

Full-Length Research Article

Modulation of Memory and Neurochemical Changes by Resveratrol and Environmental Enrichment in Rodent Model of Alzheimer's Disease

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Summary: Alzheimer's disease (AD) is the most common cause of dementia that affects one patient every seven seconds, with over 35 million people currently affected worldwide. The aim of the study was to investigate the modulation of memory and neurochemical responses by resveratrol and environmental enrichment (EE) in aluminium chloride (AlCl₃) model of Alzheimer's disease in mice. Male mice used for the study were divided into nine groups, of seven animals each. Group I (negative control): 0.2 ml normal saline/kg, Group II: 0.2 ml CMC/kg. Group III: resveratrol (200 mg/kg), Group IV: CMC and kept in EE, Group V: AlCl₃ at dose of 50 mg/kg, Group VI: resveratrol at dose of 200 mg/kg and kept in EE, Group VII: AlCl₃ (50 mg/kg) + resveratrol (200 mg/kg), Group VIII: AlCl₃ (50 mg/kg) and kept in EE, Group IX: AlCl₃ (50 mg/kg) + resveratrol (200 mg/kg) and kept in enriched environment. All treatments were oral and lasted for 8 weeks. Assessments of memory was carried out before treatment, and at weeks 4 and 8, after the first treatment. The mice were sacrificed and hippocampal samples collected for neurochemical analysis. The findings of the study suggest that AlCl₃ induced contextual fear memory deficit over time ($p < 0.05$), which was improved by resveratrol. Both A β and Nrf2 significantly ($p < 0.05$) increased in AlCl₃ + EE + resveratrol group. In conclusion, Individual treatment with either resveratrol or EE improved memory over the combined treatment in AlCl₃ model of AD by decreasing A β protein concentration.

Keywords: Resveratrol, Environmental enrichment, Aluminium chloride, Alzheimer's disease, Oxidative stress

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia that affects one patient every seven seconds, with over 35 million people currently affected worldwide (Mohandas et al., 2009; Prince et al., 2018). It has been projected that the disease will affect about 115 million people by 2050 (Prince et al., 2013), which may be caused by both genetic and environmental factors (Gatz et al., 2010). Indeed, it is the most frequent cause of dementia found in the elderly, with an estimated prevalence of 25-50% in people over the age of 85, making it one of the most important medical problem in the elderly (Hong-Qi et al., 2012). The principal histological hall-marks of the disease are the presence of aggregated amyloid-beta (A β)-laden plaques and hyperphosphorylated tau-laden neurofibrillary tangles (NFTs) (Selkoe et al., 2001). However, the neurodegeneration that occurs in AD has been proposed to arise not only from the accumulation of A β and/or aberrant modification of tau, but also from a number of other factors that include oxidative stress, inflammation, vascular disease and accumulation of metals, such as zinc and aluminium

(Al) (Mohandas et al., 2009; Armstrong, 2011; Craddock et al., 2012).

The negative impacts of aluminium toxicity include diseased ageing induced by alteration of cellular and molecular mechanisms through gene activation and silencing, eventually leading to various age-related disorders, such as AD. Interestingly, Al is an important component of many household materials, such as clays, glasses, and alum (Rui and Yongjian, 2010; Kawahara and Kato-Negishi, 2011). Exposure to Al has been associated with the impairment of mitochondrial functions and the antioxidant defence systems leading to oxidative stress and increased lipid peroxidation, which could eventually promote A β peptide formation, deposition, and AD-like amyloidosis (Niu et al., 2005; Kumar, et al., 2008; Kumar and Gill, 2009).

Resveratrol (3, 4'-5- trihydroxystilbene), which is a natural polyphenolic compound found in the skin and seeds of grapes, has been reported to possess antioxidant and anti-inflammatory properties (Das and Das, 2007; Roy et al., 2011; Poulsen et al., 2015). It induces sirtuin 1-dependent histone deacetylase (HDAC) activities (Howitz et al., 2003;

Aggarwal et al., 2005). Resveratrol is more effective in inhibiting oxidative damage than the conventional antioxidants. It scavenges ROS, such as lipid hydroperoxyl, hydroxyl and superoxide anion radicals, through modulation of glutathione biosynthesis via Nrf2 antioxidant-response-element signalling (Pervaiz, 2003; Rahman, 2008). Resveratrol possesses numerous health benefits such as increase in life-span in yeast, nematode worm, fruit fly and short-lived fish, through a direct sirtuin 1-dependent mechanism, or indirectly through activation of mitogen-activated protein kinase (Bauer et al., 2004; Viswanathan et al., 2005; Valenzano et al., 2006; Hawley et al., 2010; Wang et al., 2012). However, the life-span-prolonging effect of resveratrol has been seriously debated (Pearson et al., 2008; Miller et al., 2011), and the molecular mechanisms responsible for therapeutic potential in AD need further investigation.

