Lamotrigine enhances the neuroprotective effects of memantine on aluminum chloride-induced behavioral changes in rats

Raafat A.-B. Abdel-Aal, Hanan S.M. Farghaly, Andrew Z. Zakaria

Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Raafat A.-B. Abdel-Aal, PhD, Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt
Tel: +20 106 873 6587; Postal Code: 71526;
Fax: 0882080278;
e-mail: and00990@yahoo.com

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Background and aim
Alzheimer's disease (AD) is the most common reason for dementia in the aged population. AD increases the risk of seizures. Management of epilepsy in AD is difficult because of the possibility of drug interactions. Moreover, antiepileptic drug selection in the elderly needs special attention due to numerous pharmacokinetic factors. In the present study, the effect of lamotrigine (LTG) on the neuroprotective effect of memantine (MEM) was assessed.

Materials and methods
A total of 32 adult male Wistar rats were divided into four groups: saline-treated group, aluminum chloride ($AlCl_3$)-treated group, $AlCl_3$ + MEM-treated group, and $AlCl_3$ + MEM + LTG-treated group. AD was induced by intraperitoneal injection of $AlCl_3$ (75 mg/kg/day) for 60 days, then the rats were evaluated using the Morris water maze, radial arm maze, novel object recognition, and passive avoidance tests. After accomplishing the behavioral tests, the rats were killed and their kidneys and brains were used for estimation of acetyl cholinesterase levels and histopathological studies.

Results
$AlCl_3$ significantly impaired the performance in the Morris water maze, radial arm maze, novel object recognition test, and passive avoidance test and elevated acetyl cholinesterase levels in the cerebral cortex, hippocampus, serum, and kidneys. Moreover, the brain of $AlCl_3$-treated rats showed an increased number of damaged neurons and glial cells. Concurrent administration of MEM and LTG significantly reversed behavioral and cognitive deficits induced by $AlCl_3$.

Conclusion
LTG significantly potentiated the behavioral and cognitive improvement induced by MEM, a finding that suggests a neuroprotective profile of LTG and may hold promise in the management of dementia with epilepsy.

Keywords:
aluminum chloride, Alzheimer's disease, lamotrigine, memantine

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Introduction

Alzheimer's disease (AD) is the most common reason for dementia that is predicted to influence 13.8 million Americans by the middle of the century [1]. The economic costs of AD are estimated to exceed $277 billion by the end of 2018 [1].

In Egypt, dementia is not identified as a health challenge due to the large proportion of young people [2]. The statistical data on rates and costs of AD in Egypt are deficient [3]. However, in one study, the prevalence of dementia in Wadi Ara was reported as 20.46% for those over the age of 65 years [4].

Pathologically, the main stamps of AD are the extracellular deposition of amyloid-β protein (Aβ)-forming neuritic plaques and the intracellular accumulation of abnormal hyperphosphorylated tau proteins forming neurofibrillary tangles [5].

Patients with AD have an increased risk of developing seizures and epilepsy [6]. Furthermore, electroencephalographic interictal epileptiform discharges have been observed in the transgenic mouse model of AD with overexpressed mutated forms of amyloid-β precursor protein (AβPP) [7,8].

Aluminum is a well-identified neurotoxin [9]. It crosses the blood–brain barrier via the high-affinity transferrin receptors [10]. The distinct brain regions show variable sensitivities to aluminum caused by the differences in the blood–brain barrier mechanisms [11]. Aluminum preferentially accumulates in the hippocampus and the frontal cortex where it damages the synaptic architecture [9]. It increases the expression of AβPP [12,13] and accelerates tau protein aggregation [14]. It is a potent cholinotoxin that causes neuronal apoptosis and degeneration of

cholinergic projections [15]. These changes ultimately cause learning and memory deficits and therefore can be used as an animal model for AD [16,17].

Lamotrigine (LTG) is a second-generation antiepileptic agent that acts as a blocker of several calcium, potassium, and sodium currents [18]. Memantine (MEM) is a neuroprotective drug that acts by uncompetitive blocking of the *N*-methyl-d-aspartate (NMDA) receptor, which in turn prevents excitotoxicity caused by excessive influx of calcium [19].

Treatment of epilepsy in the elderly patients with AD is difficult and needs special attention due to numerous pharmacokinetic factors and the possibility of drug interactions [6]. In the present study, the effect of LTG on the anti-alzheimer activity of MEM has been assessed regarding the behavioral and biochemical effects as well as histopathological changes.

