Nigerian Journal of Biochemistry and Molecular Biology 2019; 34(1&2): 28-37

© 2019 Nigerian Society of Biochemistry and Molecular Biology

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.com.ng

NJBMB/013/19

Effects of Ethylacetate Leaf Extracts of *Ocimum gratissimum* and *Momordica charantia* on Memory in Scopolamine-induced Dementia Rats

***Ayinla, M. T., Uthman, Y., Bayo-Olugbami, A., Oyewole, A. L. and Owoyele, B. V.**

Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

Abstract: Lipid peroxidation, reduced anti-oxidant enzyme activities and increased DNA damage resulting from generation of free radicals, consequently leading to oxidative stress, have been implicated in the pathogenesis of various neurodegenerative conditions including Alzheimer's disease. Based on the widely reported anti-oxidative properties of *Ocimum gratissimum* (OG) and *Momordica charantia* (MC), this study evaluated the effects of Ethylacetate leaf extracts of *Ocimum gratissimum* (ELEOG), *Momordica charantia* (ELEMC) or both (ELEOG/MC) in scopolamine-induced dementia in rat model. Twenty-five male Wistar rats weighing between 110-120g were randomly distributed into five groups: Control (5ml/kg DMSO, p.o.), Scopolamine (1 mg/kg, i.p.); while the test groups were pretreated orally with ELEOG (400 mg/kg), ELEMC (400 mg/kg), and a combination of ELEOG/MC (400 mg/kg each) daily for 15 consecutive days. Amnesia was induced in all groups except control by a single injection of scopolamine (1 mg/kg, i.p) on the 15th day, following which Morris water maze (MWM) was used to assess short-term spatial memory function. Also, biochemical characterization of the brain levels of malondialdehyde (MDA) and reduced glutathione (GSH) were made. Scopolamine-treated rats with no intervention showed impaired learning and memory as depicted by increase in escape latency, increased MDA, and decreased GSH levels. Intervention with ELEOG, ELEMC or combined form ELEOG/MC decreased the escape latency, reduced MDA level and increased GSH levels. These findings show the neuro-protective efficacy of OG and MC, especially the combined form in attenuating cognitive impairment in scopolamine-induced dementia by modulating the oxidative stress pathway.

KEYWORDS: Amnesia, Ethylacetate, *Momordica charantia*, *Ocimum gratissimum*, Oxidative stress, Scopolamine

1.0 Introduction

Acquisition of skills and knowledge (learning), and the ability to retain, retrieve and remember this information (memory) are part of the essential physiological processes that make up a healthy and functioning brain. These processes are complex and involve multiple brain areas, neuronal pathways and neurotransmitters (Abel and Lattal, 2001). Alterations in these processes may result from damage to brain areas associated with learning and memory, disruption in the neurotransmission system and other factors such as aging, stress, oxidative stress, toxin exposure, mitochondrial dysfunction, and trauma can hinder the storage, retention and recollection of memory which often result in decline in memory functions, generally referred to as dementia (Tewari *et al.*, 2018).

Alzheimer's disease (AD) is the most common form of dementia considered as progressive, neurodegenerative disease characterized by cognitive dysfunction with memory impairment and behavioural disturbances (Ferri *et al.*, 2005). Although the primary cause of AD remains unclear, oxidative stress is one of the most important factors implicated in the pathogenesis of dementia (Zaki *et al.*, 2013). It causes generation of free radicals which result in lipid peroxidation, reduced antioxidant enzyme activities and increased DNA damage (Hei, 2001), leading to degeneration of brain cells and damage to the neurons involved in memory processing (Stohs, 1995; Valko *et al.*, 2007).

Scopolamine is a non-selective post-synaptic muscarinic receptor blocker. It causes cognitive

***Corresponding Author**
Tel.: +2348076029175
E-mail: gazmark@unilorin.edu.ng

impairments in rodents and humans by decreasing the effectiveness of acetylcholine in the CNS (Fibiger, 1990; Kim *et al*., 2013). It induces significant deficits in cognitive performance, which makes it a valid pharmacological model for investigating and screening potential anti-amnesic molecules (Blokland, 2005; Haider *et al*., 2016).

Medicinal plants have been used for centuries before the advent of orthodox medicine and their medicinal values have been attributed to various phytochemicals, which produce definite physiological actions in the body. These phytochemicals which are naturally occurring and biologically active compounds have been reported to be effective in combating or preventing many diseases due to their anti-oxidant properties (Akinmoladun *et al.,* 2007). The use of herbs in the treatment of various diseases has been on the increase because of their cost effectiveness, accessibility and little or no side-effects (Kumar *et al.,* 2012).

