Original Article

The Effects of Combination Telmisartan with *Rosemarinus officinalis* on Alzheimer's Outcomes in Rat's Model of Alzheimer's Disease

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Abstract

Background: Till now there is no effective treatment for Alzheimer's disease. Studies nowadays are trying to find new strategies for treating this one of the most common causes for dementia. Previous studies indicated that telmisartan has antioxidant and anti-inflammatory effects that has good impact on the patients. Rosemary also known to have these effects. In this research, we have tried to know whether combination of these two elements has a greater effect than giving each element separately. **Aim:** To study the effects of combining Telmisartan with Rosemary on inflammatory and oxidative stress biomarkers, also the behavioral changes after Alzheimer's disease induction in rats by administration of Aluminum chloride. **Materials and Methods:** Thirty adult Wistar albino male rats, aged about 12 weeks' old weighing between 210 and 290 g were gathered and habituated in an animal house of College of Medicine, Babylon University. Rats were divided into six groups as follows: Group 1: control group, Group 2: ALCL3 (10 mg/kg), Group 3: Telmisartan + ALCL3 (10 mg/kg + 10 mg/kg). Group 4: Rosemary + ALCL3 (300 mg/kg + 10 mg/kg), Group 5: the combination group, Telmisartan + Rosemary + ALCL3 (10 mg/kg + 300 mg/kg + 10 mg/kg), and Group 6: the comparison group, Donepezil + ALCL3 (5 mg/kg + 10 mg/kg). After the rats' heads were severed, the brains were taken. Interlukin-6 (IL-6), Interlukin-1 (IL-1), glutathione (GSH), tumor necrosis factor (TNF), malondialdehyde (MDA), total antioxidant capacity (TAOC), and behavioral tests (that performed before the decapitation (Y-maze spontaneous alteration test, rotarod latency to fall) were estimated using brain tissue homogenate. **Results:** In Group 2, the levels of TAOC and GSH were significantly decreased. IL-1, IL-6, TNF-α, and MDA were increased significantly compared to Group 1. TAOC and GSH were increased significantly in Groups 3 and 4 with a significant reduction in IL-1, IL-6, TNF-α, and MDA compared to Group 2. In Groups 3 and 4, the latency to fall in rotarod and spontaneous alteration percentage (SAP) in Y-maze significantly increased compared to Group 2. In Group 5, the increase in TAOC and GSH and the decrease in IL-1, IL-6, TNF-α, and MDA were greater than that in other groups. Also fall in SAP and latency increased significantly. **Conclusions:** The agents donepezil, telmisartan, the herbal rosemary and the agents together (telmisartan+rosemary), all of them have been found to have anti-inflammatory and antioxidant capacity by decreasing pro-inflammatory parameters and increasing antioxidant biomarkers, also shown to enhance spatial memory and locomotor activity,so it probably can be used for Alzheimer's disease to alleviate the symptoms.

Keywords: Aluminum chloride, Alzheimer's disease, combination, donepezil, oxidative stress, telmisartan

Introduction

The majority of dementia cases in adults aged 65 and older are caused by Alzheimer's disease, which accounts for at least two-thirds of all cases. Alzheimer's disease is a neurological illness that is characterized by a gradual onset and continuous decline in mental and behavioral abilities. Currently, there is no known treatment for Alzheimer's disease. However, there are a number of therapies that can help to reduce some of the condition's symptoms depending on the stage a person is in. Alzheimer's disease is progressive and gradual onset disease. Basically it is a neurodegenerative disease that

