthus nivalis is used in the management of neurodegenerative disorders.

Ocimum gratissimum belongs to the Lamiaceae family, it is known as African basil and its one of the best known specie [12]. It is native to Africa and Southern Asia. It is used in food as condiment and also used widely in traditional medicine such as Ayurveda, Chinese traditional medicine and other folk medicine for treating digestive system disorders, infections,

whooping cough and various types of fever [12]. Studies have reported anti-inflammatory, antinociceptive, antipyretic, gastroprotective, hepatoprotective and antimutagenic effects of O. gratissimum [13-19].

Momordical charantia is a flowering plant that belongs to the Cucurbitanceae family. It originated from Africa but it is now cultivated mostly in Asia and the Caribbeans [20]. It is known as bitter melon in English, Ejirin in Yoruba (Nigeria). It has a bitter taste which is more pronounced when it is ripe. The plant has been used in traditional and folk medicine [21] for the treatment of cancer, diabetes, hypertension, hyperlipidemia, and inflammation, viral and bacterial infections [22]. The plant is rich in phytochemicals with many health promoting effects such as terpenoids, proteins, saponins, flavonoids, phenols, essential oil, glucose [23, 24].

In the present study, we explore the potential effects of MC and OG in the management of scopolamine- or high fat diet-induced cognitive impairment in adult rats.

MATERIALS AND METHODS:

Plant materials and authentication:

Fresh leaves of both *Momordica charantia* and *Ocimmum gratissimum* were purchased from Oro, Kwara state, Nigeria. The leaves were authenticated in the Department of plant Biology, University of Ilorin, Nigeria. Fresh leaves of the two plants (*Momordica charantia* (MC) and *Ocimmum gratissimum* (OG) were air-dried at room temperature for about two weeks. An electric blender (Kenwood blender, model KP800KA) was then used to pulverize the air-dried leaves separately and they were kept in different plastic container before the commencement of the study.

Drugs and reagents**:**

All chemicals/drugs and reagents used were of analytical grade. Drug solutions were prepared freshly before use. Donepezil and Scopolamine were manufactured by Torrent Pharma Ltd, United Kingdom and Hubei Tianyao pharmaceutical Co. Ltd. Hubei, China, respectively.

Animal and experimental design**:**

Sixty male Wistar rats weighing between 120-150 g were obtained from animal holding unit of the Department of Biochemistry, University of Ilorin. They were kept in cages in the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria, and were fed with standard diet and water ad libitum. The rats were housed under standard laboratory conditions (12 hours light/dark cycle, temperature: 22 ± 3^{o}C) and acclimatized for two weeks before the commencement of the experiment. All the animals were strictly handled in conformation to the Declarations of Helsinki in 1995 (as revised in Edinburgh 2000) and the University's guidelines on Care and Use of Laboratory Animals.

Animal grouping and administration:

Two models were adopted for dementia induction, the scopolamine and the high fat diet models.

There were thirty (30) rats in each model. This was further divided into 6 groups, each made of 5 rats. Cognitive impairment was induced in all groups except control and were administered either donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG).

Scopolamine model: Cognitive impairment was induced in all groups except control by a daily single injection of scopolamine (1 mg/kg, ip). One-hour post scopolamine injection, rats were administered either donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG) as shown in Table 1. The rats were randomly divided into six groups (n = 5) as follows:

Table 1: Animal grouping

Groups	Dosage administered to each group (daily)
A (Control)	Vehicle (5 ml/kg normal saline) orally
B	Scopolamine (1 mg/kg) ip. [25].
C	Scopolamine (1 mg/kg) ip. + Donepezil (2 mg/kg) orally [25].
D	Scopolamine (1 mg/kg) ip. + MC (400 mg/kg) orally [25].
E	Scopolamine (1 mg/kg) ip. + OG (400 mg/kg) orally [25].
F	Scopolamine (1 mg/kg) ip. + MC (400 mg/kg) + OG (400 mg/kg) orally [25].

