



## OPEN Cilazapril and benazepril mitigate neurodegeneration and $\alpha$ -synuclein accumulation in a cellular model of parkinson's disease

Oznur Altunlu<sup>1</sup>✉, Ahmet Hacimuftuoglu<sup>1</sup>✉, Irmak Ferah Okkay<sup>1</sup>✉, Fatma Yesilyurt<sup>1</sup>, Mehmet Ali Yoruk<sup>2</sup> & Mustafa Ozkaraca<sup>3</sup>

Parkinson's disease (PD) is characterised by progressive neuronal degeneration and oxidative stress, both of which significantly contribute to its pathology and clinical symptoms. This study investigated the antioxidant, anti-inflammatory and neuroprotective effects of cilazapril and benazepril in an in vitro PD model induced by 6-hydroxydopamine (6-OHDA) in SH-SY5Y neuroblastoma cells. We performed molecular docking of these compounds to dopamine-related receptors (AT1, AT2, D1A, D1B and D2), as well as dynamic simulations. Morphological analysis revealed structural preservation in treated cells, and key biomarkers (MTT, TAS-TOS, IL-10, IL-1 $\beta$ , TNF- $\alpha$ , LDH, MDA, SOD and TGF- $\beta$ ) were evaluated using ELISA. The gene expression of the D1 and D2 receptors was measured using real-time PCR, and the expression of  $\alpha$ -synuclein and neuronal nitric oxide synthase was analysed using immunohistochemistry. Both drugs significantly reduced oxidative damage,  $\alpha$ -synuclein aggregation and neuroinflammation ( $*p < 0.05$ ). They modulated genetic pathways involved in dopaminergic signalling and showed high binding affinity for the AT1, AT2, D1A, D1B and D2 receptors. These findings suggest that cilazapril and benazepril exert multi-targeted protective effects that extend beyond simple antioxidant activity, and that they may be effective in treating or mitigating PD-related neurodegeneration.

**Keywords** Parkinson's disease, Cilazapril, Benazepril, Oxidative stress, Neurodegeneration, Neuroinflammation

Parkinson's disease (PD) ranks as the second most widespread neurodegenerative disorder globally, after Alzheimer's disease. Approximately 5–10% of PD cases are estimated to stem from genetic factors, which are typically correlated with advancing age. Although the prevalence of this condition is approximately 0.3% within the general population, this incidence increases to between 1% and 3% among individuals aged 65 years and older<sup>1</sup>. The motor manifestations of PD include tremors, bradykinesia, muscular rigidity, postural instability, and speech difficulties. The nonmotor manifestations include depression, dementia, and disturbances in sleep patterns<sup>2</sup>.

From a pathological perspective, cytoplasmic inclusions referred to as Lewy bodies and the degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNc) are hallmark features of the disorder. Nonetheless, the misfolding and subsequent aggregation of the protein  $\alpha$ -synuclein are instrumental in neurodegeneration in PD. Furthermore, pathophysiological mechanisms, including mitochondrial dysfunction, perturbations in protein homeostasis—most notably within the ubiquitin-proteasome and autophagy-lysosomal systems—neuroinflammation, and oxidative stress contribute to cellular impairment and promote neurodegeneration<sup>3</sup>.

Current therapeutic approaches for PD focus primarily on enhancing striatal dopaminergic neurotransmission<sup>4</sup>. The pharmacological agents employed in such treatment include levodopa (L-3,4-dihydroxyphenylalanine, L-DOPA), dopamine (DA) agonists, monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT)

<sup>1</sup>Department of Medical Pharmacology, Faculty of Medicine, Ataturk University, Erzurum 25100, Turkey.

<sup>2</sup>Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey. <sup>3</sup>Department of Pathology, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Turkey. ✉email: oznur.altunlu@atauni.edu.tr; hacimuftuoglu@gmail.com; irmakferah@atauni.edu.tr

inhibitors, and NMDA (N-methyl-D-aspartate) antagonists. However, these treatment modalities alleviate only the symptoms of the disease and do not prevent the progression of PD<sup>2,5</sup>. Typically, patients require levodopa treatment within the first few years after the onset of symptoms. However, the long-term use of levodopa has been shown to result in significant limitations to the treatment process, with an increased risk of motor complications<sup>3,4,6</sup>. One such complication is the ‘On-Off’ phenomenon, which manifests after approximately five years of levodopa treatment. This phenomenon, caused by fluctuations in dopamine levels in the striatum, can manifest as sudden cycles of mobility and immobility<sup>7</sup>. In recent years, neuroprotective therapies have been developed to slow or prevent pathological processes by targeting neurodegenerative mechanisms<sup>8</sup>. Additionally, antioxidant agents can curtail oxidative stress-induced cellular deterioration by preserving redox homeostasis within the central nervous system. The encouraging potential of agents with antioxidant and neuroprotective properties to prevent neurodegenerative processes in PD makes this field an attractive target for future therapeutic research<sup>5</sup>.

The SH-SY5Y cell line is recognized as a valuable model for investigating the molecular underpinnings of PD, as it presents characteristics reminiscent of those of dopaminergic neurons<sup>9</sup>. 6-Hydroxydopamine (6-OHDA) has been demonstrated to induce profound mitochondrial fission, a phenomenon that is particularly evident in SH-SY5Y cell cultures, providing a robust model for PD pathophysiology research. The success of established PD experimental models and studies with therapeutic agents has been supported by various biochemical, immunohistochemical, and molecular analyses, particularly in *in vivo* settings<sup>10</sup>. Behavioral tests, which are especially important in *in vivo* studies, provide important contributions to the analysis of the results obtained.

Cilazapril is recognized as a highly effective and enduring ACE inhibitor<sup>11</sup>. It has been postulated that ACE inhibitors promote mitochondrial KATP channel activation by preventing bradykinin degradation<sup>12</sup>. This proposed mechanism has been suggested to confer neuroprotective benefits.

Furthermore, the antioxidant characteristics inherent to these pharmacological agents significantly contribute to their neuroprotective capabilities<sup>13</sup>. Benazepril-HCl has demonstrated neuroprotective properties in scenarios involving intracerebral hemorrhage, excitotoxicity, and cognitive impairments associated with diabetes. Moreover, its antioxidant effects have been shown to be beneficial in the contexts of melanoma, renal ischemia reperfusion injury, and pulmonary hypertension<sup>14</sup>.

The current investigation sought to assess the neuroprotective and antioxidant effects of cilazapril and benazepril on neurodegeneration and oxidative stress phenomena, which are critical to the pathophysiology of PD. By employing an *in vitro* model of PD induced by 6-OHDA in the SH-SY5Y neuroblastoma cell line, this research aims to advance the understanding of the underlying mechanisms of PD and to clarify the therapeutic potential of these pharmacological compounds.

## Materials and methods

Cilazapril (1 mg tablet) and benazepril (10 mg tablet) were procured from a pharmacy in Erzurum, Turkey. SH-SY5Y cells were obtained from the Vaccine Development Application and Research Centre, Department of Medical Pharmacology, Atatürk University (Turkey). Dimethyl sulfoxide (DMSO) was purchased from Merck; 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Thermo and fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and 6-OHDA from Sigma Aldrich Pvt. Ltd. (purchased from India). This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Atatürk University (24.02.2022/B30).

### SH-SY5Y cell culture

The cellular suspension was procured through centrifugation at a rotational speed of 1200 revolutions per minute for 5 min. The cells were subsequently resuspended in novel DMEM/F-12, which contained 10% fetal bovine serum and a 1% antibiotic solution comprising penicillin, streptomycin, and amphotericin B. The cell suspension was subsequently transferred into 25 cm<sup>2</sup> cell culture flasks (Corning, USA) and incubated at 37 °C in an environment enriched with 5% carbon dioxide. Upon reaching a confluence of 75–80% on the flask surface, the cells were detached from the surface via a trypsin-EDTA solution (comprising 0.25% trypsin and 0.02% EDTA) and subsequently subjected to centrifugation. The resulting pellet was carefully removed, and the cell suspension was allocated into 96-well tissue culture plates at a density of 100 µL per well, which equated to approximately 10,000 cells per well.

### Parkinson's model generation with 6-OHDA

After reaching a density of 80% SH-SY5Y cells in the cell culture plates, the experimental design was conducted, and the concentrations of the substances to be utilized in the study were ascertained. The doses of cilazapril (0.32, 0.64, 1.28, 2.56, and 5.12 µM) and benazepril (0.04, 0.08, 0.16, 0.32, and 0.64 µM) were adjusted and administered to the wells. To establish the *in vitro* experimental Parkinsonian model, 200 µM 6-OHDA was applied to the indicated groups, and the cells were incubated at 37 °C with 5% CO<sub>2</sub>. A total of eight replicates were conducted for each concentration. The cells were then incubated for a period of 24 h.

### Molecular docking analysis

The 3D structures of the cilazapril (DB01340) and benazepril (DB00542) molecules were selected as ligands for molecular docking studies and downloaded from the DrugBank database (Version 5.1.10, <https://go.drugbank.com>). The target receptors were identified as D1-A (PDB ID: 7JVP), D1-B (PDB ID: 8IRV) and D2 (PDB ID: 5AER) and were obtained from the RCSB Protein Data Bank (Version 2024.10, <https://www.rcsb.org>). After this, hydrogen atoms were removed from the receptor structures, polar groups were added, and protein-ligand interactions were optimized via the Collman charge tab. The missing atoms were completed, and the receptors were ready for docking. The target proteins were then subjected to a cleaning process, with nonprotein-related

structures removed via BIOVIA Discovery Studio Visualizer software (Version 2021, Dassault Systèmes, <https://discover.3ds.com/discovery-studio-visualizer-download>). Ligand docking was performed with AutoDock Tools (ADT, Version 1.5.7, <https://autodock.scripps.edu>) software. Notably, all the molecules were close to rotation for rigid docking. The active site was defined via the UCSF Chimera (Version 1.16, <https://www.cgl.ucsf.edu/chimera/>) program, and protein–ligand docking was performed with ADT. The docking results were then analyzed via BIOVIA Discovery Studio software, and 2D and 3D molecular images were obtained<sup>15,16</sup>.

### Cell viability analysis

After a 24-h incubation period, the metabolic activities of the cells were evaluated via the MTT assay. In the assay, 100  $\mu$ L of dimethyl sulfoxide (DMSO) was added to each well to solubilize formazan crystals of MTT reduced by living cells, and the plates were then incubated for 4 h. The optical densities of the solutions were measured with a spectrophotometer at a wavelength of 570 nm.

The levels of lactate dehydrogenase (LDH) were determined via ELISA following the manufacturer's instructions (BT LAB<sup>®</sup> Cat.No. E0747Hu). First, following the preparation of the reagents and standards, appropriate samples were added to each well. The plate was subsequently washed five times, after which the substrate solutions were added. The samples were then incubated at 37 °C for 10 min. Next, a stop solution was added, and the absorbances of the samples were measured at a wavelength of 450 nm.

### Oxidative stress markers

The total antioxidant capacity (TAC) and total oxidant status (TOS) were analyzed to evaluate the level of oxidative stress, employing the Erel method (Rel Assay Diagnostics, Gaziantep, Turkey)<sup>17</sup>.

The levels were determined via ELISA according to the protocols for malondialdehyde (MDA) (BT LAB<sup>®</sup> Cat. No. E0747Hu) and superoxide dismutase (SOD) (BT LAB<sup>®</sup> Cat. No. E0168Ra). Following the preparation of standards, reagents and samples, they were distributed in each well. After the plate was washed five times, substrate solutions were added, and the mixture was incubated at 37 °C for 10 min. Finally, the addition of stop solution was followed by the measurement of OD values at 450 nm via a spectrophotometer.

### Neuroinflammatory markers

Interleukin-10 (IL-10) expression was determined via ELISA according to the kit protocol (BT LAB<sup>®</sup> Cat. No. E0108Ra) and Transforming Growth Factor Beta (TGF- $\beta$ ) (BT LAB<sup>®</sup> Cat. No. E0134Hu). Initially, all the reagents, standards, and samples were prepared. The relevant samples were then added to each well. The plate was then washed five times, after which substrate solutions were added and incubated at 37 °C for 10 min. The stop solution was subsequently added, and the optical density (OD) was measured at a wavelength of 450 nm.

