Celecoxib effect on rivastigmine anti-Alzheimer activity against aluminum chloride-induced neurobehavioral deficits as a rat model of Alzheimer's disease; novel perspectives for an old drug

Raafat A. Abdel-Aal a, Ola A. Hussein b, Reham G. Elsaady c, Lobna A. Abdelzaher d

a,c,d Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.
b Department of Histology and Cell Biology, Faculty of Medicine, Assiut University, Assiut, Egypt.

*Correspondence should be addressed to:
Lobna A. Abdelzaher
Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.
Lobna@aun.edu.eg
ORCID ID http://orcid.org/0000-0001-8438-7924

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DOI: 10.21608/JMALS.2021.210630

Abstract

Neuroinflammation plays a crucial role in Alzheimer's disease (AD) pathogenesis. Apoptosis, along with impaired neurogenesis, has been linked to AD neurodegenerative cell death, likely due to overexpression of cyclooxygenase-2 (COX-2). We investigated whether the concurrent administration of celecoxib, a selective COX-2 inhibitor, with rivastigmine, the standard anti-Alzheimer, would enhance rivastigmine anti-Alzheimer activity in the aluminum chloride (AlCl3) Alzheimer's rat model. Male rats were randomly assembled into control (Cont), AlCl3-treated (Al), rivastigmine-treated (RIVA), celecoxib-treated (Celeco), and combined rivastigmine and celecoxib-treated (RIVA+Celeco) groups. They were studied for memory, and cognitive skills, along with evaluating hippocampal acetylcholinesterase (AChE) activity. Hippocampal neuropathology, besides apoptosis, astroglial injury, and neurogenesis, were assessed through examining the expression of their related protein markers; activated caspase-3, glial fibrillary acidic protein (GFAP), and nestin. Celecoxib, rivastigmine, and their combination attenuated AlCl3-induced intellectual impairment and the associated neurodegenerative changes. However, the combination therapy had no additional neuroprotective advantage over rivastigmine alone, except for the enhancement of neurogenesis and suppression of apoptosis in the Al-intoxicated rats. As compared to rivastigmine, the efficacy of celecoxib in combination with rivastigmine confers neuroprotection only at the cellular level, enhancement of neurogenesis, and suppression of apoptosis, without having a mitigating effect on Al-induced cognitive impairment.

Keywords: Alzheimer’s disease; Celecoxib; Behavior; Caspase-3; Nestin.

Received November 10, 2021; Accepted December 18, 2021; Published December 24, 2021
1. Introduction

AD is the most prevailing cause of senile dementia, disability, and dependence among elderly over 65 years of age worldwide [1]. Acetylcholinesterase inhibitors (AChEIs), rivastigmine [2–4], and NMDA antagonist, memantine [5], are the currently FDA-approved anti-Alzheimer medications. They can, however, only offer symptomatic relief and could not delay the disease progression [6,7].

Amyloid-β (Aβ); the key component of senile plaque and neurofibrillary tangles (NFTs); the pathological insoluble aggregates of hyperphosphorylated tau proteins are the hallmark lesions of AD [8]. Aβ-induced neuronal apoptosis elicited by caspase-3 activation [9] besides impairment of adult hippocampal neurogenesis [10] mediates further cognitive decline.

COX-2 overexpression [11,12], activated microglia, and astrocyte invasion, as well as cholinergic neuronal degeneration, have all been linked to AD neuroinflammatory reactions [13]. Neuroinflammation normally starts as host defense, then becomes detrimental with subsequent neuronal degeneration [14,15]. Celecoxib, a selective COX-2 inhibitor, improves the cognitive decline in APP/PS1 transgenic mice; the rare AD familial type [16]. However, its potential neuroprotective role in the sporadic, more common AD type, which accounts for...
95% of all cases [17], has not been thoroughly investigated [17,18].

In the sporadic AD type; AlCl3 rat model, we investigated whether celecoxib, when given concurrently with rivastigmine, may enhance rivastigmine anti-Alzheimer activity.

The potential mitigating effect of celecoxib was investigated in the rivastigmine-treated Alzheimer's rat model by analyzing the rats' behavior, hippocampal histopathological changes, and AChE activity, as well as investigating the expression of inflammatory, neurogenesis, and apoptosis markers.

2. Materials and methods

2.1. Animals and experimental design
All the animal procedures were approved by the Faculty of Medicine Institutional Animal Care and Use Committee (IRB no:17100383) and can be given upon request. They were adopted following an update of the National Institute of Health Guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Every effort has been made to minimize the number of animals used and their distress. Adult male Albino Wistar rats weighing 180–220 g were purchased from the animal house of the Faculty of Medicine, Assiut University. They were housed in stainless steel cages in a well-ventilated space under a 12-h light / dark cycle. Rats had access to water and food ad libitum. Rats were divided into five classes at random (n=10):

Group 1; Cont group received daily saline injections intraperitoneal (IP) for 60 days.

Group 2; Al group received AlCl3 hexahydrate (qualikemes India), dissolved in saline, daily IP at a dose of 80 mg/Kg per injection for 60 days [19].

Group 3; RIVA group received AlCl3 and rivastigmine (reference standard drug) (Beijing Mesochem Technology co., Ltd.), dissolved in sterile water, daily IP at a dose of 1 mg/kg per injection for six weeks starting two weeks before AlCl3 administration [20,21].

Group 4; Celeco group received AlCl3 and celecoxib (Beijing Mesochem Technology co., Ltd.), dissolved in saline, daily at a dose of 20 mg/kg per injection IP for four weeks concurrently with AlCl3 [22,23].

Group 5; RIVA+Celeco group received AlCl3, rivastigmine (1 mg/kg for six weeks starting two weeks before AlCl3 administration), and celecoxib (20 mg/kg for four weeks concurrently with AlCl3).

2.2. Behavioral studies
Cognitive performance was assessed using a battery of behavioral tests measuring episodic, aversive (emotional) conditioning, and visuospatial memory via NOR, PA, and MWM tests.

2.2.1. Novel Object Recognition (NOR) test
The test is designed to evaluate rodents' exploratory nature, memory, and object recognition based on their natural preference for exploring novel objects over familiar ones. The test was performed in a room with a uniformly dim light provided by a ceiling-mounted halogen lamp. The NOR test apparatus is a 60 x 60 x 40 cm square stainless steel open field box with black walls and floor. The NOR test objects were distinct in form and color, and they were made of heavily painted wood, which rats couldn't lift. They were about 15 cm in height. The test box and objects were wiped with 70% ethyl alcohol between trials to omit behavioral tasks linked to olfactory cues. Rats were kept in the experimental room for at least 30 min before testing. They were habituated to the empty test box for 10 min per day for two sequential days. Each rat was put in the test box with two identical objects for the amount of time needed to spend a total of 15 sec exploring these two objects to exclude the possibility of random preference. Exploration was considered when, within a cut-off period of 4 min, the rat was touching, looking at, or sniffing with its head within 2 cm of the object. Rats that had explored both objects for less than 15 sec were not included in the experiment. Following the learning phase, three assessment sessions were performed to evaluate the
short, moderate, and long-term memories after a retention period of 5 min, 2 hrs, and 24 hrs. The rats were permitted 3 min to explore one of the objects they had seen during the learning process as well as a novel object. Rats with a low level of object exploration, described as less than 5 sec spent exploring novel and familiar objects, were excluded from the study. Memory performance was evaluated through the recognition index (RI), which was calculated as (time spent exploring the novel object) / (total time spent exploring both familiar and novel objects) *100 [24].