Ip-Intraperitonally, MC- Momordical charantia; OG- Ocimum gratissimum,

High fat diet model: Cognitive impairment was induced in all groups except control by free access to a high fat diet for a period of three (3) months, after which the rats were administered either Donepezil, *Momordical charantia* (MC), *Ocimum gratissimum* (OG) or a combination of *Momordical charantia* (MC) and *Ocimum gratissimum* (OG) as shown in Table 2. These administrations were done over a period of 15 days after dementia induction. The administered drugs, MC and OG extracts were suspended in vehicle (normal saline) solution and administered orally by gastric gavage once in a day for 15 days.

Table 2: Animal grouping (High fat diet)

Groups	Dosage administered to each group (daily)
CONTROL	Vehicle (5 ml/kg normal saline) orally
HFD	HFD
HFD Don	HFD + Donepezil (2 mg/kg bw) orally [25].
HFD MC	HFD + MC (400 mg/kg bw) orally [25].
HFD OG	HFD + OG (400 mg/kg bw) orally [25].
HFD MC + OG	HFD + MC (400 mg/kg bw) + OG (400 mg/kg bw) orally [25].

HFD-High fat diet, MC- *Momordical charantia*; OG- *Ocimum gratissimum*, bw- body weight.

The drug administration was carried out between the hours of 08:00 and 10:00 in the morning. Administration of treatments regimen as shown in Table 1 and 2 lasted for fifteen consecutive days. On the last day of administration, Morris water maze (MWM) and modified dark and light box were used to assess short-term spatial memory function [26]. On day 15, rats were anaesthetized and the brain was excised and then homogenized. The supernatant was then processed for biochemical analysis of Malondialdehyde (MDA) [27], Reduced glutathione (GSH) [28], Total protein [29] and Acetylcholinesterase [30].

Preparation of the extracts**:**

A known weight of the powdered *Ocimum gratissimum* (502.6 g) and *Momordical charantia* (500 g) was macerated each in 5 litres of distilled water for 24 hours. The filtrates were dried using lyophilized freeze dryer (Freeze dryer Model: HXLG10-50DG, Hunan Kaida Scientific Instruments Co. Ltd.) which yielded 52.6g of *Ocimum gratissimum* and 48.9 g of *Momordical charantia.* The dried powders were stored in separate airtight containers till use. The calculated amount of the extract was reconstituted in normal saline to give the required doses [25].

Phytochemical screening**:**

Chemical tests were carried out on the extracts (MC and OG) using the standard procedure to identify the constituents as described by Harbone [31], Trease and Evans [32] and Sofowora [33].

Behavioral Tests:

Morris Water-Maze (MWM) Test:

Spatial memory was evaluated using the Morris' water maze [25, 26]. The maze is made up of an open circular pool of about 200 cm in diameter and 70 cm deep filled with water up to about 60 cm of the pool. A hidden platform with a top surface of about 15 cm, maintained at the same position throughout the experiment was

submerged at about 1.5 cm below the water surface. The platform was made hidden by adding milk to make the water opaque thereby creating a nearly invisible platform-to-background. First, animals were trained to locate the platform. During acquisition, trial escape latency time (ELT), time taken 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 center of the pool to capture images of the swimming animal, for measures of the escape latency. The time spent by the animal in locating the hidden platform (escape latency) was noted as an index of learning.

Sample Collection**:**

On the 15th day of administration, after behavioral assessments, the rats were anaesthetized with intraperitoneal injection of ketamine (100 mg/kg). The brain tissues were isolated weighed and homogenized in 0.1 M phosphate buffer solution (pH 7.4).

The homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant were separated and used for biochemical analysis [26].

Biochemical Analysis:

Estimation of Acetylcholine esterase (AchE) level:

The cholinergic marker, acetylcholinesterase, was estimated using Acetylcholinesterase Activity Assay kit (Elabscience, China). The assay kit is an optimized version of Ellman's method [30] in which thiocholine, produced by AChE, reacts with 5, 5-dithiobis (2-nitrobenzoic acid). This homogenate was incubated for 5 min with 2.7 mL of phosphate buffer and 0.1 ml of Ellman's reagent (5, 5-dithiobis 2-nitrobenzoate, DTNB). Then, 0.1 ml of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412 nm.

Determination of Total brain protein**:**

Total amount of protein in brain was measured according to the method of Lowry et al. [29]. In this method under alkaline condition copper ion is reduced to form a complex, this complex then reduces folin-Ciocalteau reagent and the absorption was read at 650 nm.

