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Medicinal Plants Reduce Neurodegeneration and Improve Memory in Induced Alzheimer's Disease in Rat Model

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Abstract

Alzheimer's disease (AD) is a devastating and debilitating neurological brain disorder that has a multifactorial nature associated with complex pathophysiology. Thus, concerns directed to develop alternative therapies which possess multifaceted action for treatment of AD. This article was aimed to compare efficacy of *Moringa olifera* (MO), *Ginkgo biloba* (GB) and green tea (GT) extracts in managing induced Alzheimer's disease in albino rat using behavioural, biochemical, and pathological alterations. Eighty five male Wistar rats weighing 80-120g were randomly divided into five groups (17 rats for each). Control (administered with distilled water), Alzheimer disease model (ADM, administered with AlCl3), ADM + MO (administered with AlCl3 and ethanolic extract of MO), ADM + GB (administered with AlCl3 and ethanolic extract of GB), and ADM + GT (administered with AlCl3 and ethanolic extract of and ethanolic extract of MO), ADM + GB (administered with AlCl3 and ethanolic extract of GB), and ADM + GT (administered with AlCl3 and ethanolic extract of GT). All treatments were administered daily by oral gavage and persisted for seventy consecutive days. On the 60th day of the experiment, all memory tests were performed. Then the rats were humanely sacrificed using diethyl ether anesthesia, and brain samples were collected. Treatments with MO, GB, or GT successfully rescue Neuro-therapeutic abilities against AD. In addition, the used treatments restore the rats' memory and cognitive performances in the Y-maze, novel objective recognition and Morris Water maze tests. In Conclusion, MO, GB, or GT may provide a more effective strategy to lessen neurodegeneration in AD.

KEYWORDS
Alzheimer's disease, *Moringa olifera*, *Ginkgo biloba*, Green Tea, Memory Tests

INTRODUCTION

Alzheimer's disease (AD) is a chronic progressive neurodegenerative brain disorder which is linked with learning disabilities, memory impairments and it is the most concerted reason of progressive dementia in aging (Kumar and Walter, 2011; Kim and Oh, 2012). It is recognized by precipitation of amyloid plaques (β-amyloid peptide (Aβ)), and neurofibrillary tangles (NFTs) formation (Kumar and Walter, 2011). AD is a devastating and debilitating neurological brain disorder marked by enormous neuronal cell death, synaptic damage, deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles. Hence, it dramatically influences the neurological and economic soundness of our society (Selkoe, 2001; Penzes and VanLeeuwen, 2011). There is growing number of AD patients in the USA (Alzheimer's Association, 2021), 5.8 million in 2020 and will be 13.8 million by the mid-twenty-first century (Zhang *et al*., 2021). The bioavailability of aluminum absorption from the gastrointestinal tract depends on a wide variety of factors such as pH of the stomach and intestine, solubility of administering an aluminum form and some digestive, systemic and renal diseases (Długaszek *et al*., 2000).

The multifactorial nature of AD is associated with complex pathophysiology, hence concerns directed to develop new therapeutics for treatment of AD. However, recent trends focused on alternative medicine because herbs were found to possess multifaceted action, including antioxidant, anti-inflammatory, anti-apoptotic and anti-amyloid properties which can recommend their use for treatment of various neurodegenerative diseases such as AD (Kumar *et al*., 2017).

Among these herbs, *Moringa olifera* (MO), *Ginkgo biloba* (GB) and green tea (GT) were used for managing and treating AD (Rezai-Zadeh *et al*., 2008; Kim and Oh, 2012; Patave and Une, 2016). MO extract possesses a nootropic and antioxidant activity because it is rich in vitamin C and E, hence it combats oxidative stress and improve memory by using a higher concentration to treat AD (Sutalangka *et al*., 2013; Roy, 2014).GB leaves possess antioxidant activity and promote a neuroprotective effective against toxic beta-amyloid protein (Kim and Oh, 2012; Rasool *et al*., 2014). GT counteracts beta-amyloid impaired memory, including that in animals subjected to aluminum-induced neurotoxicity, and prevents or ameliorates oxidative damage in the brain, including that developed in AD (Rezai-Zadeh *et al*., 2008).

Based on our knowledge, there are different studies that examine the role of Ginseng (GS) and MO in the treatment of AD (Rezai-Zadeh *et al*., 2008; Kim and Oh, 2012; Patave and Une, 2016). However, no report compares their efficacy. Thus, we

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aimed to compare efficacy of MO, GB and GT extracts in managing induced Alzheimer's disease in albino rat using behavioural, biochemical and pathological alterations.

MATERIALS AND METHODS

Chemicals

The Aluminum chloride anhydrous (AlCl3) purchased from the Alpha Athero Company. Ethanolic *Moringa olifera* (MO) extract was prepared from dried leaves of plant, which was dried and ground to obtain a fine powder. The powder was extracted with dehydrated alcohol at room temperature for 24hrs, then the extract was filtered through Whatman filter paper and vacuum dried to obtain solid mass, which was dissolved in distilled water for final use (Ganguly and Guha, 2008).

Ethanolic *Ginkgo biloba* (GB) extract was obtained from the leaves of the GB and contains 24% flavonol glycosides, 6% terpene location and limited amounts of other substances, including proanthocyanidins and organic acid according to Ding (1999); Yamamoto *et al*. (2007). Ethanolic Green tea (GT) extract was prepared from ground tea leaves (100 g) for 72 hours with 250 ml of 99.9% ethanol at ambient conditions. Collected extracts were filtered, ethanol was evaporated. The powdered ethanol extract was kept frozen (–18°C) until further use according to Gramza *et al*. (2005); Sofy *et al*. (2014).