Environmental enrichment (EE) is a sustained and progressive increase in cognitive and sensorimotor stimuli, with cumulated voluntary physical activity and complex social interactions (Anastasia et al., 2009). It possesses many benefits such as neuroprotection both in health and disease conditions, especially in ageing and animal models, against neurodegenerative disorders (Faherty et al., 2005; Nithianantharajah and Hannan, 2006; Laviola et al., 2008). The synthesis and release of trophic factors (TFs) have been suggested to play a crucial role in the neuroprotection induced by EE. The EE has been observed to alter the expression of TFs and their receptors in several brain areas, and it induces astroglialogenesis (Spires et al., 2004; Steiner et al., 2006). It increases brain-derived neurotrophic factor (BDNF) expression in the striatum and glia cell-line-derived neurotrophic factor (GDNF) mRNA in the substantia nigra in animals (Bezard et al., 2003; Faherty et al., 2005). Furthermore, it improves spatial memory in rodents by elevating histone acetylation in the hippocampus (Levenson et al., 2004; Chwang et al., 2007; Fischer et al., 2007).

The global health burden of dementia of AD has been on the increase in an alarming rate, next to cardiovascular disease with estimated global societal economic cost of over \$604 billion per annum (Wimo and Prince, 2010). Reports indicated that unless progress is made in the management of AD, the annual national cost of managing AD will reach a projected US\$ 1.2 trillion in the US alone by 2050 (Vradenburg, 2015), which further buttress the need to carry out more researches in AD with the aim of proffering viable therapeutic approach that will ameliorate its pathophysiological progression. However, research efforts focused on elucidation of the combined role of EE and resveratrol in Al-induced AD are currently lacking in the available literature. Measures to alleviate Al-induced AD via resveratrol and EE interventions have not been investigated. This study intends to evaluate the effect of resveratrol and environmental enrichment on memory and neurochemical responses in rodent model of AD.

MATERIALS AND METHODS

Animals and management: A total of 63 male mice, 8-12 weeks of age, and weighing 22-27 g were used for this study. The animals were purchased from the Animal House Facility of the Department of Pharmacology and

Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were given free access to standard commercial grower's mash feed and water. The mice were allowed to acclimatise to the environment for two weeks before the commencement of the experiment. All experimental protocols were carried out in accordance with the Ahmadu Bello University Research policy, ethics and regulations, governing the care and use of experimental animals (NIH Publication no. 85-23, revised 1996). Ethical clearance was received from Ahmadu Bello University Animal Use and Care committee (ABUCAUC/2018/056). The experiments were conducted in a quiet laboratory from 9:00 h to 16:00 h, with light-dark cycle of about 12:12 h.

Chemicals and drugs: Resveratrol (Candlewood Stars Incorporated, Danbury, USA, Batch Number: MR 150528), Aluminium chloride (100 g), purchased from ACROS Organics, New Jersey USA (CAS: A0352086), and carboxymethyl cellulose (CMC) (Product No: 27929, BDH Laboratory Chemicals Limited Poole, England), 70% alcohol, ELISA biochemical assay kits were used in the study.

Preparation and Administration of Drugs: *Trans*-resveratrol (200 mg/kg/body weight/day), was suspended in 10 g/L carboxymethyl cellulose (CMC) because it is poorly soluble in water, and administered orally (Juan et al., 2002; Roy et al., 2011). Aluminium chloride (50 mg/kg/body weight/day) was administered orally (Bihaqi et al., 2009).

Animal Housing and management: The enriched cage (66 cm long × 46 cm wide × 38 cm high) used in keeping the mice was constructed as described by Harburger *et al.* (2007). The cage contained tubes, ramps, stairs, and different 'toys' (hard plastic balls, cubes, cones, and sticks). The toys were changed twice a week to avoid contamination by faeces and urine, and to continuously encourage exploration of the environment. The complexity (the number of objects) of the housing facility was increased progressively every two days with two to four objects added to the environment. After housing the mice in the enriched cage for ten (10) days, the complexity of the cage was expected to be maximal, but the positions of the objects were changed continuously every 2 days to ensure maximal exploration by the mice (Anastasia et al., 2009). Seven mice were housed together to allow social interactions. The standard (control) condition consisted of cages made without objects or running wheels, housing seven mice per cage.

Mice were kept in EE housing for eight weeks, while receiving the appropriate treatment as described by Steiner *et al.* (2006). The control mice were given normal saline and kept under good housing.

Animal groupings: The mice were divided into nine groups of seven animals each (Table 1). All drugs were administered via the oral route. All treatments lasted for 8 weeks. Neurobehavioral studies to assess memory and learning was carried out in three phases; 7 days before treatment, and at weeks 4 and 8 after the first treatment. The animals were sacrificed 24 hours after the last

neurobehavioral assessment and the hippocampus was collected for biochemical analysis.