Estimation of Malondialdehyde (MDA) level**:**

Malondialdehyde (MDA), marker of oxidative stress was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS) based on the method of Mihara and Uchiyama, [27]. 0.5 ml of distilled water was added with 1 ml of 10% trichloroacetic acid and was added with 0.5 ml of brain tissue homogenate. This was centrifuged at 3000 rpm for 10 min. To the mixture, 0.1 ml 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 532 nm.

Estimation of reduced glutathione (GSH) level:

Reduced glutathione was assayed according to the method of Ellman, [28]. The colorimetric assay involves carefully optimized enzymatic recycling method using glutathione reductase and Ellman's reagent; DTNB. Glutathione reductase reduces GSSG to GSH. DTNB (5-5-dithiobis (2-nitrobenzoic acid) reacts with GSH to form yellow colour chromophore, 5 – thionitrobenzoic acid (TNB) and GS- TNB. GS – TNB was further reduced to GSH and TNB by glutathione reductase. The absorbance was read at 415 nm and compared with standard curve for GSSG.

Statistical Analysis**:**

The results were expressed as mean ± 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:

Constituents of aqueous extracts of *Ocimum gratissimum* and *Momordica charantia* leaves is shown in table 3.

Effect of Ocimum gratissimum and Momordical charantia on the Morris water maze and biochemical parameters:

In Figure 1, the group B (scopolamine-treated) showed a significant increase in Escape Latency (EL) compared with the control group (A) (as this group of rats took a longer time to locate the hidden platform). This suggests that administration of scopolamine induced cognitive impairment in rats. Administration of aqueous leaves extracts of Momordical charantia to rats in group D, Ocimum gratissimum to rats in group E or a combination of Momordical charantia and Ocimum gratissimum, to rats in group F, each cause a significant decrease in escape latency when compared with group B (scopolamine treated; $p<0.05$). However, there was no significant difference in escape latency of the rats treated with donepezil (group C), the aqueous leaves extracts of Momordical charantia (group D).and Ocimum gratissimum (group E).

Table 3: Secondary metabolite constituents of aqueous extracts of Ocimum gratissimum and Momordica charantia leaves

Compounds	Ocimum gratissimum	Momordical charantia
Saponins	Absent	Present
Flavonoids	Present	Present
Tanins	Absent	Absent
Phenols	Absent	Absent
Steroids	Present	Present
Terpenoids	Present	Present
Glycosides	Present	Present
Alkaloids	Present	Absent
Proteins	Present	Present
Reducing sugar	Absent	Absent

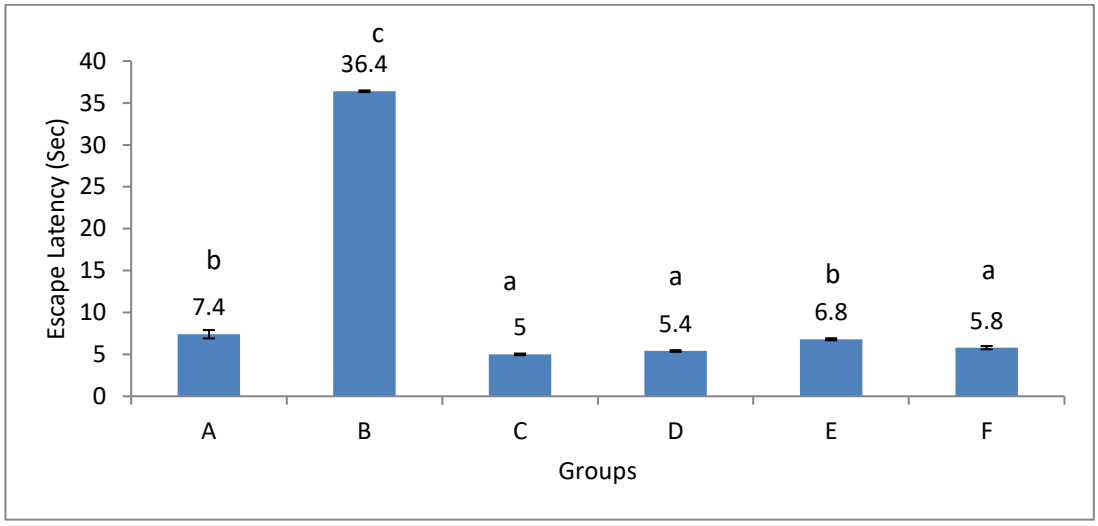

Figure 1: Effect of *Ocimum gratissimum* and *Momordical charantia* on escape latency in Morris water maze test. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ± standard error of mean, $p< 0.05$, bar with different alphabet (superscript) are significantly different from each other