Ocimum gratissimum is a perennial herb notably found in the tropical and subtropical regions of the World. It is commonly called clove basil, sweet basil, or scent leaf (Obho, 2006). It has been reported to possess diverse pharmacological properties which include; anti-oxidant (Akinmoladun *et al.,* 2007; Lukmanul *et al.,* 2008); chemotherapeutic, anti-mutagenic (Adebolu and Salau, 2005); anti-nociceptive (Rabelo *et al.,* 2003), hypotensive, hypoglycemic (Aguiyi *et al.,* 2000), anti-pyretic and anti-inflammatory (Prajapati *et al.,* 2003; Tania *et al.,* 2006) activities.

Momordica charantia is an indigenous medicinal and vegetable plant commonly known as bitter gourd or bitter melon (Lee *et al.,* 2009). Its pharmacological properties include anti-carcinogenic, hypoglycemic (Sin *et al.,* 2011), anti-viral, anti-bacterial, analgesic, anti-inflammatory (Choi *et al.,* 2012), hypotensive and anti-oxidant activities (Mahmood *et al.,* 2012; Sin *et al.,* 2012).

Based on evidence that dementia is mediated via oxidative stress, the present study investigated the neuro-protective effects of ethyl acetate leaf extracts of *O. gratissimum* and *M. charantia* against scopolamine-induced memory impairment in rat model of dementia.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant material and authentication

The leaves of *Ocimum gratissimum* (OG) and *Momordica charantia* (MC) were purchased from Oro in Kwara State, Nigeria. The leaves were authenticated by Mr Bolu Ajayi of the Department of Plant Biology, University of Ilorin, with voucher numbers: UILH/001/2019/1356 and UILH/002/2019/1292 respectively.

2.1.2 Experimental animals

Twenty-five male Wistar rats weighing between 100-120 g were obtained from the breeding colony of Department of Biochemistry, University of Ilorin, Ilorin. The animals were housed in cages placed in the Animal Holding Unit of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin. The animals were maintained on standard diet and water *ad libitum*.

2.1.3 Drug and Chemicals

Scopolamine was a product of Hubei Tianyao Pharmaceutical Co. Ltd. Hubei, China. Ethyl acetate and Dimethyl sulphoxide (DMSO) were Sigma-Aldrich, Mannheim, Germany.

2.2 Methods

2.2.1 Plant preparation

The fresh leaves of OG and MC were washed separately to remove sands and debris, air-dried at room temperature for two weeks and pulverized with an electric blender. The powdered vegetal material weighing 300 g each of OG and MC was soaked separately in ethyl acetate for 72 hours with occasional shaking. Each mixture was filtered with muslin cloth to separate the filtrate and the residue. The filtrate was then concentrated using rotatory evaporator which yielded 32.5 g of OG and 35 g of MC respectively. Two grammes each of the extracts

were reconstituted in 25 ml of DMSO to obtain the required dose of 400mg/kg of the extracts.

2.2.2 Animal Studies

2.2.2.1 Induction and assessment of amnesia

Amnesia was induced in all groups except control by a single injection of scopolamine (1 mg/kg, i.p) on the 15th day, following which Morris water maze (MWM) was used to assess short-term spatial memory function. The Morris water maze test was carried out in accordance with the method described by Morris, (1984). The MWM was used to assess short-term spatial memory following exposure to scopolamine and/or *.O gratissimum, M. charantia* (separately or combined) in Wistar rats.

Each rat was placed in a circular maze (70 cm deep ×200 cm diameter), which was filled with water and coloured with milk to make the submerged escape platform invisible. The maze was divided into four quadrants: north (N), east (E), south (S) and west (W). It contained a circular escape platform (10 cm diameter, 28 cm high) submerged (about 1.5 cm below water surface) at the center of the north-east quadrant of the maze. The water was kept at room temperature throughout the procedure.

Rats were exposed to training sessions during which each rat was placed in the maze, facing the wall and allowed to locate the hidden escape platform within 60 seconds. Rats that failed to locate the platform within this period were guided to the platform and allowed to stay on it for another 60 seconds. During each trial, escape latency which is the time taken to locate the platform was noted as the index of acquisition and this was recorded with the aid of a video system. The training session consisted of four trials daily with short resting interval after two trials for 7 consecutive days before the onset of extract administration. On the 15th day of administration, the rats were exposed to the maze for the acquisition phase, followed by a single injection of scopolamine. About 40 minutes after, rats were subjected to the actual testing phase.

2.2.2.2 Animal grouping

The rats were randomly divided into five groups (n = 5) as follows:

- Control: received DMSO (5 ml/kg orally) daily
- Scopolamine treated: received scopolamine (1mg/kg body weight, once, intraperitoneally) (Agrawal *et al.*, 2009; Pattanashetti *et al.*, 2017; Wolf *et al.*, 2017)
- MC Scop: received *Momordica charantia* (400 mg/kg body weight, orally) daily and scopolamine (1 mg/kg body weight, once, intraperitoneally)
- OG Scop: received *Ocimum gratissimum* (400 mg/kg body weight, orally) daily plus scopolamine (1 mg/kg body weight, once, intraperitoneally)
- OG+MC Scop: received a combination of *O. gratissimum* and *M. charantia* (400 mg/kg body weight, orally), daily and scopolamine (1 mg/kg body weight, intraperitoneally once)

The rats were kept under standard laboratory conditions (12h light/dark cycle, temperature: 22 ± 3°C) and acclimatized for two weeks before the commencement of the experiment. The administrations were between the hours of 08:00 and 10:00 and for fifteen consecutive days. The experiment was performed in compliance with standard guidelines for the care and use of experimental animals and as permitted by the Departmental Animal Care and Use Committee of University of Ilorin.