Experimental Animals

A total of 85 male Wistar rats weighing 40-60 grams (40 days old) were acquired from AL-Nahda University's laboratory house and then took 10 days of acclimatization. The animal home was kept at a temperature of 19-25°C with a relative humidity of 40-56 percent, and the lighting system was run on a 12-hour light/dark cycle with natural and artificial illumination. Ad-libitum feeding was available twice a day with a commercially balanced diet and a constant supply of clean fresh water. The study followed the ethical guidelines of Beni-Suef University's Institutional Animal Care and Use Committee (BSU-IACUC). The committee gave their approval to the study (018-50B).

Experimental Design

After acclimatization, rats weighing 80-120g were randomly divided into five groups (17 rats in each group). Control (administered with distilled water), Alzheimer disease model (ADM, administered with AlCl3), ADM + MO (administered with AlCl3 and ethanolic extract of MO), ADM + GB (administered with AlCl3 and ethanolic extract of GB), and ADM + GT (administered with AlCl3 and ethanolic extract of GT). All treatments were administered daily by oral gavage and persisted for seventy consecutive days.

Aluminium chloride (AlCl3) was freshly made weekly in the Alzheimer disease model group (ADM) by dissolving the powder in distilled water and keeping it in a dark bottle for oral gavage. Rats were subjected daily to oral gavage of (AlCl3) at a dose of 100 mg/kg b. wt for 70 consecutive days (Prakash and Kumar, 2013; Lakshmi *et al*., 2015). Rats in AlCl3 + MO group were subjected daily to oral gavage of the MO extract at a dose of 200 mg/kg b. wt for 70 consecutive days (Ganguly and Guha, 2008; Sutalangka *et al*., 2013). Rats in AlCl3 + GB group were subjected daily to oral gavage of the GB extract at a dose of 200 mg/kg b. wt for 70 consecutive days (Gong *et al*., 2005; Chen *et al*., 2016). Rats in AlCl3 + GT group were subjected daily to oral gavage of the GT extract at a dose of 200 mg/kg b. wt for 70 consecutive days (Sofy *et al*., 2014; Sumathi *et al*., 2015).

Memory Tests

On the 60th day of the experiment, all memory tests were performed.

Y-maze test

Working memory in rodents was assessed using the Y-maze test. The test apparatus, procedures, and the calculation equation were all carried out in accordance with the protocol outlined by Rasoolijazi *et al*. (2007) and Baluchnejadmojarad *et al*. (2012).

The apparatus was made up of three dark-colored wood arms, each measuring 40 cm long, 30 cm high, and 15 cm wide. The test days were documented with a digital camera. Each rat was placed at the end of one arm and permitted to walk around freely for eight minutes, with the maze being cleaned with 70 percent alcohol after each rat. In overlapping triplet sets, the sequence of arm entries was computed (i.e ABCCBAABC). The percentages of spontaneous alternation behaviour (SAP), alternate arm returns (AAR), and same arm returns (SAR) were calculated.

Novel object recognition (NOR)

This test was used to assess short term memory depending on animal preference to interact with novel object more than familiar object. Test apparatus, procedures and calculation equation performed according to the protocol described by Leger *et al*. (2013) and Lim *et al*. (2014). The apparatus consisted of a square arena 40x40 cm and with a height of 60 cm. A digital camera was used for recording the test days. That test consisted of two phases, familiarization phase where each rat placed for 10 minutes to identify two familiar objects which were placed in the center of the arena. The second test phase was repeated after 24hr where each rat exposed to one familiar object and other novel object for 5 minutes. The apparatus and objects wiped with 70% alcohol after removal of rat before placing the second one. Exploratory behaviour was scored when the rat directing its nose toward the object or touching it at 2 cm or less, however sitting on an object is not considered an exploratory behaviour.

Morris water maze (MWM)

The Morris water maze (MWM) is a test that evaluates spatial reference memory in rodents by employing distal cues to locate the platform. MWM is a black-painted swimming circular pool with a platform 10-12cm in diameter submerged beneath water by 2cm and a diameter of 150cm and a height of 50cm. The water temperature in the maze is regulated between 21 and 25 degrees Celsius (Qi *et al*., 2009; Chen *et al*., 2016). Test methods were carried out according to Vorhees and Williams' (2006) protocol. The test days were documented with a digital camera. The test was comprised of an acquisition phase or training phase in which rats were placed in a starting position facing the maze daily and given 60 seconds to find the platform and stand on it for 15-30 seconds before being directed if they couldn't find it. The platform was submerged in water by 2cm for the first two days of training, and then milk powder was used to make the water opaque for the remaining two days of training. Each rat was given three training trials starting from three different starting points (from three quadrants), with the platform in the fourth quadrant (target quadrant, Q4). The platform is withdrawn on the final test day, 24 hours after the last training session (probe trial), and each

rat is permitted to search for the platform for 60 seconds, with time spent in the target quadrant calculated.