Interleukin-1 $\beta$  (IL-1 $\beta$ ) levels were analyzed via ELISA according to the manufacturer's protocol (Bostonchem<sup>®</sup> Cat. No. BLS-1270Hu), and tumor necrosis factor alpha (TNF- $\alpha$ ) (Bostonchem<sup>®</sup> Cat. No. BLS-1190Hu) was used. All reagents, standards, and samples were prepared and incubated at 37 °C for 80 min. After the addition of the samples, the plate was washed three times, after which the antibody mixture was added. The plate was then incubated at 37 °C for 50 min, after which streptavidin-HRP was added, and the plate was incubated again at 37 °C for an additional 50 min. Following the addition of the substrate solutions, the plate was maintained in a dark environment at 37 °C for 20 min. In the final step, the addition of stop solution was followed by measurement of the absorbances at 450 nm.

### Immunohistochemical analysis

The primary antibodies were prepared with 500  $\mu$ L of a 0.1% BSA solution, which had been diluted 1/200, and added to the cells. These mixtures were then incubated overnight at + 4 °C. Next, the solution was removed, and the cells were washed three times with PBS. The following secondary antibodies were then applied: goat anti-mouse IgG (H + L) FITC secondary antibodies compatible with monoclonal  $\alpha$ -synuclein antibody and goat anti-rabbit IgG (H + L) Texas Red secondary antibodies compatible with polyclonal nNOS antibody. These secondary antibodies were then incubated at room temperature for a period of one hour, after which the samples were washed again with PBS. DAPI was used for nuclear staining, and the samples were examined under a fluorescence microscope. The resulting fluorescence images were then analyzed via Fiji ImageJ software (Version 2.14.0 / Fiji distribution – <https://imagej.net/software/fiji/>)<sup>18</sup>.

### Statistical analysis

The statistical analysis of the data from the tests performed with cell culture was conducted via IBM SPSS Statistics software (version 20.0; IBM Corp., Armonk, NY, USA; <https://www.ibm.com/products/spss-statistics>). The results of the experimental studies are expressed as the means  $\pm$  standard deviations. P values less than 0.05 were considered statistically significant. The significance level of the differences between the groups was determined via one-way analysis of variance (one-way ANOVA). Graphs and visual representations of the data were generated using GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA; <https://www.graphpad.com/scientific-software/prism/>).

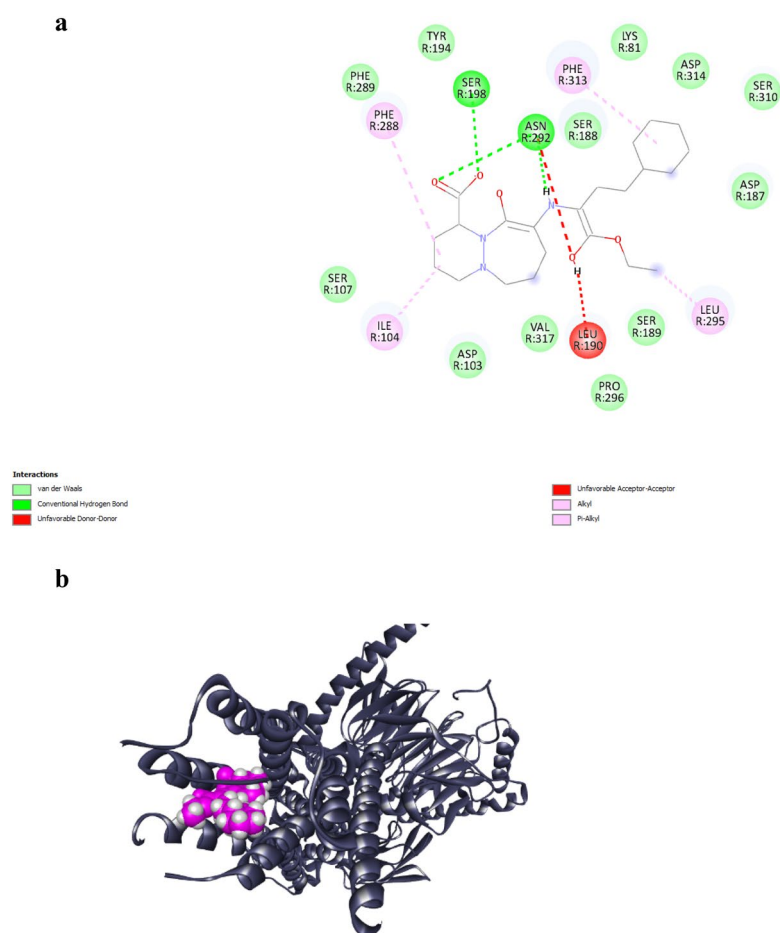
## Results and discussion

Given the critical role of oxidative stress and mitochondrial dysfunction in the progression of PD, antioxidant molecules have been explored as potential therapeutic agents. Neurotoxin-based animal models, which are widely used to study the underlying mechanisms of PD pathogenesis, serve as essential tools to assess the efficacy of different treatment strategies. The degeneration of dopaminergic neurons observed in these models closely mirrors the severe sensory and motor impairments characteristic of PD patients<sup>19,20</sup>. Oxidative stress results from an imbalance between the excessive generation of reactive oxygen species (ROS) and the availability of

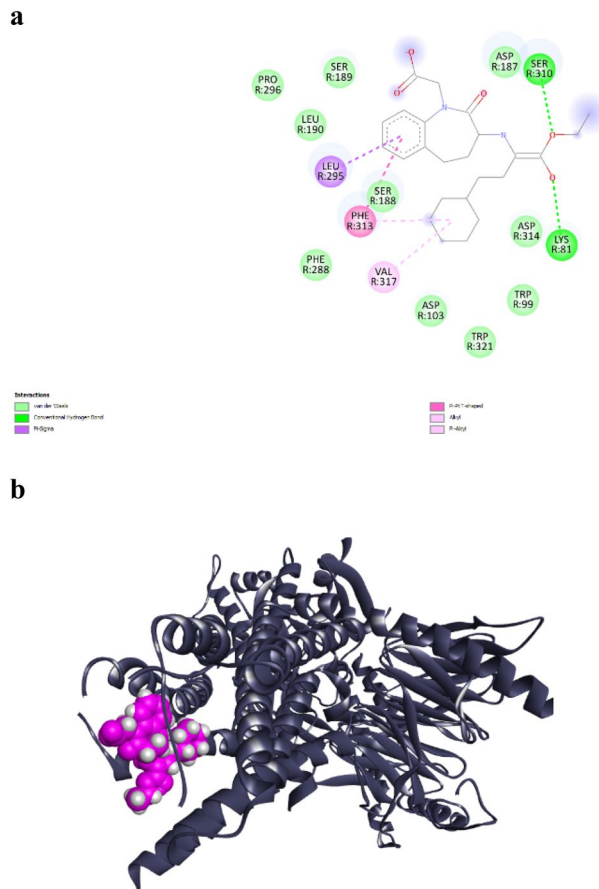
antioxidants, as well as the depletion of enzymatic or nonenzymatic ROS scavengers. The neurotoxin 6-OHDA, a hydroxylated derivative of dopamine, is taken up by cells via the dopamine transporter. Once inside the cell, the presence of  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  facilitates the nonenzymatic hydroxylation of dopamine, leading to increased formation of 6-OHDA<sup>21</sup>. The involvement of oxidative stress in 6-OHDA-induced cell death is consistent with previous findings in studies of SH-SY5Y human neuroblastoma cells<sup>22</sup>.

### Molecular docking

Cilazapril and benazepril exhibit various binding modes with receptors and interact with specific amino acids, increasing their binding stability (Figs. 1 and 2). In terms of binding energies, both molecules have the potential to interact with the D1A receptor. According to the results of the D1-Benazepril experiment, both molecules stably bound to the receptor; however, the binding sites and interaction types differed. However, cilazapril formed more hydrogen bonds with the D1A receptor, which may have contributed to its binding stability to the receptor. The interactions of benazepril in hydrophobic pockets suggest its potential to affect the dynamics of the drug-receptor complex in a different way. These findings suggest the possibility of distinguishing between the pharmacological effects of cilazapril and B. These findings offer insights into the potential impact of cilazapril and benazepril on the dopaminergic system<sup>23</sup>. In particular, the effect of C on the receptor can be said to offer a more stable profile in terms of neurodegenerative diseases, while the different binding modes of benazepril suggest that the specific pharmacodynamic effects of this drug should be investigated. These observations suggest the possibility of differential effects of both molecules on the receptor in terms of biological functions. D1-B dopamine receptors (Figs. 3 and 4) play a significant role in neurodegenerative disorders, such as PD, by exerting a critical effect on dopamine signaling<sup>24</sup>. In this context, ACE inhibitors such as cilazapril and benazepril may exert direct effects on the dopaminergic system in addition to their recognized effects on neuroinflammation and oxidative stress. Specifically, cilazapril has been found to have stronger binding sites and more stable interaction motifs with the receptor, suggesting its potential as a therapeutic candidate in the dopaminergic system. The present findings lend support to the hypothesis that the effects of ACE inhibitors on the central nervous system are not limited to the peripheral effects that have been previously reported. These findings suggest that this



**Fig. 1.** (a) D1A receptor–interaction (2D view) hydrogen bonds were observed between cilazapril and Asp187, Phe313 and Ser188. Hydrophobic interactions with surrounding residues further stabilized the binding conformation. (b) Receptor interaction (3D view). The binding pocket of cilazapril within the D1A receptor is visualized, highlighting its strong interaction via hydrogen bonding and hydrophobic forces.

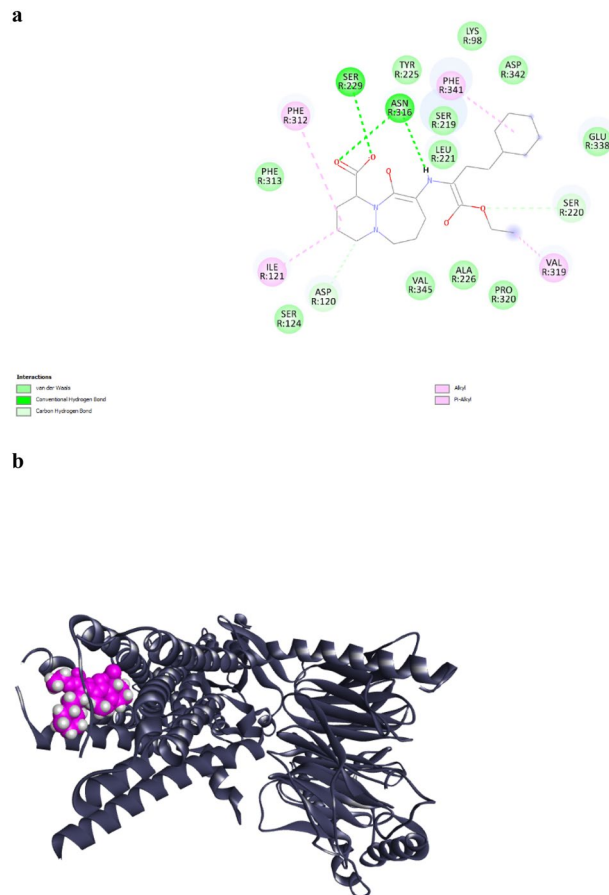


**Fig. 2.** (a) D1A Receptor-Interaction (2D View) Benazepril interacts with Asp187 and Trp321 via hydrogen bonding and differs in its binding mode compared with C. Hydrophobic interactions contribute to its stabilization at the active site of the receptor. (b) Receptor interaction (3D view) The binding site of benazepril within the D1A receptor is depicted, highlighting the key residues involved in its interaction. Compared with that of C, the binding pocket orientation emphasizes its unique conformation.

class of pharmaceuticals may be useful in the treatment of neurodegenerative disorders by acting on alternative targets<sup>25,26</sup>. The interactions of cilazapril and benazepril with the D2 dopamine receptor were investigated (Figs. 5 and 6). Cilazapril exhibited high-affinity binding to the active site of the receptor. In contrast, benazepril demonstrated a distinct binding pattern, targeting the G:41 region of the receptor and complementary regions of hydrogen bond structures. These observations offer significant insights into the potential mechanisms of action of both molecules on the receptor and their neuroprotective roles. Notably, binding to the D2 dopamine receptor plays a pivotal role in the preservation of dopaminergic neurons in PD<sup>24</sup>. These findings suggest that ACE inhibitors should be evaluated not only for their peripheral effects but also for their potential neuroprotective effects on the central nervous system<sup>27–29</sup>.