2.2.2. Passive avoidance (PA) test
The PA test is a fear-motivated test used to evaluate learning and memory in rodent models of central nervous system (CNS) disorders. It requires a combination of an aversive stimulus, mild foot shock, and a particular environmental circumstance. The apparatus consists of two chambers separated by an 8 cm communicating hole within a separating wall. A single chamber was kept lit. The rats were put individually in the illuminated chamber on the first day, and an electrical shock was administered to their feet when they entered the dark chamber. Rats were put in the illuminated chamber 24 hrs later, and the step-through latency, time lag till entry to the dark chamber, was recorded [19].

2.2.3. Morris water maze (MWM) test
The MWM test is a behavioral task that evaluates hippocampal-dependent learning and memory in rodents. MWM apparatus consists of a large circular pool (45 x 160 cm, filled to a depth of 30 cm with water at 28 ± 1 °C). Four colored light clues (lambs) were placed on each quadrant wall, dividing the tank into four equal quadrants. Since the light clues were used as a reference memory, they remained constant throughout the experiment. Each rat was exposed to four consecutive trials, considering changing the drop location for each trial, with a gap of 5 min. The rat was gently placed in the water between quadrants facing the pool rim and given 120 sec to locate the platform, during which time the latency to reach the platform was measured. The rat was given 20 sec to remain on the platform. If it did not reach within 120 sec, it was tenderly directed to the platform and left there for 20 sec. Retention trials were conducted 24 hours after the initial trials to observe spatial reference memory. The latency to reach the target area was calculated as well [25].

2.3. Acetylcholinesterase (AChE) activity
The animal groups were sacrificed, and their brains were carefully dissected at the end of the experiment. Hippocampi were weighed, homogenized in phosphate buffer saline (PBS), centrifuged for 5 min at 5000xg, and the supernatant was collected. The hippocampal AChE activity was determined using ELISA kits (Elabscience Co.) according to the manufacturer’s protocol. After the enzyme-substrate reaction was completed, the optical density (OD) was calculated using a microplate reader at a wavelength of 450 nm. By comparing the OD of the samples to the standard curve, the AChE activity was assayed.

2.4. Histopathological studies
Rats were anesthetized with thiopental sodium (50 mg/kg) IP [26], their hearts were exposed and transcardially perfused with saline till getting clear flow, then finalized with 10% formalin. Dissected hippocampal tissues were processed for light microscopy and immunohistochemical staining techniques. Some sections were stained with hematoxylin and eosin (Hx& E) [27].

2.5. Immunohistochemistry Studies
The isolated hippocampi were fixed in 10% neutral formalin, dehydrated, cleared, and paraffin-embedded. Paraffin sections were incubated overnight at 4 °C with the following primary antibodies; rabbit COX-2 antibody (1:100) (Thermo Fisher Scientific, Fremont, CA 94538, USA), rabbit antimouse caspase-3 polyclonal antibody (1:100) (Chongqing Biospes co., Ltd. China), mouse monoclonal anti-nestin antibody
(Abcam, ab22035, UK) (1:100) and anti-GFAP; Ab-1 (Clone GA-5) mouse monoclonal antibody (1:100) (Thermo Fisher Scientific Co, Ferment, California, USA). After that, sections were stained with an avidin-biotin-peroxidase system with diaminobenzidine as the chromogen (DAKO (HRP; rabbit/mouse/goat (DAB+) code no. K0679; Dako Cytomation) according to the instructions enclosed in the Dako LSAB+ System-HRP. Hematoxylin was used to counterstain the studied sections. For a negative control staining, some sections were incubated with PBS instead of the primary antibody.

2.6. Morphometric Studies

Morphometric studies were carried out with the help of image J, a Java-based open-source image processing kit. Three non-overlapped fields/five sections/three rats from each group were used to calculate the tested parameters. The number of dark cells in cornu ammonis 1 (CA1) fields were determined in the Hx&E-stained sections. The number of caspase-3 (+ve) and the number of nestin (+ve) immunostained cells in CA1 areas were calculated using an X40 lens as well.

2.7. Electron Microscopy Studies

With the aid of a dissecting microscope, the extracted hippocampi were dissected out, fixed in glutaraldehyde, and processed for transmission electron microscopy. Toluidine blue was used to stain semi-thin sections (0.5-1 μm). For the selected areas in the semi-thin sections, ultrathin sections (500-800A) were contrasted with uranyl acetate and lead citrate, examined with the JEOL (JEM-100 CXII, Tokyo, Japan) transmission electron microscope (TEM), and photographed at 80 kV in the Assiut University-Electron Microscope Unit.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by multiple t-tests, one-way and two-way repeated-measures ANOVA, followed by post hoc Tukey's test when appropriate. All statistical tests were directed using GraphPad Prism 7 software. The difference among groups was considered significant for \( p < 0.05 \).

3. Results

3.1 behavioral outcomes

3.1.1 Novel Object Recognition (NOR) test

The time spent exploring a novel object was significantly longer than that consumed for the familiar one in the Cont group at 5 min, 2 hrs, and 24 hrs. The Al group had poor memory performance, as shown by the lack of preference for a novel item compared to the Cont group (\( p < 0.001, 5 \text{ min}; 2 \text{ hrs}; p < 0.01, 24 \text{ hrs} \)) (Fig. 1). RIVA group spent substantially more time exploring the novel object relative to the Al group (\( p < 0.05, 5 \text{ min}; 2 \text{ hrs} \)) (Fig. 1). At the given time points, the Celeco group spent more time investigating the novel object compared to the Al group (\( p < 0.05, 5 \text{ min}; 2 \text{ hrs} \)) (Fig. 1). However, its concurrent administration with rivastigmine could not significantly improve memory performance than rivastigmine-only therapy (Fig. 1).

3.1.2 Passive avoidance (PA) test

Al group showed marked cognitive deficit detected as a significant decrease in step-through latency when compared to the Cont group (\( p < 0.001 \)) (Fig. 2). Rivastigmine and celecoxib improved learning and memory tasks as they significantly enhanced step-through latency of the Al-intoxicated rats (\( p < 0.01, p < 0.05 \)). However, the retention latency time was not significantly different between the RIVA+Celeco and the RIVA groups (Fig. 2).