Materials and methods

Animal groups

We utilized 32 male Wistar rats weighing 200–250 g and were divided into four groups as follows:

(1) Group I: control group which received intraperitoneal saline in equal volumes and regimens to $AlCl_3$
(2) Group II: $AlCl_3$-treated group which received $AlCl_3$ (75 mg/kg/day, intraperitoneally) for 60 days [20]
(3) Group III: $AlCl_3$ + MEM-treated group which received $AlCl_3$ (75 mg/kg/day, intraperitoneally) and MEM (10 mg/kg/day, intraperitoneally) for 60 days [20]
(4) Group IV: $AlCl_3$ + MEM + LTG-treated group which received $AlCl_3$ (75 mg/kg/day, intraperitoneally) and MEM (10 mg/kg/day, intraperitoneally) for 60 days and LTG (10 mg/kg/day, intraperitoneally) 90 min before the behavioral tests [21].

Behavioral tests

Novel object recognition test

The test was accomplished in a square stainless steel box (60 × 60 × 40 cm) with black walls and floor [22]. Each rat was placed in the test box containing two identical objects and was left to spend a total of 15 s exploring these two objects (familiarization phase) [22]. After the familiarization phases, three testing sessions (test phase) were accomplished after a retention interval of 5 min, 2 h and 24 h to asses short-term, intermediate-term, and long-term memory, respectively. Rats were placed in the box containing one of the objects previously explored during the familiarization phase and a novel one and were allowed to explore for 3 min [22]. A discrimination index was estimated as (time spent with novel object – time spent with familiar object)/(total time exploring both objects) was used to measure memory preference [22].

Passive avoidance test

The test was performed on an apparatus that was divided by a wall into two chambers (20 × 25 × 30 cm). The wall contains a connecting hole of 8 cm diameter. One chamber was maintained illuminated by a 4-watt fluorescent lamp [20]. The test was performed on 2 successive days. The acquisition trials were accomplished on the 1st day. The rats were placed individually in the brightened chamber and once entered the dark chamber, an electric shock (40 V, 0.5 A for 1 s) was delivered to their feet through the floor grid. The rats were immediately removed and returned to the cage [20]. During the retention trial performed 24 h later, the rats were placed again in the brightened chamber and the time between placement in the brightened chamber and the entrance to the dark one was recorded (step-through latency) [20].

Morris water maze

The test was performed in a circular tank made of stainless steel and filled with water at room temperature. The tank measures 160 cm in diameter and 35 cm in height. It is divided by four fixed points on its perimeter to four quadrants. It contains an escape platform of 10 × 10 × 10 cm of the same color [23]. Each rat had four trials per day separated by 10 min for 5 successive days (acquisition trials) during which three parameters were evaluated; the time latency to reach the platform, the distance traveled, and the swimming speed [23]. On the sixth day, the escape platform was removed and the rats were allowed to swim freely for 90 s (probe trials). In probe trials the latency to reach the target quadrant and the time spent in it were calculated [23].

Radial arm maze

This test was performed in an eight-arm maze made of wood. Each arm is 15 × 15 × 80 cm radiating from a circular platform which is 30 cm in diameter and of the same level as the arms [20]. Each rat had given two daily trials, 6 days/week for a total of 2.5 weeks. In each trial, time was recorded and the rat was free to explore [20]. The following parameters were estimated: working memory errors (the number of repeated entries to the baited arms) and reference memory errors (the number of entries to the unbaited arms). The score was expressed as the mean number of reference and working memory errors for each group, with data averaged over five blocks,

each of six trials. The mean time required to complete the task in all trials was also calculated [20].

Estimation of AChE concentration

The kidney and the brain were obtained from each animal after being killed at the end of the behavioral tests. The concentration of acetyl cholinesterase (AChE) is assayed using micro-ELISA strip plate provided within a rat AChE ELISA kit (Bioneovan Co. Ltd, Daxing Industry Zone, Beijing, China) according to the manufacturer's protocol [24,25].

Statistical analysis

Statistical analysis was done with the one-way analysis of variance and the two-way analysis of variance (for the time-course data) followed by Bonferroni post-hoc test using GraphPad Prism Software Inc. (San Diego, California, USA). The results were represented as mean ± SEM. A P value of less than 0.05 was considered significant.

Results

Effect of $AlCl_3$, MEM, LTG, and their combination on the rat exploration time in the novel object recognition test in rats

There was no significant difference between the groups in the total exploration time during the familiarization phase between groups (Fig. 1; $P > 0.05$).