In figure 2, the total protein in the brain increased significantly in the scopolamine treated rats (group B) when compared with control (group A). Conversely, administration of aqueous leaf extract of *Momordica charantia to rats in* group D*, aqueous leaf extract of Ocimum gratissimum* to rats in group E or a combination of *Momordical charantia and Ocimum gratissimum,* to rats in group F, each cause a significant reduction in the total protein concentration in the brain ($p<0.05$) when compared with the scopolamine treated rats (group B). This is similar to the effect observed in the donepezil-treated rats (group C).

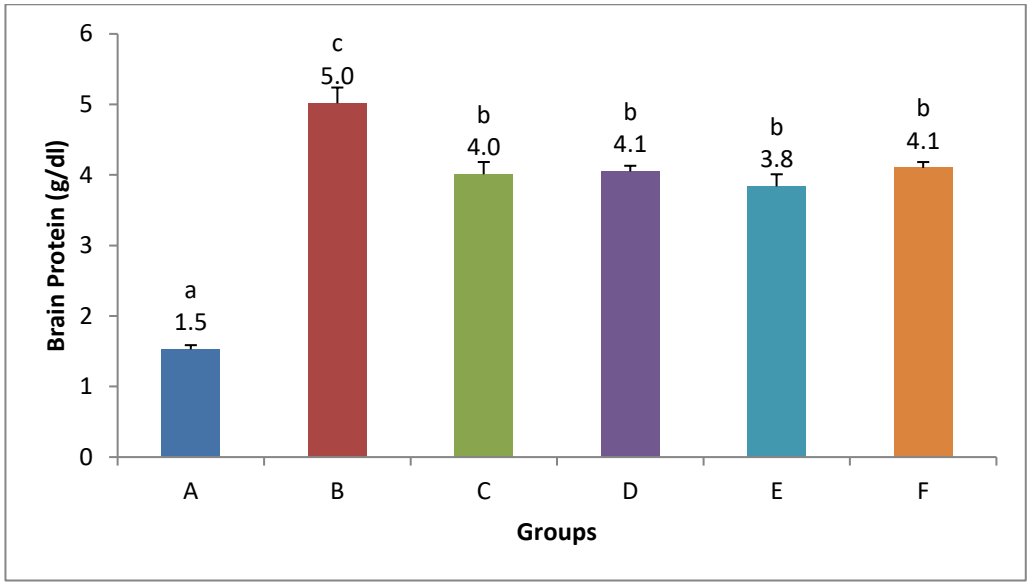

Figure 2: Effect of Ocimum gratissimum and Momordical charantia on brain protein levels. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, $p < 0.05$, bar with different alphabet (superscript) are significantly different from each other

Figure 3 shows that the brain MDA level (an index of lipid peroxidation) was significantly increased in group B (scopolamine treated rats) when compared with the control (group A). Treatment with either aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* (group D, E and F; $p<0.05$) significantly attenuated the level of MDA compared with the scopolamine treated rats (group B). The group E (treated with aqueous leaf extract of *Ocimum gratissimum)* compared favourably with group C (the donepezil treated rats)

The activity of AchE (an enzyme which breaks down Ach neurotransmitter) was significantly increased in the scopolamine treated rats (group B) when compared with control (group A) (figure 4). But upon administration of aqueous leaves extracts of Momordical charantia or/and Ocimum gratissimum to rats in group D, E and F, there was a significant reduction in its activity when compared with group B (the scopolamine treated rats). The reduction in the activity of AchE in the combined treatment group follow a similar pattern with group C (the donepezil treated rats). (figure 4).