Table 1:

Animal Groupings

S/No	Groups	Treatment	Duration
1	Group I	0.2 ml normal saline/kg body weight (negative control)	8 weeks
2	Group II	0.2 ml CMC/kg body weight	8 weeks
3	Group III	Resveratrol (200 mg/kg) orally	8 weeks
4	Group IV	CMC + Enriched housing	8 weeks
5	Group V	AlCl ₃ (50 mg/kg)	8 weeks
6	Group VI	Resveratrol (200 mg/kg) + Enriched environment	8 weeks
7	Group VII	AlCl ₃ (50 mg/kg) + Resveratrol (200 mg/kg)	8 weeks
8	Group VIII	AlCl ₃ (50 mg/kg) + Enriched environment.	8 weeks
9	Group IX	AlCl ₃ (50 mg/kg) + Resveratrol (200 mg/kg) + Enriched environment	8 weeks

Neurobehavioral assessments

Assessment of contextual fear memory using passive avoidance test: The passive avoidance chamber (40 cm × 25 cm × 25 cm) as described by Zhu et al. (2001) and modified by Ambali et al. (2010), was used for the evaluation of emotional memory, based on contextual fear conditioning learning (Altman et al., 1987). The floor of the chamber consisted of parallel 2-mm-calibre stainless steel bars, copper rods spaced 1 cm apart, and a well-insulated 2.5 cm × 8 cm × 25 cm wooden platform placed on the extreme left of the chamber. An electric shock was delivered through the floor bars. Each mouse was placed in the chamber for a three-minute adaptation period and then placed on the platform. The latency to step-down on the grid with all four paws was measured. Upon stepping down on the copper bars, each mouse received an immediate mild electrical shock (40-volt foot shock). To avoid the shock, the mouse demonstrated an instinctive reaction to jump back onto the platform. Each mouse was tested in this manner for 5 minutes (Shuchang et al., 2009). The number of times the mouse stepped down from the platform within 5 minutes was considered acquisition errors. This procedure was repeated 24 hours later, and the step-down latency was used as a measurement of memory retention. The number of times the mouse stepped down from the platform within the 5-minute interval was recorded as retention errors.

Biochemical Assessments: All the mice were humanely sacrificed. The hippocampus was removed from each brain sample using surgical incision and rinsed with ice-cold isotonic saline. The hippocampus was then ground in a cold glass mortar and homogenised with ice-cold 100 mM phosphate buffer (pH 7.4; 1 g of tissue/9 mL). The homogenates (10 % w/v) were then centrifuged at 1000g for 20 min and the supernatants were used for the following biochemical analyses.

Quantification of mouse amyloid beta peptide 1-42 concentration:

The Mouse Amyloid Beta Peptide 1-42 Elisa assay kit (GA-E0181MS) was used to assess the concentration of Aβ1-42 in mouse hippocampus, based on the principle of biotin double antibody sandwich technology (Song et al., 2011). Exactly 40 µL of the sample, 10 µL of mouse Aβ1-42 antibody and 50 µL streptavidin-HRP were added to wells, pre-coated with Aβ1-42 monoclonal antibody. Mouse Aβ1-42 antibody (10 µL) was added to the blank well, with no sample added to it. The standard well contained 50 µL of the standard + 50 µL streptavidin-HRP (no biotin antibody added). The plate was covered with a seal and shaken gently to mix. It was incubated at 37°C for 60 minutes. Thereafter, the plate was removed and drained off liquid. Each well was filled with washing solution and allowed to stand for 3 seconds, after which it was blot off. The washing was repeated five times. Chromogen A (50 µL) and Chromogen B (50 µL) were added to the blank, standard and sample wells, respectively. The plate was shaken to mix the solution, and incubated at 37°C for 10 minutes away from light for colour development. The plate was removed after 10 minutes and 50 µL stop solution was added to each well to stop the reaction. Colour changes were observed from blue to yellow. The absorbance of each well was measured one by one under 450 nm, 10 minutes after adding stop solution. The optical density was measured and concentration of the samples was determined using MyAssays software.

Quantification of malondialdehyde concentration:

The Mouse malondialdehyde (MDA) Elisa assay kit (GA-E0638MS) was used to assess the concentration of MDA in mouse hippocampus on the basis of biotin double antibody sandwich technology (Janero et al., 1990), according to the manufacturer's protocol.

Quantification of nuclear factor E -related factor 2 (Nrf2) concentration:

The Mouse Nrf2 Elisa assay kit (GA-E3955MS) was used to assess the concentration of Nrf2 in mouse hippocampus on the basis of biotin double antibody sandwich technology (Taguchi et al., 2011), according to the manufacturer's protocol.

Statistical Analyses

The analysis of data was carried out using SPSS version 22 (NY: IBM Corp, 2013) and values obtained were expressed as Mean ± SEM. All analyses were done using one-way analysis of variance (ANOVA) for biochemical parameters and mixed analysis of variance for neurobehavioural evaluation, followed by Tukey's and Bonferroni *post-hoc* tests in order to evaluate the significance of the differences between the means, respectively. Values of *P* < 0.05 were considered significant.

RESULTS

Assessment of memory and learning deficits induced by AlCl₃ in mice

Acquisition error (learning)-induced deficits in mice:

Table 1 shows the effect of resveratrol and EE on AlCl₃-induced acquisition error deficits using passive avoidance test in mice. A significant (*p* < 0.05) difference was

observed in acquisition error across the three phases of the study: [$F(2, 42) = 6.47$; $p = 0.004$]. A significant increase in acquisition error was observed in AlCl_3 + EE treatment group (6.33 ± 1.42) in phase 2, when compared to the baseline mean score of the same treatment group (1.67 ± 0.64). There was no significant [$F(8, 21) = 2.99$; $p = 0.2$] difference between groups, and there was no interaction between groups and time in all the three phases of the study: [$F(16, 42) = 0.67$; $p = 0.80$].

