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Possible combined effect of perindopril and Azilsartan in an experimental model of dementia in rats



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ABSTRACT

Renin-angiotensin system exerted deleterious effects on learning and cognitive functions through different mechanisms. The present study has been designed to evaluate the protective effect of perindopril and azilsartan as monotherapy or in combination on aluminum chloride (AlCl₃) induced neurobehavioral and pathological changes in Alzheimeric rats. Male Wistar rats were divided into nine groups (n = 6); negative control, AlCl₃ treated, vehicle, AlCl₃ and Azilsartan (3.5 mg/kg, 7 mg/kg) co-treated, AlCl₃ and perindopril (0.5 mg/kg, 1 mg/kg) co-treated, AlCl₃ and (Azilsartan 3.5 mg/kg + perindopril 0.5 mg/kg), and AlCl₃ and (Azilsartan 7 mg/kg + perindopril 1 mg/kg), all groups were treated for consecutive 60 days. Then, memory function was evaluated by the Y- maze test. Amyloid Peptide – 42 (Aβ-42), Acetylcholinesterase (AChE), Malondialdehyde (MDA), Tumor necrosis factor (TNF-α) and Nitric Oxide (NO) levels in the hippocampus were assessed with (ELISA) kits. The histopathological studies of the hippocampal dentate gyrus (DG) and Cornu Ammonis-3 (CA3) were also performed. Oral administration of either azilsartan and perindopril alone or in combined for 60 days have shown; improvement of cognitive function, significant reduction in the hippocampal levels of Aβ-42, Acetylcholinesterase, Malondialdehyde (MDA), Tumor necrosis factor (TNF-α) and reserved most of histopathological changes in dentate gyrus (DG) and Cornu Ammonis-3 (CA3) that mediated by AlCl₃. Our behavioral, biochemical, and histopathological studies indicate that perindopril and azilsartan have neuroprotective effects on the AD model of rats induced by AlCl₃, suggesting that perindopril and azilsartan may be a candidate drugs for the treatment of AD.

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1. Introduction

Dementia is a broad term for multiple brain disorders caused by neurodegenerative and vascular diseases (WHO, 2018, Prince et al., 2013). It is characterized by gradual, progressive impairment in the cognitive and behavioral functions such as learning skills, daily social or living activities, language, and memory (Prince et al., 2013). In 2015 approximately 50 million people globally were diagnosed with dementia and this number is expected to rise

(Prince et al., 2016). Regionally, the prevalence of dementia in the kingdom of Saudi Arabia (KSA) is uncertain due to the paucity in the epidemiological studies (Brookmeyer et al., 2018). However, based on the Saudi Alzheimer's Disease Association there are about 130,000 cases of dementia in KSA.

Renin-Angiotensin System (RAS) is a hormonal system that has crucial regulatory and homeostatic actions through the body involving blood pressure and fluid balance; apart from peripheral functions, it has been also documented to be found in the central nervous system (CNS) and play an important role in various neurodegenerative diseases. In the RAS, both centrally and locally, the angiotensinogen is cleaved by renin enzyme to form angiotensin I (Ang I). After that, the Ang I is converted to angiotensin II (Ang II), a potent vasoconstrictor, by the action of the angiotensin-converting enzyme (ACE). Ang II has two receptors, including the angiotensin type 1 receptor (AT1R) and angiotensin type 2 receptor (AT2R) (Miller et al., 2019). Many studies have revealed that RAS exerted deleterious effects on learning and cognitive functions

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through different mechanisms including a deposition of amyloid β , aggregation of tau protein, inflicting of oxidative stress, neurotransmitters abnormalities, and neuroinflammation (Mogi et al., 2012). Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptors blockers (ARBs) are the main drug classes that interfering with RAS.

Besides the roles of ACEIs and ARBs on treating different cardiovascular diseases (CVS), Findings from many previous studies provide convincing evidence on the functions of ACEIs and ARBs in different body system rather than cardiovascular system, involving nervous system (O’Caoimh et al., 2014).

ACEIs can be classified according to their ability to cross the BBB. Captopril, Ramipril, and perindopril penetrate the BBB and are referred to as centrally acting ACEI (CACE-Is), while Imidapril, Lisinopril, and Enalapril do not and are regarded as non-CACE Is (Fazal et al., 2017). Treatment with CACE-Is are associated with a reduced incidence of dementia compared to those taking non-CACEIs (Solfrizzi et al., 2013). Also, CACEIs show slower rates of cognitive decline in patients with established AD compared with patients not currently prescribed CACEIs (O’Caoimh et al., 2014b). Also, the inhibition of RAS by ARBs (valsartan, losartan and candesartan) has been suggested to prevent the onset of AD by attenuate oligomerization of A β peptides and AT1-initiated oxidative stress. Also, ARBs restore Cerebral blood flow (CBF), Ach level, decreased AChE activity and MDA level (Gebre et al., 2018). It is worth mentioning that the impact of azilsartan on cognitive function has not yet been evaluated.

To date, the management of dementia is limited to offer symptomatic effects rather than curative, and there are only a few drugs in clinical use which comprised of choline esterase inhibitors (ChEIs) and N-methyl-d-aspartate receptor antagonist (NMDA), so there is an urgent requirement to find alternative approaches that conferring radical cure for such syndromes (Ulep et al., 2018).

Recently, the focus on ACE-Is and ARBs has been intensified to target dementia as well as other neurodegenerative disorders and reported promising results (Rygiel, 2016).