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is caused by neuronal cell death. Usually it starts in the entorhinal cortex of the hippocampus and then spreads to other regions. The exact mechanism of Alzheimer's disease is still not clear, but the accumulation of aberrant neurofibrillary tangles and neurotic plaques is what distinguishes Alzheimer's disease. Several theories include the most accepted ones which are plaques formation and tangles formation. Amyloid is deposited around meningeal, cerebral, and grey matter arteries. Plaques are multifocal formations made up of interconnected grey matter deposits. Other theory includes the neurofibrillary tangles formation, Because of the buildup of extracellular amyloid peptide, which results in the aggregation of tau aggregates, there will be hyperphosphorylation of tau protein in Alzheimer's disease. Oxidative stress and neuroinflammation have major roles in the development of Alzheimer's disease. Although reactive oxygen species (ROS) are essential for metabolism, cell communication, and the proper development of learning and memory processes in a healthy state,[1] too much of them can cause oxidative stress.[2] NADPH-oxidase (NOX) produces ROS as a primary byproduct, and numerous other enzymatic activities, such as those involving xanthine oxidase, cyclooxygenases, uncoupled NOS, and the mitochondrial electron transport chain, also produce ROS as secondary byproducts.[3] In addition, accumulating ROS can damage mitochondrial structure, reduce adenosine triphosphate synthesis, and result in the creation of even more ROS from the mitochondria. It is well established that mitochondrial-derived ROS may cause cellular malfunction by making their way to the cytoplasm, exhibiting further negative effects. The majority of nitrogen oxide (NOX)-derived ROS in the brain is contributed by microglial cells. NOX2 is the main producer of extracellular ROS in microglia among the NOX isoforms.[4] The activation, proliferation, and release of pro-inflammatory signals by microglia are likewise impacted by NOX-derived ROS intracellular signaling pathways. Through the NF-k pathway, Rho-kinase activation promotes AT1R expression in microglial cells. Microglial RhoA/Rho kinase pathway activation, a crucial regulator of the actin cytoskeleton, mediates microglial polarization and neurodegeneration.[5] ARBs can stop BBB deterioration and lessen the influx of inflammatory mediators seen in many neurodegenerative diseases such as Alzheimer's disease (AD).[6] Previous studies indicated that telmisartan has antioxidant and antiinflammatory effects that has good impact on the patients.[7] Rosemary also known to have these effects.[8] However, recent studies have shown PPAR-modulation to have neuroprotective characteristics that are independent of AT1 blockage. Telmisartan, for instance, protected against the neurotoxicity induced by high glutamate concentrations and prevented apoptosis in neuronal cells exposed to starvation. Telmisartan also lessens inflammatory processes in astrocytes, microglia, and cerebrovascular endothelial cells, which increases neuroprotection in various conditions,[9] which is a promising treatment for amyloidogenesis and cognitive decline,[10] and has a protective effect against cuprizone-induced demyelination and behavioral dysfunction in mice.[11] Additionally, it can function as a potential neuroprotective drug shielding individuals with vascular dementia from cognitive decline.[12] On the other hand, positive outcomes have been seen in numerous clinical investigations including the use of rosemary essential oil for memory loss and, in particular, Alzheimer's disease. Sadly, a large number of these investigations omit to specify which specific chemotype of rosemary essential oil was utilized. Its actions on the disease include the anti-inflammatory, direct, and indirect antioxidant actions. The majority of the brain's resident innate immune inflammatory cells are called microglial cells, and when activated, they can release proinflammatory cytokines such as IL-1, IL-6, and TNF-α. CA was discovered to block mouse microglia's LPS-induced activation, hence reducing the release of inflammatory cytokines including IL-1β and IL-6 in an *in vitro* model of brain infection. Additionally, it has been claimed that human AD brains exhibit an upregulation of microglial-mediated inflammation. The therapeutic potential of CA for a number of neurodegenerative illnesses, including AD, has been proposed based on several inflammatory models studies.[13] Oxidation processes and the defense of cells against oxidative cell death have been used to demonstrate the antioxidant effects of CA. For instance, it is believed that oxidative damage via ROS causes the catechol form of CA to change into an ortho-quinone. In addition to these direct antioxidant mechanisms, CA also has considerable indirect antioxidant actions, which are primarily transcriptional in nature. The activation of phase 2 antioxidant enzymes is a crucial aspect of the cell's defense against the oxidative stress brought on by these chemicals.[14]

Materials and Methods

Animals

Thirty male adult albino rats weighing between 210 and 290 g were kept. These rats were housed in an environment with 14-hour light–dark cycles, a typical commercial feed, and water, and at a temperature of 25°C with a relative humidity of 60%–65%.

Chemicals and reagents

Aluminum chloride was obtained THOMAS BAKER LTD Company (UK). Telmisartan was purchased from Boehringer Ingelheim Pharmaceuticals (USA), phosphate buffer saline was from HiMedia (Mumbai, Maharashtra, India), and formaldehyde was AL-Jubail (Saudia Arabia). Donepezil was obtained from Pfizer (New York, NY, USA), and rosemary liquid extract was obtained from HAWAII PHARM LLC (USA). ELISA kits rat IL-1, rat

IL-6, and rat TNF-α, used sandwich -ELISA principle. Lipid peroxidation kits were purchased from Bilişim Destek Hizmetleri (Turkey) obtained by measuring aldehyde breakdown products of lipid peroxidation.

The kits that used for biochemical analysis in this study, were from Elabscience biotechnology (USA).

Study design and experimental protocols

Animals

The selected rats were divided randomly into six groups with five rats in each group as follows.