Docking analyses of the interactions of cilazapril and benazepril with D1 (A), D1 (B) and D2 dopamine receptors revealed that these molecules have significant differences in terms of binding affinities (Table 1). At the D1(A) receptor, benazepril surpassed cilazapril by 9.9-fold, with a docking score of -10.4. This finding suggests that benazepril has a greater binding affinity for the D1(A) receptor, indicating a stronger interaction with this receptor. These findings suggest the potential of benazepril to modulate dopaminergic activity. In the case of the D1(B) receptor, cilazapril demonstrated a greater binding affinity than benazepril (-9.8), with a docking score of -10.4. This strong binding of cilazapril to the D1(B) receptor suggests that this molecule may provide neuroprotective effects, especially in diseases where dopaminergic dysfunction is at the forefront, such as PD. In contrast, analyses of the D2 receptor indicated that the binding scores of both molecules were relatively low. While benazepril demonstrated a greater binding affinity for the D2 receptor than cilazapril, with a docking score of -8.3, this interaction was more constrained than that of other receptors. This finding raises the question of whether the molecules may not have a significant effect on the D2 receptor compared with the other molecules. In conclusion, cilazapril and benazepril molecules exhibited high binding affinities for dopamine D1 receptor subtypes (D1(A) and D1(B)), whereas their binding scores for the D2 receptor were lower. These findings suggest that the therapeutic effects of these molecules may be specific to dopamine D1 subtypes and may be considered targetable biomolecules in neurological disorders affecting the dopaminergic system, such as PD<sup>30</sup>.

The molecular docking results (Table 2) revealed that benazepril has the highest docking score with AT1 (-9.0 kcal/mol) and a slightly lower score with AT2 (-7.8 kcal/mol), indicating a strong interaction with both

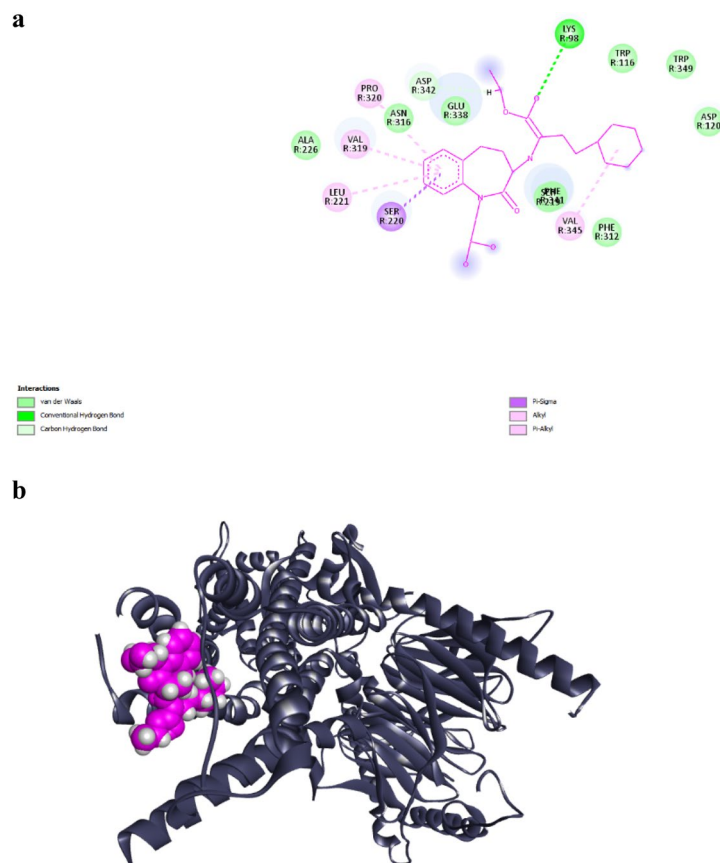


**Fig. 3.** (a) D1B receptor–cilazapril interaction (2D view) Cilazapril forms hydrogen bonds, particularly with critical amino acids such as Ser220 and Val319, whereas (b) D1B receptor–cilazapril interaction (3D view) displays the binding pocket of cilazapril within the D1A receptor, highlighting its strong interaction through hydrogen bonding and hydrophobic forces.

receptor subtypes. Similarly, silazapril had high docking scores for AT1 (-8.7 kcal/mol) and AT2 (-8.1 kcal/mol), confirming its receptor inhibition potential. These results are consistent with previous studies showing that both cilazapril and benazepril have strong binding affinities for AT1 and AT2 receptors and regulate them, leading to vasodilation and blood pressure reduction. Notably, the docking results suggest that benazepril may have a slightly greater affinity for AT1 than cilazapril does, which may translate into different pharmacokinetic and pharmacodynamic properties<sup>31,32</sup>.

The results of the MTT assay demonstrated that cilazapril significantly increased cell viability ( $*p < 0.05$ ) compared with that of the control group, particularly at a concentration of 2.5  $\mu\text{M}$ . In contrast, benazepril significantly mitigated 6-OHDA-induced neurodegenerative impairment (Fig. 7a–b). This effect was particularly pronounced at a dose of 0.16  $\mu\text{M}$ , where a significant increase in cell viability was observed ( $*p < 0.05$ ). This effect may be attributed to benazepril-mediated suppression of neuroinflammation and regulation of nitric oxide (NO) production.

The levels of LDH (Fig. 8a–b) markedly increased in the 6-OHDA-treated group, suggesting the potential for cellular damage and membrane disruption. This increase can be considered an indicator of cellular damage and disruption of membrane integrity. 6-OHDA has been demonstrated to induce oxidative stress and apoptosis in cells<sup>33</sup>. Conversely, a substantial decrease in LDH levels was evident in the benazepril- and cilazapril-treated groups (Fig. 8a–b). Notably, low doses of benazepril significantly suppressed LDH release and reduced cellular damage. The suppressive effect of ACE inhibitors on cellular damage may be associated with the suppression of oxidative stress caused by angiotensin II. Angiotensin II has been demonstrated to stimulate the production of ROS through the augmentation of NADPH oxidase activation. An increase in ROS levels may lead to mitochondrial dysfunction and damage to the cell membrane<sup>34</sup>. The findings of this study demonstrate that benazepril and cilazapril can effectively decrease LDH levels, suggesting a protective effect on cell membrane integrity and potential prevention of oxidative stress-induced cellular toxicity. These results suggest that benazepril and cilazapril can suppress 6-OHDA-induced neuroinflammation and oxidative stress responses. The mechanisms through which ACE inhibitors exert these effects appear to be multifaceted and involve a decrease in inflammatory cytokine production and the suppression of oxidative stress. ACE inhibitors have been reported to have therapeutic potential in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease<sup>35</sup>.

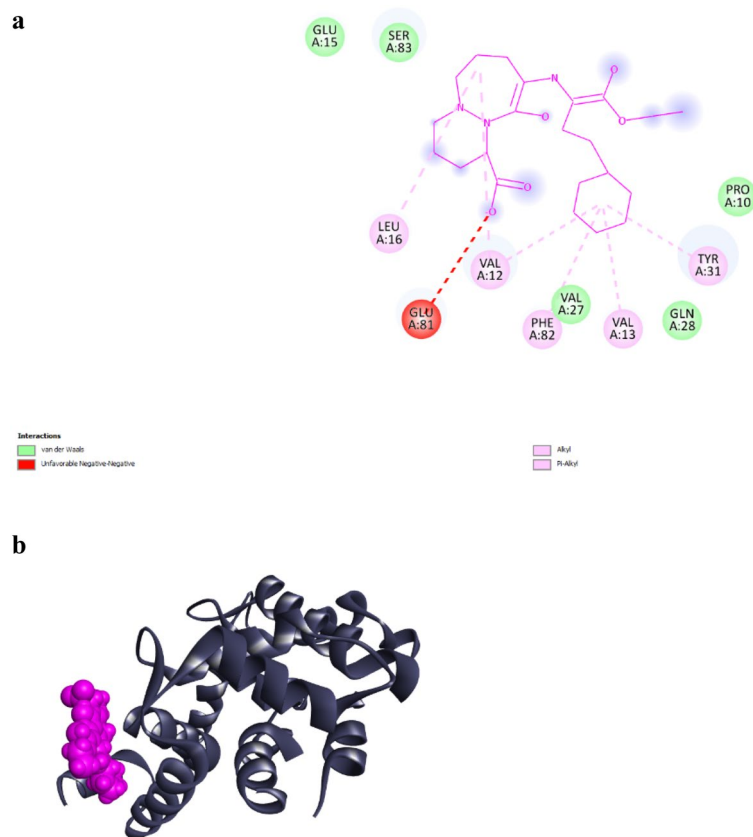


**Fig. 4.** (a) D1B Receptor-Interaction (2D View) Benazepril is more oriented toward binding sites such as Glu121 and Tyr218. (b) D1B receptor– interaction (3D view) The binding site of benazepril within the D1A receptor is depicted, highlighting the key residues involved in its interaction. The binding pocket is visualized, highlighting its strong interaction through hydrogen bonding and hydrophobic forces.

### Oxidative stress markers

Oxidative stress has been identified as a precursor of numerous pathologies and disorders. Consequently, the antioxidant activity of pharmaceuticals has emerged as a pharmacologically significant factor. Numerous clinical reports have indicated that the administration of angiotensin-converting enzyme inhibitors (ACE-Is) can enhance the condition of patients afflicted with neurodegenerative disorders and potentially decelerate inflammatory processes<sup>36,37</sup>. In a particular study, an electron paramagnetic resonance-based comparative analysis of the antioxidant properties of cilazapril, ramipril, imidapril, lisinopril, perindopril and quinapril was conducted, in which electron paramagnetic resonance was used to investigate specific ACE-I interactions with a free radical model (Fig. 9a-b). All the drugs tested exhibited antioxidant properties, with quinapril demonstrating the strongest interaction in the MTT assay (Fig. 7), and a dose of 2.5  $\mu\text{g}/\text{mL}$  was identified as effective. Upon further examination of the relationships between these results and the oxidant parameters, a difference in the total antioxidant parameters was detected at the same dose, and a correlation was established. However, these results did not align with those observed for the total oxidant parameters, which were not statistically significant. Benazepril, an inhibitor of the renin-angiotensin-aldosterone system (RAAS), has been demonstrated to possess antioxidant properties. When the TAS results of the benazepril and the 6-OHDA groups were compared, the 0.16  $\mu\text{M}$  and 0.32  $\mu\text{M}$  benazepril groups presented the highest antioxidant levels, with values of 1.48 and 1.27, respectively. Consequently, benazepril had a beneficial effect at a drug dose of 0.16  $\mu\text{M}$ , and this effect was associated with antioxidant parameters (Fig. 9c-d).

MDA, an end product of lipid peroxidation, is an important biomarker of oxidative stress. The role of oxidative stress in PD pathophysiology is well documented<sup>38</sup>. The present study examined the effects of ACE inhibitors (benazepril and cilazapril) on MDA levels in a 6-OHDA-induced Parkinson's disease model (Fig. 10a-b). The significantly increased MDA levels in the 6-OHDA group revealed severe oxidative stress, which is consistent with previously reported findings in the literature<sup>39</sup>. However, a significant decrease in the MDA content was observed in the cilazapril- and benazepril-treated groups. These findings support the potential of ACE inhibitors to reduce oxidative stress. The lower MDA levels observed in the cilazapril group suggest that this drug may be more effective at inhibiting lipid peroxidation than benazepril is. The mechanism by which ACE inhibitors reduce oxidative stress may be related to the reduction in ROS levels due to the decrease in angiotensin II<sup>40</sup>. The ability of angiotensin II to increase NADPH oxidase activity plays a fundamental role in ROS production. Therefore, ACE inhibitors may reduce oxidative stress levels by breaking this chain. These



**Fig. 5.** (a) D2 receptor–cilazapril interaction (2D view). Cilazapril forms hydrogen bonds, particularly with critical amino acids such as Ser220 and Val319. (b) D2 Receptor–cilazapril interaction (3D view). The binding pocket of cilazapril within the D1A receptor is visualized, highlighting its strong interaction through hydrogen bonding and hydrophobic forces.