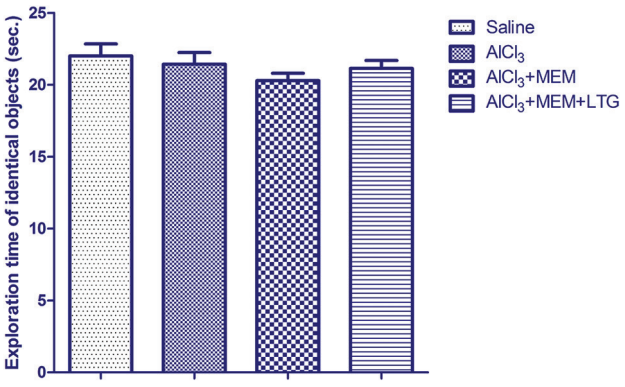
Effect of $AlCl_3$, MEM, LTG, and their combination on the discrimination index of the novel object recognition test in rats

The $AlCl_3$-treated rats showed a significant decline in the discrimination index after 5 min, 2 h, and 24 h (0.03521 ± 0.0387, −0.01697 ± 0.04520, and −0.01816 ± 0.03329, respectively), compared with the saline-treated group at the same allocated time (0.056 ± 0.02734, 0.2005 ± 0.02956, and 0.1231 ± 0.01471, respectively) (Fig. 2; $P < 0.01$, <0.001, <0.001, respectively). Combined treatment with MEM significantly increased the discrimination index after 5 min, 2 h, and 24 h (0.1613 ± 0.01665, 0.1096 ± 0.02117, and 0.07373 ± 0.01420, respectively) compared with the $AlCl_3$-treated rats (Fig. 2; $P < 0.01$, <0.05, <0.05, respectively). The $AlCl_3$ + MEM + LTG-treated rats showed a significant increase in the discrimination index after 5 min, 2 h, and 24 h (0.2238 ± 0.01304, 0.1374 ± 0.01647, and 0.1124 ± 0.01645, respectively) compared with the $AlCl_3$-treated rats (Fig. 2; $P < 0.001$, <0.01, <0.01, respectively). There was no significant difference between the $AlCl_3$ + MEM + LTG and the $AlCl_3$ + MEM-treated rats in the discrimination index after 5 min, 2 h, and 24 h (Fig. 2; $P > 0.05$).

Effect of $AlCl_3$, MEM, LTG, and their combination on the passive avoidance test in rats

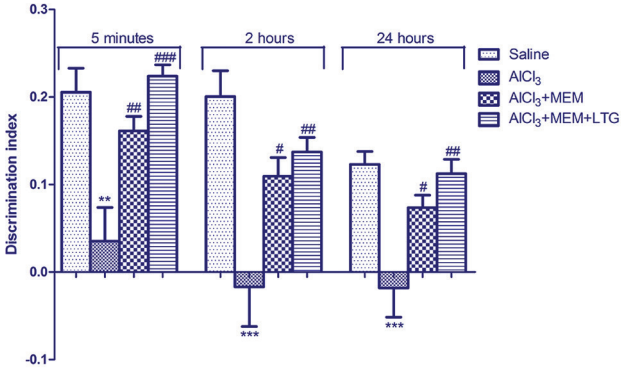
$AlCl_3$-treated rats had significantly decreased the step-through latency compared with the saline-treated rats (Fig. 3; $P < 0.001$). The $AlCl_3$ + MEM-treated rats as well as the $AlCl_3$ + MEM + LTG-treated rats showed a significant increase in step-through latency compared with the $AlCl_3$-treated rats (Fig. 3; $P < 0.05$, <0.001, respectively). There was no significant difference in the step-through latencies between the $AlCl_3$ + MEM-treated rats and the $AlCl_3$ + MEM + LTG-treated rats (Fig. 3; $P > 0.05$).

Figure 1



Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on the total exploration time spent in investigating identical objects in the learning trial of novel object recognition task. Values are represented as means±SEM of eight observations. LTG, lamotrigine; MEM, memantine.

Figure 2



Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on discrimination index (%) after 5 min, 2 h, and after 24 h. Discrimination index is calculated as the difference in exploration time between the novel and familiar objects divided by the total time spent exploring both objects. Values are represented as means±SEM of eight observations, $^{**}P<0.01$ versus saline grou$P$ values, $^{***}P<0.001$ versus saline grou$P$ values, $^{\#}P<0.05$ versus $AlCl_3$ grou$P$ values, $^{\#\#}P<0.01$ versus $AlCl_3$ grou$P$ values, and $^{\#\#\#}P<0.001$ versus $AlCl_3$ grou$P$ values. LTG, lamotrigine; MEM, memantine.

Effect of $AlCl_3$, MEM, LTG, and their combination on the Morris water maze test in rats

Acquisition trials

Throughout the 5 successive days of acquisition trials, there was a significant increase in the escape latency [Fig. 4a] [F (1, 56)=245.15, P < 0.0001] and the mean traveled distance [Fig. 4b] [F (1, 60)=199.39, P < 0.0001] in the $AlCl_3$-treated rats compared with the saline-treated group. The $AlCl_3$ + MEM-treated rats showed enhanced performance in the form of significant reduction in escape latency [Fig. 4a] [F (1, 56)=65.05, P < 0.0001] and the mean distance traveled [Fig. 4b] [F (1, 60)=62.93, P < 0.0001] compared with the $AlCl_3$-treated rats. The $AlCl_3$ + MEM + LTG-treated rats showed a significant further reduction in escape latency [Fig. 4a] [F (1, 56)=134.07, P < 0.0001] and the mean traveled distance [Fig. 4b] [F (1, 60)=48.27, P < 0.0001] compared with the $AlCl_3$-treated rats. There was no significant difference in the escape latency [Fig. 4a] [F (1, 60)=13.49, P > 0.05] and the mean distance traveled [Fig. 4b] [F (1, 60)=1.97, P > 0.05] between the $AlCl_3$ + MEM-treated rats and the $AlCl_3$ + MEM + LTG-treated rats.