The present study was aimed to examine whether perindopril and azilsartan as monotherapy or in combination have a protective role against Aluminum chloride-induced neurotoxicity in rats. Moreover, possible underlying mechanisms were also studied.

2. Materials and methods

2.1. Statement

All procedures conducted according to the ethical guidelines of the medical ethics committee of King Abdul-Aziz University (KAU). The research ethics committee approved the animal Protocol with Approval number (306-18).

2.2. Chemicals and reagents

Perindopril and azilsartan were procured from Sigma Aldrich, (CO., Saint Louis, MO, USA). Sodium- Carboxymethyl cellulose (Na-CMC) was obtained from the faculty of pharmacy at King Abdulaziz University. Aluminum chloride ($AlCl_3$) was obtained from KFCMR at King Abdulaziz University. Rat ELISA kits for the determination of beta-amyloid Peptide - 42 (A β -42) and Nitric Oxide (NO) were bought from (SunLong Biotech Co. China). Tumor necrosis factor (TNF- α), Malondialdehyde (MDA) and Acetylcholinesterase (AChE) were purchased from (Elabscience Co. USA)

2.3. Drugs doses and preparations

Perindopril was prepared daily in sterile water and orally administered by gavage at doses of (0.5 and 1 mg/kg/day).

Azilsartan was freshly prepared in suspension form and given orally by gavage at doses (3.5 and 7 mg/kg/day). The doses of perindopril and azilsartan were obtained based on the human therapeutic dose using the formula of converting human dose to rat dose (Nair et al., 2016). $AlCl_3$ was daily prepared and dissolved in normal saline, and given at the dose of (100 mg/kg); the dose was selected based on previous reports (Lakshmi et al., 2015, Thenmozhi et al., 2015).

2.4. Animal and housing

Fifty- four adult male Wister rats, aged 6–8 weeks, weighing (180–220 g) were procured from the faculty of Pharmacy - King Saud University. Animals were housed in groups of six animals per cage and kept at a controlled environment at a temperature of 23.2 °C, 12:12-h light/dark cycle, relative humidity between 50% 70% and free access to water and food.

2.5. Experimental design

Fifty-four rats were randomly divided into nine groups (n = 6) as follows: Group I: rats treated with p.o saline and used as negative control, group II: rats treated with oral Na-CMC; vehicle group , group III: rats given $AlCl_3$ (100 mg/kg, p.o) which considered as positive control, group IV: as group III + Azilsartan dose 1 (3.5 mg/kg, p.o), group V: as group III + Azilsartan dose 2 (7 mg/kg, p.o), group VI: as group III + perindopril dose 1 (0.5 mg/kg, p. o), group VII: as group III + perindopril dose 2 (1 mg/kg, p.o), group VIII: as group III + (Azilsartan dose 1 + Perindopril dose 1) and group IX: as group III + (Azilsartan dose 2 + Perindopril dose 2). Saline, Na-CMC, $AlCl_3$, Azilsartan, and perindopril were administrated once daily for consecutive 60 days. Groups IV, V, VI, VII, VIII, and IX were pretreated with azilsartan and perindopril in three hours interval from the administration of $AlCl_3$.

At the end of the treatment period, the Y-Maze test was performed, and then all rats were deprived of food for 12 h then sacrificed by decapitation under humane conditions.

2.6. Assessment of cognitive function

2.6.1. Y-Maze test

Short-term memory was assessed by spontaneous alternation behavior (SAB) in the Y-maze task. The Y-maze used in the present study has consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when all four limbs of the rat were completely within the arm. Also, the maze was cleaned with alcohol-free disinfectant wipes between each trial. SAB was defined as the entry into all three arms on consecutive choices. The number of maximum SAB was calculated manually as the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) \times 100 (Kraeuter et al., 2019).

2.7. Tissues preparation and biochemical analyses

After the Y-maze task was finished, the whole brain of each rat was rapidly extracted, washed with isotonic saline, dried, and sagittally divided into two halves; the hippocampus of the left side was dissected and kept in -80 °C for homogenate preparation and the right-sided cerebral hemisphere for histopathological examination.

For preparing of hippocampus homogenate, tissue samples with ice-cold 0.3 M phosphate buffer (pH 7.4) were homogenized using a TissuLyser II homogenizer (Qiagen, Germany) at oscillation fre-

quencies (180 – 1800 oscillations per minute). The homogenates were then centrifuged at 15 000 rpm at 4 °C for 15 min using a refrigerated centrifuge. The clear supernatants obtained were used to estimate the hippocampal levels of; A β -42, NO, TNF- α , MDA and AchE and were determined by quantification ELISA kits following the company's recommended procedures (Konstantinou, 2017).

2.8. Histopathological examination

The second portion of each brain was fixed in phosphate buffer formalin (10%) for 24 h. The brains were processed and embedded in paraffin blocks. Sections (4 μ m thick) were stained with hematoxylin and eosin stains stain (Amin et al., 2013). Sections were examined and photographed using an Olympus light microscope (model: BX51TF- Japan). Photomicrograph was done at a magnification of (x4, x 200 and x600).

2.9. Data analysis

The experimental results were expressed as means \pm SEM. The intergroup variation between various groups were analyzed statistically using one-way analysis of variance (ANOVA) using Statistical Package for the Social Science software package (SPSS) version 23.0, followed by Tukey's post hoc test. Results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Effects of azilsartan and perindopril on the cognitive dysfunction

As shown in (Table 1) the existence of cognitive decline in rats was evidenced by a significant decrease ($p < 0.001$) of spontaneous alternation percentage (SAP) in the AlCl₃-treated group compared to the negative control and vehicle groups. Multiple comparisons of the present results showed a significant increase ($p < 0.001$) of SAP in each of perindopril, azilsartan either alone or in combination groups compared to the positive control group.