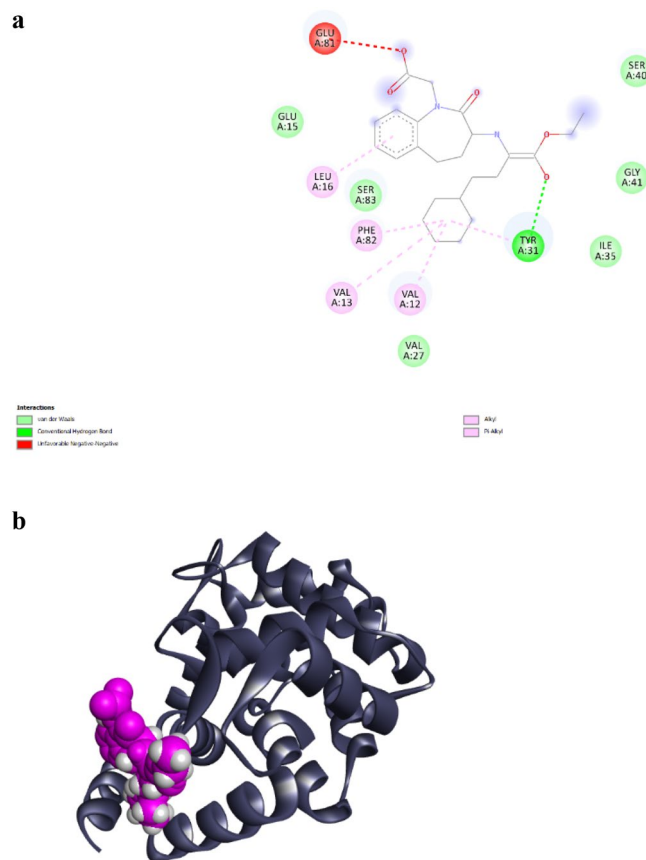
findings support the therapeutic potential of ACE inhibitors in the pathogenesis of PD. In particular, cilazapril appears to have a marked effect on suppressing lipid peroxidation. However, the effects at different doses need to be studied in more detail to better understand these effects.

SOD is an enzyme that reduces oxidative stress and specifically detoxifies ROS<sup>41</sup>. Decreased SOD activity in PD may accelerate neurodegeneration by causing the accumulation of free radicals<sup>42</sup>. This study examined the potential of ACE inhibitors to increase SOD levels in a 6-OHDA-induced Parkinson's disease model (Fig. 11a–b). SOD levels were significantly lower in the 6-OHDA group than in the control group. These findings support previous studies showing that 6-OHDA activates oxidative stress mechanisms<sup>43</sup>. In the groups treated with cilazapril or benazepril, a significant increase in SOD activity was observed. These findings suggest the potential of ACE inhibitors to reduce ROS levels and support antioxidant systems. The difference observed between benazepril and cilazapril may be related to the biochemical structure and pharmacological effects of both molecules. The higher SOD activity observed in the Cilazapril group may indicate that this drug more effectively supports antioxidant mechanisms. This finding suggests that the potential for therapeutic use of cilazapril may be greater than that of benazepril<sup>44</sup>. This increase in SOD activity suggests that ACE inhibitors play an active role, especially in the mechanisms of protecting mitochondrial functions and reducing oxidative damage.

### Markers of neuroinflammation

IL-10 levels were also able to reverse the decline observed in the 6-OHDA group. IL-10 is known to limit inflammation and reduce damage to tissues. In this study, the IL-10 levels decreased in the 6-OHDA group, and the levels in the 1.25, 2.5 and 5  $\mu$ M cilazapril groups were significantly greater. All doses of benazepril had high IL-10 levels, and these values were statistically significant (Fig. 12a–b). These findings suggest that benazepril may have anti-inflammatory and neuroprotective effects in addition to its antioxidant effects.

IL-1 $\beta$ , an inflammatory cytokine, plays a direct role in neurodegeneration processes. In our study, 6-OHDA administration significantly increased IL-1 $\beta$  levels. These findings confirm that inflammatory responses are an essential component of Parkinson's disease pathogenesis<sup>45</sup>. Benazepril and cilazapril both suppressed these inflammatory effects by significantly decreasing IL-1 $\beta$  levels (Fig. 13a–b). The reductions in the low-dose benazepril (B-0.04) and cilazapril (C-0.32) groups suggest that these drugs may target early-stage inflammatory responses. However, high-dose administrations (B-0.32 and C-1.28) had a stronger effect, and the IL-1 $\beta$  levels



**Fig. 6.** (a) D2 receptor- interaction (2D view). Benazepril is more oriented toward binding sites such as Glu121 and Tyr218. (b) D2 Receptor-B interaction (3D view) The binding site of benazepril within the D1A receptor is depicted, highlighting the key residues involved in its interaction. The binding pocket is visualized, highlighting its strong interaction through hydrogen bonding and hydrophobic forces.

decreased to levels close to those of the control group. These findings support the dose-dependent effect of ACE inhibitors in suppressing proinflammatory cytokines<sup>46</sup>. Furthermore, it has been suggested that ACE inhibitors regulate microglial activation by suppressing neuroinflammation. This mechanism may contribute to protecting dopaminergic neurons and slowing the neurodegenerative process<sup>47</sup>.

TNF- $\alpha$  levels were significantly increased after 6-OHDA administration. This finding is consistent with previous findings that 6-OHDA triggers oxidative stress and inflammatory responses in dopaminergic cells<sup>48</sup>. A significant decrease in TNF- $\alpha$  levels was observed with cilazapril and benazepril administration (Fig. 14a-b). In particular, at low doses of benazepril (0.04 and 0.08  $\mu$ M), the TNF- $\alpha$  levels decreased to levels close to those in the control group. Cilazapril was effective at higher doses, which revealed a dose-dependent effect of both ACE inhibitors. The neuroprotective effect of ACE inhibitors may be attributed to their ability to inhibit the effects of angiotensin II, which increases inflammatory cytokine production. Angiotensin II increases the release of TNF- $\alpha$  and other proinflammatory cytokines by activating the NF- $\kappa$ B pathway<sup>49</sup>. The reduction in TNF- $\alpha$  levels by benazepril and cilazapril indicates the anti-inflammatory potential of these inhibitors. Moreover, previous animal studies also indicated that ACE inhibitors suppress microglial activation and reduce neuroinflammation<sup>49</sup>, indicating that ACE inhibitors provide neuroprotective effects by reducing angiotensin II levels in the brain. The findings of this study suggest that ACE inhibitors should be considered a potential treatment strategy for PD.

Transforming growth factor beta (TGF- $\beta$ ) is a cytokine that plays a critical role in neuroinflammation and the immune response of the nervous system. 6-OHDA administration in a PD model caused a significant increase in TGF- $\beta$  levels, indicating that oxidative stress and inflammation are important in disease pathogenesis<sup>50</sup>. In our study, ACE inhibitors regulated the TGF- $\beta$  levels in both the benazepril and cilazapril groups compared with those in the control group (Fig. 15a-b). Benazepril and cilazapril may suppress inflammatory processes by acting through the renin-angiotensin system (RAS). Indeed, previous studies have shown that these drugs can reduce peripheral inflammation and oxidative stress indicators<sup>51</sup>. In particular, at high doses (C-1.28 and B-0.32), the TGF- $\beta$  levels were significantly lower than those in the 6-OHDA group. These findings suggest that the anti-inflammatory effects of ACE inhibitors work through a dose-dependent mechanism. In conclusion, the regulation of TGF- $\beta$  levels by ACE inhibitors supports their neuroprotective properties. These findings suggest that RAS inhibitors could be used as potential therapeutic targets in PD.

According to the findings of real-time PCR, both molecules significantly altered the expression of the dopamine D1 (Fig. 16a-b) and D2 (Fig. 17a-b) receptors. In particular, cilazapril had a more pronounced effect

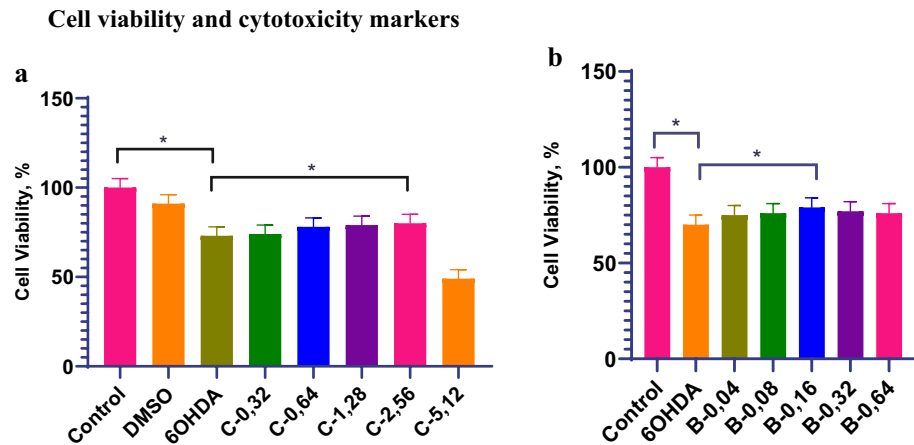
Docking Scores	
Cilazapril	Benazapril
Receptor D1(A)	
-9.9	
-8.9	-10.4
-8.4	-9.4
-8.3	-9.1
-8.1	-8.5
-7.4	-8.1
-7.2	-7.6
-7.2	-7.4
-7.2	
Receptor D1(B)	
	-9.8
	-8.2
-10.4	-7.7
-9.6	-7.5
-8.1	-7.4
-7.4	-7.3
-7.4	-7.3
	-7.2
	-7.2
Receptor D2	
-7.4	-8.3
-7.3	-7.4
-7.2	-7.1
-7.1	-7.0
-7.0	-7.0
-6.7	-7.0
-6.6	-6.9
-6.6	-6.9
-6.6	-6.8

**Table 1.** D1 and D2 receptor Docking scores.

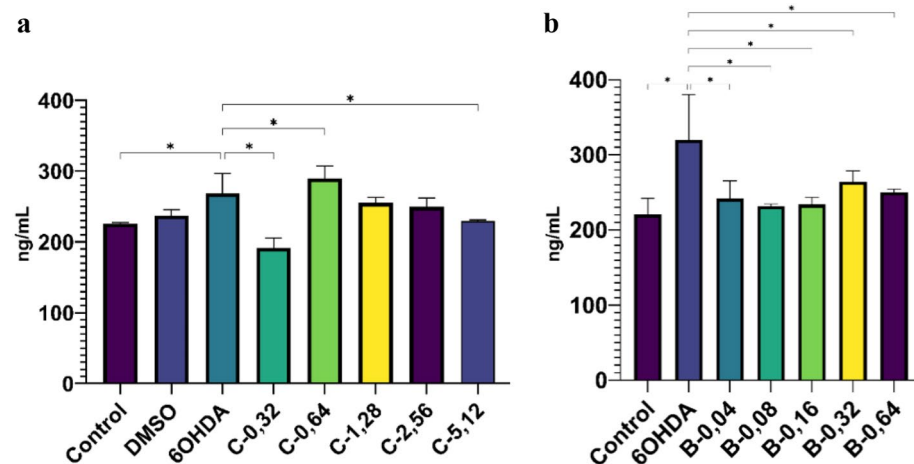
Cilazapril	Benazapril
AT1	
-8.7	-9.0
-7.6	-8.9
-7.4	-8.7
-7.3	-8.7
-7.2	-8.6
-7.1	-8.5
-6.9	-8.5
-6.8	-8.4
-6.8	-8.3
AT2	
-8.1	-7.8
-7.9	-7.7
-7.9	-7.6
-7.8	-7.5
-7.8	-7.4
-7.6	-7.3
-7.6	-7.1
-7.6	-7.0
-7.5	-6.7

**Table 2.** AT1 and AT2 receptor Docking scores.

on the D1(B) receptor, whereas benazepril had a stronger effect on the D1(A) receptor. This finding suggests that the two molecules may exhibit different binding affinities and mechanisms. Impact on D1 Receptors: The effects of cilazapril and benazepril at various concentrations on D1(A) and D1(B) receptors were analyzed. The results demonstrated that cilazapril significantly increased the expression of the D1(B) receptor ( $p < 0.001$ ), whereas its effect on the D1(A) receptor was more limited. These findings suggest that cilazapril may have therapeutic potential in the regulation of dopamine receptors in PD. A similar trend was observed with benazepril, which also increased D1(A) receptor expression, although its effect on D1(B) was comparatively weaker. These results suggest that the effects of ACE inhibitors on the dopaminergic system in PD are receptor specific. This assertion is further substantiated by recent studies that support the dopaminergic effects of ACE inhibitors in the brain. For example, ACE inhibitors have been shown to reduce neuroinflammation and lower oxidative stress levels in the brain<sup>52</sup>. These studies suggest that ACE inhibitors may have neuroprotective effects not only on the cardiovascular system. Effects on D2 Receptors: The effects of cilazapril and benazepril on D2 receptor expression were less pronounced than those on D1 receptor expression. However, cilazapril was able to modulate D2 receptor expression even at low doses, suggesting potential neuroprotective effects in PD. Benazepril, on the



**Fig. 7.** The results of the MTT assay demonstrated that (a) cilazapril and (b) benazepril significantly increased cell viability ( $*p < 0.05$ ) compared with that of the control group.