Step down latency (memory-induced deficits in mice):

The effect of resveratrol and EE on AlCl_3 -induced latency (memory) deficits using passive avoidance test in mice showed a significant [$F(2, 42) = 39.65$; $p = 0.001$] difference across the three phases of the study (Table 2). A significant ($p < 0.05$) decrease in latency (s) was recorded across the three phases in: normal saline; phases 2 (13.00 ± 27.54) and 3 (27.25 ± 46.82), CMC; phases 2 (22.00 ± 31.80) and 3 (2.67 ± 54.06), EE; phases 2 (39.00 ± 31.80) and 3 (20.00 ± 54.06) treatment groups, when compared to the mean scores of their respective phase 1 (base-line). A significant decrease in latency (s) was also recorded in AlCl_3 ; phase 3 (13.33 ± 54.06), EE + resveratrol; phase 2 (6.75 ± 27.54); AlCl_3 + resveratrol; phases 2 (155.67 ± 31.80) and 3 (124.00 ± 54.06); AlCl_3 + EE; phase 2 (5.00 ± 31.80), and AlCl_3 + EE + resveratrol; phase 2 (67.75 ± 27.54) treatment groups, when compared to the mean scores of their respective phase 1 (base-line). However, significant decrease in latency (s)

was also observed in AlCl_3 -treatment group in phase 3 (13.33 ± 54.06), when compared to phase 2 (235.33 ± 31.80), EE + resveratrol in phase 2 (6.75 ± 27.54), when compared to phase 3 (172.25 ± 46.82); and AlCl_3 + EE in phase 2 (5.00 ± 31.80), compared to phase 3 (208.33 ± 54.06), respectively.

A significant difference was observed between groups: [$F(8, 21) = 3.7$; $p = 0.01$], in phases 1 and 2 of the study. The latency(s) in resveratrol group was lower (144.67 ± 42.22), when compared to that of AlCl_3 + EE + resveratrol (251.25 ± 36.57) group at base-line (phase 1). In phase 2 of the study, a significant decrease in latency (s) was recorded in the control (13.00 ± 27.54), CMC (22.00 ± 31.80) EE (39.00 ± 31.80), EE + Resveratrol (6.75 ± 27.54), AlCl_3 + EE (5.00 ± 31.80) and AlCl_3 + EE + resveratrol (67.75 ± 27.54) treatment groups, compared to resveratrol (217.00 ± 31.80) or AlCl_3 (235.33 ± 31.80) groups, respectively.

A significant interaction between groups and time was observed in latency (s) in the study: [$F(16, 42) = 3.29$; $p = 0.001$]. Using Bonferroni *post-hoc* test, a significant increase ($p < 0.001$) was obtained in latency (s) of AlCl_3 (235.33 ± 31.80) group in phase 2, when compared to normal saline (13.00 ± 27.54), CMC (22.00 ± 31.80), EE (39.00 ± 31.80), EE + Resveratrol (6.75 ± 27.54), AlCl_3 + EE (5.00 ± 31.80) and AlCl_3 + EE + resveratrol (67.75 ± 27.54) treatment groups, respectively. The latency (s) of AlCl_3 decreased over time in phase 3 (13.33 ± 54.06).

Table 1:

Effect of resveratrol and environmental enrichment on acquisition error in aluminium chloride-induced cognitive deficits using passive avoidance test

Groups	Phase 1 (Base-line)	Phase 2 (Four weeks)	Phase 3 (Eight weeks)
Normal Saline	2.75 ± 0.55	5.00 ± 1.23	3.25 ± 0.85
CMC	1.67 ± 0.64	3.33 ± 1.42	3.33 ± 0.98
Resveratrol	2.33 ± 0.64	4.33 ± 1.42	3.67 ± 0.98
EE	3.67 ± 0.64	5.33 ± 1.42	2.33 ± 0.98
AlCl_3	1.67 ± 0.64	2.00 ± 1.42	1.33 ± 0.98
EE + Resveratrol	2.00 ± 0.55	4.75 ± 1.23	2.50 ± 0.85
AlCl_3 + Resveratrol	1.33 ± 0.64	2.00 ± 1.42	2.00 ± 0.98
AlCl_3 + EE	1.67 ± 0.64	$6.33 \pm 1.42^*$	2.33 ± 0.98
AlCl_3 + EE + Resveratrol	1.75 ± 0.55	2.00 ± 1.23	2.50 ± 0.85

* Indicate significance ($p < 0.05$) when compared to phase 1 (base-line). CMC = Carboxymethyl cellulose, EE = Environmental enrichment, AlCl_3 = Aluminium chloride, $n = 7$

Table 2:

Effect of resveratrol and environmental enrichment on aluminium chloride-memory deficits using passive avoidance test in mice

Groups	Step-down latency (s) Phase 1 (Base-line)	Step-down latency (s) Phase 2 (Four weeks)	Step-down latency (s) Phase 3 (Eight weeks)
Normal Saline	247.75 ± 36.57	$13.00 \pm 27.54^{*,b,c}$	$27.25 \pm 46.82^*$
CMC	180.00 ± 42.22	$22.00 \pm 31.80^{*,b,c}$	$2.67 \pm 54.06^*$
Resveratrol	144.67 ± 42.22^a	217.00 ± 31.80	88.33 ± 54.06
EE	266.67 ± 42.22	$39.00 \pm 31.80^{*,b,c}$	$20.00 \pm 54.06^*$
AlCl_3	297.67 ± 42.22	235.33 ± 31.80	$13.33 \pm 54.06^{*#}$
EE + Resveratrol	261.50 ± 36.57	$6.75 \pm 27.54^{*,b,c}$	172.25 ± 46.82
AlCl_3 + Resveratrol	300.00 ± 42.22	$155.67 \pm 31.80^*$	$124.00 \pm 54.06^*$
AlCl_3 + EE	284.33 ± 42.22	$5.00 \pm 31.80^{*,b,c}$	208.33 ± 54.06
AlCl_3 + EE + Resveratrol	251.25 ± 36.57	$67.75 \pm 27.54^{*,b,c}$	191.75 ± 46.82

* Indicates significant ($p < 0.05$) difference when compared to phase 1 (base-line), # Indicates significant ($p < 0.05$) difference compared to phase 2, + Indicates significant ($p < 0.05$) difference compared to phase 3, ^{a, b and c} Indicates significant ($p < 0.05$) difference compared to AlCl_3 + EE + resveratrol, Resveratrol and AlCl_3 treatment groups, respectively. CMC = Carboxymethyl cellulose, EE = Environmental enrichment, AlCl_3 = Aluminium chloride, s = seconds, $n = 7$

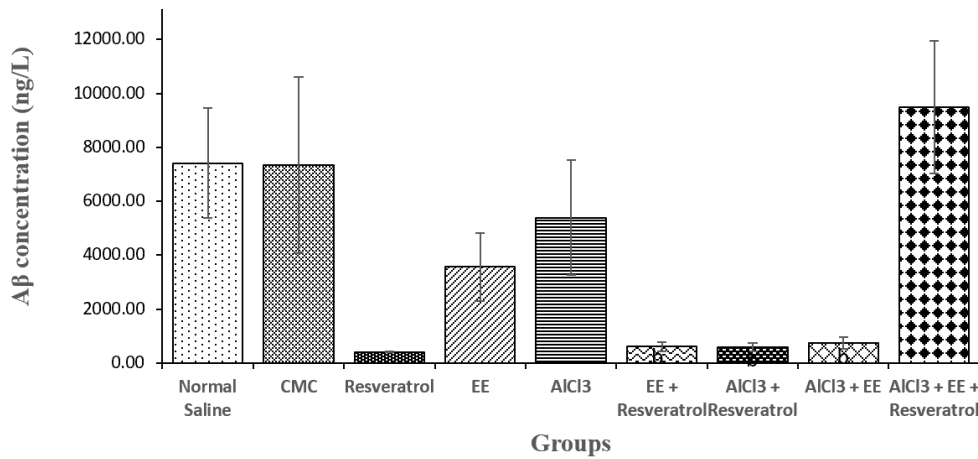


Figure 1: Effect of resveratrol and environmental enrichment on amyloid-beta concentration in aluminium chloride model of Alzheimer's disease.

^b Indicate significant ($p < 0.05$) difference, when compared to AlCl₃ + EE + Resveratrol. CMC = Carboxymethyl cellulose, EE = Environmental enrichment, AlCl₃ = Aluminium chloride, $n = 4$

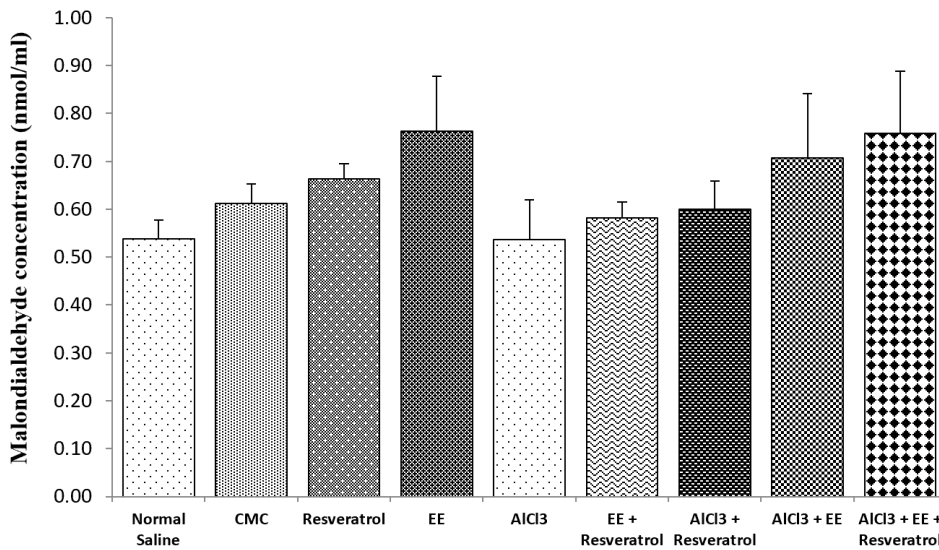


Figure 2: Effect of resveratrol and environmental enrichment on malondialdehyde concentration in aluminium chloride model of Alzheimer's disease in mice