3.2. Effects of azilsartan and perindopril on the hippocampal level of A β -42

Table 2 illustrated that A β -42 was significantly high in the AlCl₃ treated group ($p < 0.001$) compared with negative and vehicle groups and this elevation was significantly mitigated ($p < 0.001$) in perindopril and azilsartan groups either alone or in combination compared to the positive control group. However, no significant difference of the A β -42 level was detected between perindopril and azilsartan groups either alone or in combination and control,

Table 1

Effects of azilsartan, perindopril and combined groups on Y-maze test in AlCl₃ -induced Alzheimer's disease in rats.

Groups	Spontaneous Alternation Percentage %
Negative Control	72 \pm 0.85*
Vehicle	72.16 \pm 0.87*
AlCl ₃ (100 mg/kg) - Positive control	45 \pm 0.68
Azilsartan 1 (3.5 mg/kg)	71.33 \pm 0.49*
Azilsartan 2 (7 mg/kg)	72.66 \pm 0.84*
Perindopril 1 (0.5 mg/kg)	73.16 \pm 0.70*
Perindopril 2 (1 mg/kg)	72.16 \pm 0.94*
Combined 1 (Azilsartan 1 + Perindopril 1)	69.83 \pm 0.60*
Combined 2 (Azilsartan 2 + Perindopril 2)	70.66 \pm 1.20**

Values are expressed as means \pm SEM, n = 6 rats.

* $P < 0.001$ compared with the corresponding AlCl₃ group values; by one way ANOVA and Tukey HSD post hoc test.

Table 2

Effects of azilsartan, perindopril and combined groups on the hippocampal level of A β -42 in AlCl₃ -induced Alzheimer's disease in rats.

Groups	A β -42 (pg/mg of protein)
Negative Control	74 \pm 2.04*
Vehicle	70.58 \pm 1.21*
AlCl ₃ (100 mg/kg) - Positive control	118 \pm 5.01
Azilsartan 1 (3.5 mg/kg)	70 \pm 2.30*
Azilsartan 2 (7 mg/kg)	72 \pm 1.12*
Perindopril 1 (0.5 mg/kg)	70 \pm 3.10*
Perindopril 2 (1 mg/kg)	68 \pm 2.33*
Combined 1 (Azilsartan 1 + Perindopril 1)	66.40 \pm 1.17*
Combined 2 (Azilsartan 2 + Perindopril 2)	63 \pm 1.94*

Values are expressed as means \pm SEM, n = 6 rats.

* $P < 0.001$ compared with the corresponding AlCl₃ group values; by one way ANOVA and Tukey HSD post hoc test.

vehicle groups ($p > 0.05$). While combined groups have shown the lowest reduction in the A β -42 level.

3.3. Effect of perindopril and azilsartan on the hippocampal level of AchE

As showed in Table 3, AlCl₃ correlated with a significant increase ($p < 0.05$) in the level of AchE versus negative control and vehicle groups. This overexpression of AchE level was significantly reduced ($p < 0.05$) in perindopril and azilsartan either monotherapy or in combination. However, no significant difference of the AchE level was observed between perindopril and azilsartan either monotherapy or in combination and control, vehicle groups ($p > 0.05$).

3.4. Effect of perindopril and azilsartan on hippocampal level of MDA

In the present work, as shown in Table 4, a significantly high level of MDA was detected ($p < 0.001$) in the AlCl₃ group versus negative control and vehicle groups. This elevation was significantly reserved ($p < 0.001$) in perindopril and azilsartan either alone or in combination compared with the positive control group. However, no significant difference of the MDA level was observed in perindopril and azilsartan either alone or in combination, negative control & vehicle groups ($p > 0.05$).

3.5. Effect of perindopril and azilsartan on the hippocampal level of TNF- α

Table 5 clarified that the level of the TNF- α was significantly elevated ($p < 0.001$) in the AlCl₃ group versus negative control and vehicle groups. This overexpression of the level of TNF- α was

Table 3

Effects of azilsartan, perindopril and combined groups on the hippocampal level of AchE in AlCl₃ -induced Alzheimer's disease in rats.

Groups	AchE (ng/mg of protein)
Negative Control	0.62 \pm 0.08*
Vehicle	0.66 \pm 0.01*
AlCl ₃ (100 mg/kg) - Positive control	1.39 \pm 0.14
Azilsartan 1 (3.5 mg/kg)	0.73 \pm 0.07*
Azilsartan 2 (7 mg/kg)	0.68 \pm 0.09*
Perindopril 1 (0.5 mg/kg)	0.72 \pm 0.06*
Perindopril 2 (1 mg/kg)	0.75 \pm 0.08*
Combined 1 (Azilsartan 1 + Perindopril 1)	0.80 \pm 0.07*
Combined 2 (Azilsartan 2 + Perindopril 2)	0.73 \pm 0.05*

Values are expressed as means \pm SEM, n = 6 rats.

* $P < 0.001$ compared with the corresponding AlCl₃ group values; by one way ANOVA and Tukey HSD post hoc test.

Table 4Effects of azilsartan, perindopril and combined groups on the hippocampal level of MDA in AlCl_3 -induced Alzheimer's disease in rats.