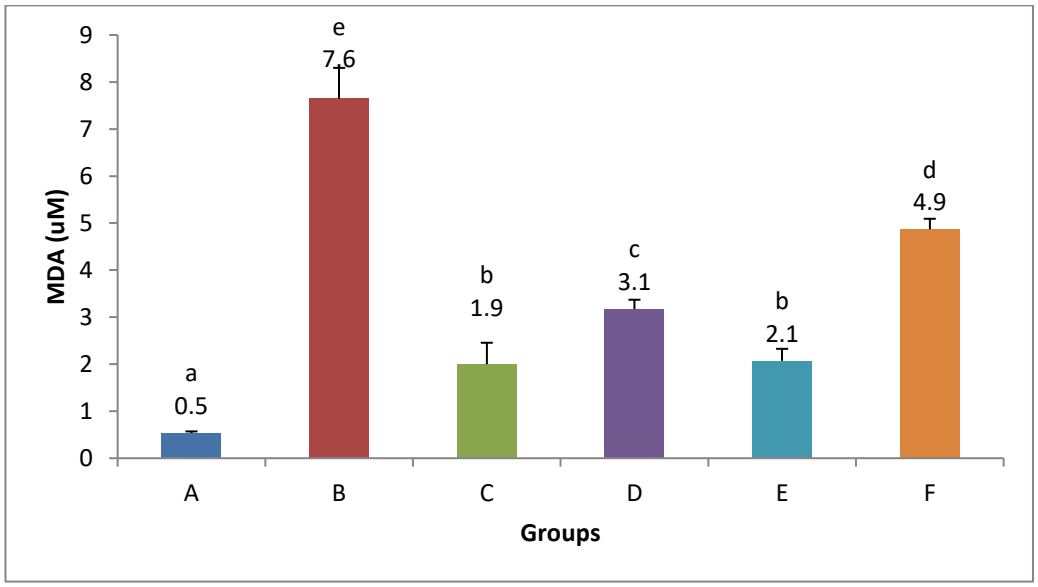

Figure 3: Effect of Ocimum gratissimum and Momordical charantia on MDA levels. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

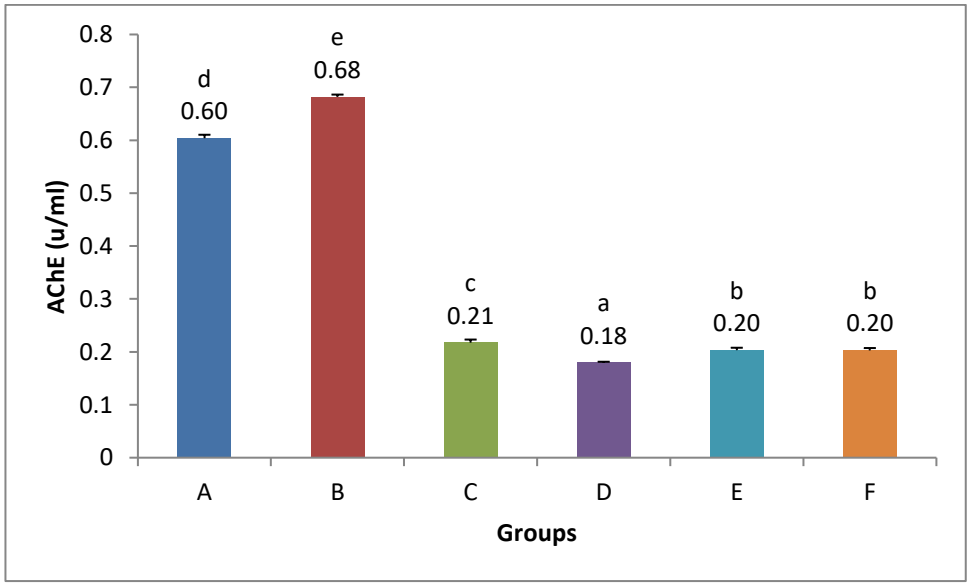

Figure 4: Effect of Ocimum gratissimum and Momordical charantia on AChE activity. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

In Figure 5, the rats in group B (scopolamine treated) showed an increase in the tissue level of GSH compared with group A (control rats). Administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* to rats in group D, E and F led to a more pronounced (P<0.05) increase in GSH when compared with group B (scopolamine treated). In addition, there was no significant difference in the level of GSH in the three intervention groups.