3.1.3 Morris water maze (MWM) test

The MWM test assessed spatial learning and memory through the acquisition and probe trials, respectively. AICl3 administration produced significantly increased time to reach the platform in all four quadrants compared to the Cont group (\( p < 0.001 \)) (table 1). RIVA, Celeco, and RIVA+Celeco groups showed significantly decreased time to reach the platform in all the four quadrants when compared to the Al group.
(p < 0.05) (table 1). In probe trials, the Al group had significantly longer escape latency to the platform site than the Cont group (p < 0.01). In contrast, the treated groups had significantly shorter escape latency compared to the Al group in all four quadrants (p < 0.05) (table 2). Therefore, celecoxib may have the ability to rescue the learning and the memory deficit induced by AlCl3 in the AD rat model without any added benefits of the combination therapy over rivastigmine treatment.

3.2 Acetylcholinesterase (AChE) activity
AChe activity was evaluated in the hippocampi of the studied groups. Hippocampal AChE activity was significantly enhanced, an indication of cholinergic impairment, in the Al group when compared to the Cont group (p < 0.001) (Fig. 3). RIVA and Celeco groups dramatically reduced hippocampal AChE activity compared to the Al group (p < 0.05) (Fig. 3). RIVA+Celeco group showed a non-significant decrease in hippocampal AChE activity than the RIVA group (Fig. 3). Collectively, celecoxib could ameliorate cholinergic dysfunction, the hallmark of AD, through downregulating AChE activity; however, it could not confer additive effect when concurrently administered rivastigmine compared to rivastigmine only therapy.

3.3 Histopathological studies
Examination of the H&E-stained sections of the Cont group brain revealed the normal structure of the rat hippocampus. It was made up of the outer (Ob) and inner (Ib) blades of the dentate gyrus (DG), as well as the CA1, CA2, and cornu ammonis 3 (CA3) fields of Ammon’s horn (AH) (Fig. 4a). Cont CA1 field showed stratum oriens (SO), stratum pyramidale (SP), and stratum radiatum (SR) that extended from the alveus to the hippocampal fissure (HF) (Fig. 4a). The pyramidal neurons (P), the principal cell type in the CA1 field, were characterized by their triangular perikarya, medium-sized round vesicular nuclei (N), and basophilic cytoplasm. Their processes extended to stratum radiatum (SR) (Fig. 4b). The Al group showed multiple intensely stained irregular pyramidal neurons surrounded by empty spaces (Fig. 4c). Most pyramidal neurons (P) appeared normal with round vesicular nuclei (N), yet few cells still seemed irregular and deeply stained in the RIVA group (Fig. 4d). Celco group showed some pyramidal neurons (P) with a regular appearance and round vesicular nuclei (Fig. 4e). However, most pyramidal neurons (P) seemed regularly shaped with round vesicular nuclei in the RIVA+ Celco group (Fig. 4f). Our morphometric results revealed a significant increase in the number of dark cells (p < 0.0001) in the Al group compared to the Cont group. A significant decrease in their numbers, however, was observed in the treated groups compared to the Al group (p < 0.0001) (Fig. 4g). Remarkably, the RIVA+Celeco group had a significantly lower number of dark cells compared to the RIVA group, indicating the additive neuroprotective effect of celecoxib when combined with rivastigmine (p < 0.05) (Fig. 4g).

3.4 Immunohistochemical Studies
3.4.1 COX-2 expression (hippocampus; CA1 field)
Cont group revealed few COX-2 (+ve) immunostained cells (Fig. 5a). A marked increase in COX-2 (+ve) immunostained cells was noticed in the Al group compared to the Cont group (Fig. 5b). RIVA, Celeco, and RIVA+ Celeco groups showed a marked reduction in COX-2 (+ve) immunostained cells compared to the Al group (Figs. 5c-5e). Morphometric results revealed a significant increase in COX-2 (+ve) immunostained cells in the Al group compared to the Cont group (p < 0.0001). In contrast, treated groups showed a significant reduction in COX-2 (+ve) immunostained cells compared to the Al group (p < 0.0001). There was also a substantial reduction in COX-2 (+ve) immunostained cells in the RIVA+Celeco group relative to the RIVA group (p < 0.001) (Fig. 5f), suggesting that the combination
therapy has a stronger anti-inflammatory effect compared to the rivastigmine only therapy.

3.4.2 Active caspase-3 expression (hippocampus; CA1 field)

Caspase-3 is one of the key proteases of the caspase cascade that is involved in apoptosis. Cont group revealed few caspase-3 (+ve) immunostained cells (Fig. 6a). A marked increase in caspase-3 (+ve) immunostained cells was noticed in the Al group compared to the Cont group (Fig. 6b). RIVA, Napro, and RIVA+Napro groups showed a marked reduction in caspase-3 (+ve) immunostained cells compared to the Al group (Figs. 6c-6e). Morphometric results revealed a significant increase in caspase-3 (+ve) immunostained cells in the Al group compared to the Cont group ($p < 0.0001$). Treated groups, however, showed a significant reduction in caspase-3 (+ve) immunostained cells compared to the Al group ($p < 0.0001$). Furthermore, the RIVA+Celeco group had a significantly lower number of caspase-3 (+ve) immunostained cells than the RIVA group ($p < 0.05$) (Fig. 6f), indicating that the combination therapy has an additive antiapoptotic effect.

3.4.3 Nestin expression (hippocampus; CA1 & DG fields)

Decreased nestin (+ve) immunostained cells were observed in the Al group (Fig. 7b) relative to the Cont group (Fig. 7a), indicating impaired neurogenesis. RIVA, Celeco, and RIVA+Celeco groups showed increased nestin (+ve) immunostained cells (Figs. 7c-7e) compared to the Al group indicating enhanced neurogenesis. Statistically, a significant reduction in nestin (+ve) immunostained cells was observed in the Al group compared to the Cont group ($p < 0.05$). The RIVA, the Celeco and the RIVA+Celeco groups showed a substantial increase in nestin (+ve) immunostained cells ($p < 0.01$, $p < 0.001$, $p < 0.05$) relative to the Al group (Fig. 7f). However, the combination therapy had enhanced nestin expression compared to the RIVA group ($p < 0.05$). Immunohistochemically stained sections of DG fields revealed few nestin (+ve) immunostained cells in the Al and the RIVA groups (Figs. 7h&7i). In contrast, the RIVA+Celeco group showed a marked increase of nestin (+ve) immunostained cells (Fig.7j) compared to the RIVA group. As a result, when celecoxib is combined with rivastigmine, neurogenesis in the hippocampal CA1 and DG fields is likely to be enhanced relative to rivastigmine alone.