Group	MDA (ug/mg of protein)
Negative Control	1.08 ± 0.12*
Vehicle	1.11 ± 0.51*
AlCl_3 (100 mg/kg) - Positive control	2 ± 0.12
Azilsartan 1 (3.5 mg/kg)	1.17 ± 0.02**
Azilsartan 2 (7 mg/kg)	1.15 ± 0.04*
Perindopril 1 (0.5 mg/kg)	1.23 ± 0.10*
Perindopril 2 (1 mg/kg)	1.16 ± 0.03*
Combined 1 (Azilsartan 1 + Perindopril 1)	1.18 ± 0.07*
Combined 2 (Azilsartan 2 + Perindopril 2)	1.18 ± 0.03*

Values are expressed as means ± SEM, n = 6 rats.

* P < 0.001 compared with the corresponding AlCl_3 group values; by one way ANOVA and Tukey HSD post hoc test.**Table 5**Effects of azilsartan, perindopril and combined groups on the hippocampal level of TNF- α in AlCl_3 -induced Alzheimer's disease in rats.

Group	TNF- α (pg/mg of protein)
Negative Control	20.22 ± 0.66*
Vehicle	19.19 ± 1.19*
AlCl_3 (100 mg/kg) - Positive control	30.54 ± 1.7
Azilsartan 1 (3.5 mg/kg)	19.87 ± 1.2*
Azilsartan 2 (7 mg/kg)	21.21 ± 1.5*
Perindopril 1 (0.5 mg/kg)	18.33 ± 0.97*
Perindopril 2 (1 mg/kg)	16.45 ± 0.62*
Combined 1 (Azilsartan 1 + Perindopril 1)	20.01 ± 1.7*
Combined 2 (Azilsartan 2 + Perindopril 2)	21 ± 1.4*

Values are expressed as means ± SEM, n = 6 rats.

* P < 0.001 compared with the corresponding AlCl_3 group values; by one way ANOVA and Tukey HSD post hoc test.

significantly decreased ($p < 0.001$) in the perindopril and azilsartan as monotherapy or in combination compared with a positive control group. Also, post hoc analysis showed that perindopril groups (0.5 mg/kg and 1 mg/kg), produced the lowest reduction in the TNF- α level.

3.6. Effect of perindopril and azilsartan on hippocampal level of NO

Table 6 showed no significant change ($p > 0.05$) in the level of NO in the AlCl_3 group versus negative control and vehicle groups. Moreover, no significant group difference was observed ($p > 0.05$) in perindopril and azilsartan either alone or in combination groups versus the AlCl_3 -treated group.

3.7. Effect of azilsartan and perindopril on hippocampal histology

In the present work, two major components of the hippocampus were studied, dentate gyrus (DG) and CA3.

Table 6Effects of azilsartan, perindopril and combined groups on the hippocampal level of NO in AlCl_3 -induced Alzheimer's disease in rats.

Group	NO (μmol /mg of protein)
Negative Control	42.40 ± 1.03
Vehicle	41.66 ± 1.26
AlCl_3 (100 mg/kg) - Positive control	37 ± 1.06
Azilsartan 1 (3.5 mg/kg)	39.80 ± 2.13
Azilsartan 2 (7 mg/kg)	39.73 ± 1.51
Perindopril 1 (0.5 mg/kg)	34.43 ± 2.60
Perindopril 2 (1 mg/kg)	37.03 ± 3.74
Combined 1 (Azilsartan 1 + Perindopril 1)	30.46 ± 0.98
Combined 2 (Azilsartan 2 + Perindopril 2)	35.34 ± 0.83

Values are expressed as means ± SEM, n = 6 rats.

The intergroup variation between various groups was performed using one-way ANOVA.

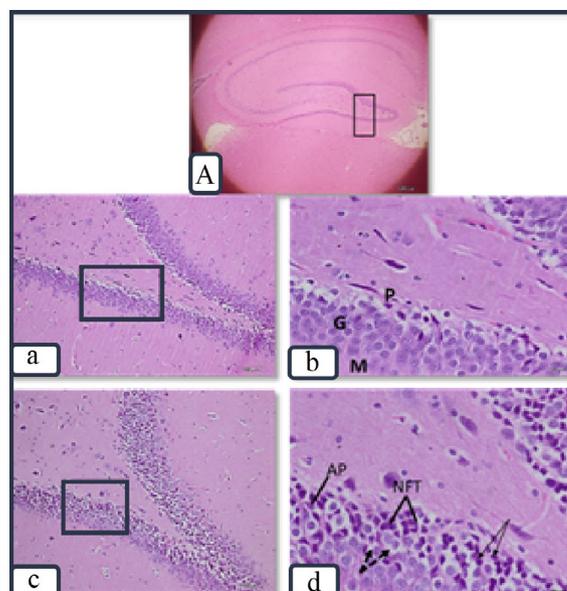


Fig. 1. Photomicrographs showing the hippocampus at the DG in the negative control group (a & b) with its layers; molecular (M) Granular (G) & Polymorphic (P). The granular cells are polyhedral with vesicular nuclei. In the positive control group (c & d), granular cells are vacuolated (dashed arrows), or shrunken with pyknotic nuclei (arrows). Neurofibrillary tangles (NFTs) and amyloid plaques (AP) are noted. Figure (A) shows low magnification of the hippocampus with DG marked ($\times 4$) (H&E stain. a & c $\times 200$) (b & d $\times 600$).

Examination of DG of the hippocampus region in the negative control group exhibited three layers; molecular, granular cell, and polymorphic layers. The polyhedral granular cell layer the major constituent and had vesicular nuclei as illustrated in (Fig. 1a, b). While, in the positive control group, granular cells were disorganized, with marked vacuolation. Others were shrunken with pyknotic nuclei. The pathological hallmarks of AD were also evident; neurofibrillary tangles (NFTs) and amyloid plaques (AP) as presented in (Fig. 1c, d).