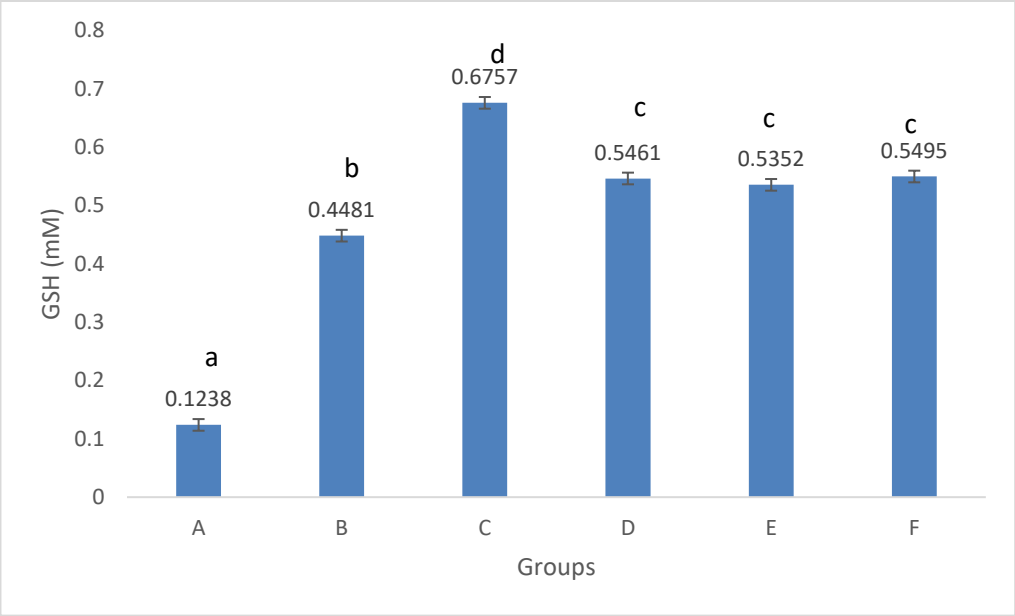

Figure 5: Effects of aqueous leaves extracts of Momordica charantia and Ocimum gratissimum on reduced glutathione (GSH) level. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E- Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

The rats in group B (scopolamine treated) showed a significant decrease in the level of plasma protein compared with group A (control rats) (figure 6). However, the administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* to rats in group D, E and F, led to a significant (P<0.05) increase in plasma protein level when compared with group B. The plasma protein level of the rats in group E (treated with aqueous leaf extract of *Ocimum gratissimum)* compares favorably with group C (the donepezil treated rats) figure 6.

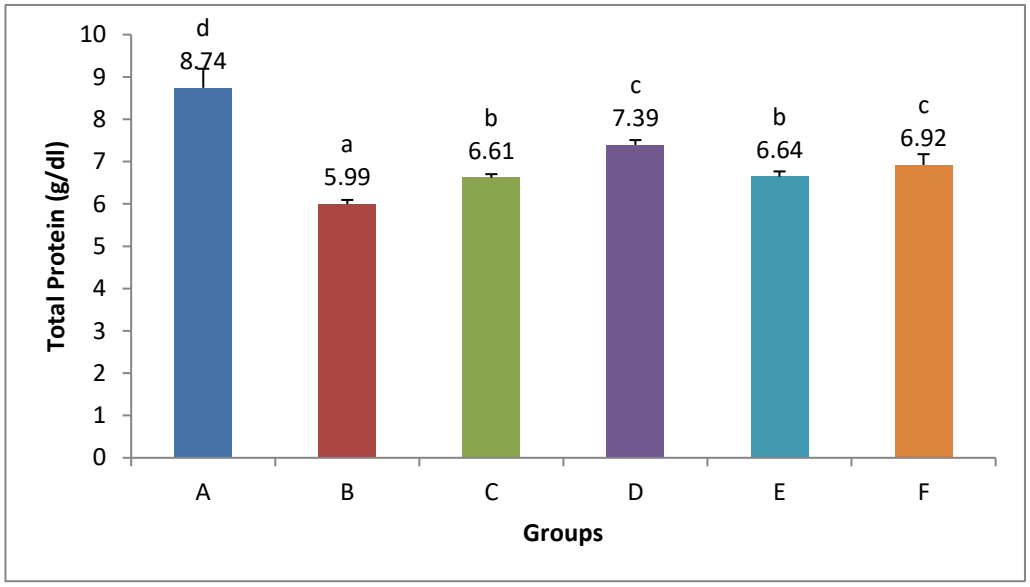

Figure 6: Effects of aqueous leaves extracts of Momordica charantia and Ocimum gratissimum on total plasma protein level. A - Control; B- Scopolamine treated; C-Scopolamine + Donepezil; D- Scopolamine +MC; E-Scopolamine +OG; F- Scopolamine +MC +OG. Data are presented as mean ±standard error of mean, p< 0.05, bar with different alphabet (superscript) are significantly different from each other