**Fig. 8.** Levels in the experimental PD model treated with cilazapril and benazepril. LDH levels: A significant increase in LDH levels was observed as a result of disruption of cellular membrane integrity by 6-OHDA administration. (a) Cilazapril and (b) benazepril had positive effects on membrane stabilization and significantly decreased LDH levels ( $*p < 0.05$ ).

other hand, induced a significant change in D2 receptor expression at relatively high concentrations ( $*p < 0.01$ ). These findings are likely associated with the critical role of D2 receptors in PD. The literature emphasizes that D2 receptors play a fundamental role in the regulation of motor functions and control of dopamine release<sup>53,54</sup>.

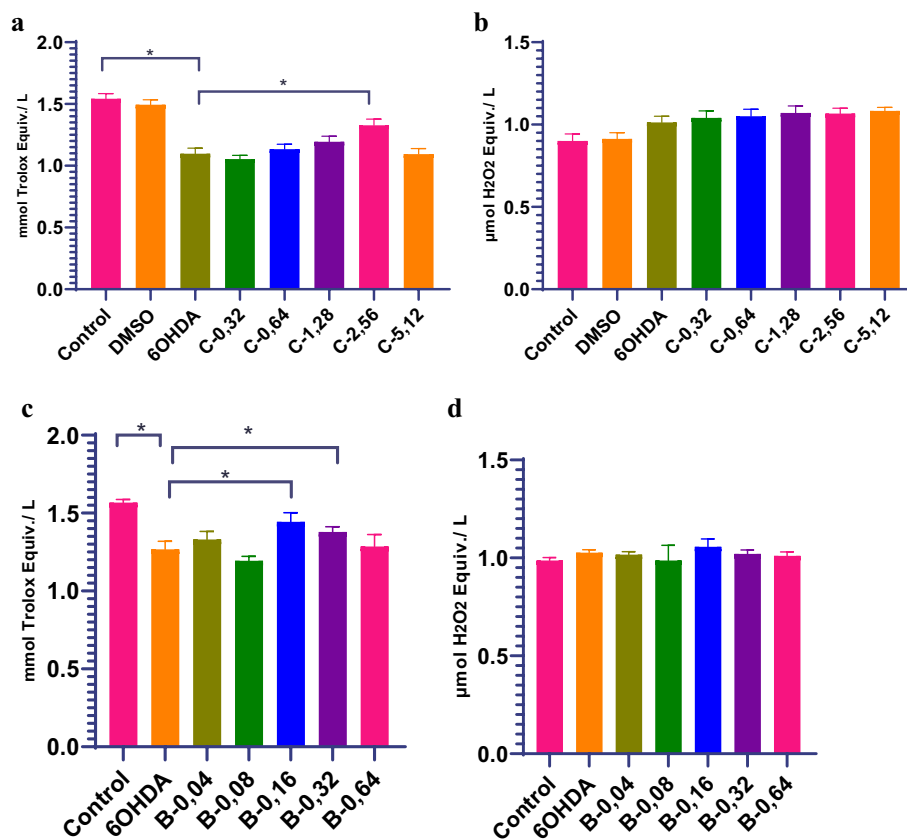
### Immunohistochemical analysis

Significant differences were detected between the groups in terms of  $\alpha$ -synuclein and nNOS expression in the S and benazepril groups (Tables 2 and 3). While mild positivity was detected in the control and DMSO groups and severe positivity was detected in the 6-OHDA group, different levels of positivity were detected in the other treatment groups depending on the drug used.

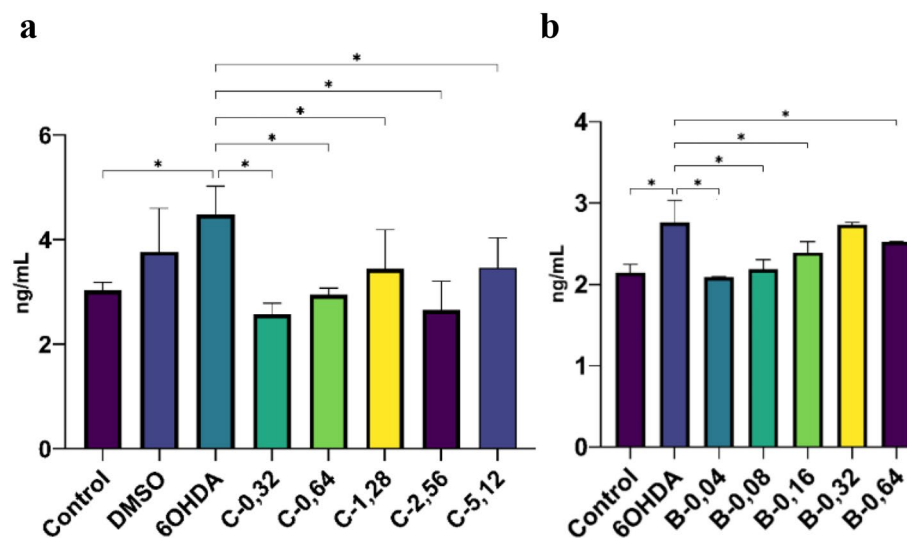
The S-0.32 and S-5.12 groups were severely positive, the S-0.64 and S-1.28 groups were moderately positive, and the S-2.56 group was mildly positive (Table 4, Fig. 19).

In the staining performed with Benazepril,  $\alpha$ -synuclein and nNOS positivity was severe in the B-0.04 group; moderate in the B-0.08, B-0.32 and B-0.64 groups; and mild in the B-0.16 group (Table 3; Fig. 18).

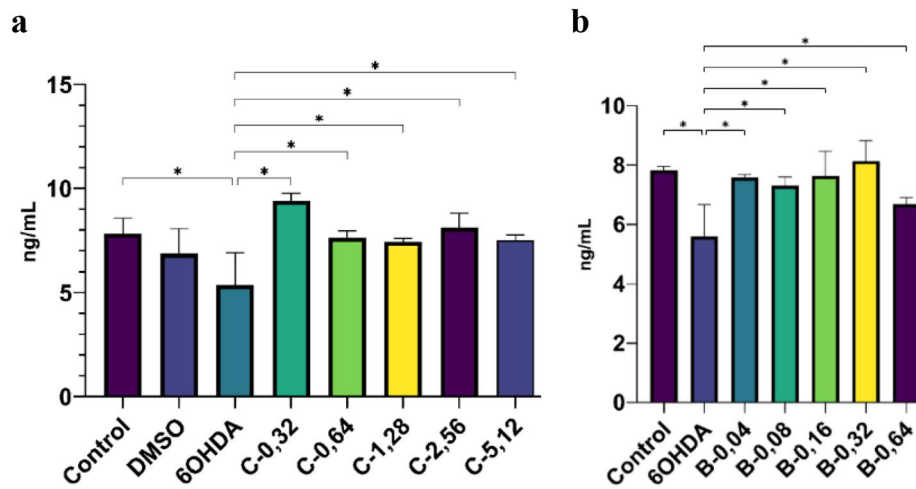
$\alpha$ -Synuclein is a protein that plays a pivotal role in the pathophysiology of PD, and its accumulation has been shown to trigger toxic mechanisms that contribute to neurodegeneration<sup>55</sup>. Immunohistochemical analyses demonstrated that the administration of cilazapril and benazepril led to a significant reduction in  $\alpha$ -synuclein positivity (Table 3; Fig. 18). These findings support the hypothesis that cilazapril and benazepril may exert neuroprotective effects by reducing  $\alpha$ -synuclein accumulation. In the literature, evidence suggests that ACE inhibitors may alleviate  $\alpha$ -synuclein pathology by reducing oxidative stress and the inflammatory response<sup>56,57</sup>. nNOS is responsible for the production of nitric oxide (NO), which plays a significant role in neuroinflammation. Increased nNOS activity has been linked to toxic damage to dopaminergic neurons<sup>58,59</sup>.



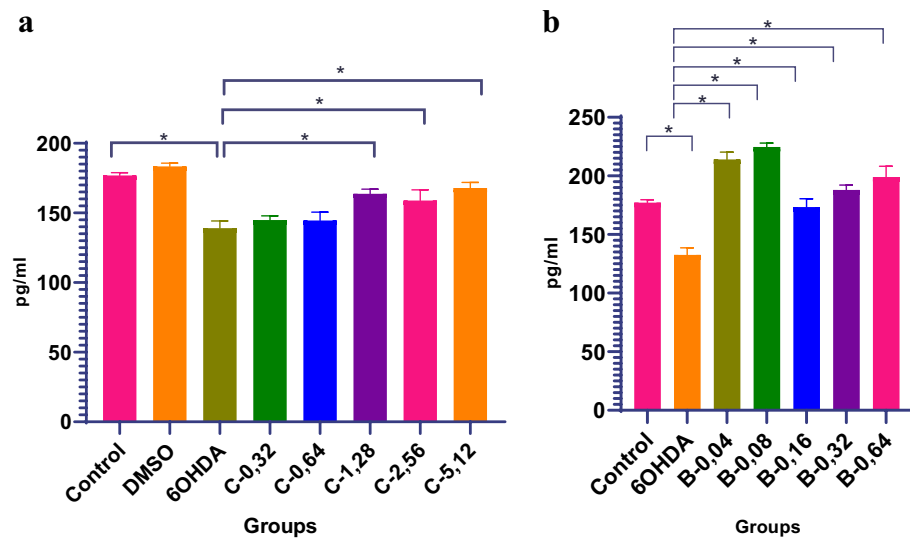
**Fig. 9.** (a) Total antioxidant capacity (TAC) results for cilazapril, which were significantly lower than those of the control group ( $*p < 0.05$ ). (b) Total oxidant status (TOS) results for cilazapril, showing no statistically significant differences among the groups. (c) TAC results for benazepril, indicating a significant decrease relative to the control group ( $p < 0.05$ ). (d) TOS results for benazepril, revealing a dose-dependent increase in oxidative status.



**Fig. 10.** MDA levels in the experimental PD model treated with cilazapril and benazepril. The effects of different doses (0.32 μM–5.12 μM) of (a) cilazapril and (b) benazepril on the cellular MDA (malondialdehyde) levels were evaluated. In the 6-OHDA-induced oxidative stress model, significantly lower MDA levels were observed in the ACE inhibitor groups than in the control, DMSO and 6-OHDA groups ( $*p < 0.05$ ),  $**p < 0.01$ ).