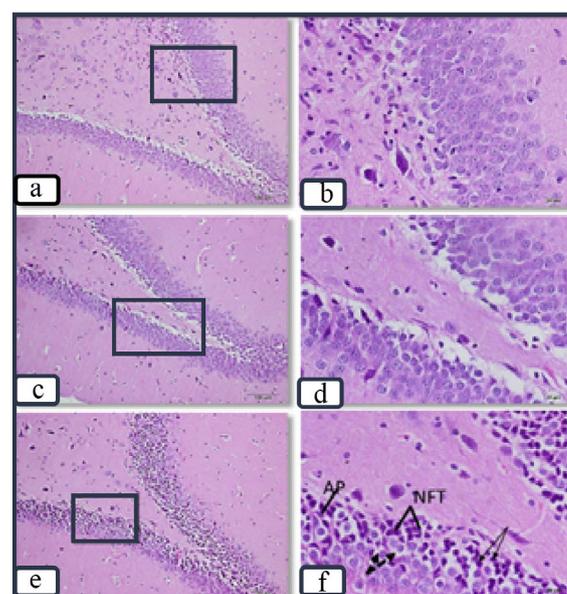


Fig. 2. Photomicrographs of sections of the hippocampus at DG showing a normal histological structure, and normal granular cells with vesicular nuclei in groups treated with Perindopril dose 1 (0.5 mg/kg) (a & b) and perindopril dose 2 (1 mg/kg) (c & d) compared to sections hippocampus from the positive control group (e&f). (H & E stain . a,c & e $\times 200$) (b,d & f $\times 600$).

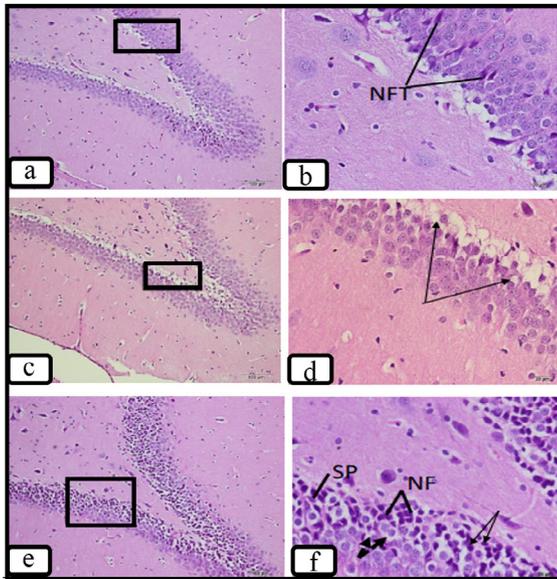


Fig. 3. Photomicrographs of sections of the hippocampus at DG showing few scattered NFTs in groups treated with azilsartan dose 1 (3.5 mg/kg) (a & b) and only vacuolated cells (arrows) in group treated with azilsartan dose 2 (7 mg/kg) (c & d) compared to sections hippocampus from the positive control group (e & f). (H&E stain. a, c & e $\times 200$) (b, d & f $\times 600$).

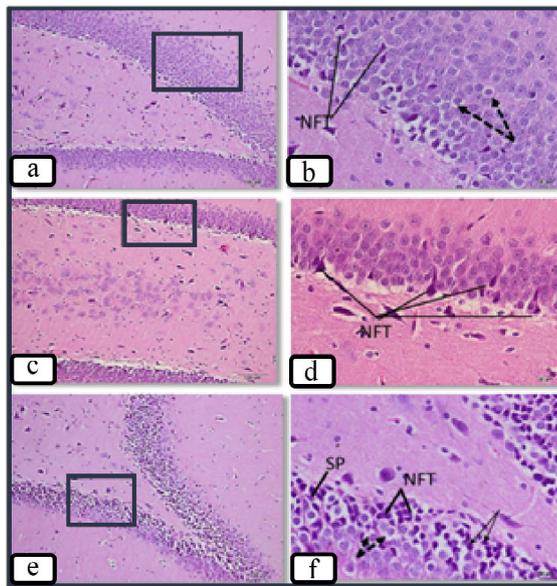


Fig. 4. Photomicrographs of sections of the hippocampus at DG showing scattered NFTs and vacuolated cells (dashed arrows) among the normal granular cells in combined Group 1 (azilsartan 3.5 mg/kg + perindopril 0.5 mg/kg) (a & b), on administrating (azilsartan 7 mg/kg + perindopril 1 mg/kg) in combined group 2 multiple NFTs were noted (c & d). This was in comparison to sections hippocampus from the positive control group (e & f). (H & E stain. a, c & e $\times 200$) (b, d & f $\times 600$).