AD = Alzheimer's disease, CMC = Carboxymethyl cellulose, EE = Environmental enrichment, AlCl₃ = Aluminium chloride, MDA = Malondialdehyde, $n = 4$

Determination of Amyloid beta Peptide (Aβ)

Concentration: Results of Aβ peptide concentration (ng/L) obtained during the study are represented in Figure 1. There was no significant difference in concentration (ng/L) of Aβ protein across all the treatment groups, when compared to the control (normal saline). However, a significant ($p < 0.05$) decrease was observed in concentration (ng/L) of Aβ protein in resveratrol (412.35 ± 25.84), EE + resveratrol (617.35 ± 171.38), AlCl₃ + resveratrol (590.30 ± 150.75) and AlCl₃ + EE (741.76 ± 219.13) groups, compared to AlCl₃ + EE + Resveratrol (9495.00 ± 2462.40) groups, respectively, [$F(8, 21) = 4.71$; $p = 0.02$].

Assessment of oxidative stress biomarkers in aluminium chloride model of Alzheimer's disease

Determination of malondialdehyde (MDA)

concentration: Results of MDA concentration (nmol/ml) obtained during the study are represented in Figure 2. There was no significant difference in MDA concentration (nmol/ml) in all the treatment groups, when compared to the controls, [$F(8, 24) = 0.86$; $p = 0.56$].

Determination of nuclear factor erythroid 2-related factor 2 (Nrf2) concentration:

Results of Nrf2

concentration (ng/ml) obtained during the study are represented in Figure 3. There was a significant [$F(8, 23) = 4.59$; $p = 0.002$] difference in concentration (ng/ml) of Nrf2 in Resveratrol (5.62 ± 0.31), AlCl₃ + Resveratrol (5.92 ± 0.53) and AlCl₃ + EE (6.96 ± 0.31) groups, compared to the controls (13.24 ± 0.72). The concentrations (ng/ml) of Nrf2 in Resveratrol (5.62 ± 0.31) and AlCl₃ + Resveratrol (5.92 ± 0.53) groups were lower ($P < 0.05$), when compared to that of AlCl₃ + EE + Resveratrol (12.61 ± 0.78).

DISCUSSION

The result of the study demonstrated that resveratrol and environmental enrichment modulate memory and neurochemical changes in AlCl₃ model of AD in mice.

The results of the current study agreed with the findings that environmental stimuli such as physical exercise, pollutants, life-style, chemicals, pesticides, nutrition, physical stress, behavioural stress and exposure to metals such as aluminium affect the normal inherited methylome throughout the life-span of an organism, leading to either healthy or diseased ageing (Nicolia et al., 2015). The negative impacts of these stressors such as Al toxicity have been shown to lead to diseased ageing by altering cellular and molecular mechanisms through gene activation and silencing.