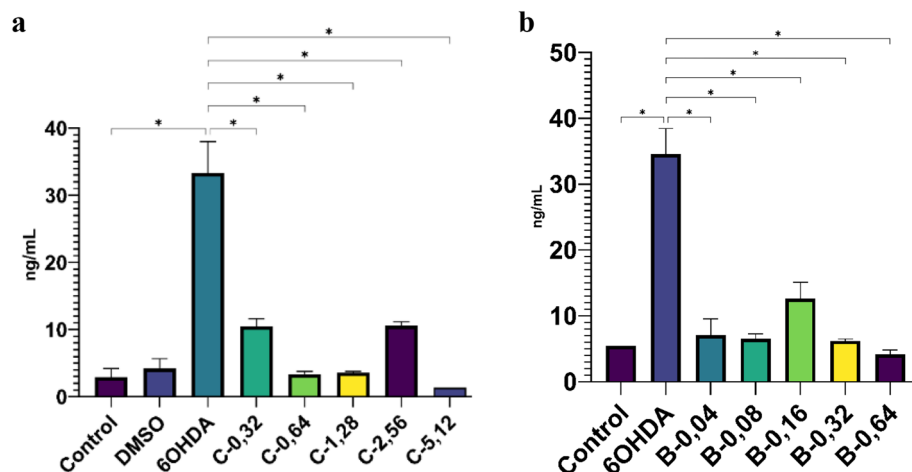


**Fig. 11.** SOD levels (ng/mL) measured in SH-SY5Y cells treated with increasing concentrations of cilazapril (C; 0.32, 0.64, 1.28, 2.56 and 3.12  $\mu$ M) after 6-OHDA induction. The control, DMSO and 6-OHDA groups were compared. (a) Significantly greater SOD activity was detected in the cilazapril-treated groups (C-0.32 and C-0.64) than in the 6-OHDA group ( $*p < 0.05$ ). SOD levels (ng/mL) in SH-SY5Y cells treated with increasing concentrations of (b) benazepril (B; 0.04, 0.08, 0.16, 0.32 and 0.64  $\mu$ M) after 6-OHDA induction. Compared with those in the 6-OHDA group, the SOD levels in the benazepril-treated groups (B-0.16 and B-0.32) were significantly greater ( $*p < 0.05$ ).

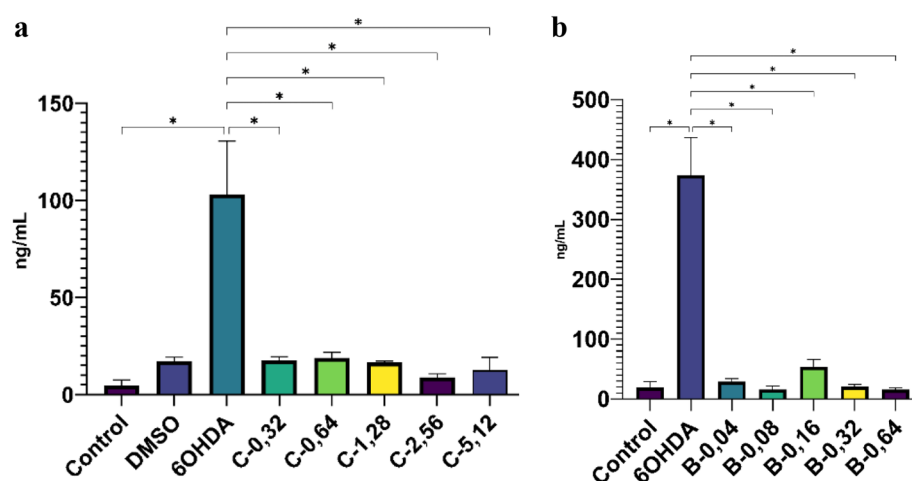


**Fig. 12.** Effects of cilazapril and benazepril on the 6-OHDA-induced increase in IL-10 levels. (a) Cilazapril reversed the 6-OHDA-induced decrease in IL-10 levels to a moderate extent in a dose-dependent manner. The C-1.28 and C-5.12 groups showed significant improvement ( $*p < 0.05$ ). (b) Benazepril significantly restored the IL-10 levels, particularly in the B-0.08 and B-0.16 groups. This resulted in values closer to those of the control group ( $*p < 0.05$ ).

The findings of the immunohistochemistry study revealed a significant reduction in nNOS positivity in subjects treated with cilazapril and benazepril (Table 4; Fig. 19). The effect of cilazapril treatment on this decrease was more pronounced, whereas the effect of benazepril increased in a dose-dependent manner. In the extant literature, ACE inhibitors have been reported to have neuroprotective potential in nNOS regulation<sup>60</sup>. The differential effects of cilazapril and benazepril on  $\alpha$ -synuclein and nNOS expression suggest that these molecules may target various pathological mechanisms in PD. The findings of this study indicate that cilazapril has notable efficacy, particularly in the management of  $\alpha$ -synuclein-related pathologies. Conversely, benazepril's capacity to curtail nNOS activity at lower doses indicates its potential to target the neuroinflammatory aspects of Parkinson's disease. In conclusion, the findings of immunohistochemistry analyses of  $\alpha$ -synuclein and nNOS positivity support the therapeutic potential of cilazapril and benazepril in a Parkinson's disease model. Further translational studies are needed for a more comprehensive clinical evaluation of these findings. The ability of ACE inhibitors to target



**Fig. 13.** Effects of cilazapril and benazepril on the 6-OHDA-induced increase in the IL-1 $\beta$  level. (a) Cilazapril suppressed the increase in IL-1 $\beta$  in a dose-dependent manner, and the IL-1 $\beta$  levels in the S-2.56 and S-5.12 groups decreased to values close to those in the control group ( $*p < 0.05$ ). (b) Benazepril also significantly decreased the IL-1 $\beta$  level, especially in the B-0.32 and B-0.64 groups ( $*p < 0.05$ ).

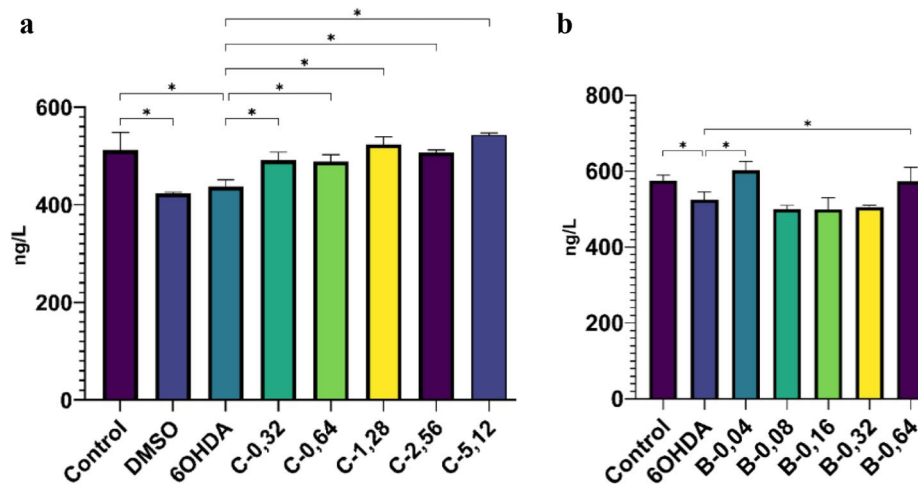


**Fig. 14.** TNF- $\alpha$  levels in the experimental PD models treated with cilazapril and benazepril (a-b) TNF- $\alpha$  levels. The effects of different doses (0.32  $\mu$ M–5.12  $\mu$ M) of cilazapril and benazepril on the 6-OHDA-induced inflammatory response were evaluated. Compared with those of the control and other groups, the TNF- $\alpha$  levels of the 6-OHDA group were significantly greater ( $*p < 0.05$ ). Significant decreases in TNF- $\alpha$  levels were observed after treatment with both ACE inhibitors ( $*p < 0.05$ ).

these pathological mechanisms may provide important contributions to the development of new approaches for Parkinson's disease treatment.

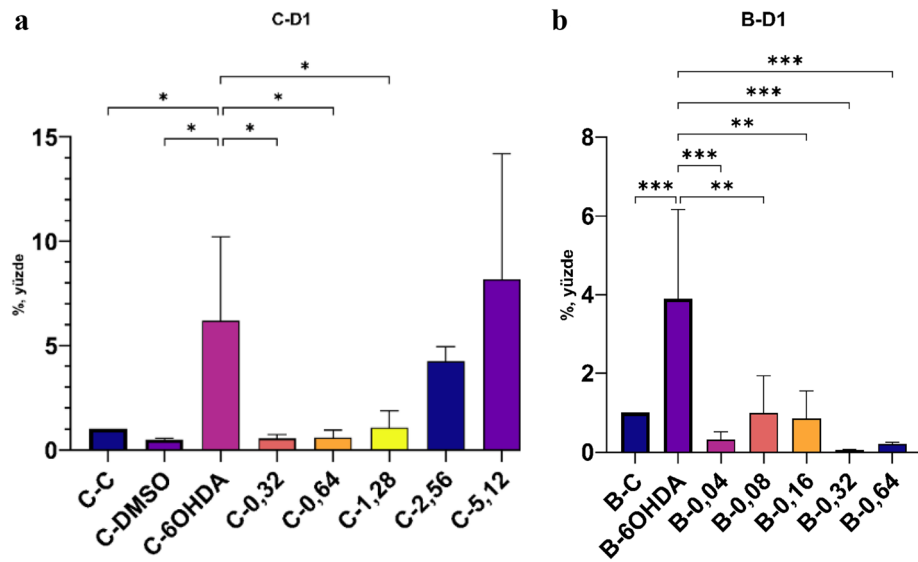
## Conclusion

This study systematically investigated the neuroprotective, antioxidant and anti-inflammatory properties of the compounds cilazapril and benazepril in an in vitro model of 6-OHDA-induced PD. Molecular docking and dynamic simulations revealed strong binding affinities of both compounds for the dopamine D1 and D2 receptors. In vitro experiments further revealed that treatment with cilazapril and benazepril significantly attenuated 6-OHDA-induced oxidative stress,  $\alpha$ -synuclein aggregation and nNOS activity in neuronal nitric oxide synthase. In addition, their anti-inflammatory effects were demonstrated by a reduction in the levels of proinflammatory cytokines, including IL-1 $\beta$ , TNF- $\alpha$  and TGF- $\beta$ . The structural improvements in cell morphology observed in the treatment groups further support the neuroprotective potential of cilazapril and benazepril, as confirmed by PCR and other analytical results. These results suggest that ACE inhibitors may play a critical role in modulating PD-related pathological processes and highlight their potential for clinical translation as multifunctional therapeutic agents in the treatment of PD. This study provides a strong foundation for future research aimed at elucidating the therapeutic benefits of ACE inhibitors.

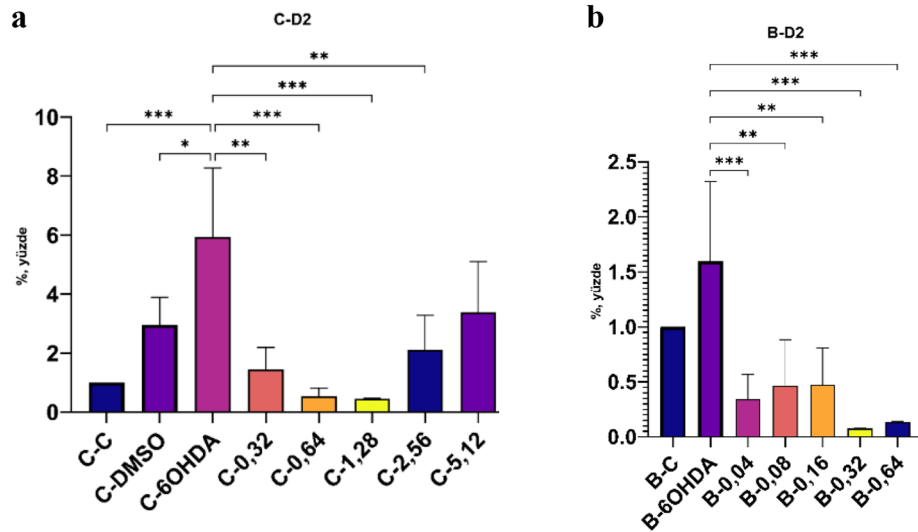


**Fig. 15.** Effects of ACE inhibitors on the TGF-β levels and the TGF-β levels in the cilazapril groups. Cilazapril dose-dependently suppressed the increase in the TGF-β levels caused by 6-OHDA administration and decreased the TGF-β levels to levels close to those of the control group at high doses (C-1.28, C-2.56). (b) TGF-β levels in the benazepril groups: benazepril effectively reduced the increase in the TGF-β level caused by 6-OHDA, with a significant reduction at low doses (B-0.04, B-0.08). The values are presented as the means ± standard errors of the means (\**p* < 0.05).

**Real-time PCR analysis of D1 and D2 dopamine receptor genes**



**Fig. 16.** Real-time PCR analysis of D1 and D2 dopamine receptor gene expression. (a) Cilazapril-D1: Effect of cilazapril on D1 receptor expression. (b) Benazepril-D1: Effect of benazepril on D1 receptor expression. The effects of different doses (0.04 μM–5.12 μM) of cilazapril and benazepril on D1 and D2 dopamine receptor gene expression were evaluated.