Measurements of biochemical parameters

After the end of the behavioural tests, rats were humanely euthanized via intraperitoneal injection of ketamine (90 mg/kg) and xylazine (5mg/kg) combination, brain samples were washed in saline and stored in a deep freezer at -80°C. Then, at the National Organization for Drug Control and Research in Giza, Egypt, a brain homogenate was prepared, and following measurements were taken as follows:

Determination of acetylcholinesterase activity

Acetylcholinesterase activity (AchE) was measured according to method of Ellman *et al.* (1961) and modified method by Gorun *et al.* (1978).

Estimation of oxidative stress and antioxidant

The level of malondialdehyde (MDA) was determined using the technique of Uchiyama and Mihara (1978). The level of reduced glutathione (GSH) was evaluated using the Van Doorn *et al.* (1978) Method, and catalase (CAT) activity was determined using the method of Aebi (1984). The technique of Lowry *et al.* (1951) was used to determine total protein level.

Determination of the monoamine Levels

The fluorometric approach established by Ciarlone (1978) was used to estimate the levels of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in the entire brain of rats.

Examination of brain pathology

Study of hippocampus histology

Rats were anesthetized with anesthetic ether and then underwent a cardiac perfusion procedure in which 4 percent paraformaldehyde in 0.1 M phosphate buffer was injected into the left ventricle. The brains were extracted and immersed in 4% paraformaldehyde for 48 hours. Dehydration, clearing, embedding, and microtomy of sagittal sections of brains were among the routine histological methods used. A rotary microtome was used to make Microtomy of 5 µm sagittal sections, which were then stained with hematoxylin and eosin (Gage *et al.*, 2012; Barkur and Bairy, 2016). The carcasses of the rats were disposed in a sanitary manner at Beni- Suef University's Faculty of Veterinary Medicine's incineration unit.

Morphometric analysis

A light microscope was used to perform morphometric analysis (Olympus Pvt. Ltd., Germany). For the calculations, six sections from each rat were used. In the dentate gyrus, the number of degenerated neurons was quantified (Barkur and Bairy, 2016).

Statistical Analysis

The data obtained from the biochemical analysis of different groups are presented as Mean ± Standard Deviation (Mean ± SD). The significance of the difference between the groups was analyzed by one way analysis of variance (ANOVA) followed by Duncan test for multiple comparisons at $P<0.05$, using the SPSS-PC computer software package version 20. The percentage of change representing the percent of variation in concentration with respect to control or AlCl3-treated values were also calculated:

% change = treated value – control value (or AlCl3-treated values) / control value (or AlCl3-treated values) X 100

RESULTS

Based on statistical analysis, significant (P= 0.03) decrease in SAP (- 15.6%) in the ADM group in comparison with the control group, and increased AAR in ADM group (30.1%). Treatment with MO, GB, and GT extracts improved memory impairment induced by AlCl3 by significantly (P= 0.00, 0.01, 0.01, respectively) increasing SAP (32.7%, 22.5%, 19.1%, respectively) compared to ADM group respectively. Moreover, treatment with MO and GT significantly (P= 0.008, 0.01, respectively) decreased AAR (- 36.7%, - 32.9%, respectively) in relation to ADM group as shown in (Table 1).

Table 2 proved that recognition memory was impaired in the ADM group that noticed by a significant (P= 0.000) reduction in DS, DI and DR (-208.5%, -54.3%, -47.3%, respectively) in comparison with the control group. Treatment with MO, GB and GT extracts improved the impaired short term memory induced by AlCl3 by a significant (P= 0.000) increase in DS (175.6%, 210.1%, 249.5%, respectively), DI (118.8%, 137.5%, 131.3%, respectively) and DR (70.8%, 90.5%, 75.8%, respectively) in relation to ADM group. However, treatment with GT extract significantly (P= 0.02) increased the DS compared to MO group.

Figure 1 demonstrated that ADM rats showed a significant (P= 0.0001, 0.008, 0.001) decrease in the retention latency TQ4 (less time spent in Q4) (12.75 sec) in comparison with control, MO, GB and GT groups (22.92, 18.25, 20.00, 21.92 sec) respectively. However, TQ4 decreased significantly (P= 0.02) in the MO

Table 1. Effect of the investigated medicinal plants on impaired spontaneous alternation behaviour in Y-maze induced by AlCl3.

Groups \ Parameters	Behavioural scoring in Y-maze test					
	SAP		SAR		AAR	
	Mean± SD	% Change	Mean± SD	% Change	Mean± SD	% Change
Control	64.31±2.86^{a}		0.44±0.14^{a}		26.95±3.89^{ab}	
ADM ($AlCl_3$ orally)	54.25±3.70^{b}	-15.6	0.67±0.19^{a}	52.3	35.06±3.01^{a}	30.1
ADM + MO	71.98±3.65^{a}	11.9 (32.7)	0.42±0.19^{a}	- 4.5 (- 37.3)	22.19±2.54^{b}	- 17.7 (- 36.7)
ADM + GB	66.48±3.48^{a}	3.4 (22.5)	0.58±0.19^{a}	31.8 (- 13.4)	27.00±3.59^{ab}	0.2 (- 23)
ADM + GT	66.56±2.98^{a}	3.5 (19.1)	0.83±0.24^{a}	88.6 (23.9)	23.53±3.33^{b}	- 12.7 (- 32.9)

Results are expressed as means ± standard deviation (Each value represents a mean of 12 values). $^{a\&b}$ Superscripts in the same column, values with different letters are significant at $P< 0.05$. % changes represent a comparison between treated and control groups. (%) changes represent a comparison between treated and AlCl3-groups.