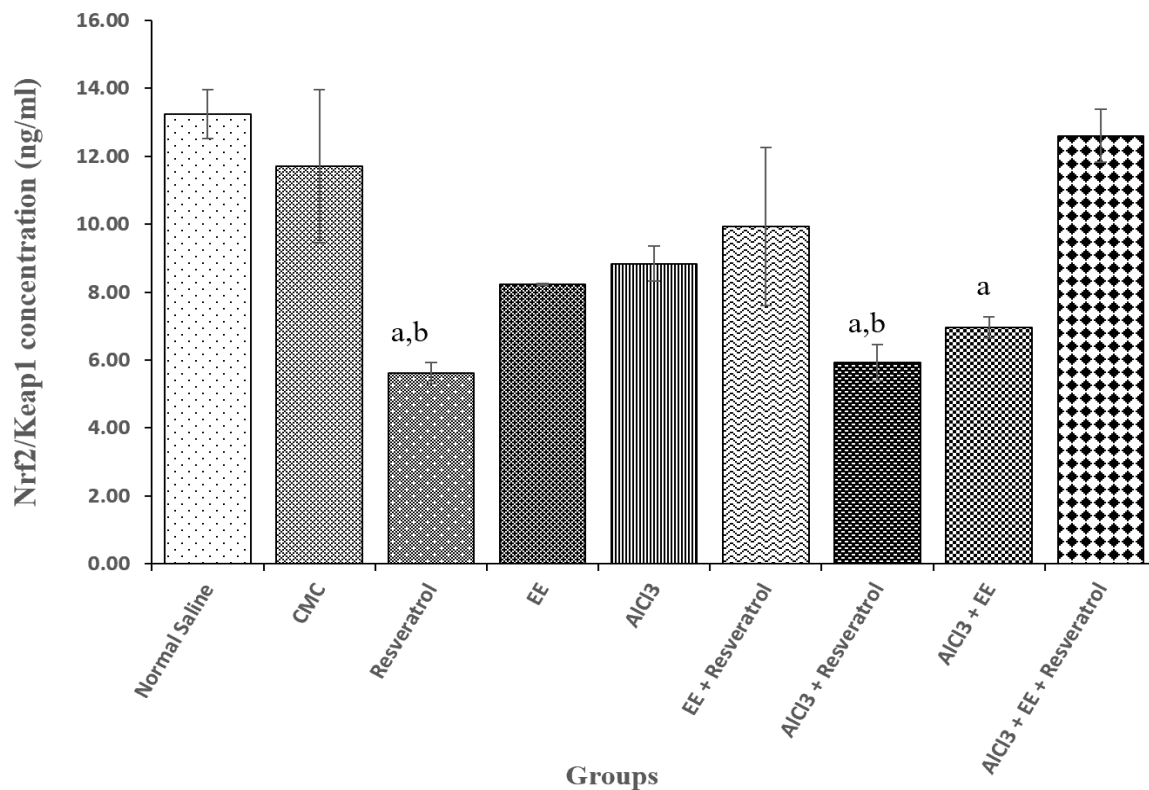


Figure 3:

Effect of resveratrol and environmental enrichment on nuclear factor erythroid 2-related factor 2 concentration aluminium chloride model of AD in mice

a and b = significant ($p < 0.05$) difference, when compared to the control and $\text{AlCl}_3 + \text{EE} + \text{Resveratrol}$, respectively. AD = Alzheimer's disease, CMC = Carboxymethyl cellulose, EE = Environmental enrichment, AlCl_3 = Aluminium chloride, Nrf2/keap-1 = Nuclear factor erythroid 2-related factor 2/keap-1, $n = 4$

The impacts eventually lead to the manifestation of various aged-related disorders such as Alzheimer's disease. Environmental enrichment and dietary supplement of resveratrol have been shown to act as epigenetic factors that regulate gene activation and silencing (Kawano et al., 2015; Nicolai et al., 2015). Experimental models have unveiled numerous pathways; thus, providing novel insights into the mechanisms of their epigenetic control, especially in pathological conditions. Therefore, modulation of neurobehavioral and cellular responses obtained in the present study by resveratrol and EE may be of prophylactic and therapeutic potentials in the management of AD.

The significant increase observed in acquisition error in $\text{AlCl}_3 + \text{EE}$ group in the fourth week of the study, when compared to the mean base-line score demonstrated the effect of time on learning process. The significant decrease in latency (memory) of the animals to stay on the safe platform over time across the various groups using the passive avoidance test indicated the effect of time and ageing on cognitive functions. However, in EE + resveratrol and $\text{AlCl}_3 + \text{EE}$ treatment groups, the significant decrease in latency observed in at the fourth week, compared to the base-line was followed by significant increase in latency at the eighth week of the study. This finding may be attributed to improvement in cognitive function, elicited by both resveratrol and EE. This finding was further corroborated by significant decrease in $\text{A}\beta$ concentration in the same treatment groups. Both treatments have been reported to improve cognitive functions in ageing and animal models of AD by reducing oxidative stress, neuroinflammation, and CREB pathway; thereby decreasing the levels of $\text{A}\beta$ -

precursor protein and promoting the synthesis of brain derived neurotrophic factor, consequently leading to improved memory and learning (Kawano et al., 2015; Yazir et al., 2015; Grinan-Ferre et al., 2015; Zhang et al., 2016).

The significant decrease in latency of the animals to stay on the safe platform observed in the resveratrol group, when compared to $\text{AlCl}_3 + \text{EE} + \text{resveratrol}$ group at base-line, could be as a result of behavioural variation due to anxiety of animals exposed to novel environment. Although the animals were well acclimatised to the passive avoidance test apparatus prior to the commencement of the experiment, anxiety may play a role in the decline in latency observed in the resveratrol group, compared to $\text{AlCl}_3 + \text{EE} + \text{resveratrol}$ treatment group at base-line, as animals responses to novelty differ. After four weeks of the study (phase 2), a significant decrease in latency of the animals to stay on the platform was observed in the controls, CMC, EE, EE + Resveratrol, $\text{AlCl}_3 + \text{EE}$ and $\text{AlCl}_3 + \text{EE} + \text{resveratrol}$ groups, compared to resveratrol and AlCl_3 groups, respectively. The significant increase in latency observed in the resveratrol group, compared to the various treatment groups may be due to its ability to improve cognitive functions, which was further corroborated by significant decrease in $\text{A}\beta$ concentration in the same treatment group. Resveratrol has been reported to improve memory and learning in humans and animal models of AD by activating sirt-1-dependent mechanism, increasing hippocampal production of insulin-like growth factor-1 via sensory neurone stimulation, reducing cellular level of iNOS and lipid peroxidation by increasing haeme oxygenase-1 (HO-1) production and reducing the expressions of miR-134 and miR-124, thus in

turn up-regulating CREB levels, thereby promoting BDNF synthesis and decreasing A β concentration (Harada et al., 2011; Huang et al., 2011; Zhao et al., 2013; Yazir et al., 2015). These are possible mechanisms via which resveratrol improves memory and learning as observed in the present study.