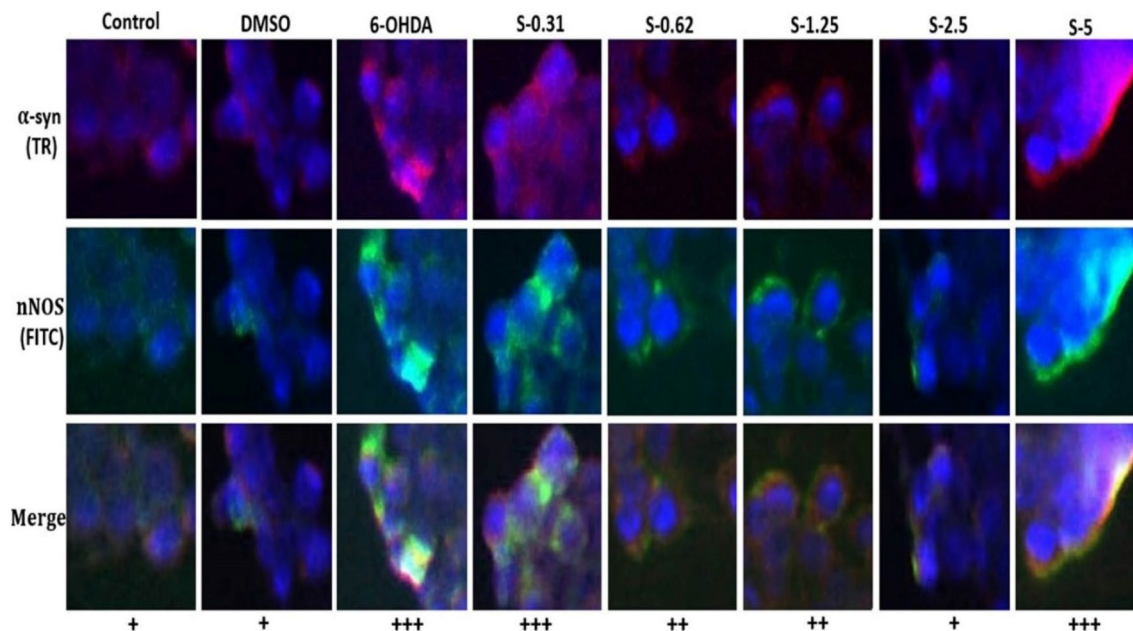
**Fig. 17.** (a) Cilazapril-D2: Effect of cilazapril on D2 receptor expression. (b) Benazepril-D2: Effect of benazepril on D2 receptor expression. The data were compared with those of the control groups (cilazapril and benazepril). Statistical significance levels: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

	$\alpha$ -syn	nNOS
Control	16,84 $\pm$ 2,41 <sup>a</sup>	19,59 $\pm$ 3,61 <sup>a</sup>
DMSO	17,11 $\pm$ 4,02 <sup>a</sup>	20,81 $\pm$ 3,45 <sup>a</sup>
6-OHDA	36,43 $\pm$ 7,12 <sup>b</sup>	38,04 $\pm$ 5,53 <sup>b</sup>
C-0.32	35,61 $\pm$ 4,82 <sup>b</sup>	37,31 $\pm$ 3,64 <sup>b</sup>
C-0.64	28,67 $\pm$ 3,34 <sup>c</sup>	30,19 $\pm$ 3,73 <sup>c</sup>
C-1.28	27,50 $\pm$ 5,09 <sup>c</sup>	29,83 $\pm$ 4,23 <sup>c</sup>
C-2.56	18,49 $\pm$ 3,93 <sup>a</sup>	21,06 $\pm$ 5,17 <sup>a</sup>
C-5.12	37,06 $\pm$ 6,49 <sup>b</sup>	39,41 $\pm$ 6,35 <sup>b</sup>

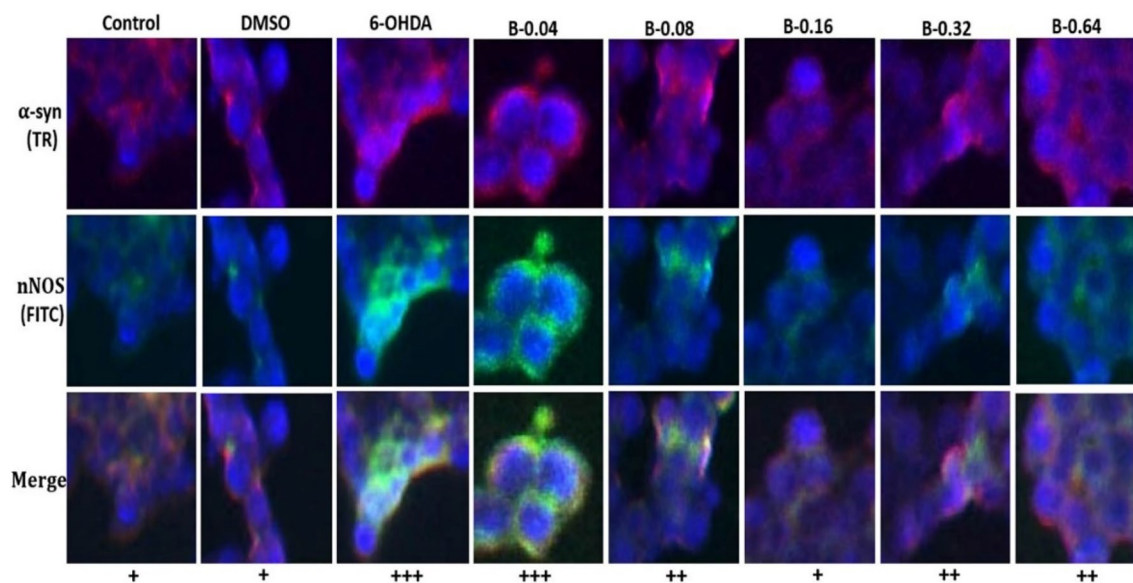
**Table 3.** Cilazapril. Different letters in the same column indicate differences between groups (\* $p < 0.001$ ).

	$\alpha$ -syn	Nnos
Control	20,45 $\pm$ 3,51 <sup>a</sup>	21,20 $\pm$ 1,29 <sup>a</sup>
DMSO	21,04 $\pm$ 3,82 <sup>a</sup>	23,84 $\pm$ 3,44 <sup>a</sup>
6-OHDA	43,16 $\pm$ 4,83 <sup>b</sup>	45,93 $\pm$ 6,75 <sup>b</sup>
B-0.04	41,92 $\pm$ 6,48 <sup>b</sup>	44,03 $\pm$ 5,40 <sup>b</sup>
B-0.08	34,81 $\pm$ 5,16 <sup>c</sup>	35,70 $\pm$ 4,02 <sup>c</sup>
B-0.16	22,09 $\pm$ 3,48 <sup>a</sup>	22,57 $\pm$ 3,28 <sup>a</sup>
B-0.32	35,08 $\pm$ 3,06 <sup>c</sup>	34,08 $\pm$ 8,46 <sup>c</sup>
B-0.64	33,97 $\pm$ 4,11 <sup>c</sup>	35,06 $\pm$ 5,28 <sup>c</sup>

**Table 4.** Benazepril drug. Different letters in the same column indicate differences between groups (\* $p < 0.001$ ).



**Fig. 18.** Mild  $\alpha$ -syn positivity (TR: Texas Red) and nNOS positivity (FITC: fluorescein isothiocyanate) in the control, DMSO, and S-2.5 groups; severe  $\alpha$ -syn positivity in the 6-OHDA, C-0.31, and C-5.12 groups; and moderate  $\alpha$ -syn positivity in the C-0.62 and C-1.25 groups.



**Fig. 19.** Mild  $\alpha$ -syn positivity (TR: Texas Red) and nNOS positivity (FITC) in the control, DMSO, and B-0.16 groups; severe  $\alpha$ -syn positivity in the 6-OHDA and B-0.04 groups; and moderate  $\alpha$ -syn positivity in the B-0.08, B-0.32, and B-0.64 groups.

### Data availability

Data is available from the corresponding author upon reasonable request.

Received: 17 July 2025; Accepted: 20 November 2025

Published online: 06 December 2025

### References

1. Raza, C., Anjum, R. & Shakeel, N. U. A. Parkinson's disease: Mechanisms, translational models and management strategies. *Life Sci.* **226**, 77–90. <https://doi.org/10.1016/j.lfs.2019.03.057> (2019).