2.2.2.3 Preparation of brain homogenate

At the end of the behavioural test, the animals were anaesthetized with ketamine (100mg/kg). The brain was perfused with phosphate buffered saline (PBS) after which the brain was excised and hippocampus was isolated. The hippocampal tissue was homogenized and then centrifuged at 503 x g for 10 minutes. The supernatant from the homogenate was separated into plain bottles and kept in the freezer for biochemical analysis.

2.2.2.4 Determination of biochemical parameters

The levels of malondialdehyde (MDA) was determined indirectly as thiobarbituric acid reactive substances (TBARS) according to the thiobarbituric acid reaction of Mihara and Uchiyama, (1978) whereas reduced glutathione level was determined using the spectrophotometric procedure described by Ellman (1959).

2.2.2.5 Data analysis

All Data were analyzed using one-way analysis of variance (ANOVA) and subjected to post hoc Duncan's multiple comparison tests. The results were expressed as mean ±SEM using SPSS software (version 21). Values of $p<0.05$ were considered statistically significant.

3.0 Results

Scopolamine treated rats had a significant ($p<0.05$) delay in escape latency (EL), [the time required to locate the hidden platform in MWM task] when compared with the control group. However, interventions with either OG, MC or a combination of both significantly ($p<0.05$) shortened the EL when compared with Scop-treated rats. Interestingly, the EL in the three intervention groups was similar to control. In addition, though there was no significant difference ($p>0.05$) in the EL across the intervention groups, but the combined treatment of OG and MC was biologically most effective in reducing the escape latency (Figure 1).

Scopolamine caused a significant ($p<0.05$) increase in the level of MDA compared with control. While treatments with MC alone or a combination of OG and MC caused a significant ($p<0.05$) decrease in the activity of MDA. Administration of OG alone resulted in a considerable but insignificant reduction in MDA level compared with rats that received scopolamine only. Treatments with MC or OG+MC reversed MDA to a level comparable with control group but administration of OG alone significantly ($p<0.05$) increased its level compared to control rats. Similarly, across the three treatment groups, the level of MDA was significantly ($p<0.05$) lower in rats treated with either MC or OG+MC compared with OG treated group (Figure 2).

The level of GSH in Scopolamine-treated rats was not altered compared to control group. Treatment with either OG, MC or OG+MC significantly ($p<0.05$) increased the level of GSH compared with scop-treated group. However, there was no significant alteration in the level of GSH across the treatment groups. Surprisingly, treatments with OG, MC or OG+MC caused a marked increase in GSH level compared with control group. Biologically, rats pre-treated with MC prior to Scopolamine-induced dementia show the highest level of GSH across the whole groups (Figure 3).

4.0 Discussion

Although growing evidences have indicated the therapeutic potentials (especially the antioxidant capacities) of *O. gratissimum* (OG) and *M. charantia* (MC), individually on different biological processes, yet, no study is available on the potential neuro-protective impact of the combination of these two extracts on dementia, a neurological dysfunction that is linked majorly to oxidative stress. Hence, this study investigated the neuro-protective effects of the combination of the ethyl acetate extracts of OG and MC on scopolamine-induced dementia using behavioural and biochemical parametres.

Scopolamine has been severally reported to produce detrimental actions on cognitive function. Also there are various studies indicating the role of oxidative stress in several disorders including dementia. Additionally, data have indicated that oxidative stress is one of the foremost events in the pathogenesis of memory impairment (Halliwell, 2001; Butterfield *et al.,* 2002; Silva *et al.,* 2004; Suzuki *et al.,* 2006). This cognitive deficit associated with dementia and other diseases is well linked to oxidative damage through imbalance between pro-oxidants and antioxidants resulting into oxidative stress (Castellani *et al.,* 2001; Halliwell, 2001; Bradley *et al.,* 2010).