3.4.4 GFAP expression (hippocampus; CA1 field)

Immunostained sections of the CA1 field revealed few GFAP (+ve) immunostained cells in the Cont group (Fig. 8a). Al group revealed an increased GFAP (+ve) immunostained cells compared to the Cont group (Fig. 8b). The RIVA, the Celeco, and the RIVA+Celeco groups showed a persistent increase of GFAP (+ve) immunostained cells (Figs.8c-8e).

3.5 Electron microscopy studies

Electron microscopic examination of stratum pyramidale (SP) of the CA1 field of the Cont group revealed large pyramidal cells with large oval euchromatic nuclei. Their voluminous electrulcent cytoplasm contained multiple rER, mitochondria, and ribosomes (Fig. 9a). The majority of the cells had degenerated rarified cytoplasm with small involute nuclei and heterochromatin in the Al group (Fig. 9b). Some cells tended to be electron-dense (Fig. 9b). Ultrastructure examination of the RIVA, the Celeco, and the RIVA+Celeco groups showed that most pyramidal cells were more or less similar to the Cont group as they appeared electrulcent with oval euchromatic nuclei (Figs. 9c-9e).
Table 1 Effect of rivastigmine, celecoxib and their combination on the acquisition trials of Morris Water Maze (MWM) test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Quadrant 1 (latency in s)</th>
<th>Quadrant 2 (latency in s)</th>
<th>Quadrant 3 (latency in s)</th>
<th>Quadrant 4 (latency in s)</th>
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<tbody>
<tr>
<td>Cont</td>
<td>14.9 ±3.6</td>
<td>18.3 ± 3.5</td>
<td>8.9 ± 1</td>
<td>9.3 ± 1</td>
</tr>
<tr>
<td>Al</td>
<td>74.3 ±18.4***</td>
<td>91.8 ± 12.4****</td>
<td>31.3 ± 6.3***</td>
<td>69.7 ± 14.3****</td>
</tr>
<tr>
<td>RIVA</td>
<td>21.2 ± 4#</td>
<td>19.1 ± 3.4###</td>
<td>14.3 ± 1.9#</td>
<td>18 ± 2.5###</td>
</tr>
<tr>
<td>Celeco</td>
<td>26 ± 6.1##</td>
<td>25.1 ± 3.9###</td>
<td>14.5 ± 2.2##</td>
<td>10.6 ± 1.2###</td>
</tr>
<tr>
<td>RIVA+ Celeco</td>
<td>24.4 ± 3.3##</td>
<td>26.6 ± 6.6###</td>
<td>13.5 ± 3.3##</td>
<td>10.3 ± 1.6###</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SEM. Statistical analysis was performed by one-way repeated-measures ANOVA followed by a post hoc Tukey’s test. All statistical tests were directed using GraphPad Prism 7 software. ***P<0.001 compared with Cont; ****P<0.0001 compared with Cont; #P<0.05 compared with Al; ##P<0.01 compared with Al; ###P<0.001 compared with Al.

Table 2 Effect of rivastigmine, celecoxib and their combination on the probe trials of Morris Water Maze (MWM) test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Quadrant 1 (latency in s)</th>
<th>Quadrant 2 (latency in s)</th>
<th>Quadrant 3 (latency in s)</th>
<th>Quadrant 4 (latency in s)</th>
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<tr>
<td>Cont</td>
<td>13.7 ± 3.1</td>
<td>8.7 ± 1.6</td>
<td>9.4 ± 2.6</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>Al</td>
<td>72 ± 18.9***</td>
<td>40.9 ± 5.7****</td>
<td>45.6 ± 14.4**</td>
<td>41.2 ± 7.5****</td>
</tr>
<tr>
<td>RIVA</td>
<td>29.3 ± 4.4##</td>
<td>14.4 ± 1.9###</td>
<td>13 ± 2.8##</td>
<td>9.9 ± 0.7##</td>
</tr>
<tr>
<td>Celeco</td>
<td>34.9 ± 7.3#</td>
<td>19.8 ± 1.9###</td>
<td>18 ± 4.3#</td>
<td>11.7 ± 2.3###</td>
</tr>
<tr>
<td>RIVA+ Celeco</td>
<td>28.3 ± 2.9##</td>
<td>17.3 ± 2.8###</td>
<td>12 ± 1.1##</td>
<td>9.1 ± 0.4###</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SEM. Statistical analysis was performed by one-way repeated-measures ANOVA followed by a post hoc Tukey’s test. All statistical tests were directed using GraphPad Prism 7 software. **P<0.01 compared with Cont; ***P<0.001 compared with Cont; ****P<0.0001 compared with Cont; #P<0.05 compared with Al; ##P<0.01 compared with Al; ###P<0.001 compared with Al.
Fig 1. Effect of rivastigmine, celecoxib, and their combination on the recognition index (RI) of the novel object recognition (NOR) test in the AlCl3-induced Alzheimer’s in rats. The data are expressed as mean ± SEM. *p < 0.05, ##p < 0.01, ###p < 0.001. ##, ###: a significant difference from the Cont group. *: a significant difference from the Al group.

Fig 2. Effect of rivastigmine, celecoxib, and their combination on the step-through latency of passive avoidance (PA) test in the AlCl3-induced Alzheimer’s in rats. The data are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
Fig 3. Effect of rivastigmine, celecoxib, and their combination on the acetylcholinesterase (AChE) in the AlCl3-induced Alzheimer’s in rats. The data are expressed as mean ± SEM. *p < 0.05, ***p < 0.001.

Fig 4. Photomicrographs of Hx&E stained sections of rat hippocampus (CA1 field). a) Cont hippocampus; showing V-shaped structure, inner blade (Ib) and outer blade (Ob) of the dentate gyrus (DG), CA1 of Ammon’s horn (AH), and hippocampal fissure (Hf) x40. b) Cont CA1; showing stratum pyramidale (SP), medium-sized pyramidal cells (curved arrow), round vesicular nuclei, and their processes extend to stratum radiatum (SR) x400. c) Al CA1; much irregular, dark shrunken pyramidal cells (curved arrows) surrounded by empty spaces (*). Few of them appear pale stained (P) x400. d) RIVA CA1; multiple pale stained pyramidal (P) cells with round vesicular nuclei, few of which are still dark (curved arrows) x400. e) Celeco CA1; pyramidal cells with round vesicular nuclei (P), shrunken and deeply stained cells are seen (curved arrows) x400. f) RIVA+Celeco CA1; most pyramidal cells (P) are well arranged and have round vesicular nuclei, dark cells (curved arrow) x400. g) Statistical analysis of the number of dark cells in CA1 fields. The data are expressed as mean ± SEM. *p < 0.05, ****p < 0.0001.
Fig 5. Photomicrographs of Cox-2 immunostained sections of the rat hippocampus (CA1 field). a) Cont CA1; few Cox-2 immunostained cells (curved arrows) x400. b) Al CA1; multiple Cox-2 immunostained cells (curved arrows) x400. c) RIVA CA1; some Cox-2 immunostained cells (curved arrows). d) Celeco CA1; few Cox-2 immunostained cells (curved arrows) x400. e) RIVA+Celeco CA1; few Cox-2 immunostained cells (curved arrow) x400. f) Statistical analysis of the number of Cox-2 immunostained cells in CA1 fields. The data are expressed as mean ± SEM. ***p < 0.001, ****p < 0.0001.
Fig 6. Photomicrographs of active caspase-3 immunostained sections of the rat hippocampus (CA1 field). a) Cont CA1; few caspase-3 immunostained cells (↑) x400. b) Al CA1; multiple caspase-3 immunostained cells (↑) x400. c) RIVA CA1; few caspase-3 immunostained cells (↑) x400. d) Celeco CA1; few caspase-3 immunostained cells (↑) x400. e) RIVA+Celeco CA1; few caspase-3 immunostained cells (↑) x400. f) Statistical analysis of the number of caspase-3 immunostained cells in CA1 fields. The data are expressed as mean ± SEM. *p < 0.05, ****p < 0.0001.
**Fig 7. Photomicrographs of nestin immunostained sections of the rat hippocampus (CA1 & DG fields).**