SAP: Spontaneous Alternation Percent; SAR: Same Arm Return; AAR: Alternative Arm Return; ADM: Alzheimer Disease Model.

group (18.25 sec) in relation to control group (22.92 sec).

The results illustrated in Table 3 showed that malondialdehyde level (MDA) increased significantly (P= 0.000) in ADM group (111.2%) in comparison with the control group. Treatment with MO, GB and GT significantly (P= 0.001, 0.000, 0.005, respectively) decreased the elevated MDA level (- 33.5%, - 41.6%, - 27.3%, respectively) in relation to ADM group. However, MDA increased significantly (P= 0.04, 0.009, respectively) in MO and GT groups (40.5%, 53.4%, respectively) compared to control group.

In additions, significant difference could be detected between groups in the glutathione level, where GSH level decreased significantly in ADM, MO and GT groups (P= 0.000, 0.001, 0.002, respectively) compared to control group (- 42.5%, - 33.1%, - 27.2%, respectively). Where, GSH level increased significantly (P= 0.002) in GB group (46.5%) compared to ADM group. In additions, the catalase activity decreased significantly (P= 0.000) in the ADM group (- 50%) in relation to control group. Treatment with MO, GB and GT significantly (P= 0.000) increased catalase activity (128.6%, 100%, 85.7%, respectively) when compared to ADM group.

Table 4 demonstrated that dopamine level decreased significantly (P= 0.03) in the ADM group (-30.5%) compared to control group. Treatment with GT significantly (P= 0.01) increased the dopamine level (50.9%) in relation to ADM group, also treatment with MO and GB increased the dopamine level (14%, 29.8%, respectively) but the difference was not significant in relation to ADM group. Moreover, norepinephrine level increased significantly (P= 0.01) in MO, GB and GT groups (69.2%, 68.4%, 64.7%, respectively) compared to ADM group.

Serotonin level as decreased significantly (P= 0.03) in the ADM group (-23.7%) in comparison with the control group. Treatment with GT significantly (P= 0.006) increased serotonin level (43.1%) compared to ADM group, also treatment with MO and GB increased the serotonin level (17.2%, 13.8%, respectively) but the difference was not significant compared to ADM group.

In additions, acetylcholinesterase activity (AchE) was decreased significantly (P= 0.001) in ADM rats (-24.7%) in comparison with the control group. Treatment with MO, GB, and GT extracts ameliorated the reduction in AchE activity. The enzyme activity was significantly (P= 0.005, 0.02, 0.000, respectively) increased in MO, GB and GT groups (26.3%, 19.9%, 39.8%, respectively) when compared to ADM group. However, GT extract significantly (P= 0.002) increased the enzyme activity when compared to GB group.

Figure (2) showed presence of characteristic areas of hippocampus particularly in the dentate gyrus. Sections from the con-

Table 2. Effect of the investigated medicinal plants on impaired recognition in novel object recognition test induced by Alcl3.

Groups \ Parameters	Behavioural scoring in novel object recognition test					
	DS		DI		DR	
	Mean ± SD	%Change	Mean ± SD	%Change	Mean ± SD	%Change
Control	9.14±1.99^{ad}		0.70±0.04^{a}		69.13±3.47^{a}	
ADM ($AlCl_3$ orally)	-9.92±3.37^{c}	-208.5	0.32±0.03^{b}	-54.3	36.45±3.24^{b}	-47.3
ADM + MO	7.50±1.20^{bd}	- 17.9 (175.6)	0.70±0.02^{a}	0.0 (118.8)	62.25±3.70^{a}	- 10.0 (70.8)
ADM + GB	10.92±1.55^{ad}	19.5 (210.1)	0.76±0.04^{a}	7.9 (137.5)	69.44±4.21^{a}	0.4 (90.5)
ADM + GT	14.83±2.30^{a}	62.3 (249.5)	0.74±0.02^{a}	5.7 (131.3)	64.08±2.73^{a}	- 7.3 (75.8)

Results are expressed as means ± standard deviation (Each value represents a mean of 12 values). $^{a, b \& c}$ Superscripts in the same column, values with different letters are significant at P<0.05 and similar second letter ^{d} superscript in the same column are non-significant. % changes represent a comparison between treated and control groups. (%) changes represent a comparison between treated and AlCl3-groups.
DS: Difference Score; DI: Discrimination Index; DR: Discrimination Ratio Percent (frequency); ADM: Alzheimer Disease Model.

Table 3. Effect of the investigated medicinal plants on alterations of brain oxidative stress biomarkers induced by AlCl3.