The significant increase in latency of the animals to stay on the platform in the AlCl₃ group, observed in the fourth week of the study, decreased over time after the eighth week of the study period (phase 3). This demonstrated a cognitive decline induced by AlCl₃ at the eighth week and further shows that, the increased latency observed over time was transient in the AlCl₃ treatment group at the fourth week, indicating a significant interaction between group and time in the present study.

Results of A β peptide concentration obtained during the study showed no significant difference in concentration of A β protein across all the groups, when compared to the control (normal saline) group. However, a significant decrease in concentration of A β protein was observed in resveratrol, EE + resveratrol, AlCl₃ + resveratrol and AlCl₃ + EE treatment groups, compared to AlCl₃ + EE + Resveratrol treatment group. The increase in concentration of A β protein observed in AlCl₃ + EE + Resveratrol implies that the combined treatment of resveratrol and EE with AlCl₃ did not decrease A β protein concentration, rather it increased the value far beyond the value obtained in the AlCl₃ treatment group.

Available data suggest that both caloric restriction and EE exert similar beneficial effects on neurones in the brain by inducing a mild stress, thereby sending signals that increase the production of cell survival-promoting molecules, including growth factors (insulin like growth factors, brain derived neurotrophic factors, and nerve derived neurotrophic factor), neurotransmitters (glutamate, serotonin), protein chaperones (heat-shock proteins 70 and glucose-regulated protein 78), antioxidants (superoxide dismutase, reduced glutathione), increased calcium influx and gene expression and enhanced energy metabolism (electron-transport chain and glycolysis). These factors consequently lead to enhanced cognitive function, disease resistance, increased insulin sensitivity and improved lipid metabolism (Mattson et al., 2001; Bordone and Guarente, 2005; Mattson et al., 2005; Sinclair, 2005). Resveratrol acting as an established CR mimetic, could act via similar mechanism to elicit its beneficial role in AD (Markus and Morris, 2008). However, the combination of resveratrol and EE in AlCl₃ model of AD may reverse the beneficial role of the individual treatment by exaggerating the beneficial mild stress response, elicited by the individual treatments in the AD model. This may lead to oxidative stress that consequently increased A β protein production as observed in the AlCl₃ + EE + Resveratrol group. Decreasing the dose of resveratrol from below 200 mg/kg/body weight may result in better response in the combined treatment.

The results of oxidative stress biomarkers obtained from the study showed that there was no significant difference in MDA concentrations in all the groups, when compared to the controls. However, the Nrf2 concentrations obtained during the study showed significant decrease in the concentration in Resveratrol, AlCl₃ + Resveratrol and AlCl₃ + EE groups, compared to the controls. This finding showed that both resveratrol and EE did not to up-regulate

the concentration of Nrf2 independently and in combination with AlCl₃ above those of the controls, and AlCl₃ group. This is in disagreement with the findings of Tamaki et al. (2014) and that of Yang et al. (2015), who reported an increase in Nrf2 protein level of rats exposed to chronic cerebral hypo-perfusion and periodontitis by EE and resveratrol, respectively, thereby improving the antioxidant status of the animals. Both resveratrol and EE may probably exert their beneficial effect via alternate pathway in the present study, improving oxidative status by either increasing the synthesis of high molecular weight endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase enzymes or constitutive nitric oxide which has unpaired electron in its outermost shell thereby making it more attractive to ROS and sparing Nrf2 for other scavenging roles (Das and Maulik, 2006).

The increase in Nrf2 concentration observed in AlCl₃ + EE + Resveratrol compared to Resveratrol and AlCl₃ + Resveratrol could be adaptive in order to contain the oxidative stress-induced neuropathology. The increase, apparently, resulted from increased MDA concentration observed in the same group, with a trend towards significance, which consequently led to increase in A β concentration. This result demonstrated the synergistic role of resveratrol and EE in up-regulating Nrf2 concentration to convert the increased oxidative stress level and A β protein concentration, thereby suggesting the antioxidant role of the combined treatments in AlCl₃ model of AD. This cascade could be via activation of SIRT1-dependent mechanism as both resveratrol and treadmill exercise, which is an important component of EE, were reported to increase the level of Nrf2 via SIRT1-dependent mechanism (Tamaki et al., 2014; Koo et al., 2017).

In conclusion and based on the results of this study, it was concluded that time significantly influenced memory in the various groups at the fourth week of the study. Resveratrol administration improved contextual fear memory. Resveratrol and EE treatments alone significantly lowered A β concentration over the combined treatment in AlCl₃ model of AD. Combined treatment of resveratrol and EE enhanced the antioxidant status by upregulating Nrf2 concentration in AlCl₃ model of AD.

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