- **a)** Cont CA1; nestin immunostained cells, especially in glial cells (curved arrows) x400.
- **b)** Al CA1; few nestin immunostained cells (curved arrows) x400.
- **c)** RIVA CA1; multiple nestin immunostained cells (curved arrows) x400.
- **d)** Celeco CA1; multiple nestin immunostained cells (curved arrows) x400.
- **e)** RIVA+Celeco CA1; multiple nestin immunostained cells (curved arrows) x400.
- **f)** Statistical analysis of the number of nestin immunostained cells in CA1 fields. The data are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
- **g)** Cont DG; nestin immunostained cells (curved arrows) x400.
- **h)** Al DG; few nestin immunostained cells (curved arrows) x400.
- **i)** RIVA DG; few nestin immunostained cells (curved arrows) x400.
- **j)** RIVA+Celeco DG; multiple nestin immunostained cells (curved arrows) x400.
Fig 8. Photomicrographs of GFAP immunostained sections of the rat hippocampus (CA1 field). a) Cont CA1; few immunostained cells (curved arrows) x400. b) Al CA1; multiple GFAP immunostained cells (curved arrows) with long processes (↑) x400. c) RIVA CA1; multiple GFAP immunostained cells (curved arrows) x400. d) Celeco CA1; multiple GFAP immunostained cells (curved arrows) with long processes (↑) x400. e) RIVA+Celeco CA1; multiple GFAP immunostained cells (curved arrows) x400.
Fig 9. Transmission electron micrographs (TEM) of the rat hippocampus (CA1 fields; pyramidal cells). a) Cont pyramidal cells; multiple medium-sized pyramidal cells (P) with large oval nuclei (N) and abundant cytoplasm that contains mitochondria (m) and rER x3600. b) Al pyramidal cells; multiple pyramidal cells (P) with small involute oval nuclei (N1&N2) contain heterochromatin and rarefied cytoplasm (*). Electron-dense cells can be detected x3600. c) RIVA pyramidal cells; a group of pyramidal cells (P) with euchromatic oval or rounded nuclei (N), abundant electrolucent cytoplasm contains mitochondria (m) and rER x2900. d) Celeco pyramidal cells; electrolucent pyramidal cell (P), nuclei (N), mitochondria (m), and ribosomes (r) x3600. e) RIVA+Celeco pyramidal cells; a group of electrolucent pyramidal cells (P) with oval nuclei (N), mitochondria (m), and rER x3600.

Highlights

- AlCl3 Alzheimer’s rat model represents the sporadic type, the most common.
- Rivastigmine, a cholinesterase inhibitor, is the standard anti-Alzheimer drug.
- Celecoxib, a selective COX-2 inhibitor, ameliorated cholinergic dysfunction, mitigated behavioral insults, and hippocampal neuropathology in the Alzheimer’s rat model.
- When given concurrently with rivastigmine, celecoxib improved rivastigmine anti-Alzheimer activity at the cellular level, enhanced neurogenesis & suppresses apoptosis.
- Celecoxib given concurrently with rivastigmine did not have an extra mitigating effect on Alzheimer's rat cognitive impairment compared to rivastigmine-only therapy.
4. Discussion

The current study was the first to display the celecoxib additive neuroprotective impact when combined with rivastigmine, the standard anti-Alzheimer drug, in the sporadic AD; AlCl3-induced Alzheimer’s rat model. Celecoxib enhanced the anti-Alzheimer activity of rivastigmine by restoring the AD disrupted neuronal turnover through suppressing apoptosis, caspase-3 downregulation, and enhancing neurogenesis; nestin upregulation consequent to its anti-inflammatory effect. Even though celecoxib reduced cognitive deficits and enhanced memory efficiency in the AD rat model, it could not confer an additive impact when paired with rivastigmine compared to rivastigmine alone therapy, implying that it should be given earlier or for a longer period.

AD is a common irreversible, progressive neurodegenerative disorder manifested by gradual loss of memory, judgment, visuospatial skill, and executive functions [28,29]. Complex behavioral and psychological problems develop as the illness progresses, posing a significant burden on the patients, their caregivers, and society [30]. Collectively, AD typically progresses slowly in three general stages: (1) the asymptomatic “preclinical” stage, (2) mild cognitive impairment, which manifests as changes in mood commonly associated with confusion, and some memory loss, and (3) dementia, which exhibits multiple cognitive domains significant enough to cause loss of function [31–33]. The currently available medications merely ease the AD-associated symptoms. Curative therapies, therefore, are yet to be discovered.

AD pathology often begins several years preceding the onset of the clinical signs [34,35] and is already irreversible at diagnosis [17]. Extracellular senile plaques formed of deposits of hydrophobic Aβ peptides and intracellular aggregates of NFTs are the disease hallmark [36]. Aβ is the cleavage product of amyloid precursor protein (APP) via α-, β-, and γ-secretases [37]. The accumulated β-amyloid plaques function as a toxin for neurons, disrupting neuron-neuron contact [38]. Furthermore, tau tangles cause poor nutrient transport triggering AD-associated neuronal loss [38].

Synaptic dysfunction and eventually neurodegeneration, particularly within the hippocampus, the entorhinal, the polymodal association cortices, and the basal forebrain [39], are common AD associations. Early in the disease, cholinergic neuron loss, particularly within the basal forebrain, occurs and is a major contributor to cognitive impairment [40–42], besides being strongly linked to the severity of dementia [43,44].