In groups treated with perindopril doses 1 & 2 (0.5 mg/kg and 1 mg/kg, respectively), the granular cells retained normal criteria and normal vesicular nuclei were prominent as depicted (Fig. 2a, b, c, d) simultaneously, compared to the positive control (Fig. 2e, f). In treated groups with azilsartan dose 1 (3.5 mg/kg), most of the granular cells retained normal structure but still scattered NFTs were evident as shown in (Fig. 3a, b). While on administration of azilsartan dose 2, (7 mg/kg), vacuolated granular cells were only evident as observed in (Fig. 3c, d). Moreover, the perindopril and

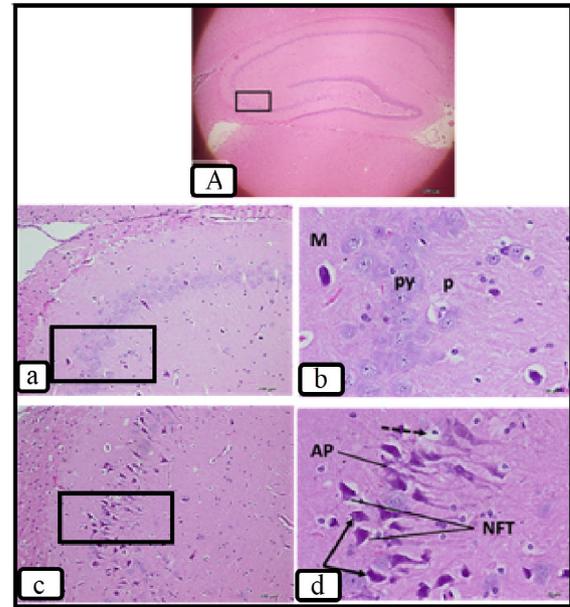


Fig. 5. Photomicrographs showing hippocampus at the CA3 in the negative control group (a & b) with its layers; molecular (M), pyramidal (py) & Polymorphic (p). The main polyhedral pyramidal cells show vesicular nuclei. In the positive control group (c & d), pyramidal cells are vacuolated (dashed arrows), or shrunken with pyknotic nuclei (arrows). NFTs and AP are noted. Figure (A) shows low magnification of the hippocampus with CA3 marked (H&E stain. a & c $\times 200$) (b & d $\times 600$).

azilsartan combined group retained most of the normal histopathological changes compared to the positive control group as illustrated in (Fig. 4a, b, c, d).

The CA3 part of the hippocampus in the negative control group was composed of 3 layers; polymorphic, pyramidal and molecular cell layers. The pyramidal cell layer packed in 4–5 layers, held the principal cell bodies. They have large vesicular nuclei and basophilic cytoplasm as showed in (Fig. 5a, b). While, in sections of the positive control group, the hippocampus at CA3 revealed the NFTs and AP. Some pyramidal cells were vacuolated while others were shrunken with pyknotic nuclei as shown in (Fig. 5c, d). Moreover,

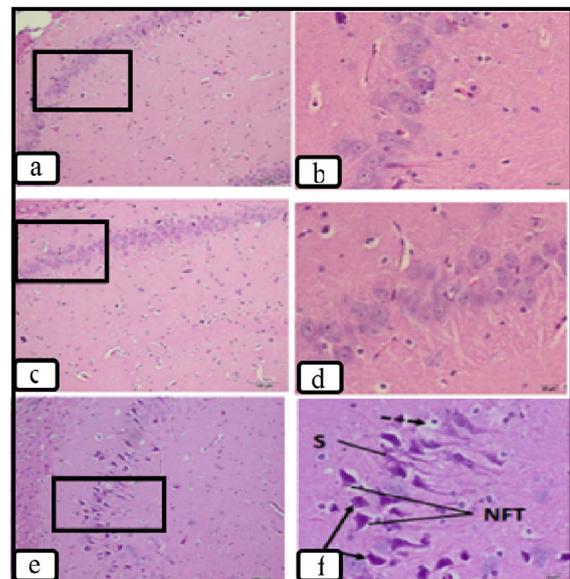


Fig. 6. Photomicrographs of sections of the hippocampus at CA3 showing a normal histological structure in groups treated with Perindopril dose 1 (0.5 mg/kg) (a & b) and perindopril dose 2 (1 mg/kg) (c & d) compared to sections hippocampus from the positive control group (e & f). (H & E stain. a, c & e $\times 200$) (b, d & f $\times 600$).

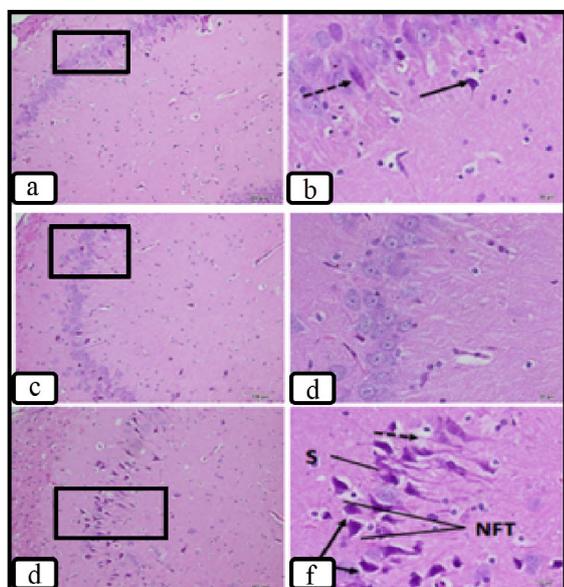


Fig. 7. Photomicrographs of sections of the hippocampus at CA3 showing few scattered NFTs in groups treated with azilsartan dose 1 (3.5 mg/kg) (a & b). While, in group treated with azilsartan dose 2 (7 mg/kg) (c & d) pyramidal cells retained vesicular nuclei compared to sections hippocampus from the positive control group (e & f). (H & E stain. a, c & e $\times 200$) (b, d & f $\times 600$).