Groups \ Parameters	Malondialdehyde *(nM/mg protein)*		Glutathione *(µM/mg protein)*		Catalase *(IU/min/mg protein)*	
	Mean± SD	% Change	Mean± SD	% Change	Mean± SD	% Change
Control	1.16±0.22^{cd}		42.35±3.60^{a}		0.014±0.001^{a}	
ADM ($AlCl_3$ orally)	2.45±0.12^{a}	111.2	24.37±1.69^{b}	-42.5	0.007±0.001^{b}	-50
ADM + MO	1.63±0.10^{b}	40.5 (- 33.5)	29.35±1.85^{bc}	- 33.1 (20.4)	0.016±0.001^{a}	14.3 (128.6)
ADM + GB	1.43±0.14^{bd}	23.3 (- 41.6)	35.71±1.56^{ac}	- 15.7 (46.5)	0.014±0.001^{a}	0.0 (100)
ADM + GT	1.78±0.14^{b}	53.4 (- 27.3)	30.81±2.17^{bc}	- 27.2 (26.4)	0.013±0.000^{a}	- 7.1 (85.7)

Results are expressed as Means ± Standard Deviation (Each value represents a mean of 5 values). $^{a, b \& c}$ Superscripts in the same column, values with different letters are significant at P<0.05 and similar second letters $^{c\&d}$Superscripts in the same column are non-significant. % changes represent a comparison between treated and control groups. (%) changes represent a comparison between treated and AlCl3-groups.
ADM: Alzheimer Disease Model.

Table 4. Effect of the investigated medicinal plants on alterations of brain monoamine and acetylcholinesterase induced by AlCl3.

Groups \ Parameters	Dopamine *(µg/g tissue)*		Norepinephrine *(µg/g tissue)*		Serotonin *(µg/g tissue)*		Acetylcholinesterase *(µMSH/min/g tissue)*	
	Mean ± SD	% Change	Mean± SD	% Change	Mean± SD	% Change	Mean± SD	% Change
Control	0.82±0.14^{a}		1.96±0.34^{ab}		0.76±0.05^{a}		39.30±2.40^{ad}	
ADM ($AlCl_3$ orally)	0.57±0.03^{b}	-30.5	1.33±0.18^{b}	-32.1	0.58±0.03^{b}	-23.7	29.61±1.52^{c}	-24.7
ADM + MO	0.65±0.02^{ab}	- 20.7 (14.0)	2.25±0.25^{a}	14.8 (69.2)	0.68±0.04^{ab}	- 10.5 (17.2)	37.39±1.54^{ad}	- 4.9 (26.3)
ADM + GB	0.74±0.04^{ab}	- 9.8 (29.8)	2.24±0.19^{a}	14.3 (68.4)	0.66±0.05^{ab}	- 13.2 (13.8)	35.51±2.01^{bd}	- 9.6 (19.9)
ADM + GT	0.86±0.07^{a}	4.9 (50.9)	2.19±0.15^{a}	11.7 (64.7)	0.83±0.10^{a}	9.2 (43.1)	41.40±0.95^{a}	5.3 (39.8)

Results are expressed as Means ± Standard Deviation (Each value represents a mean of 5 values). a& b superscripts in the same column, values with different letters are significant at P< 0.05. % changes represent a comparison between treated and control groups. (%) changes represent a comparison between treated and AlCl3-groups. ADM: Alzheimer Disease Model.

trol group showed dentate gyrus, which formed of small granule cells. The outward continuation of CA1 region is called subiculum. The molecular layer comprises the areas in among compact zones, which consists of neuronal processes, including axons and dendrites, glial cells, and scattered nerve cells (fig 2A). Sections from ADM and MO groups (Figs2 B, C) was severely affected and showed marked disorganization associated with neuronal cell loss in different parts. Moderate changes could be found in GB and GT groups. (Figs 2D, E) in a hematoxylin and eosin stained hippocampus sections. In addition to positive detection of amyloid plaques in the hippocampus in ADM group (Figures 2F).

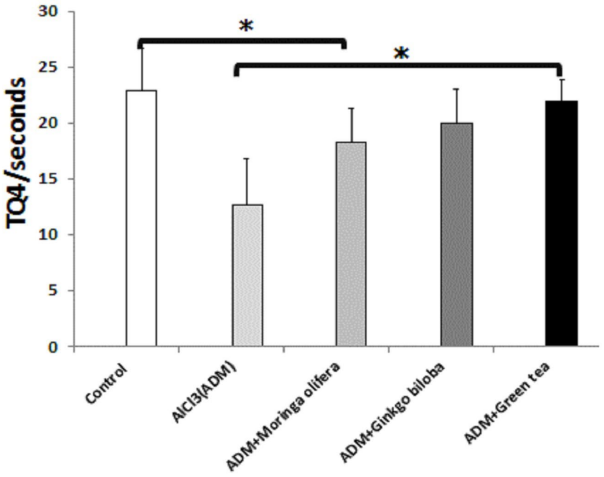

Fig. 1. Effect of some medicinal plants on impaired target quadrant Q4 (TQ4) induced by Alcl3 AD rat model. Results are expressed as means ± standard Deviation (Each value represents a mean of 12 values).