According to the cholinergic hypothesis, the onset of AD symptoms is primarily caused by structural changes in cholinergic synapses, the loss of particular subtypes of acetylcholine (ACh) receptors, the death of ACh-producing neurons with consequent weakening of cholinergic neurotransmission [45]. As a result, the ACh-hydrolyzing enzyme acetylcholinesterase (AChE) accumulates. Therefore, the enzyme activity can be used as a marker for the central cholinergic status [46–55]. Jha et al. [56] showed a marked decrease in AChE activity with increasing age. However, intense AChE activity appears in the neuritic plaques and neurofibrillary tangles [57]. AChE can promote Aβ assembly [58] and form stable complexes with Aβ fibrils [59] that are more toxic than Aβ fibrils alone [60]. A significant correlation between AChE inhibition and cognitive improvement in AD patients was observed [51]. Therefore, AChEIs [2] are a significant of the currently approved AD medications. Rivastigmine is a unique cholinesterase inhibitor (ChEIs) with both AChE and butyrylcholinesterase inhibitory activity [61,62]. It displays specific activity for the central AChE over the peripheral one [63]. Rivastigmine tends to have the same effectiveness in inhibiting cholinesterases (ChEs) in plaques and tangles as it
does in neurons and axons [64]. It provides only symptomatic relief and a moderate disease-modifying effect as it can dramatically reduce agitated behavior and improve cognitive tasks in patients with dementia [65].

Al-induced neurotoxicity and its link to AD pathophysiology have been reported [66–70]. Al is considered the third most abundant element in the earth's crust, with high human exposure anticipated by cooking tools, food antacids, and various industrial applications [71] to produce rubber, lubricants, paints, wood preservatives, pesticides, antiperspirants, and pharmaceuticals. Al is a potentially dangerous neurotoxic metal that can pass through the blood-brain barrier through the iron-binding protein transferrin and is widely distributed in different brain areas [43]. Higher levels of Al have been found in the brains of AD patients, especially in the hippocampus, indicating that it may play a role in the disease progression [72,73]. Al has been found in senile plaques and NFT-bearing neurons [74] and is particularly abundant in areas rich in cholinergic synapses [75]. It has a robust ability to induce epigenetic changes [76] due to its strong affinity for interaction with biological molecules, including DNA, RNA, and proteins [77]. Al can induce changes in APP gene expression and cause misfolding of cytoskeleton protein, which contributes to increased APP transcription and enhanced Aβ plaque and neurofibrillary tangles deposition in the brain [78–81]. Collectively, Al has been linked to oxidative brain damage, neuronal death, cholinergic neuron degradation, and amyloid deposition, all of which have been associated with intellectual disability in AD cases [82–84].

Al is considered a potent cholinotoxin, causing increased ChE activities [85,86] with consequent impairment of the brain cholinergic transmission. After being exposed to low levels of Al, various mouse brain regions showed a rise in AChE activity [87]. ACl3 Alzheimer rat model may experimentally simulate the critical aspects of AD pathology and disease processes [17]; hence, it is widely accepted that ACl3 can be used to induce neurodegenerative changes in animals to mimic AD [88].

Our findings revealed a substantial increase in hippocampal AChE activity in the AD rat, consistent with other studies [89,90] indicating significant cognitive deficits. According to Gulya et al. [91], an increase in AChE activity after Al exposure is due to the allosteric interaction between cation (Al⁺⁺) and anionic sites of AChE, which leads to alteration in its secondary structure and activity. The second proposed mechanism [92] is the Al's ability to facilitate the accumulation of insoluble Aβ (1-42) [93], which has a direct inhibitory effect on nicotinic acetylcholine receptors (nAChR) with consequent enhancement of AChE activity. On the other hand, other research found decreased AChE activity in the cerebellum and hippocampus on long-term exposure to Al [94]. The conflicting results could be attributed to the various molecular forms of AChE or the Al biphasic influence on the AChE activity [94] mediated through the formation of reversible/irreversible Al complex [94]. Rivastigmine inhibited ACl3-enhanced hippocampal AChE activity, resulting in a partial enhancement in cholinergic neurotransmission, which is in line with previous studies [95–97]. However, in advanced cases, rivastigmine works by inhibiting butyrylcholinesterase (BuChE) activity [98,99].

Our study showed that Al-intoxicated rats had a week exploratory preference, impaired spatial and retention memory, and shortened step-through latency as assessed by NOR, MWM, and PA tests, consistent with previous literature [25]. ACl3-treated animals have displayed increased retention latency and decreased RI percentage [100], indicating reduced short, intermediate, and long-term memory [101,102]. Intracerebral ACl3 administration caused learning deficits in rabbits (93). Rivastigmine improved spatial and retrieval memory impairments caused by ACl3
administration, which is in line with previous studies (94). It has reversed the scopolamine-induced spatial learning deficits as well [103]. RIVA group spent considerably more time investigating the novel object (5 min & 2 hrs). It exhibited a significant increase in step-through latency than the Al group reflecting improvement in learning and memory tasks. Decreased escape latency and increased discrimination index reported in the streptozotocin rat model of AD have been attenuated through rivastigmine administration [104]. Moreover, rivastigmine exerted positive effects on learning and memory in the chronic d-galactose-induced accelerated aging rodent model [105].

The hippocampus is considered the neurobiological root [106] of spatial learning, the working, and the episodic memory skills, which deteriorate with age progression [107]. DG, CA3, and CA1 are the three distinct subregions of the hippocampus. Hippocampal hyperactivity has been identified in people who have a genetic or familial risk of AD [108,109] and asymptomatic and minimally impaired older individuals with Aβ deposition [110]. Its degree correlated with the memory decline [111] and was previously thought to be a compensatory reaction for worsening neuronal circuitry [108]. New research, however, indicates that hippocampal hyperactivity may be a sign of neuronal excitotoxicity, a pathological mechanism in which neurons are destroyed by excessive activation. Since CA1 neurons are highly sensitive to excitotoxicity and more vulnerable to loss in AD, they are perhaps the most studied of all the hippocampal subregions in rodents [112]. Our findings revealed that Al-intoxicated rats had a substantial increase in degenerated pyramidal cells within the CA1 region, which appeared shrunken with ill-defined organelles and nuclei, which is in line with previous research [90,113,114]. The RIVA group revealed a rise in the number of regenerating pyramidal cells in line with other research [115] confirming the rivastigmine mitigating impact on AlCl3-induced oxidative damage and neurotoxicity.

Recent studies have uncovered the role of neuroinflammation and aberrant gliosis in AD [15]. When the neuronal loss is still absent, pathological neuroinflammatory reactions, astroglisis, and microglial activation were observed [116–118]. Microglia reacts more quickly to injury or pathological insult than astrocytes [119]. Once pathologic Aβ deposition begins, microglia activation or reactive astrogliosis may be required to improve clearance of excess toxic amyloid [120], thereby limiting Aβ plaques build-up [121,122] through the expression of type III intermediate filament (IF) protein; GFAP (a marker for astrogliosis) [123,124]. However, as plaques form, microglia and astrocyte stimulation can further worsen the disease [125,126]. They are known to release reactive oxygen species (ROS), nitric oxide (NO), and inflammatory cytokines that encourage low-grade neuroinflammation [127–129], enhance APP, Aβ production [130–132], and tau hyperphosphorylation. Microglia and astrocytes' role, therefore, in AD is still controversial. Nonetheless, the degree of astrogliosis is often linked to the cognitive decline in AD [133–135].