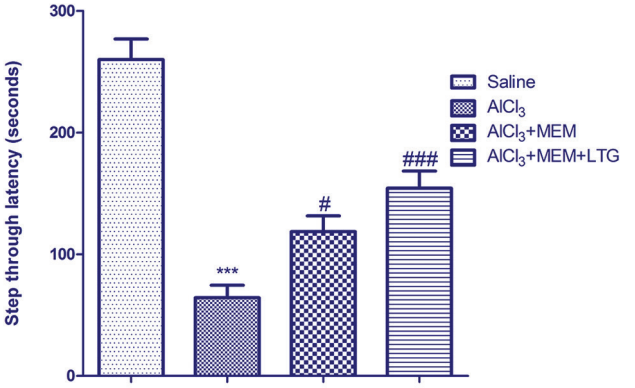
Probe trials

The $AlCl_3$-treated rats exhibited a significant increase in the time required to reach the hidden platform (Fig. 4c; P < 0.001) and reduced time spent in the target quadrant compared with the saline-treated group (Fig. 4d; P < 0.001). The $AlCl_3$ + MEM-treated rats showed a significant reduction in the time required to reach the hidden platform (Fig. 4c; P < 0.01) and increased time spent in the target quadrant (Fig. 4d; P < 0.05) compared with the $AlCl_3$-treated group. In addition, the $AlCl_3$ + MEM + LTG-treated rats showed a significant reduction in the time required to reach the hidden platform (Fig. 4c; P < 0.001) and increased time spent in the target quadrant compared with the $AlCl_3$-treated group (Fig. 4d; P < 0.001). Results have shown that the $AlCl_3$ + MEM + LTG-treated rats showed a significant increase in the time spent in the target quadrant (Fig. 4d; P < 0.05) compared with the $AlCl_3$ + MEM-treated rats. There was no significant difference in the time required to reach the hidden platform between the $AlCl_3$ + MEM and the $AlCl_3$ + MEM + LTG-treated rats (Fig. 4c; P > 0.05).

Effect of $AlCl_3$, MEM, LTG, and their combination on the performance of rats in the radial arm maze

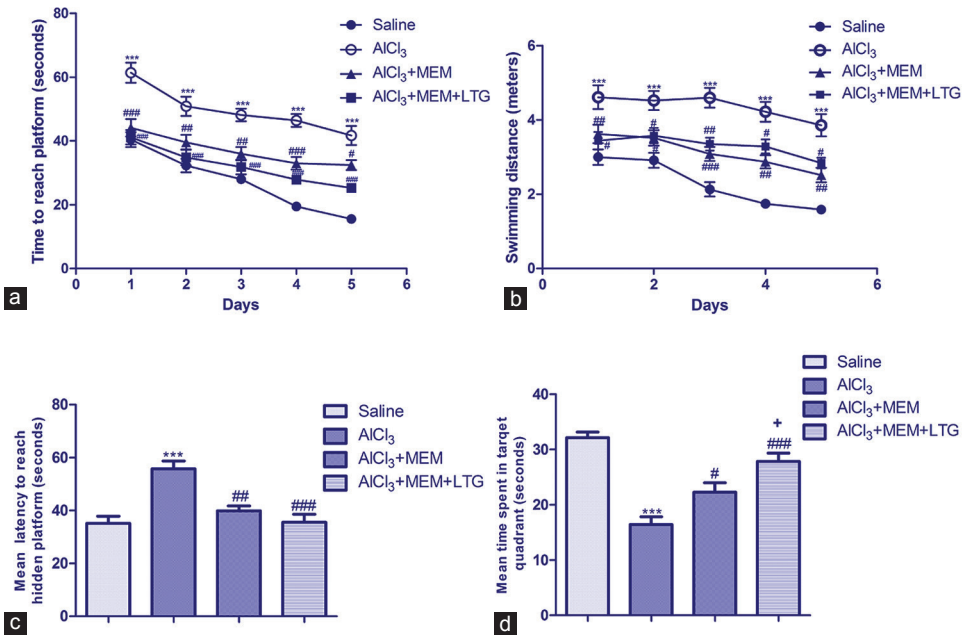
There is a significant increase in the number of reference errors [Fig. 5a] [F (1, 60)=1126.08, P < 0.0001] as well as working errors [Fig. 5b] [F (1, 54)=323.05, P < 0.0001] in blocks 2 (t = 6.654, P < 0.001), 3 (t = 10.72, P < 0.001), 4 (t = 12.01, P < 0.001) and 5 (t = 14.94, P < 0.001) in the $AlCl_3$-treated group compared with the saline-treated group. The $AlCl_3$ + MEM-treated group showed a significantly lower number of reference errors [Fig. 5a] [F (1, 60)=264.37, P < 0.0001] and working errors [Fig. 5b] [F (1, 60)=50.50, P < 0.0001] in blocks 3 (t = 3.585, P < 0.01), 4 (t = 3.076, P < 0.05), and 5 (t = 6.152, P < 0.001) compared with the $AlCl_3$-treated group. Combined treatment with LTG and MEM significantly reduced the number of reference errors [Fig. 5a] [F (1, 60)=668.76, P < 0.0001] and working errors [Fig. 5b] [F (1, 60)=157.67, P < 0.0001] compared with the $AlCl_3$-treated group. Results have shown that the $AlCl_3$ + MEM + LTG-treated group showed a significantly lower number of reference errors [Fig. 5a] [F (1, 60)=90.49, P < 0.0001] in blocks 2 (t = 4.158, P < 0.001), 3 (t = 4.653, P < 0.001), 4 (t = 4.988, P < 0.001), and 5 (t = 4.814, P < 0.001) and working errors [Fig. 5b] [F (1, 60)=40.23, P < 0.0001] in blocks 2 (t = 3.958, P < 0.01) and 5 (t = 4.328, P < 0.001) compared with the $AlCl_3$ + MEM-treated group. The $AlCl_3$-treated group showed a significant increase in latency (time to consume all four rewards) compared with the saline-treated group (Fig. 5c; P < 0.001). In the $AlCl_3$ + MEM as well as the $AlCl_3$ + MEM + LTG-treated group, the latency decreased significantly compared with the $AlCl_3$-treated group (Fig. 5c; P < 0.01, P < 0.001, respectively). The $AlCl_3$ + MEM + LTG-treated group showed a significant decrease in latency compared with the $AlCl_3$ + MEM-treated group (Fig. 5c; P < 0.001).