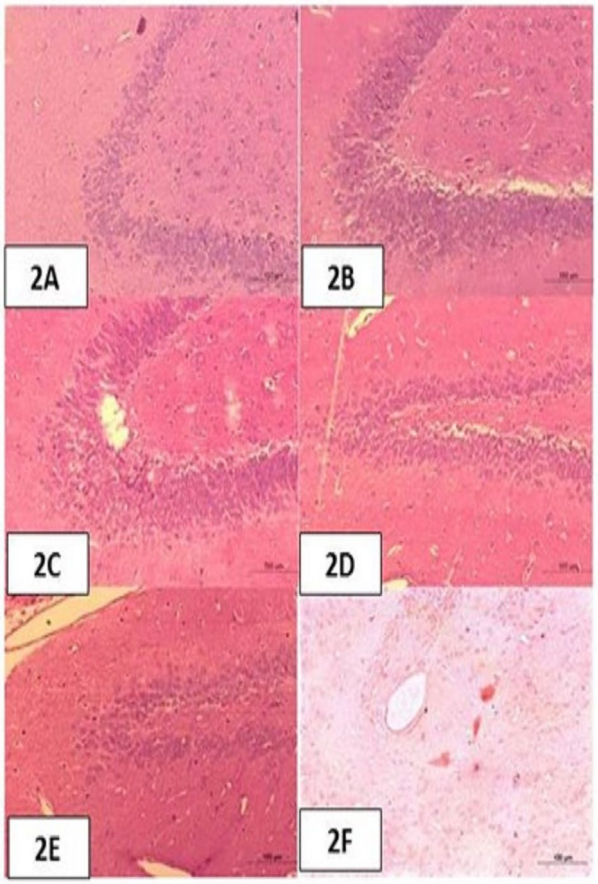

Fig. 2. Examination of hematoxylin and eosin stained sections revealed the presence of characteristic areas of hippocampus particularly in the dentate gyrus. Sections from the control group showed dentate gyrus, which formed of small granule cells. The outward continuation of CA1 region is called subiculum. The molecular layer comprises the areas in among compact zones, which consists of neuronal processes, including axons and dendrites, glial cells, and scattered nerve cells (Fig. 2A). Sections from ADM and MO groups (Figs2 B, C) was severely affected and showed marked disorganization associated with neuronal cell loss in different parts. Moderate changes could be found in GB and GT groups. (Figs 2D, E). In addition to positive detection of amyloid plaques in the hippocampus in ADM group (Figures 2F).

DISCUSSION

The findings of this investigation revealed that chronic administration of AlCl3 caused behavioural, biochemical, and histopathological changes that were similar to those seen in Alzheimer's disease that expressed by impaired memory and cognition performances (Cao *et al*., 2017).

In the present study, the induction of AD using AlCl3 rat model of AD was confirmed by degeneration of dentate gyrus neurons of hippocampus and presence of β- amyloid plaques (Aβ), assessed by histopathological examination. β- amyloid plaques, are intracellular neurofibrillary tangles composed predominantly of hyperphosphorylated tau proteins (De Strooper and Karran, 2016). And considered the most pathological hallmark expected in AD. Science, hippocampus is the first and most affected area in AD (Cao *et al*., 2017), the observed histopathological lesions confirm the induction of AD.

Degeneration of dentate gyrus hippocampal neurons and formation of β-amyloid plaques resulted from AlCl3 toxin caused impairments of memory performance in Y-maze testes and cognition performances in novel objective recognition and Morris Water Maze testes. These behavioural performances are displayed by significant reduction in SAP, and non-significant elevation in SAR and AAR (Table 1); significant reduction in DS, DI, and DR and TQ4. These data are in parallel with earlier reports (Iqbal *et al*., 2016); Xing *et al*., 2018). The above findings indicate that AlCl3 toxin is more probable mimics some of the most essential markers expected in AD (Cao *et al*., 2017).

On the other hand, treatment of medicinal plant (MO, GB, and GT) recovered the AlCl3 induced hippocampal neuronal damage and formation of β- amyloid plaques as well as enhancement of rats' memory and recognitive performances. These results are supported by previous studies that treatment of MO, GB, or GT improved memory and cognitive deficits and prevented the hippocampal neuronal damage in the experimentally AlCl3 rat model (Thenmozhi *et al*., 2016; Ma *et al*., 2020). Wang *et al*. (2013) Found that treatment with the GB leaf extract improved D-galactose impaired memory in the Y - maze test in a dose dependent manner. Schimidt *et al*. (2017) Revealed that green and red tea supplementation increased the decreased DI in object recognition test in AD rat model, and Ma *et al*. (2020) reported that GT improved cognition in AD patients. Gumay *et al*. (2017) Reported that administration of (-) epigallocatechin-3-gallate (EGCG) to D-galactose dementia mice model improved their spatial memory in MWM by increasing time spent in target quadrant in probe trial of the test.

Our findings showed that treatment with the medicinal plants enhanced the impaired working memory in Y-maze test (Omotoso *et al*. 2018; Abdelghany *et al*. 2019) demonstrated that administration of MO increased alternation percent in a Y - maze in an induced memory decline in rat model. Kim *et al*. (2004) reported that administration of GT polyphenols improved the impaired spontaneous alternation behaviour induced by scopolamine in mice in a Y - maze test.

Medicinal plant treatment ameliorated the inhibitory effect of AlCl3 on recognition memory. These data coincided with the study of Patave and Une (2016) and Mahmoud *et al*. (2017) Found that treated with ethanolic extract of MO (100, 200 and 600 mg) caused the rats and mice spend more time exploring novel object more than familiar object. Walesiuk *et al*. (2005) revealed that administration of the GB extract (EGB 761) increased time of exploration of new object and DI significantly in comparison with stress and cortisol treated rats.

In our study, AlCl3 impaired spatial reference memory rates

while, MO and GB mitigated this effect. Sutalangka *et al*. (2013) Found that administration of the MO extract (100, 200, 400 mg) decreased escape latency and increased retention time in MWM test in animal models of Age-Related Dementia.

In additions, Xu *et al*. (2010) spent in the target quadrant in the test day in rats suffered from chronic hypoperfusion and several essential factors have been postulated to describe the etiopathogenic of AD and the neurotherapeutic effects produced by medicinal plant (MO, GB, and GT) in the experimental rats AlCl3 model of AD.