Group 1: the control group; in this group the rats exposed to the same condition and each rats received i.p, normal saline only for 2 months. Group 2: the rats received i.p ALCL3 10 mg/kg daily for 2 months. Group 3: the rats received Telmisartan (10 mg/kg) orally for 2 months with i.p ALCL3. Group 4: Rosemary group, the rats received oral liquid extract of rosemary (300 mg/kg) daily for 2 months along with i.p ALCL3. Group 5 (the combination group) oral telmisartan (10 mg/kg + p.o 300 mg/kg rosemary) for 2 months with i.p ALCL3, and Group 6: the rats received Donepezil as the FDA approved drug of Alzheimer's disease to make comparison with Telmisartan results. The dose was 5 mg/kg orally daily for 2 months along with i.p ALCL3.

Behavioral evaluation by Y-maze spontaneous alteration test and Rotarod apparatus

The Y-maze was used to test rats' spatial memory, and the spontaneous alteration percentage (SAP) was calculated using the following equation:

SAP % = [(number of alterations)/(total arm entries – 2)] × 100.

Also rats are placed on a horizontal rod that rotates along its length in the rotarod device, which is used to assess rodents' balance and motor coordination. The rat has to move forward in order to keep itself upright and avoid falling off, and the latency to fall will be calculated in seconds.

Preparation of sample and decapitation of rat brain

Isolation of the brain

The animals were decapitated after the experiment's 60-day period and the conclusion of the behavioral tests. Four rats from each group had their hippocampi dissected after their skulls had been dissected, and the brains had been delicately removed from the rat skulls. Phosphate buffer solution was used to rinse the hippocampi. Then, these samples were frozen at –20°C. A phosphate buffer with the same pH was also used to homogenize the hippocampi (10%, w/v). The entire brain of one randomly chosen rat from each group was also fixed in 10% formalin before being sent for histopathological analysis.

Statistical analysis

The mean value along with the standard deviation (SD) were used to illustrate the results. SPSS version 1.0.0.1406 (IBM, Chicago, IL, USA) was used for the statistical analysis, which used either a one-way or two-way ANOVA. Dennett's adjustment was used for multiple comparisons. At $P < 0.05$, differences were deemed statistically significant.

Ethical approval

According to document number 4-3, the publishing ethics committee granted approval for the study at the College of Medicine, University of Babylon, Iraq, on July 6, 2022.

Results

Oxidative stress biomarker

GSH

Aluminium chloride, telmisartan, rosemary, combination and donepezil on glutathione level (µg/mL), after 2 months daily administration as shown in Figure 1.

MDA biomarker

The effects of daily administered ALCL3, Telmisartan, Rosemary, combination and Donepezil on MDA level (µmol/L) for 2 months in rat's brain as explain in Figure 2.

T-AOC biomarker

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil, on T-AOC level (µmol/g) in rat's brain after daily administration for 2 months as explain in Figure 3.

Inflammation biomarkers

TNF-α biomarker

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil on TNF-α level (µg/L) in rat's brain after daily administration for 2 months as explain in Figure 4.

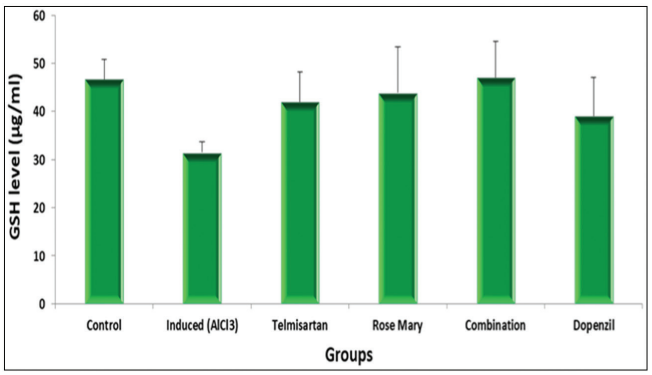

Figure 1: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3, on GSH level (µg/mL) in rat's brain. Results ($n = 5$) have been stated as the average (±SD)

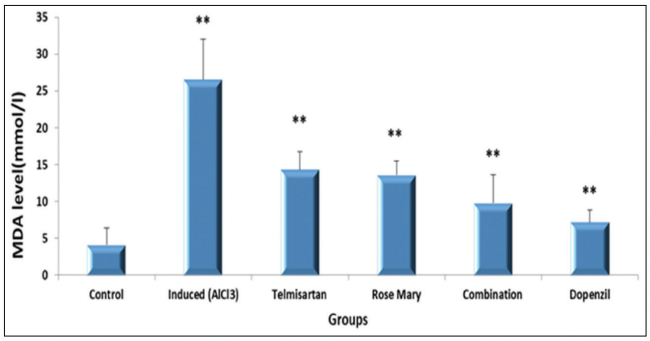

Figure 2: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3, on MDA level (μmol/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD), (*$P < 0.05$), (**$p < 0.001$)