Figure 3



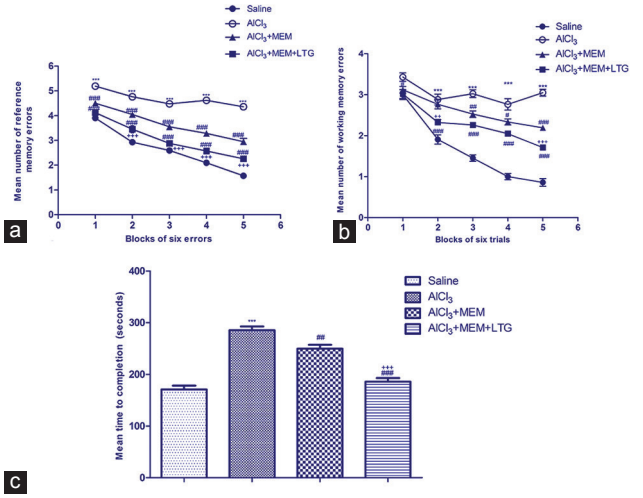
Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on the passive avoidance test. On day 1, the rats received a footshock, and 24 h later, the step-through latency, the time between placement in illuminated chamber and entry to the dark room as a test for the retention memory was recorded in seconds (s) with a 300 s cutoff time. Values are represented as means±SEM of seven observations. ***P<0.001 versus the saline-treated grou$P$ values, $^{\#}P$<0.05 versus $AlCl_3$-treated grou$P$ values, and $^{\#\#\#}P$<0. 001 versus the $AlCl_3$-treated grou$P$ values. LTG, lamotrigine; MEM, memantine.

Figure 4



Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on the acquisition trials; time (s) to reach platform (panel a) and swimming distance (meters) (panel b). Probe trials; time (s) to reach hidden platform quadrant (panel c) and the time (s) spent in hidden platform quadrant (panel d). Values are represented as means±SEM of eight observations. ***P<0.001 versus saline grou$P$ values, #P<0.05 versus $AlCl_3$ grou$P$ values, ##P<0.01 versus $AlCl_3$ grou$P$ values, ###P<0.001 versus $AlCl_3$ grou$P$ values, and+P<0.05 versus $AlCl_3$+MEM. LTG, lamotrigine; MEM, memantine.

Figure 5



Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on the reference memory (panel a), the working memory (panel b), and the time (s) required to end the task in the radial arm maze test (panel c). Values are represented as means±SEM of eight observations. ***P<0.001 versus saline grou$P$ values, #P<0.05 versus $AlCl_3$-treated grou$P$ values, ##P<0.01 versus $AlCl_3$-treated grou$P$ values, ###P<0.001 versus $AlCl_3$-treated grou$P$ values, ++P<0.01 versus $AlCl_3$+MEM-treated grou$P$ values, and +++P<0.001 versus $AlCl_3$+MEM-treated grou$P$ values. LTG, lamotrigine; MEM, memantine.