Numerous reports have shown that there is cholinergic transmission blockage and cognitive impairment with a single intraperitoneal

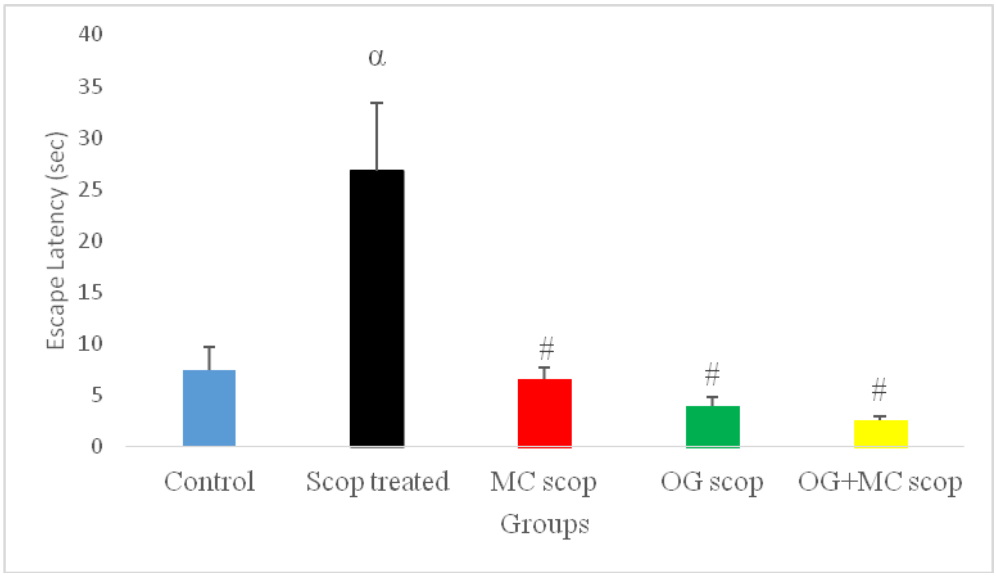

Figure 1: Ethyl acetate extracts of OG or/and MC reduced the escape latency in scopolamine-induced amnesia. $^{\alpha}P < 0.05$ compared with control, $^{\#}P < 0.05$ compared with Scop-treated. Values are expressed as mean ± SEM of 5 rats per group. Control = Vehicle; Scop-treated = Scopolamine only; OG-scop = *Ocimum gratissimum* + Scopolamine; MC-scop = *Momordica charantia* + Scopolamine; OG + MC scop = *Ocimum gratissimum* and *Momordica charantia* + Scopolamine

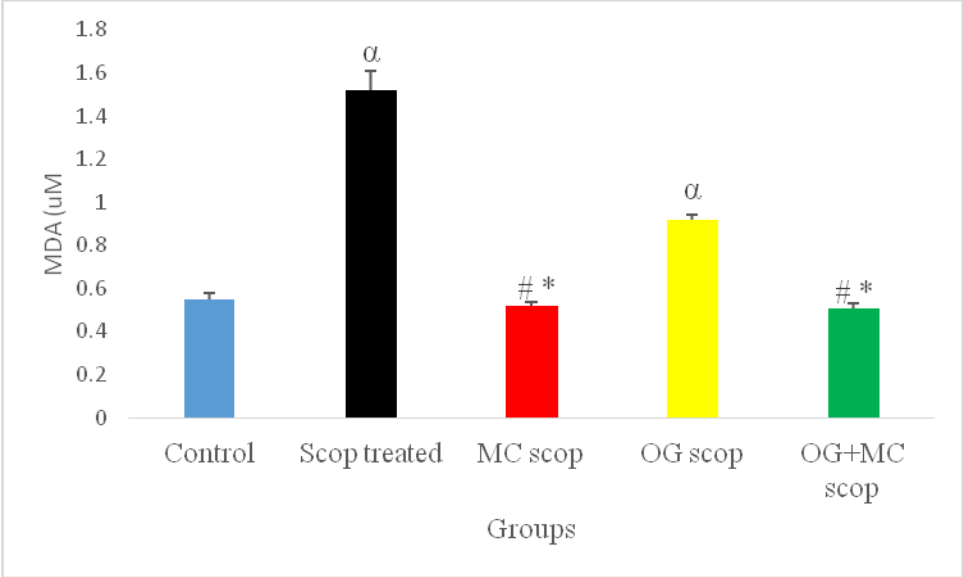

Figure 2: Ethyl acetate leaf extracts of MC or OG+MC reduced MDA level in scopolamine-induced amnesia. $^{\alpha}P<0.05$ compared with control; $^{\#}P<0.05$ compared with Scop-treated; *P<0.05 compared with OG Scop. Values are expressed as mean ± SEM of 5 rats per group. Control = Vehicle; Scop-treated = Scopolamine only; OG-scop = *Ocimum gratissimum* + Scopolamine; MC-scop = *Momordica charantia* + Scopolamine; OG+MC scop = *Ocimum gratissimum* and *Momordica charantia* + Scopolamine