Oxidative stress has long been implicated in the initial and later stages of neuronal degeneration (Melo *et al*., 2011). In the present study, oral administration of AlCl3 caused a significant increase in brain MDA compared to control rats (Table 3). This was consistent with other investigators (Al-Amin *et al*., 2016; Saied *et al*., 2019) whose reported that oral or intraperitoneal injection of AlCl3 induced significant increase in MDA level in various areas of brain.

In in vitro and in vivo study, Ahmed *et al*. (2013) suggested that aluminum exposure caused an increase in ROS, including H_2O_2 production, which subsequently initiate the process of LPO in different brain regions. The brain has been highly susceptible to LPO in the presence of increased ROS level, due to an abundance of PUFA in brain membranes and lower endogenous anti-oxidative defense system (Valko *et al*., 2007). Overproduction of free radicals and the decrease in antioxidant defense potential in AlCl3 exposure may be a key factor to push the cell towards oxidative stress in the brain leading to tissue injury and dysfunction (Amjad and Umesalma, 2015).

The finding of the current study also revealed that the increase in the level of MDA in AlCl3-treated rats was accompanied by concomitant decreases in the level of GSH and activity of CAT. This result is consistent with (Al-Amin *et al*., 2016; Saied *et al*., 2019). These enzymes are closely related to direct elimination of ROS (Sahoo *et al*., 2008). Therefore, the reduction in the level and activity of these enzymes may be contributed to the increase utilization of these enzymes in scavenging and neutralizing the free radicals and lipid peroxides that are generated during AlCl3 exposure (Kumar *et al*., 2009). Inhibition of the enzymatic activities may also contribute to Al+3-induced decline in the mRNA expression of endogenous antioxidants (Gonzalez *et al*., 2007).

The GSH is present in all mammalian tissues (Skelly, 2008) and plays an important role in many biological processes of cells, including synthesis of proteins, DNA, and against oxidative damage (Liu and Pravia, 2010). GSH can act as a non-enzymatic antioxidant by direct interaction with ROS or it can be involved in the enzymatic detoxification reaction for ROS, as a cofactor or coenzyme (Selvakumar *et al*., 2005). Therefore, any alteration in its level may predispose the tissue to oxidative stress. The reduction in the activity of CAT may reflect the inability of cells to eliminate the H_2O_2 that may lead to accumulation of H_2O_2, as CAT is responsible for the conversion of H_2O_2 to H_2O. This reduction may be attributed to enzyme inactivation caused by excess ROS production (Katyal *et al*., 1997).

Medicinal plants with antioxidant activities have been used traditionally in the treatment of several diseases in different parts of the world (Yassin *et al*., 2013; Kumar *et al*., 2017). In the present study, co-supplementation of MO or GB or GT extract with Alcl3 significantly enhanced the tissue level of antioxidants, by reducing MDA level compared to AlCl3-treated rats (Table 3), suggesting that either MO, GB, or GT modulate impaired redox homeostasis status and show challenging role in quenching free radicals, as shown in previous findings (Patave and Une, 2016; Kumar *et al*., 2017).

MO extract possesses a nootropic and antioxidant activities because it is rich in vitamin C and E, hence it combats oxidative stress and improves memory by using a higher concentration to treat AD (Sutalangka *et al*., 2013; Roy, 2014). Administration of aqueous extract or ethanolic extract of MO at a dose of 250 mg/kg b. wait for two weeks increased catalase and GSH activities in a dose dependent manner and decreased MDA level in brain tissue of AD rat model by infusion of colchicine (Roy and Das, 2013; Roy, 2014).

GB leaves possess antioxidant activity and promote a neuroprotective effective against toxic beta-amyl aid protein (Kim and Oh, 2012; Rasool *et al*., 2014). Bridi *et al*. (2001) Found that EGb761 extract can increase catalase activity and decrease lipid peroxide level in striatum, hippocampus and substantia nigra in rats. GB extract (EGb761) restored the decreased glutathione peroxidase and superoxide dismutase activity and reduced the elevated MDA level in rat hippocampus after high sustained positive Gz (+Gz) exposure (Chen *et al*., 2016).

GT counteracts beta-amyloid impaired memory, including that in animals subjected to aluminium-induced neurotoxicity, and prevents or ameliorates oxidative damage in the brain, including that developed in AD (Haque *et al*., 2008). Assuncão *et al*. (2011) and Schimidt *et al*. (2017) recorded that GT administration reduced the increased MDA level during hypoxia exposure and aging. In additions, Schimidt *et al*. (2014) reported that GT supplementation in ischemic rats reversed the reduction in GSH concentration and enhanced the catalase activity in rat prefrontal cortex and reduced LPO in rat prefrontal cortex and hippocampus.

In the present study, rats challenged by AlCl3 exhibited significant decreases in AchE activity compared to the control group. These results were in agreement with Kumar *et al*. (2009). However, combined treatments with GB, MO, or GT and AlCl3 increased the reduction in AchE compared to Alcl3-intoxicated rats. Treatment with MO leaf extract ameliorated the adverse effect of colchicine and increased choline acetyltransferase and AchE activity in AD animal model (Roy, 2014). Zaki *et al*. (2015) Reported that administration of the GB extract (100 mg) increased the reduced brain cholinesterase activity resulted from exposure to radiation or lead toxicity or combination of both. Jelenković *et al*. (2014) found that injection of the GT extract before AlCl3 in rat hippocampus reversed the decreased AchE activity induced by AlCl3 administration in the different brain areas.