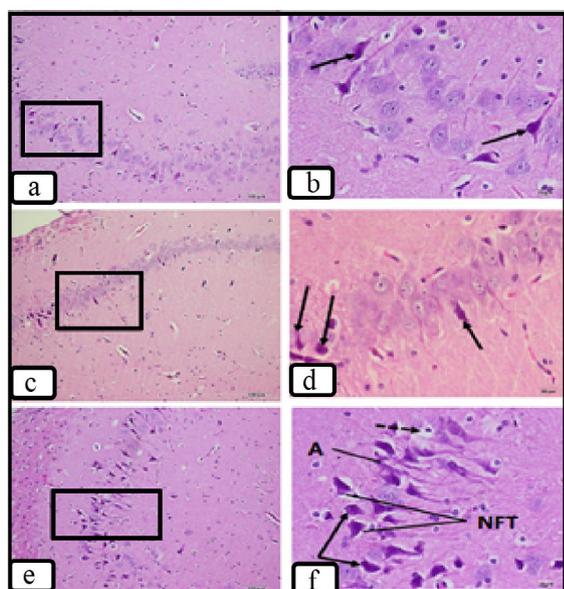


Fig. 8. Photomicrographs sections of the hippocampus at CA3 in the combined group 1 (azilsartan 3.5 mg/kg + perindopril 0.5 mg/kg) (a & b) and combined group 2 (azilsartan 7 mg/kg + perindopril 1 mg/kg) (c & d) showing few NFT (arrows) among normal pyramidal cells. This is in comparison to sections of hippocampus in the positive control group (e & f). (H&E stain. a, c & e $\times 200$) (b, d & f $\times 600$).

In groups treated with perindopril 1&2 (0.5 mg/kg and 1 mg/kg, respectively), the pyramidal cells were comparable to that of the negative control group as depicted in (Fig. 6a,b,c,d). Also, groups treated with azilsartan dose 1 (3.5 mg/kg) still showed scanty NFTs and SP (Fig. 7a, b) but those treated with azilsartan dose 2 (7 mg/kg) (Fig. 7c, d) almost retained the normal structure of pyramidal cells compared to positive control (Fig. 7e,f). Furthermore, perindopril and azilsartan combined group maintained most of the normal histopathological changes compared to the positive control group as depicted (Fig. 8a, b, c, d).

4. Discussion

The main findings of this work were as follows: (A) AlCl_3 produced significant cognitive impairment, with an increased hippocampal level of $\text{A}\beta$ -42, also, significant elevation of AChE, MDA, $\text{TNF-}\alpha$ level in the hippocampus, while no change was found on the hippocampal level of NO. (B) Chronic treatment with perindopril and azilsartan for 60 days produced a significant improvement of cognitive decline which was evaluated by the Y-Maze test. (C) Perindopril and azilsartan administration alone or in combination for 60 days significantly decreased each of $\text{A}\beta$ -42, AChE, MDA, $\text{TNF-}\alpha$ level in the hippocampus, with no change detected in the hippocampal NO level. It is worth noting that no significant change was obtained between either perindopril & azilsartan groups and combined groups. (D) Perindopril and azilsartan-received groups retained most of the histopathological changes in different regions of the hippocampus irrespective of doses compared to the AlCl_3 group.

In the current study, the existence of cognitive dysfunction in rats received AlCl_3 was confirmed by a significant decrease ($p < 0.001$) in spontaneous alteration percentage (SAP) compared to the negative control group. In agreement with the present study, Zghari et al. (2018), stated that SAP was significantly lowered in AlCl_3 -treated group versus the negative control group ($p < 0.001$).

Moreover, multiple comparisons of the present results showed a significant increase ($p < 0.001$) of SAP in each of perindopril, azilsartan either alone or in combination groups compared to the positive control group which suggested the essential role of perindopril and azilsartan in the improvement of hippocampus-dependent learning and memory. In accordance with the results of the present work, the previous study documented that perindopril was able to enhance memory and learning function in behavioral tests in colchicine-induced memory impairment in animals (Awasthi et al., 2012). In addition, perindopril exhibited an improvement in the Y-Maze through the increase in the SAP in the transgenic animal model of AD (Dong et al., 2011). Moreover, valsartan was reported to improve cognitive impairment in the animal models of AD evaluated by WMT (Yang et al., 2014).

The amyloid-beta deposition is one of the most important hallmarks of AD, in the present work, the level of $\text{A}\beta$ -42 in the hippocampus was significantly increased in the positive control group ($p < 0.001$) versus a negative control group. In agreement with the current finding, the previous study reported that AlCl_3 -induced oxidative stress and resulted in $\text{A}\beta$ formation and accumulation in the transgenic animal model of AD (Praticò et al., 2002). While in perindopril and azilsartan groups the level of $\text{A}\beta$ was significantly reduced ($p < 0.001$) compared to the positive control group. This goes hand with the previous report which documented that perindopril exerted an inhibitory effect on $\text{A}\beta$ deposition in lipopolysaccharide (LPS)-induced cognition impairment and amyloidogenesis in mice (Ali et al., 2016). In addition, Captopril provided a neuroprotective action via preventing amyloidogenic pathways of APP consequently, inhibited the deposition of $\text{A}\beta$ (AbdAlla et al., 2013). Furthermore, telmisartan has enhanced cognitive functions in STZ-induced AD in mice through decreasing the level $\text{A}\beta$ -42, APP and beta-secretase 1 (BACE1) (Du et al., 2014).