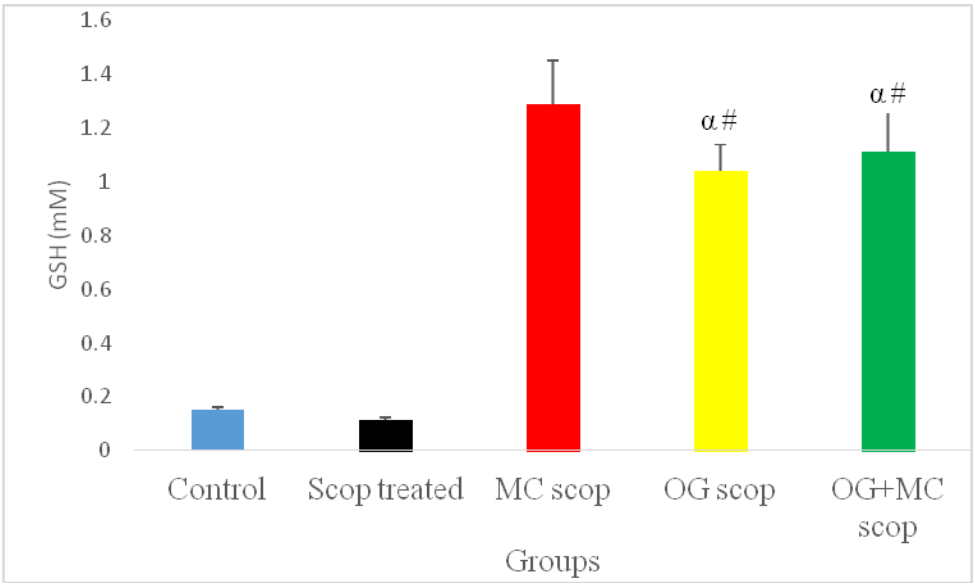

Figure 3: Ethyl acetate leaf extracts of OG or/and MC increased the level of Glutathione (GSH) in scopolamine-induced amnesia. $^{\alpha}P<0.05$ compared with control; $^{\#}P<0.05$ compared with Scop-treated. Values are expressed as mean ± SEM of 5 rats per group. Control= Vehicle; Scop-treated= Scopolamine only; OG-Scop = *Ocimum gratissimum* + Scopolamine; MC-Scop = *Momordica charantia* + Scopolamine; OG+MC Scop = *Ocimum gratissimum* and *Momordica charantia* + Scopolamine.

injection of scopolamine (Alikatte *et al.*, 2012; Zaki *et al.*, 2013). In agreement with that, findings from this present study show that intraperitoneal injection of scopolamine impaired learning and memory as depicted by increase in escape latency observed in rats treated with scopolamine compared to control and other treatment groups. Similarly, decline in cognition due to injection of scopolamine was reportedly accompanied with changes in oxidative stress in the brain (Wang *et al.*, 2014).

In the present study, oxidative stress markers were estimated to investigate the mechanisms by which the cognitive deficit recorded in the scopolamine treated group occurred. Our results showed that it was due to increased lipid peroxidation as depicted by elevated level of MDA and decreased level of reduced glutathione within the brain. *Ocimum gratissimum* and *M. charantia* have both been reported to have neuro-protective (Nerurkar *et al.*, 2011; Malik *et al.*, 2011) and antioxidant activities (Akinmoladun *et al.*, 2007). In line with our results, a study reported that orally administered aqueous extract of OG leaf could reduce oxidative and toxicant activity and enhance specific activities of hepatic antioxidant enzymes in rats (Ighodaro and Ebuehi, 2009). Similarly, another study ascribed the beneficial effect of aqueous extract of OG to its capacity to increase serum catalase and DPPH radical scavenging activities (all of which reduce oxidative stress) in a chemical-induced toxin (CCl_4) model of hepatic injury, hence, its hepato-protective effect (Chiu *et al.*, 2012). In addition, the study of Bora *et al.*, (2011), the cerebro-protective potential of *OG* in focal ischemia and reperfusion-induced cerebral injury was mediated through its antioxidant activities, where in it led to a significant reduction in cerebral infarct volume, lipid peroxidation, and increased levels of SOD and glutathione peroxidase in the brain. Likewise, in a monosodium glutamate-induced neurotoxicity model, OG reportedly showed an increase in superoxide dismutase, glutathione and glutathione peroxidase activities (Imosemi and Okori, 2017).

The significant reduction in the escape latency observed in the groups treated with ethyl acetate leaf extracts of *O. gratissimum* and *M. charantia* shows the neuroprotective effect of these leaves via antioxidant mechanism. This neuroprotective effect can be attributed to the decreased malondialdehyde (MDA) and increased reduced glutathione (GSH) activities. The neuroprotective and memory-enhancing effect of the extracts may be due to the active metabolites such as saponins, tannins, glycosides, polysaccharides, flavonoids steroids, lipids and especially polyphenolic compounds which have been reported to be present in these plants (Akinmoladun *et al.,* 2007; Choi, 2012 ; Xu *et al.,* 2015; Zhang *et al.,* 2016). Polyphenols from plant extracts have been indicated as being major therapeutic components for oxidative stress. Although the cellular mechanisms underlying the actions of polyphenols and their metabolites have not been completely interpreted, it is believed that their properties including antioxidant activity, free radical scavenging, and anti-inflammation could be involved (Rajadurai and Prince 2006).