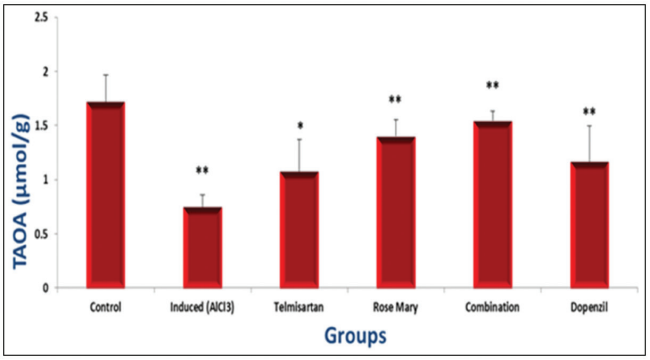

Figure 3: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3, on TAO-C level (μmol/g) in rat's brain. Results ($n = 5$) have been stated as the average (±SD) (*$P < 0.05$), (**$P < 0.001$)

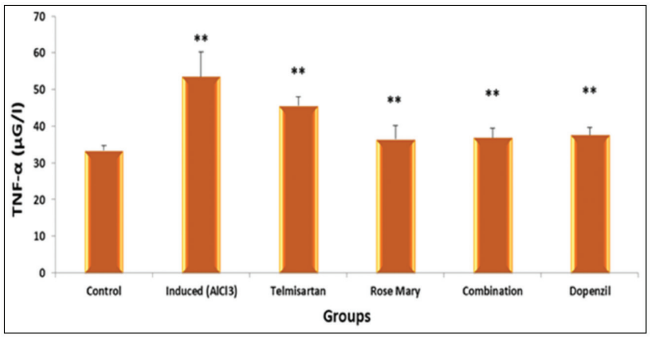

Figure 4: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3 on TNF-α level (μg/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$)

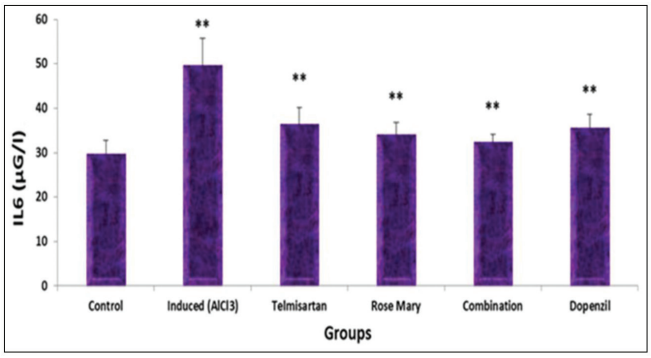

Figure 5: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3 on IL-6 level (μg/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$)

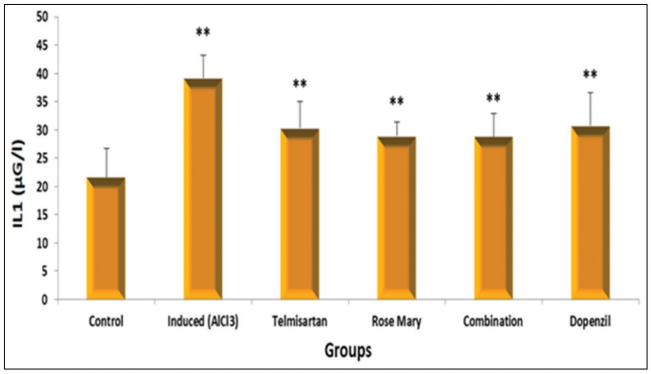

Figure 6: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3 on IL-1 level (μg/L) in rat's brain. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$)

IL-6 biomarker

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil on IL-6 level (µg/L) in rat's brain after daily administration for 2 months as explain in Figure 5.

IL-1 biomarker

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil on IL-1 level (µg/L) in rat's brain after daily administration for 2 months as explain in Figure 6.

Behavioral tests results

Rotarod latency

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil, on latency to fall in rotarod apparatus (in seconds) after daily administration for 2 months as explain in Figure 7.