The results obtained from the present study showed that oral administration of AlCl3 was associated with marked reductions of DA, NE, and 5-HT compared to control group (Table 4). These observations are consistent with the study of Cao *et al*. (2017) who reported that treatment of rats with AlCl3 resulted in a significant decrease in hippocampus monoaminergic neurotransmitters particularly DA and NE. Furthermore, Said *et al*. (2013) revealed that administration of AlCl3 (35 mg/kg, orally) for 4 weeks, resulted in brain tissue damage, featured by a significant decrease in 5-HT, DA, and NE. The authors added that, the decreases in monoamines content following AlCl3 exposure could be attributed to AlCl3-induced oxidative stress and oxidation of monoamines. Oxidative stress and inflammation cause deficiencies of many of the major neurotransmitters, 5-HT, Ach, and DA (Crockett *et al*., 2008).

Said and Elmenofy (2015) reported that AlCl3 is a neurotoxicant potentially affecting ionic, cholinergic, and dopaminergic neurotransmission in the CNS and these alterations are known to be associated with learning and memory abilities. AlCl3 destroys the microtubules integrity of the neurons, thereby causing degeneration in the brain cells and this can be associated with a significant reduction of monoamines levels in various brain regions. Delgado *et al*. (2000) suggested that the decrease in monoamines content might be attributed to decreased synthesis, resulting from AlCl3-induced damage to the ileal mucosa and reduction in net ilea absorption, where a decrease in the absorption of L-tyrosine may diminish the production of DA and NE, while a decrease in absorption of tryptophan would reduce the synthesis of 5-HT (Martin *et al*., 2001).

The obtained decrease in monoamines levels in our study was explained by Foster (2000) who mentioned that aluminum neurotoxicity elevates neopterin levels in the brain of AD patients and at the same time induces reduction of brain neurotransmitters such as DA, NE and 5-HT via depressing cerebrospinal fluid tetrahydrobiopterin levels which is required for the synthesis of

those neurotransmitters.

The decrease in the content of monoamines (DA, NE, and 5-HT) induced by AlCl3 was significantly prevented by simultaneous administration of MO, GB, or GT compared to AlCl3-treated group (Table 4). Being powerful antioxidants and have high scavenging capacities, MO, GB and GT were able to prevent the changes seen with neurotransmitters under the action of AlCl3. As shown in our study the protective effects of MO, GB, or GT against learning and memory impairment could be attributed to their free radical scavenging activities (Iqbal *et al*., 2016; Schimidt *et al*., 2017).

The aforementioned results revealed that exposure to aluminum via the oral route, led to severe and marked histopathological alterations in the subiculum and dentate gyrus of the hippocampus, that represented by neuronal degeneration in different regions of the hippocampus, this was similar to the previous recorded by the findings of Buraimoh *et al*. (2011) and Kamel and Mostafa (2013) who reported that exposure of rats to oral AlCl3 administration induced neurodegenerative changes in hippocampus. Furthermore, Congo red staining showed a minute positive reaction of amyloid plaques which could be seen in the cerebral cortex in ADM group, this agreed with Cao *et al*. (2017) who revealed presence of amyloid plaques in AlCl3 treated rats.

Moreover, neurodegeneration induced by Alcl3 decreased with MO treatment at a mild degree, as well as treatment with GB and GT reduced the observed neurodegenerative changes at a moderate degree which could be supported by Jessberger *et al* (2009) who observed increased neurogenesis in the dentate gyrus throughout the life may contribute significantly to the learning and memory integrity, especially recognition memory and spatial reference memory.

The obtained results were to some extent inconsistent with Alazzouni *et al*. (2016) who found that treatment with MO showed normal tissue histoarchitecture with hematoxylin and eosin stain. In additions, Yin *et al*. (2013) reported that induction of AD by intrahippocampal injection of beta-amyloid (Aβ25–35) induced degeneration and nuclear condensation in hippocampal and frontal cortex neurons and with bilobalide treatment neuronal degeneration reduced. Moreover, Ortiz-López *et al*. (2016) found a dose-dependent relation between the effects of hippocampal neurogenesis providing that EGCG possesses a pro-oxidant and pro-apoptotic at higher doses, while possessing a neuroprotective action at lower doses. In additions to that EGCG increased survival and decreased apoptosis of hippocampal neurons without affecting cell proliferation.

CONCLUSION

Exposure of rats to neurotoxin; Alcl3 induces AD signs, represented by degeneration of dentate gyrus hippocampal neurons, formation of β- amyloid plaques and impairment of rats' memory and cognitive performances. Treatments with MO, GB, or GT successfully rescue Neuro-therapeutic abilities against AD most likely through limitation of oxidative burden, manifested by the decline of MDA level and restoration of GSH level and CAT activity, subsequently preventing reduction of monoamines. Therefore, it is suggested that MO, GB, or GT may provide a more effective strategy to lessen neurodegeneration in AD, hence ensure the integrity of dentate gyrus hippocampal neurons and abolish the formation of β- amyloid plaques as well as restore the rats' memory and cognitive performances in the Y-maze, novel objective recognition and Morris Water maze testes. Further studies are required to investigate different doses of testing extracts and for longer duration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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