Based on the fact that the etiology of dementia is multifactorial, utilization of multiple drugs can bring about better result outcomes than single therapy (Youdim and Buccafusco, 2005). For instance, the combined effects of seleginile (an irreversible inhibitor of monoamine Oxidase-B) and meloxicam (cyclooxygenase-2 inhibitor) was reported to be more effective against scopolamine-induced dementia (Goverdhan *et al.,* 2012). Deiana *et al.,* also showed that combined therapies give better result as compared to single drug administration (Deiana *et al.,* 2009). These reports are similar to that observed in the present study in which the co-administration of OG and MC extracts attenuated cognitive impairment in scopolamine-induced dementia.

Conclusion

The present study demonstrates that treatment of rats with ethylacetate leaf extracts of *Ocimum gratissimum* and *Momordica charantia* mitigated scopolamine-induced behavioural and biochemical changes. This could be attributed to their antioxidant properties. Hence, a combination of these extracts has anti-amnestic potential. More studies would be needed to unravel the molecular mechanism underlying the neuro-protective activities of these herbal extracts.

References

Abel, T. and Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology 11:180-187.

Adebolu, T. T. and Salau, S. O. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria in Southwest Nigeria. African Journal of Biotechnology 4: 682-684.

Agrawal, R., Tyagi, E., Saxena, G. and Nath, C. (2009). Cholinergic influence on memory stages: A study on scopolamine amnesic mice. Indian Journal of Pharmacology 41(4):192-196.

Aguiyi, J. C., Obi, C. I., Gang, S. S. and Igweh, A. C. (2000). Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapia 71: 444–446.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M. and Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Scientific Research and Essay 2: 163-166.

Alikatte, K. L., Akondi, B. R., Yerraguntu, V. G., Veerareddy, P. R. and Palle, S. (2012). Antiamnestic activity of *Syzygium cumini* against scopolamine induced memory impairments in rats. Brain Development 34: 844-851.

Beloin, N., Gbeassor, M., Akpagana, K., Hudson, J., de Soussa, K., Koumaglo, K. and Arnason, J. T. (2005). Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. Journal of Ethnopharmacology 96: 49-55.

Blennow, K., de Leon, M. J. and Zetterberg, H. (2006). Alzheimer's disease. Lancet. 368: 387-403.

Blokland, A. (2005). Scopolamine-induced deficits in cognitive performance: A review of animal studies. Scopolamine Review. 1: 1-76.

Bora, K. S, Shri, R. and Monga, J. (2011). Cerebroprotective effect of *Ocimum gratissimum* against focal ischemia and reperfusion-induced cerebral injury. Pharmaceutical Biology 49(2): 175-181.

Bradley, M. A., Markesbery, W. R. and Lovell, M. A. (2010). Increased levels of 4-hydroxynonenal and acrolein in the brain in preclinical Alzheimer

disease. Free Radical Biology and Medicine 48: 1570-1576.

Butterfield, D. A., Griffin, S., Munch, G. and Pasinetti, G. M. (2002). Amyloid β-peptide and amyloid pathology arer central to the oxidative stress and inflammatory cascades under which Alzheimer's disease brain exists. Journal of Alzheimers Disease 4: 193-201.

Castellani, R. J., Harris, P. L., Sayre, L. M., Fujii, J. and Taniguchi, N. (2001). Active glycation in neurofibrillary pathology of Alzheimer disease: N^{ε}-(carboxymethyl) lysine and hexitol-lysine. Free Radical Biology and Medicine 31:175-180.

Chiu, C. C., Huang, C. Y., Chen, T. Y., Kao, S. H., Liu, J. Y., Wang, Y. W., Tzang, B. S. and Hsu, T. C. (2012). Beneficial effects of *Ocimum gratissimum* aqueous extract on rats with CCl_4 - induced acute liver injury. Evidence Based Complement Alternative Medicine doi.org/10.1155/2012/736752

Cho, E. J., Sin, S. M., Choi, J. M., Lee, S., Cho, K. M. and Kim, H. Y. (2012a). Protective effects of the active fraction of bitter melon (*Momordica charantia*) on nitric oxide-induced oxidative stress in LLC-PK1 cell. Journal of Medicinal Plant Research 6:4968-4973.

Choi, J. S., Kim, H. Y., Seo, W. T., Lee, J. H. and Cho, K. M. (2012). Roasting enhances antioxidant effect of bitter melon (*Momordica charantia* L.) increasing in flavan-3-ol and phenolic acid contents. Journal of Food Science and Biotechnology 21: 19-26.