In AlCl3-intoxicated rats, we detected an enhanced hippocampal GFAP immunoreactivity, indicating extreme astrocytic activation, probably, induced by Al uptake. Our findings are in line with those of other studies [136–139], which found increased GFAP immunolabelling in the hippocampus after chronic Al intoxication in rats and rabbits [140]. However, unchanged GFAP immunoreactivity was still reported [141]. In contrast, a decrease in GFAP immunoreactivity was detected, reflecting astrocytes' vulnerability to Al-induced neurotoxicity [114,136]. RIVA group showed enhanced GFAP immunoreactivity indicating persistent astrogliosis, which contradicts other studies [142] that displayed...
reduced immunoreactivity by 45–50%. Rivastigmine has also been shown to reduce memory loss in T2DM-AD model mice by suppressing gliosis [143]. The diversity of the AD models could lie behind the conflicting outcomes.

NSAIDs, cyclooxygenase (COX) inhibitors have been studied to reduce the risk, help delay the progression of AD [144–149] by suppressing the associated inflammatory response [150–152]. They can lower the production of Aβ in senile plaques and phosphorylated tau in NFTs [120], signifying COX activity's involvement in the cascade of AD development. Both COX isoforms, COX-1 and COX-2, were found to be constitutively expressed in the brain [153,154]. COX-2 expression is the highest in the hippocampus and cerebral cortex, suggesting the enzyme role in the regulation of the plasticity mechanisms that sustain learning and memory processes [155,156]. Preclinical studies have shown that treatment with specific COX-2 inhibitors impairs cognitive functions in hippocampal-dependent paradigms [157].

On the other hand, a piece of evidence has indicated that COX-2 aberrant activation under pathological circumstances may cause the associated cognitive dysfunction [158,159]. COX-2 expression is rapidly upregulated in response to a variety of neurotoxic stimuli [155]. Therefore, it has been linked to the pathogenesis of neurodegenerative diseases like multiple sclerosis, Parkinson’s disease, traumatic brain injury, ischemia-induced neuronal damage, epileptogenesis, and AD [160].

COX-2 is induced by various inflammatory molecules as IL-1, IL-2, and TNF-α and is expressed differently in different stages of AD [161]. Previous literature has reported an association between COX-2 induction and the development of amyloid plaques [162] and neurodegeneration [163]. COX-2 overexpression decreased the learning ability of Tg mice via increasing the production of Aβ [164–168]. It is considered the rate-limiting enzyme in the synthesis of prostaglandins (PGs) such as PGE2, PGD2, PGI2, PGF2, and thromboxane A2 (TXA2), which are important components in brain cell damage associated with AD [169]. Activated microglia have been identified as the major producers of the prostaglandin PGE2 via the COX-2 pathway [170–172]. COX-2 and PGE2 regulate the expression of APP, α-, β-, and γ-secretases [120,173]. PGD2 has been described as the most abundant eicosanoid in the brain [174,175]. Activation of the DP2 receptor in astrocytes or Th2 cells enhanced inflammatory cytokines production [176,177], which are crucial in the progression of AD [120].

COX-2 expression was significantly higher in the CA1 area of the Al group, which is consistent with other studies [137,178] that revealed elevated hippocampal COX-2 mRNA [179] and protein expression in correlation with amyloid plaque density [12]. COX-2 has been shown to have high expression within pyramidal neurons [11,180–184] in the early stages of AD, which could be related to regeneration and cell cycle regulation [167,185,186], suggesting that it may be used as a functional indicator of clinical dementia [180]. Whereas, as the disease progresses, the expression of COX-2 is observed to decrease, most likely due to selective degeneration of COX-2-expressing neurons and loss of synaptic activity [161]. These findings suggest that COX-2 may play a role in the early stages of AD but is unlikely to play a role later, emphasizing the importance of early intervention.

RIVA group showed a substantial decrease in COX-2 expression compared to the Al group, which is consistent with other studies showing a reduction of COX-1 and COX-2 mRNA and proteins expression within macrophages fluoride-influenced model [187] that may be attributed to rivastigmine anti-inflammatory properties. Its anti-inflammatory activity can be explained by its effect on nAchRs and
transmitter-gated ion channels involved in the cognitive processes [188–190].

The most appropriate AD prevention seems to be achieved by the specific inhibitors of COX-2, namely celecoxib and rofecoxib [191]. Selective COX-2 inhibitors can attenuate amyloid-β-mediated suppression of memory and synaptic plasticity by preventing PGE2 response at synapses [192]. Restoration of memory in Tg2576 mice over-expressing APP by selective COX-2 inhibitors was previously detected as well [192]. Patients with AD who were given celecoxib for 18 months and 4 to 5 years had their learning ability preserved [193–195]. However, in large-scale clinical trials, selective COX-2 inhibitors did not reveal any benefit in Alzheimer’s patients [196–198]. A one-year regimen with celecoxib did not slow cognitive deterioration in 285 patients with AD [199].

Moreover, celecoxib has been reported to raise the level of the Aβ1-42 segment in cell culture study and the brains of transgenic and non-transgenic mice [200]. These conflicting results have recently been attributed to the hypothesis that the effects of COX-2 inhibitors may vary depending on the stage of AD development. Celecoxib is ineffective once the Aβ deposition begins. It may be detrimental due to its inhibitory effect on the chronically activated microglia, which may slow down Aβ clearance (38), accelerating the disease process [201]. Failure of selective COX-2 inhibitors to treat advanced stages of AD may be explained by the fact that the expression of the COX-2 enzyme in AD hippocampal tissue correlates with the disease severity, with increased immunoreactivity in early AD and decreased activity in advanced stages [155]. Our study revealed that the RIVA+Celeco group displayed significant suppression of COX-2 compared to the RIVA group. The concurrent administration of celecoxib with rivastigmine, therefore, can provide a further anti-inflammatory effect that could probably enhance rivastigmine anti-Alzheimer activity.

Persistently enhanced hippocampal GFAP immunoreactivity was detected in the RIVA, Celeco, and RIVA+Celeco groups. On the contrary, other studies showed that celecoxib had reduced the number of activated astrocytes and the percentage of GFAP immunostaining, thus attenuating LPS-induced brain inflammation in neonatal rats [23,202]. The variations in the model and treatment period may be to blame for the conflicting results.

Celecoxib has been shown to inhibit the enzyme AChE in a non-competitive manner, possibly due to its aromatic structural motives [203]. In agreement with a previous study [204], our study showed that celecoxib suppressed AChE activity previously enhanced in Al-intoxicated rats that ensure its mitigating effect in learning and memory deficit [205]. When combined with rivastigmine, celecoxib had no additional AChE inhibitory effect relative to rivastigmine alone. Other studies [203], on the other hand, found that the celecoxib AChE inhibitory effect is too weak to trigger major AChE inhibition and that it can be used as an additive inhibitor to a standard medication as rivastigmine.