Table 4: Effects of aqueous leaves extracts of *Momordica charantia* and *Ocimum gratissimum* on Morris water maze and biochemical parameters of High fat diet-induced Spatial Memory Impairment

Parameters/Groups	Control	HFD	HFD Don	HFD MC	HFD OG	HFD MC+OG
Escape latency (s)	5.8±0.2^{b}	18.0±1.2^{c}	1.8±0.37^{a}	2.4±0.27^{a}	1.6±0.24^{a}	1.6±0.40^{a}
GSH (mM)	1.69±0.11^{a}	1.82±0.06^{a}	2.59±0.13^{b}	2.39±0.12^{b}	2.60±0.21^{b}	2.69±0.18^{b}
MDA (uM)	0.89±0.08^{a}	2.41±0.00^{c}	1.93±0.00^{b}	1.00±0.09^{a}	1.18±0.14^{a}	1.05±0.07^{a}
Protein (mg/dl)	2.46±0.00^{b}	2.63±0.02^{d}	2.53±0.01^{c}	2.40±0.01^{a}	2.47±0.02^{b}	2.36±0.02^{a}
ACHE (U/ml)	0.078±0.02^{a}	0.118±0.00^{b}	0.09±0.01^{a}	0.080±0.00^{a}	0.070±0.00^{a}	0.082±0.01^{a}
Cholesterol	138.2±0.58^{b}	163.4±4.7^{c}	158.8±0.97^{c}	124.2±2.24^{a}	138.8±2.47^{b}	126.2±5.52^{a}

HFD- High fat diet group, HFD Don- High fat diet+Donepezil group, HFD MC- High fat diet+*Momordical charantia* group, HFD OG- High fat diet+*Ocimum gratissimum* group, HFD MC+OG- High fat diet+*Momordical charantia*+*Ocimum gratissimum* group. Data are presented as mean ±standard error of mean, p< 0.05, mean in the same row with different alphabet (superscript) are significantly different from each other

As seen in Table 4, the HFD group exhibited a significant increase in escape latency (time taken to locate the hidden platform relative to the control. Administration of either OG or/and MC cause a significant decrease in the time taken to locate the hidden platform (decrease in Escape latency) when compared with the HFD group. Similarly, there is no significant difference in the escape latency among HFD + extract treated groups. The HFD + Donepezil Group compares favorably with all the HFD + extract treated groups.

A significant increase in the brain MDA of the HFD group was observed in this study. But upon administration of OG and MC either singly or in combination causes a significant decrease in the brain MDA when compared with the HFD group. The HFD + extract treated groups and the HFD +Donepezil groups are not significantly different $p<0.05$.

In Table 4, the HFD group showed a slight increase in the level of GSH compared with control rats (but not significant). The administration of aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* led to a significant ($P<0.05$) increase in GSH when compared with HFD group.

Acetylcholine esterase (AchE) activity in the brain was significantly increase in the HFD group, however, administration of the aqueous extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* cause a decrease in acetylcholine esterase (AchE) activity similar to the HFD +Donepezil group.

The total protein in the brain increased significantly in the HFD group when compared with control group. Treatment with aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* reduced the total protein concentration in the brain ($p<0.05$) when compared with the HFD group. The reduction in the total protein concentration in the brain. ($p<0.05$) in all the extracts (MC and OG) treatment groups followed a similar pattern with the Donepezil-treated group.

The total cholesterol level increased in the HFD group when compared with control group. Treatment with aqueous leaf extract of *Momordica charantia* or/and aqueous leaf extract of *Ocimum gratissimum* reduced the total cholesterol level ($p<0.05$) when compared with the HFD group. The reduction in the total cholesterol concentration ($p<0.05$) in all the extracts (MC and OG) treatment groups followed a similar pattern with the control group.