Deiana, S., Harrington, C. R., Wischik, C. M. and Riedel, G. (2009). Methylthioninium chloride reverses cognitive deficits induced by scopolamine: Comparison with rivastigmine. Psychopharmacology (Berl). 202: 53-65.

Ellman, G. L. (1959). Tissue sulfhydryl groups. Arch Biochemistry and Biophysics 82:70-77.

El-Sherbiny, D. A., Khalifa, A. E., Attia, A. S. and Eldenshary, E. E. D. S. (2003). *Hypericum perforatum* extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnesic dose of scopolamine. Pharmacology Biochemistry and Behaviour 76: 525-533.

Fan, Y., Hu, J., Li, J., Yang, Z. and Xin, X. (2005). Effect of acidic oligosaccharide sugar chain on scopolamine-induced memory impairment in rats and its related mechanisms. Neuroscience Letters 374:222-226.

Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hali, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., Menezes, P. R., Rimmer, E. and Scazufca, M. (2005). Global prevalence of dementia: A Delphi consensus study. Lancet. 366(9503): 2112-2117.

Fibiger, H. C. (1990). Central cholinergic systems and memory. In: Squire, L. R, Lindenlaub, E. editors. The biology of memory. Symposia Medica Hoechst, Springer-Verlag. New York, pp. 381-398.

Freeman, B. A. and Capro, J. D. (1982). Biology of disease: Free radicals and tissue injury. Laboratory Investigation 47: 412-426.

Goverdhan, P., Sravanthi, A. and Mamatha, T. (2012). Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. International Journal of Alzheimers Disease 2012: 974013.

Grover, J. K. and Yadav, S. P. (2004). Pharmacological actions and potential uses of *Momordica charantia*: A review. Journal of Ethnopharmacology 93:123-132.

Haider, S., Tabassum, S., Perveen, T. (2016). Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: a comparative study. Brain Research Bulletin 127: 234–247.

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. Journal of Neurochemistry 59:1609-1623.

Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. Drugs & Aging 18:685-716.

Ighodaro, O. M. and Ebuehi, O. A. (2009). Aqueous leaf extract of *Ocimum gratissimum* potentiates activities of plasma and hepatic antioxidant enzymes in rats. Nigerian Quarterly Journal of Hospital Medicine. 19(2):106–109.

Imosemi, I. O. and Okori, S. O. (2017). Neuroprotective effects of ethanolic Leaf extract of *Ocimum gratissimum* on monosodium glutamate-induced oxidative stress in developing Wistar rat cerebellum. African Journal of Medicine and Medical Science 46(4):463-472.

Kim E. J., Jung I. H., Jeong J. J., Kim N. J., Kim D. H. (2013). Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice. Journal of Ethnopharmacology 146: 294-299.

Kumar, H., More, S. V., Han, S. D., Choi, J. Y. and Choi, D. K. (2012). Promising therapeutics with natural bioactive compounds for improving learning and memory-A review of randomized trials. Molecules 17:10503-10539

Lee, M. J., Chen, H. M. and Tzang, B. S. (2011). *Ocimum gratissimum* aqueous extract protects H9c2 myocardiac cells from H_2O_2-induced cell

apoptosis through Akt signalling. Evidence-Based Complementary and Alternative Medicine Doi.org/10.1155/2011/578060.

Lee, S. Y., Eom, S. H., Kim, Y. K., Park, N. I. and Park, S. U. (2009). Cucurbitanetype triterpenoids in *Momordica charantia* Linn. Journal of Medicinal Plant Research 3:1264-1269.

Liu, X. R, Deng, Z. Y., Fan, Y. W., Li, J. and Liu, Z. H. (2010). Mineral elements analysis of *Momordica charantia* seeds by ICP-AES and fatty acid profile identification of seed oil by GC-MS. Guang Pu Xue Yu Guang Pu Fen Xi. 30(8):2265-2268.

Lukmanul, H. F., Girija, A. and Boopathy, R. (2008). Antioxidant property of selected Ocimum species and their secondary metabolites content. Journal of Medicinal Plant Research 2:250-257.

Mahmood, A., Raja, G. K., Mahmood, T., Gulfraz, M. and Khanum, A. (2012). Isolation and characterization of antimicrobial activity conferring component(s) from seeds of bitter gourd (*Momordica charantia*). Journal of Medicinal Plant Research 6:566-573.

Malik, Z. A., Singh, M. and Sharma, P. L. (2011). Neuroprotective effect of *Momordica charantia* in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. Journal of Ethnopharmacology 133:729-734.

Martin, J. B. (1999). Molecular basis of the neurodegenerative disorders. New England Journal of Medicine 340:1970-1980.

Mihara, M. and Uchiyama, M. (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry 86:271-278.

Mishima, K., Tsukikawa, H., Miura, I., Inada, K. and Abe, K. (2003). Ameliorative effect of NC-1900, a new AVP_{4-9} analog, through vasopressin V_{1A} receptor on scopolamine-induced impairments of spatial memory in the eight-arm radial maze. Neuropharmacology 44:541-552.