Celecoxib attenuated the Al-induced cognitive deficit, which is in line with previous research [193,194]. Importantly, the Celeco group showed a decreased time to reach the platform, whereas increased step-through latency and time exploring novel objects implied enhanced spatial and retention memory in the AD rat model. Celecoxib did, however, impair memory acquisition and retrieval [206], probably mediated through late short-term celecoxib therapy, which increased Aβ-42 deposition. Importantly, our study found that the simultaneous administration of celecoxib with rivastigmine had no other mitigating impact on the memory and cognitive decline of the Al-intoxicated rats. Still, it did effectively attenuate Al-induced hippocampal
neurodegeneration compared to rivastigmine medication alone.

AD neuronal cell death can be attributed to apoptosis and DNA fragmentation [207–210]. Caspase-3 is considered the final executor of apoptosis [211] that mediates cytoskeletal and nuclear proteins [212]. Increased Aβ is likely to result in a higher likelihood of oligomer formation, intermediate assemblies, as soluble [213] as well as insoluble aggregates, that possess toxic effects [214–216] through several canonical apoptotic pathways, including caspase-3 [217]. Studies have revealed the apoptogenic role of AChE [218], which may lie behind the beneficial role of AChEIs in the early stages of AD [219]. Furthermore, PGE2 and PGF2α have been shown to promote cortical neuronal apoptosis and damage (135, 136) during the late stages [120].

Al is thought to cause cell death by triggering apoptotic pathways mediated by stress processes in the mitochondria or endoplasmic reticulum [220]. In Al-intoxicated rats, the protein level of activated caspase-3 was significantly increased relative to the Cont group, consistent with other studies [137,221–226]. The RIVA group displayed a significant reduction of the activated caspase-3 expression compared to the AL group, consistent with other research findings [227,228]. Similarly, Al-mediated caspase-3 overexpression was significantly attenuated by celecoxib administration, which agrees with previous studies that showed celecoxib's ability to inhibit the Aβ-induced apoptosis of neurons through inhibiting PGD2-induced apoptosis in AD [16]. Moreover, celecoxib was found to be neuroprotective against ethanol-induced neurodegeneration [229] and renoprotective against gentamicin-induced nephrotoxicity [230]. Caspase inhibition is recognized as a Cox-independent anti-inflammatory mechanism for NSAID drugs with a consequent decrease in cell death and pro-inflammatory cytokine production [231].

On the contrary, celecoxib has been studied to have an antitumor and chemo-sensitizing effect [232,233] through enhancing caspase-3 activity. The conflicting results may be related to the variability in the tissue type, nature of the experiment, the dose, and duration of administration. Celecoxib decreased Al-induced activated caspase-3 overexpression significantly when combined with rivastigmine compared to rivastigmine alone, indicating that celecoxib may improve rivastigmine anti-Alzheimer role.

Recent studies have suggested the implication of neurogenesis in neurodegenerative disorders [234–237]. Deficits in adult neurogenesis may contribute to tau hyperphosphorylation in new neurons, compromised hippocampal circuitry, and cognitive impairments in AD [238]. Therefore, through pharmacological and genetic approaches, induction of neurogenesis can slow down disease progression [239]. The neurogenesis process has been well acknowledged in two brain regions; the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus DG [234]. Several studies have shown that impaired neurogenesis within the hippocampus is linked to the development of AD [240,241]. The disturbance of the equilibrium between neurogenesis and neurotoxicity caused by COX-2 and PG expression hastens the development of AD [126]. Conversely, It has been reported that the proliferation of neuroprogenator stem cells (NPCs) is increased in the hippocampus of AD patients [242] early in the disease as a compensatory mechanism for Aβ-induced apoptosis [243]. Therefore, enhancing neurogenesis in the earliest stages of AD could provide a potentially powerful disease-modifying treatment strategy for AD [244].

Nestin, a type VI intermediate filament, is considered an important component of the
cytoskeleton and is thought to be a marker for neural stem cells; neurogenesis [245]. Our study showed a significant reduction of nestin immunoreactivity in the Al group, indicating impaired cellular proliferation compared to the Cont group that is consistent with other studies [246–248]. However, concomitant administration of rivastigmine increased nestin immunoreactivity, presumably, due to the compensation for the cholinergic deficits [249]. Similarly, other research results [250] showed enhanced expression of the brain nestin gene by 65.2% through rivastigmine administration relative to the untreated AD population. Similarly, celecoxib administration-induced nestin protein overexpression may represent a therapeutic option to restore adult neurogenesis in AD patients. Other studies, similarly, revealed celecoxib's ability to enhance neurogenesis and reduce apoptosis in APP/PS1 transgenic mice [16] and alleviate Aβ-reduced neurogenesis in transgenic Alzheimer mouse model [16]. Previous studies [251] showed that celecoxib increased the number of neural stem cells in the lesion zone in spontaneous intracerebral hemorrhage. When combined with rivastigmine, celecoxib significantly increased nestin expression in Al-intoxicated rats relative to rivastigmine alone, suggesting that celecoxib can boost neurogenesis and possibly enhance rivastigmine anti-Alzheimer activity.

**Conclusion**

AD is the primary cause of dementia in the middle-aged and elderly worldwide. Rivastigmine, the standard anti-Alzheimer drug, can only provide symptomatic relief and has a moderate disease-modifying effect. COX-2 overexpression has been related to the formation of senile amyloid plaques and subsequent neurodegeneration, highlighting the role of neuroinflammation in AD pathogenesis. Our study displayed that celecoxib, a selective COX-2 inhibitor, has attenuated cognitive deficits and improved memory performance in the AlCl3-induced Alzheimer's rat model. On the other hand, celecoxib was unable to boost rivastigmine anti-Alzheimer activity when given concurrently, despite being able to restore AD-disrupted neuronal turnover via suppressing apoptosis and enhancing neurogenesis relative to rivastigmine alone medication. More study is needed to reveal whether celecoxib, when given over a longer period or earlier in the course of the disease, may increase the efficacy of rivastigmine in treating Alzheimer's disease.

**Limitations of the study**

Our study has several limitations. First, we had better study other signaling mechanisms that may be implicated in celecoxib neuroprotective effect as peroxisome proliferator-activated receptor γ (PPARγ), γ-secretase enzyme, NF-κB, and mTOR. Second, to test its comparable efficacy, we should have a study group that receives celecoxib prophylactically in a rivastigmine-treated Alzheimer rat model.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Acknowledgments**

This work was supported by Assiut Medical School Grants Office (grant code 20171121002R1) Assiut University, Assiut, Egypt.

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