Effect of $AlCl_3$, MEM, LTG, and their combination on AChE levels in the serum, kidney, hippocampus, and cerebral cortex in rats

The $AlCl_3$-treated rats showed a significant increase in the AChE activity in the serum, kidneys, hippocampus, and the cerebral cortex compared with the saline-treated animals (Fig. 6; P < 0.001). The AChE activity significantly decreased in the serum, kidneys, hippocampus, and the cerebral cortex of the $AlCl_3$ + MEM as well as the $AlCl_3$ + MEM + LTG-treated groups compared with the $AlCl_3$-treated group (Fig. 6; P < 0.001). AChE activity significantly decreased in the serum and hippocampus of the $AlCl_3$ + MEM + LTG-treated group compared with the $AlCl_3$ + MEM-treated group (Fig. 6; P < 0.05 and < 0.01, respectively).

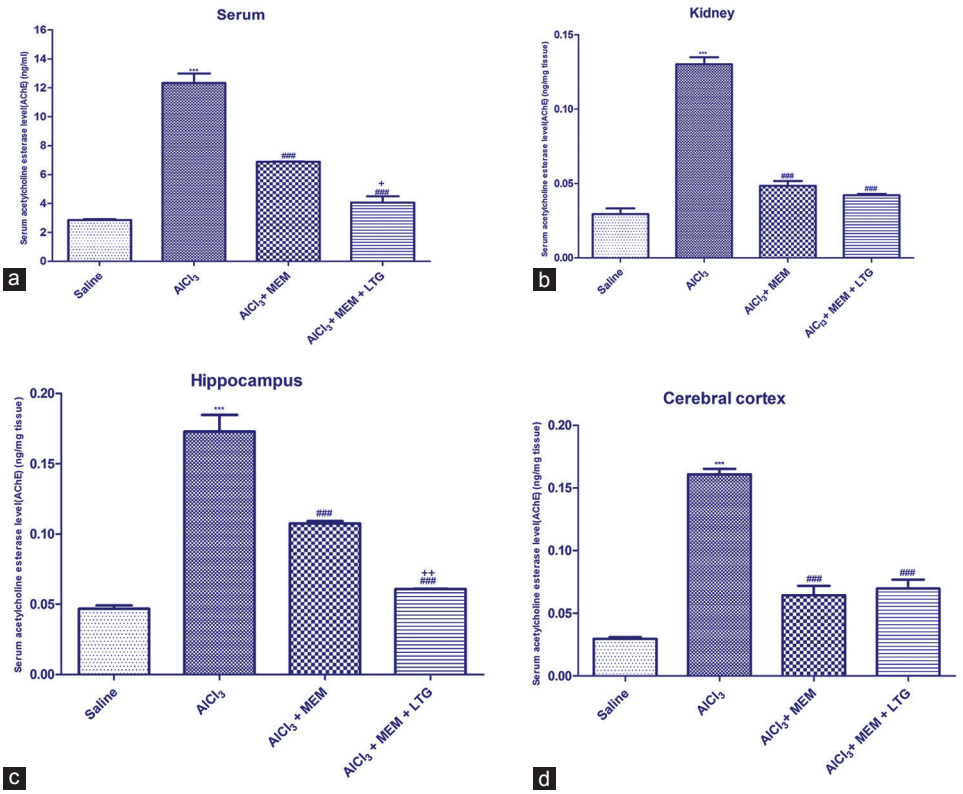
Effect of $AlCl_3$, MEM, LTG, and their combination on the histopathology of the cerebral cortex

Sections of the cerebral cortex from the $AlCl_3$-treated rats showed degenerated cerebral cortex neurons, that is, pyramidal cells (P) with vacuolation and increased number of glial cells (G) accompanied by cellular infiltration. The cerebral cortex of the $AlCl_3$ + MEM and the $AlCl_3$ + MEM + LTG-treated rats showed a marked reduction in the number of the damaged neurons together with the appearance of a large number of intact neuronal cells with less glial cells (Fig. 7).