DISCUSSION

Lifestyle and drug abuse are some of the risk factors of dementia. Despite enormous effort that has been directed towards the discovery of drugs that could be used in the management of dementia; there has been no definite means of treating dementia. In the present study, we assessed two established model of dementia/cognitive impairment and show that

administration of MC and/or OG prevent the spatial memory disruption induced by scopolamine or long time consumption of high fat diets in adult rats.

Specifically, our data indicated that both plants extracts were able to maintain the cholinergic activities of the brain reduce the plasma cholesterol level and prevent the oxidant-anti-oxidant imbalance induced by scopolamine or HFD. Acetylcholine is a well-studied neurotransmitter in the brain [34, 35]. It acts on the cholinergic receptors which are widely distributed in the brain to promote memory-related functions. Acetylcholine activity has been the focus of many neuroscientists in recent years [25, 26, 35]. This is because, impairment of the cholinergic transmission that result from either alteration in the levels of acetylcholine or AChE acitivity may lead to learning and memory deficit which mimics the conditions in AD patients [36,37].

Scopolamine is a nonselective acetylcholine muscarinic receptor antagonist. It induces cognitive dysfunction by disrupting the cholinergic signaling [36, 38, 39]. Scopolamine can enhance the AchE activity which in turn blocks the nerve impulses that are mediated by acetylcholine [39-41]. Studies have also shown that scopolamine administration can increase oxidative stress in the brain [40]. Increased level of oxidative stress markers has also been linked to memory deficits. MWM test was an experimental method designed by British psychologist Morris in the early 1980s [42]. Nowadays, it has become the most widely recognized method for evaluating learning and memory in rodent experiments. In the present study, we observed that treatment with scopolamine alone increases the latency to find the hidden platform in MWM experiment. This effect was reversed by the administration of donepezil. This suggests that scopolamine administration lead to impairment in spatial memory formation. Similarly, we found a significant increase in brain AChE activity in scopolamine treated rats. AChE is cholinergic enzyme which hydrolyzes ACh into acetic acid and choline. Increase AChE activity usually leads to memory dysfunction. Scopolamine can induce memory impairment by promoting brain oxidative stress. In this study we found that scopolamine-treated rats show a significant increase in brain MDA level. In this study, we observed a significant increase in the brain GSH level in scopolamine-treated group. Although, we would expect a decrease in level of GSH which is an antioxidant in nature. Instead, we observed a traumatic increase in the brain GSH level, suggesting that the enhanced oxidative stress triggers a cascade of activities to increase the brain anti-oxidant activities to prevent the brain against oxidative damage. Whether the increased brain GSH level was enough to resist the damaging effect of increased MDA/oxidative stress cannot be ascertained in this study. Collectively, memory impairment observed in the scopolamine treated rats study can be attributed to dysfunction in brain cholinergic

system and partial increase in the brain oxidative stress. Our result shows that treatment of rats with MC and/or OG prevent scopolamine-induced memory impairment, restores cholinergic dysfunction and oxidative stress. This could be due to the presence of different phytoconstituents, including flavonoids, sterols, and phenolic compounds in these plants [43,44]. Furthermore, evidence from the literature suggests that chronic HFD intake causes metabolic disorder which results in cognitive impairment, particularly in AD [45]. Different mechanisms that underlie the HFD-induced cognitive impairment in experimental animals have been reported. These include excessive production of reactive oxygen species (ROS), such as MDA and reduces the antioxidant enzyme levels [46,47].

Similarly, abnormal cholesterol accumulation has been associated with increased Aβ in cellular and most animal models of AD, and drugs that inhibit cholesterol synthesis have been shown to lower Aβ in these models [48,49]. Hence, we used HFD model which relies on comorbid effect of excessive weight gain and metabolic disorders to cause memory deficit in experimental animals and human. In our study, HFD rats shows pronounced hypercholesterolemia, increased AChE activity, increased oxidative stress as shown by enhanced MDA level in the brain, and spatial memory deficit in MWM task. Treatment with MC/OG alone or in combination did not only prevent the cognitive impairment in HFD-rats, but also reduced the plasma cholesterol level and restore the AChE activity.

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