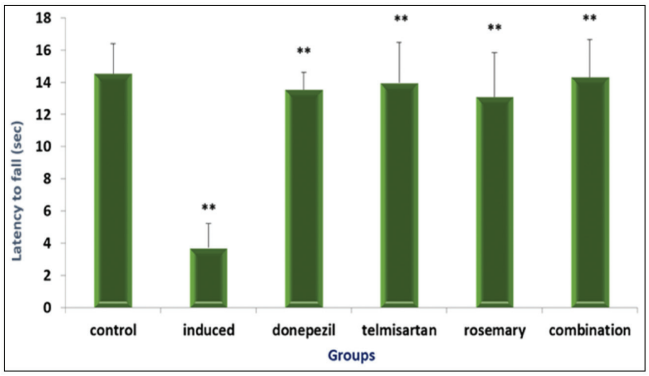

Figure 7: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3, on latency (secs) in rat's brain. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$)

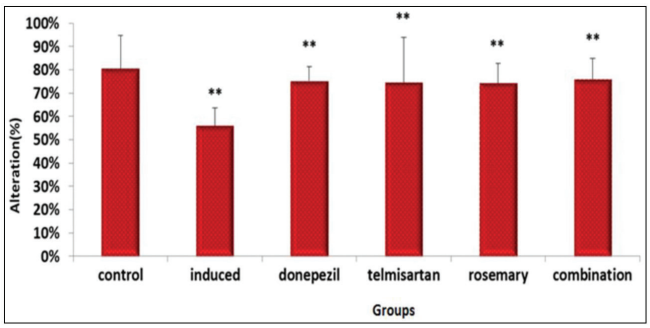

Figure 8: Effects of ALCL3 i.p (10 mg/kg), p.o TEL (10 mg/kg) + i.p ALCL3. p.o Rosemary (300 mg/kg) + i.p ALCL3, combination (telmisartan 10 mg/kg + rosemary 300 mg/kg) p.o + i.p ALCL3, Donepezil (5 mg/kg) p.o + i.p ALCL3 on SAP. Results ($n = 5$) have been stated as the average (±SD). (*$P < 0.05$), (**$P < 0.001$)

SAP

Effects of ALCL3, Telmisartan, Rosemary, combination, Donepezil on SAP in Y-maze after daily administration for 2 months as explain in Figure 8.

Discussion

The combination group effect on behavioral parameters and biomarkers

In this research, we focused on the combination group, that is, the rats group that received both telmisartan doses with rosemary liquid extract at the same time. We have noticed that all biomarkers including the behavioral and biochemical types were better than when we gave each agent alone. In rotarod test that gave an idea about the motor activity and coordination, the results were much better than the groups of telmisartan and rosemary individually. The average of seconds that spent on the apparatus indicated the latency time was about 40.598 s as compared with telmisartan group which is about 38.312 s and rosemary group about 7.266 s. It was very near to donepezil group result which is about 47.926 s. The SAP result has showed that, combination group has better effect than the separated groups (telmisartan and rosemary) alone. SAP test indicate the spatial memory in rats that is affected by the neuro degeneration. We have found that SAP values for combination group were better than the results of each group alone. It was about 76%. The result of telmisartan alone SAP was about 74.60% and for rosemary liquid extract SAP was 74%. That indicated the better enhancement in cognitive functions in combination group. In the biochemical analysis, the proinflammatory cytokines' values also indicated the preference for the combination group. Several previous studies have indicated that rosemary diterpenoids components such as carnosol and carnosic acid will have anti-inflammatory and antioxidant characteristics. They interfere and inhibit the important inflammation pathways. The chemical components in rosemary herbal have the ability to suppress NF-kB pathway which control the inflammation process, also these components suppress proinflammatory cytokines like IL-1b and TNF-α.[15] The property in which rosemary sharing with telmisartan is that rosemary also has shown to have agonist activity on PPAR-y, the ligand-activated transcription factor, a member of nuclear receptor superfamily that control the cytokines-mediated signal transduction and production. The control of this transcription factor is caused by cross talking with other transcription factors by specific mechanism, so the production of proinflammatory cytokines will be negatively effected and the actions of them will be prevented. CNS, which is the main part, that is effected in Alzheimer's disease, will be protected from the harmful effects of these cytokines and the neurodegeneration will be decreased to some level; telmisartan was the most potent angiotensin receptor blocker in this property of partial agonist activity toward PPARy.[16] As we mentioned before, we have found that both agents can work on this point and their positive action will be duplicated in most biochemical test results also in behavioral results and that supports the main thought of this research.

Conclusions

This study indicated that the co-administration of telmisartan with rosemary liquid extract at the same time has greater antioxidant and anti-inflammatory effects than the usage of only one of them, via the decreasing of oxidative stress biomarkers and inflammatory parameters (GSH, TAOC, MDA, TNFα, IL-1, IL-6) that induced by using aluminum chloride. Telmisartan and *Rosemarinus officinalis* together at the same time improved the locomotor activity and the spatial memory of rats.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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