Effect of $AlCl_3$, MEM, LTG, and their combination on the histopathology of the hippocampus

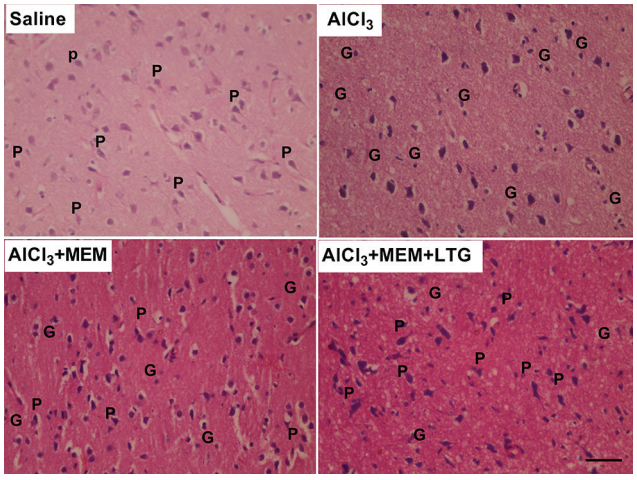
The hippocampus of the $AlCl_3$ and the $AlCl_3$ + MEM + LTG-treated rats showed dark neurons with dark nuclei associated with vacuolation (Fig. 8).

Figure 6



Effects of $AlCl_3$ and its combined treatment with MEM and MEM+LTG on AChE levels in serum (panel a), kidney (panel b), hippocampus (panel c), and cerebral cortex (panel d) in rats. Values are represented as means±SEM of eight observations. $^{***}P$<0.001 versus saline-treated grou$P$ values, $^{###}P$<0.001 versus $AlCl_3$-treated grou$P$ values, ^{+}P<0.05 versus $AlCl_3$+MEM-treated grou$P$ values, and ^{++}P<0. 01 versus $AlCl_3$+MEM-treated grou$P$ values. LTG, lamotrigine; MEM, memantine.

Figure 7



Photomicrograph sections of the cerebral cortex of rats from various groups, that is saline-treated group, $AlCl_3$-treated group, $AlCl_3$+MEM-treated group, and $AlCl_3$+MEM+LTG-treated group. Brain sections from aluminum chloride-treated rats stained with hematoxylin and eosin stain (×40) showing degenerated pyramidal cells (p) with vacuolation and increased number of glial cells (g). Scale bar 100 μm. LTG, lamotrigine; MEM, memantine.

Combined treatment with MEM resulted in marked reduction in the number of damaged neurons together with the appearance of a large number of intact neuronal cells (Fig. 8).

Effect of $AlCl_3$, MEM, LTG, and their combination on the histopathology of the cerebellum

The cerebellum of the $AlCl_3$-treated rats showed irregular outlined nuclei with degeneration of Purkinje cells (Fig. 9). On the other hand, the cerebellum of the $AlCl_3$ + MEM-treated rats and the $AlCl_3$ + MEM + LTG-treated rats showed no vacuolation with a regular arrangement of Purkinje cells with prominent nuclei (Fig. 9).

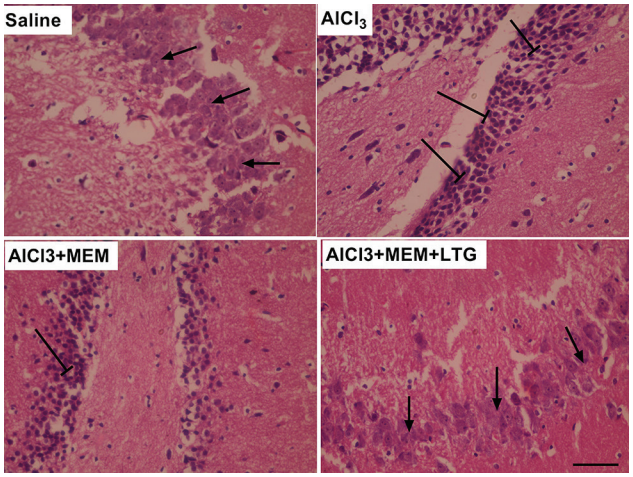
Discussion

Aluminum impaired the performance of rats in the novel object recognition task, Morris water maze, radial arm maze, and passive avoidance tests. It caused a significant increase in AChE levels in the hippocampus, cerebral cortex, serum, and kidney of the treated rats.

The $AlCl_3$ + MEM-treated group showed improved performance in the behavioral tests, compared with the $AlCl_3$-treated rats. Concurrent administration of LTG significantly potentiated MEM-induced behavioral enhancement.

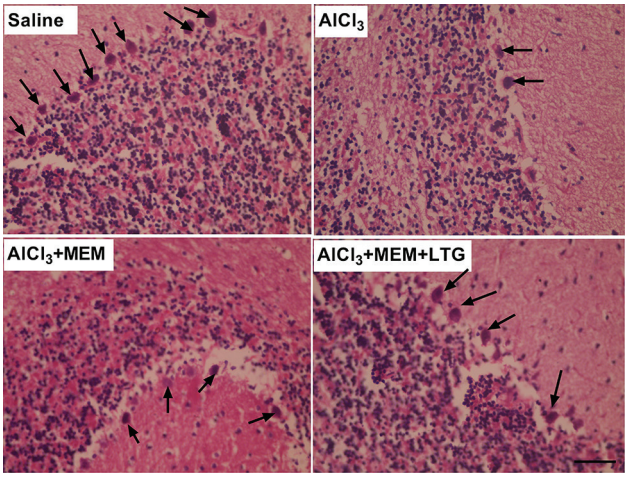
Regarding AChE concentrations, the $AlCl_3$ + MEM-treated rats and the