Morris, R. G. M. (1984). Developments of water maze producer of studying spatial learning in the rats. Journal of Neuroscience Methods 11:47-60

Nerurkar, P. and Ray, R. B. (2010). Bitter melon: antagonist to cancer. Pharmacology Research 27: 1049-1053.

Obho, G. (2006). Antioxidant and antimicrobial properties of ethanolic extract of *Ocimum gratissimum* leaves. Journal of Pharmacology and Toxicology 1:47-53

Pattanashetti, L. A., Taranalli, A. D., Parvatrao, V., Malabade, R. H. and Kumar, D. (2017). Evaluation of neuroprotective effect of quercetin with donepezil in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology 49(1): 60-64.

Prajapati, N. D., Purohit, S. S., Sharma, A. K. and Kumar, T. (2003). Agro's dictionary of medicinal plants. 1st ed. Agrobios: India.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., Ferri, C. P. (2013). The global prevalence of dementia: a systematic review and meta-analysis. Alzheimers Dementia 9(1):63-75.e2.doi: 10.1016/j.jalz.2012.11.007.

Rabelo, M., Souza, E. P., Soares, P. M. G., Miranda, A. V., Matos, F. J. A. and Criddle, D. N. (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Brazillian Journal of Medicine and Biological Research 36:521-524.

Rajadurai, M. and Prince, P. S. M. (2006). Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: biochemical and histopathological evidences. Toxicology 228(2-3):259-268.

Reiter, R. J. (1995). Oxidative processes and antioxidative defense mechanisms in the aging brain. FESEB Journal 9:526-533.

Ross, C. A. and Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease. Natural Medicine 10 Suppl, S10-17.

Shi, J., Liu, Q., Wang, Y. and Luo, G. (2010). Coadministration of huperzine A and ligustrazine phosphate effectively reverses scopolamine-induced amnesia in rats. Pharmacology, Biochemistry and Behaviour 96:449-453.

Silva, R. H., Abilio, V. C., Takatsu, A. L., Kameda, S. R. and Grassl, C. (2004). Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. Neuropharmacology 40:895-903.

Sin, S. M., Mok, S. Y., Lee, S., Cho, K. M., Cho, E. J. and Kim, H. Y. (2011). Protective effect of bitter melon (*Momordica charantia*) against oxidative stress. Cancer Prevention Research 16: 86-92.

Sin, S. M., Mok, S. Y., Lee, S., Cho, K. M., Cho, E. J. and Kim, H. Y. (2012). Anti-inflammatory effect of bitter melon (*Momordica charantia*) in RAW 264.7 cell. Cancer Prevention Research 17: 56-61.

Stohs, S. J. (1995). The role of free radicals in toxicity and disease. Journal of Basic Clinical Physiology and Pharmacology 6:205-228.

Suzuki, Y., Jain, V., Park, A. and Day, R. (2006). Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. Free Radical Biology and Medicine 40:1683-1692.

Tania, U., Nakamura, R. R. and Mandonca, F. (2006). Antileishmanial activity of Eugenol-rich essential oil from *Ocimum gratissimum*. Parasitology International 55:99-105.

Tewari, D., Stankiewicz, A. M., Mocan, A., Archana, N. S., Nikolay, T. T., Lukasz, H., Jarosław, O. H. and Atanas, G. A. (2018). Ethnopharmacological approaches for dementia therapy and significance of natural products and herbal drugs. Frontier Aging Neuroscience. doi.org/10.3389/fnagi.2018.00003.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 39(1): 44-84.

Wang, X., Wang, W., Li, L., Perry, G., Lee, H. G. and Zhu, X. (2014). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochemistry Biophysics Acta 1852(8): 1240-1247.

Wolf, A., Bauer, B., Abner, E. L, Ashkenazy-Frolinger, T. and Hartz, A. M. (2017). A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PLOS. https://doi.org/10.1371/journal.pone.0147733

Xu, X., Shan, B., Liao, C. H., Xie, J. H., Wen, P. W. and Shi, J. Y. (2015). Anti-diabetic properties of *Momordica charantia* L. polysaccharide in alloxan-induced diabetic mice. Int J Biol Macromol. 81:538–543.

Youdim, M. B. and Buccafusco, J. J. (2005). CNS Targets for multi-functional drugs in the treatment of Alzheimer's and Parkinson's diseases. Journal of Neural Transmission. 112(4): 519-537.

Zafar, R. Neerja. (1991). *Momordica charantia*-a review. Hamdard. Med. 34(3): 49-61.

Zaki, H. F., Abd-El-Fattah M. A, Attia A. S. (2013). Naringenin protects against scopolamine-induced dementia in rats. Bulletin of Faculty of Pharmacy, Cairo University. 52: 15-25. DOI: 10.1016/j.bfopcu.2013.11.001