In AD patients, the cholinergic neurons severely degenerated and the activity of acetylcholine neurotransmitter is diminished, the present investigation showed significant AChE increase ($p < 0.001$) in the positive control group compared with the negative control group. In consistence with the current study, Auti et al. (2019), posited that chronic administration of AlCl_3 increases the activity of AChE. In contrast, in the present work, treatment with perindopril and azilsartan whether alone or in combination significantly decreased the level of AChE, this was supported by previous

research determined that perindopril improved cholinergic transmission by decreasing AChE level and consequently, enhancing Ach action in scopolamine-induced memory impairment in mice (Tota et al., 2012). In addition, lisinopril and telmisartan were found to reduce the activity of AChE in STZ-induced AD in mice and enhanced memory functions (Singh et al., 2013).

Oxidative stress has been hypothesized to be involved in the pathogenesis of many CNS diseases such as AD, and it has been associated with amyloidosis and tauopathy (Gandhi et al., 2012). In the present study, the MDA level was significantly enhanced ($p < 0.001$) in the $AlCl_3$ group compared to the negative control group, this finding was in agreement with previous literature which reported that the administration of $AlCl_3$ resulted in significant high level of MDA compared to the treated group (Gol et al., 2019). While perindopril and azilsartan groups whether alone or in combination the MDA significantly lowered compared to the positive group. This is in accordance with the previous study which documented that perindopril exhibited antioxidant effect by significantly increased the activities of SOD and GSH-Px, which decreased the MDA level in the mice model of AD induced (Yang et al., 2013). In addition, captopril administration in animals showed a significant decrease in MDA and increase in SOD and GSH (Bild et al., 2013). Furthermore, treatment with telmisartan in animals produced antioxidant effects via decreasing MDA, total oxidant status (TOS) (Erdi et al., 2016).

Several experimental and clinical evidence has reported the presence of neuroinflammation in the brain of AD due to the activation of microglia as well as other CNS immune cells (Kinney et al., 2018). In the present investigation, $TNF-\alpha$ was significantly elevated ($p < 0.001$) in the positive control group versus the negative control. Further confirmatory evidence was obtained by Tsunoda and Sharma (1999), who reported a significant increase in the mRNA expression of $TNF-\alpha$ in mice brains that exposed to $AlCl_3$. This was further supported by another researcher who documented that a higher level of $TNF-\alpha$ was observed after 10 weeks of exposure to $AlCl_3$ in drinking water in the brain of mice (Campbell et al., 2004). In the current work, the level of $TNF-\alpha$ significantly reduced in perindopril and azilsartan either alone or in combination compared to the positive control group, this finding suggested the crucial role of perindopril and azilsartan as anti-inflammatory agents. In accordance with the present data, Ali et al., (2016), have demonstrated that perindopril showed an anti-inflammatory effect by decreasing the level of $TNF-\alpha$ significantly in an animal model of AD. Moreover, Candesartan showed strong anti-inflammatory actions evidenced by attenuating the release of different proinflammatory markers in the brains of animal models (Goel et al., 2018).

In AD, the roles of NO are controversial either neuroprotective or neurotoxic (Balez et al., 2016). In the present work, the hippocampal level of NO was insignificantly changed ($p > 0.05$) in all treated groups versus positive control. This finding has consisted with a previous study reported that the level of NO was insignificantly changed after treatment with perindopril in scopolamine-induced memory impairment in mice (Tota et al., 2012).

Regarding the histopathological studies, two major components of the hippocampus were studied, dentate gyrus (DG) and cornu ammonis 3 (CA3). These areas in the hippocampus exert essential roles in learning, memory, and spatial coding (Jonas et al., 2014). In the present study, histopathological changes in different areas of the hippocampus including DG and CA3 regions were noted in the $AlCl_3$ group compared to the negative control group, which were indicated by the presence of disorganized granular and pyramidal cells with marked vacuolation. Others were shrunk with pyknotic nuclei. Moreover, the pathological hallmarks of AD were also evident; NFT and AP. These findings are consistent with previ-

ous research, Chronic administration of Al via drinking water produced injurious effects on pyramidal neurons manifested by cellular shrinking and disorganized pyramidal cells in the CA3 region of the hippocampus in rats (Liaquat et al., 2019, Sethi et al., 2008). While, treatment with perindopril and azilsartan retained most of the histopathological alternations induced by $AlCl_3$ and showed normal criteria and morphology, in accordance with present results, administration of Lisinopril and telmisartan reserved histopathological damage by decreasing $A\beta$ deposits in the brain of an animal model of AD induced by STZ (Singh et al., 2013). Treatment with captopril and valsartan mitigated histological changes in the C1A region of the hippocampus (Abbassi et al., 2016).

5. Conclusion

In the present study, either azilsartan and perindopril alone or in combined have shown significant ameliorative effects on different aspects of AD pathogenesis, producing improvement of cognitive function, significant reduction in the hippocampal levels of $A\beta$ -42 and AChE, antioxidant activity by decreasing the hippocampal level of MDA, anti-inflammatory effect evidenced by significant reduction in the level of $TNF-\alpha$ in the hippocampus and reserved most of histopathological changes that mediated by $AlCl_3$. It is worth mentioning that in this study we found no significant effect in the combination of perindopril and azilsartan compared to mono-therapy, also, different doses have been used from both perindopril and azilsartan produced insignificant effects (i.e. there effects were non-dose dependent). Lack of combined effect could be explained by the need for prolonged treatment since the AD is a chronic disease. Moreover, higher doses may be needed to detect the difference between combined and monotherapy if present.

5.1. Recommendations

Further evidence from well-designed and adequately powered clinical trials is required to establish the roles of perindopril and azilsartan in preventing or treating AD. Moreover, Due to the prevalence of CVD in the KSA which are considered to be established risk factors for AD, it is recommended that better control of these diseases could consequently lower or prevent AD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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