Figure 8



Photomicrograph sections of the hippocampus of rats stained with hematoxylin and eosin stain (×40) from various groups, that is the saline-treated group, $AlCl_3$-treated group, $AlCl_3$+MEM-treated group, and $AlCl_3$+MEM+LTG-treated group. The arrow heads point to the vacuolation within the hippocampal neurons. Scale bar 100 μm. LTG, lamotrigine; MEM, memantine.

Figure 9



Photomicrograph sections of the cerebellum of rats stained with hematoxylin and eosin stain (×40) from various groups, that is the saline-treated group, $AlCl_3$-treated group, $AlCl_3$+MEM-treated group, and $AlCl_3$+MEM+LTG-treated group. Arrow heads point to the regularly arranged Purkinje cells. Scale bar 100 μm. LTG, lamotrigine; MEM, memantine.

$AlCl_3$ + MEM + LTG-treated rats showed a significant decrease in AChE levels in the serum, cerebral cortex, hippocampus, and the kidneys compared with the $AlCl_3$-treated rats.

Sections from different brain regions of the $AlCl_3$ + MEM-treated rats and the $AlCl_3$ + MEM + LTG-treated rats showed decreased number of damaged neurons and glial cells with increased number of intact well-defined neuronal cells compared with the $AlCl_3$-treated rats.

The choice of chronic rather than acute administration of a dose of 10 mg of MEM given by the intraperitoneal route was based on several previous studies. For example, Danysz *et al.* [26] found that acute administration of big doses of MEM (20–30 mg/kg) may build up very high plasma C_{max} levels. Moreover, other authors found that acute administration of large doses of MEM can produce undesirable effects like ataxia, abnormal stereotypical behavior, and learning dysfunction [27–29].

There are many theories which explain the neuroprotective effects of MEM in aluminum-induced neurotoxicity. Rosi *et al.* [30] found that MEM to a degree stabilized information processing in the hippocampus, and when administered during the early phases of the pathology, it provided neuronal and cognitive protection and indirectly prevented pathological microglial activation. Furthermore, MEM protected proteins of the cerebral cortex and the hippocampus against oxidative stress-induced damage [31]. It was capable of preserving memory during neuronal inflammation [30,32]. In other studies, MEM enhanced attention and memory of rats injected with Aβ peptides [33], and was found to protect the neurons of the basal forebrain involved in acetylcholine release [34].

The blockage of NMDA receptor-mediated excitotoxicity contributes to preserving the normal neuronal structure and function [35,36]. An anti-excitotoxic drug must block excessive NMDA receptor activation that causes neuronal excitotoxicity while leaving the normal NMDA function relatively intact to avoid adverse effects [37]. MEM which is relatively low-affinity open-channel blockers goes into the channel only when it is opened by the agonist [37,38]. The relatively fast off-rate prevents MEM from sequestrating inside the ion channels and consequently interfering with normal synaptic transmission [37,38].

The results of the current study suggest a favorable cognitive profile of LTG when concurrently administered with MEM; a finding that was matched with other studies. Acute LTG administration prevented behavioral disruption [39] and reduced the number of injured cortical neurons in rats treated with NMDA antagonists, MK-801 [40]. It was able to prevent disruption of reversal learning in rodents caused by D-amphetamine [41].

The mechanism by which LTG may exert its neuroprotective effects may be related to blockade of voltage-sensitive sodium channels [42–44]. LTG binds to and stabilizes the inactivated state of the

different subtypes of voltage-gated sodium channels. The action of LTG on the sodium channels may inhibit the excessive presynaptic release of glutamate, which may synergize the NMDA receptor-blocking activity of MEM [39]. Moreover, LTG may inhibit arachidonic acid metabolic cascade mediated by the NMDA receptors in the rat brain [45]. It has also an indirect inhibitory effect on the N-type and R-type voltage-activated calcium channels, but not the T-type calcium channels in the recombinant cell lines [46–48]. The effect of LTG on the recombinant hyperpolarization-activated cyclic nucleotide-gated ion channels in the hippocampus [49] can participate in the neuroprotective effects mediated by LTG [50].

Limitations of the study

A clear-cut analysis of our results is limited by the use of one-dose regimen for each drug. Our future studies will take into consideration the estimation of acetylcholine and glutamate levels in the brain as they are involved in learning and memory.

Conclusion

Combining MEM and LTG have a positive neuroprotective outcome, a result that may hold promise in the treatment of dementia with epilepsy.

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Conflicts of interest

There are no conflicts of interest.

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