2. Poewe, W. et al. Parkinson disease. *Nat. Rev. Dis. Primers.* **3**, 17013. <https://doi.org/10.1038/nrdp.2017.13> (2017).
3. Almezgagi, M. et al. Diacerein: recent insight into Pharmacological activities and molecular pathways. *Biomed. Pharmacother.* **131**, 110594. <https://doi.org/10.1016/j.biopha.2020.110594> (2020).
4. LeWitt, P. A. Levodopa therapy for Parkinson's disease: Pharmacokinetics and pharmacodynamics. *Mov Disord.* **30** (1), 64–72. <https://doi.org/10.1002/mds.26082> (2015).
5. Ferah Okkay, I. et al. Neuroprotective effect of Bromelain in 6-hydroxydopamine induced *in vitro* model of parkinson's disease. *Mol. Biol. Rep.* **48** (12), 7711–7717. <https://doi.org/10.1007/s11033-021-06779-y> (2021).
6. Goetz, C. G. The history of parkinson's disease: early clinical descriptions and neurological therapies. *Cold Spring Harb Perspect. Med.* **1** (1), pa008862. <https://doi.org/10.1101/cshperspect.a008862> (2011).
7. Erdal, K. J. Depression and anxiety in persons with parkinson's disease with and without On–Off phenomena. *J. Clin. Psychol. Med. Settings.* **8** (4), 293–299. <https://doi.org/10.1023/A:1011972930917> (2001).
8. Carrera, I. & Cacabelos, R. Current drugs and potential future neuroprotective compounds for parkinson's Disease. *Curr. Neuropharmacol.* **17** (3), 295–306. <https://doi.org/10.2174/1570159x17666181127125704> (2019).
9. Xicoy, H., Wieringa, B. & Martens, G. J. The SH-SY5Y cell line in Parkinson's disease research: a systematic review. *Mol Neurodegener.* **12** (1), 10. <https://doi.org/10.1186/s13024-017-0149-0> (2017).
10. Yürük, M. A. et al. Behavioral tests used in experimental animal models. *Anatol. J. Biology.* **3** (2), 14–22 (2022).
11. Kleinbloesem, C. H., van Brummelen, P., Francis, R. J. & Wiegand, U. W. Clinical pharmacology of cilazapril. *Drugs* **41** (Suppl 1), 3–10. <https://doi.org/10.2165/00003495-199100411-00003> (1991).
12. Sengul, G. et al. Neuroprotective effect of ACE inhibitors in glutamate - induced neurotoxicity: rat neuron culture study. *Turk Neurosurg* **21** (3), 367–71. <https://doi.org/10.5137/1019-5149.Jtn.4313-11.0> (2011).
13. Juszcak, A., Szczolko, W., Ramos, P., Pilawa, B. & Stanisz, B. Evaluation of antioxidant properties of angiotensin-converting enzyme inhibitors-interactions with free radicals model examined by EPR spectroscopy. *Hosp. Pharmacol. - Int. Multidisciplinary J.* **8**, 25–32. <https://doi.org/10.15406/ppij.2020.08.00276> (2020).
14. Zhan, L. et al. Benazepril hydrochloride protects against doxorubicin cardiotoxicity by regulating the PI3K/Akt pathway. *Exp. Ther. Med.* **22** (4), 1082. <https://doi.org/10.3892/etm.2021.10516> (2021).
15. Natarajan, K., Chandrasekaran, R., Sundararaj, R., Joseph, J. & Asaithambi, K. Neuroprotective assessment of nutraceutical (Betanin) in neuroblastoma cell line SHSY-5Y: an in-Vitro and in-Silico Approach. *Neurochem Res.* **50** (1), 54. <https://doi.org/10.1007/s11064-024-04312-8> (2024).
16. Ram, T. S. et al. In silico evaluation of the compounds of the ayurvedic drug, AYUSH-64, for the action against the SARS-CoV-2 main protease. *J Ayurveda Integr Med* **13** (1), 100413. <https://doi.org/10.1016/j.jaim.2021.02.004> (2022).
17. Erel, O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin. Biochem.* **37** (2), 112–119. <https://doi.org/10.1016/j.clinbiochem.2003.10.014> (2004).
18. He, Q. et al. The Synergistic Effect Study of Lipopolysaccharide (LPS) and A53T- $\alpha$ -Synuclein: Intranasal LPS Exposure on the A53T- $\alpha$ -Synuclein Transgenic Mouse Model of Parkinson's Disease. *Mol Neurobiol* **61** (9), 7046–7065. <https://doi.org/10.1007/s12035-024-04020-y> (2024).
19. Bové, J., Prou, D., Perier, C. & Przedborski, S. Toxin-induced models of Parkinson's disease. *NeuroRx* **2** (3), 484–94. <https://doi.org/10.1602/neuroRx.2.3.484> (2005).
20. Terzioglu, M. & Galter, D. Parkinson's disease: genetic versus toxin-induced rodent models. *Febs j.* **275** (7), 1384–1391. <https://doi.org/10.1111/j.1742-4658.2008.06302.x> (2008).
21. Linert, W. et al. Dopamine, 6-hydroxydopamine, iron, and dioxygen—their mutual interactions and possible implication in the development of parkinson's disease. *Biochim. Biophys. Acta.* **1316** (3), 160–168. [https://doi.org/10.1016/0925-4439\(96\)00020-8](https://doi.org/10.1016/0925-4439(96)00020-8) (1996).
22. Jordán, J., Galindo, M. F., Tornero, D., González-García, C. & Ceña, V. Bcl-x L blocks mitochondrial multiple conductance channel activation and inhibits 6-OHDA-induced death in SH-SY5Y cells. *J. Neurochem.* **89** (1), 124–133. <https://doi.org/10.1046/j.1471-4159.2003.02299.x> (2004).
23. Watanabe, H., Ogura, T., Hosoya, M., Nishida, N. & Ota, Z. Diuretic effect of cilazapril and dopamine system in the spontaneously hypertensive rat. *Acta Med Okayama.* **49** (5), 247–52. <https://doi.org/10.18926/amo/30400> (1995).
24. Latif, S. et al. Dopamine in parkinson's disease. *Clin. Chim. Acta.* **522**, 114–126. <https://doi.org/10.1016/j.cca.2021.08.009> (2021).
25. Bateman, B. T. et al. Angiotensin-Converting Enzyme Inhibitors and the Risk of Congenital Malformations. *Obstet Gynecol.* **129**(1), 174–184. <https://doi.org/10.1097/aog.0000000000001775> (2017).
26. Kaur, P., Muthuraman, A. & Kaur, M. The implications of angiotensin-converting enzymes and their modulators in neurodegenerative disorders: current and future perspectives. *ACS Chem Neurosci* **6**(4), 508–21. <https://doi.org/10.1021/cn500363g> (2015).
27. Antony, P. et al. Molecular insights into the Inhibition of angiotensin-converting enzyme 1 by hemopressin peptides. *Sci. Rep.* **14** (1), 28726. <https://doi.org/10.1038/s41598-024-78893-3> (2024).
28. Halimi, M. & Hajipasha, A. Pharmacophore modeling, Docking and molecular dynamic simulation studies in the discovery of potential human Renin inhibitors. *J. Mol. Graph Model.* **116**, 108272. <https://doi.org/10.1016/j.jmgm.2022.108272> (2022).
29. Liu, X. et al. Molecular dynamics investigation on the interaction of human angiotensin-converting enzyme with tetrapeptide inhibitors. *Phys. Chem. Chem. Phys.* **23** (11), 6685–6694. <https://doi.org/10.1039/d1cp00172h> (2021).
30. Martel, J. C., Gatti, S. & McArthur Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia. *Front Pharmacol.* **11**, 1003. <https://doi.org/10.3389/fphar.2020.01003> (2020).
31. Regulski, M. et al. Chemistry and pharmacology of Angiotensin-converting enzyme inhibitors. *Curr Pharm Des.* **21** (13), 1764–75. <https://doi.org/10.2174/138161282066614112160013> (2015).
32. Maiti, S., Banerjee, A. & Kanwar, M. In silico Nigellidine (N. sativa) bind to viral spike/active-sites of ACE1/2, AT1/2 to prevent COVID-19 induced vaso-tumult/vascular-damage/comorbidity. *Vascul Pharmacol* **138**, 106856. <https://doi.org/10.1016/j.vph.2021.106856> (2021).
33. Chang, K. H. & Chen, C. M. The Role of Oxidative Stress in Parkinson's Disease. *Antioxidants (Basel)* **9**(7), <https://doi.org/10.3390/antiox9070597> (2020).
34. Ha, T. S., Seong, S. B., Ha, D. S. & Kim, S. J. Upregulation of NADH/NADPH oxidase 4 by angiotensin II induces podocyte apoptosis. *Kidney Res. Clin. Pract.* **42** (2), 202–215. <https://doi.org/10.23876/j.krccp.22.198> (2023).
35. Ghalayini, J. & Boulianne, G. L. Deciphering mechanisms of action of ACE inhibitors in neurodegeneration using Drosophila models of Alzheimer's disease. *Front Neurosci* **17** 1166973. <https://doi.org/10.3389/fnins.2023.1166973> (2023).
36. Santiago, T. C. et al. Angiotensin-converting enzymes as druggable features of psychiatric and neurodegenerative disorders. *J. Neurochem.* **166** (2), 138–155. <https://doi.org/10.1111/jnc.15806> (2023).
37. Abiodun, O. A. & Ola, M. S. Role of brain Renin angiotensin system in neurodegeneration: an update. *Saudi J. Biol. Sci.* **27** (3), 905–912. <https://doi.org/10.1016/j.sjbs.2020.01.026> (2020).
38. de Farias, C. C. et al. Highly specific changes in antioxidant levels and lipid peroxidation in parkinson's disease and its progression: disease and staging biomarkers and new drug targets. *Neurosci. Lett.* **617**, 66–71. <https://doi.org/10.1016/j.neulet.2016.02.011> (2016).
39. Tuon, T. et al. Behavior and oxidative stress parameters in rats subjected to the animal's models induced by chronic mild stress and 6-hydroxydopamine. *Behav Brain Res.* **406**, 113226. <https://doi.org/10.1016/j.bbr.2021.113226> (2021).
40. Vajapey, R., Rini, D., Walston, J. & Abadir, P. The impact of age-related dysregulation of the angiotensin system on mitochondrial redox balance. *Front. Physiol.* **5**, 439. <https://doi.org/10.3389/fphys.2014.00439> (2014).

41. Jiménez, A., Correa, S. & Sevilla, F. Identification of superoxide dismutase (SOD) isozymes in plant Tissues. *Methods Mol. Biol.* **2798**, 205–212. [https://doi.org/10.1007/978-1-0716-3826-2\\_14](https://doi.org/10.1007/978-1-0716-3826-2_14) (2024).
42. Pandey, S., Singh, B., Yadav, S. K. & Mahdi, A. A. Novel biomarker for neurodegenerative diseases- motor neuron disease (MND), cerebellar ataxia (CA) and parkinson's disease (PD). *Clin. Chim. Acta.* **485**, 258–261. <https://doi.org/10.1016/j.cca.2018.07.021> (2018).
43. Asanuma, M. et al. Region-Specific neuroprotective features of astrocytes against oxidative stress induced by 6-Hydroxydopamine. *Int. J. Mol. Sci.* **20** (3). <https://doi.org/10.3390/ijms20030598> (2019).
44. Waterfall, J. F. A review of the preclinical cardiovascular pharmacology of cilazapril, a new angiotensin converting enzyme inhibitor. *Br J Clin Pharmacol* **27** (Suppl 2, no. Suppl 2), 139s–150s. <https://doi.org/10.1111/j.1365-2125.1989.tb03475.x> (1989).
45. Adamu, A., Li, S., Gao, F. & Xue, G. The role of neuroinflammation in neurodegenerative diseases: current understanding and future therapeutic targets. *Front Aging Neurosci* **16**, 1347987. <https://doi.org/10.3389/fnagi.2024.1347987> (2024).
46. Oosthuizen, D. & Sturrock, E. D. Exploring the Impact of ACE Inhibition in Immunity and Disease. *J Renin Angiotensin Aldosterone Syst.* **2022**, 9028969. <https://doi.org/10.1155/2022/9028969> (2022).
47. Pike, A. F. et al. Dopamine signaling modulates microglial NLRP3 inflammasome activation: implications for parkinson's disease. *J. Neuroinflammation.* **19** (1), 50. <https://doi.org/10.1186/s12974-022-02410-4> (2022).
48. Mishra, J. et al. BBPT attenuated 6-OHDA-induced toxicity by modulating oxidative stress, apoptotic, and inflammatory proteins in primary neurons and rat models of Parkinson's disease. *Neurotoxicology* **105**, 67–81. <https://doi.org/10.1016/j.neuro.2024.08.008> (2024).
49. Yang, M. H. et al. Utilizing Proteomic Approaches to Uncover the Neuroprotective Effects of ACE Inhibitors: Implications for Alzheimer's Disease Treatment. *Molecules.* **28**, 16. <https://doi.org/10.3390/molecules28165938> (2023).
50. Kashima, R. & Hata, A. The role of TGF- $\beta$  superfamily signaling in neurological disorders. *Acta Biochim. Biophys. Sin (Shanghai).* **50** (1), 106–120. <https://doi.org/10.1093/abbs/gmx124> (2018).
51. Labandeira-Garcia, J. L., Labandeira, C. M., Guerra, M. J. & Rodriguez-Perez, A. I. The role of the brain renin-angiotensin system in Parkinson's disease. *Transl Neurodegener* **13** (1), 22. <https://doi.org/10.1186/s40035-024-00410-3> (2024).
52. Gouveia, F. et al. Targeting brain Renin-Angiotensin System for the prevention and treatment of Alzheimer's disease: Past, present and future. *Ageing Res Rev.* **77**, 101612. <https://doi.org/10.1016/j.arr.2022.101612> (2022).
53. Märtin, A. et al. A Spatiomolecular Map of the Striatum. *Cell Rep.* **29** (13), 4320–4333.e5. <https://doi.org/10.1016/j.celrep.2019.11.096> (2019).
54. Liu, C. & Kaeser, P. S. Mechanisms and regulation of dopamine release. *Curr Opin Neurobiol* **57**, 46–53. <https://doi.org/10.1016/j.conb.2019.01.001> (2019).
55. Ganguly, U., Singh, S., Chakrabarti, S., Saini, A. K. & Saini, R. V. Chapter Ten - Immunotherapeutic interventions in Parkinson's disease: Focus on  $\alpha$ -Synuclein, in *Advances in Protein Chemistry and Structural Biology*, vol. 129, R. Donev Ed.: Academic, 381–433. (2022).
56. Jo, Y., Kim, S., Ye, B. S., Lee, E. & Yu, Y. M. Protective effect of Renin-Angiotensin system inhibitors on parkinson's disease: A nationwide cohort Study. *Front. Pharmacol.* **13**, 837890. <https://doi.org/10.3389/fphar.2022.837890> (2022).
57. Ray, B. et al. Effects of Telmisartan, an AT1 receptor antagonist, on mitochondria-specific genes expression in a mouse MPTP model of Parkinsonism. *Front. Biosci. (Landmark Ed).* **26** (8), 262–271. <https://doi.org/10.52586/4942> (2021).
58. Park, S. Y., Kang, M. J. & Han, J. S. Neuronal NOS Induces Neuronal Differentiation Through a PKC $\alpha$ -Dependent GSK3 $\beta$  Inactivation Pathway in Hippocampal Neural Progenitor Cells. *Mol Neurobiol.* **54** (7), 5646–5656. <https://doi.org/10.1007/s12035-016-0110-1> (2017).
59. Alboani, A., Kar, S. & Kavdia, M. Computational modeling of neuronal nitric oxide synthase biochemical pathway: A mechanistic analysis of tetrahydrobiopterin and oxidative stress. *Free Radic Biol Med* **222**, 625–637. <https://doi.org/10.1016/j.freeradbiomed.2024.07.011> (2024).
60. Agrawal, S., Kumari, R., Sophronea, T., Kumari, N. & Luthra, P. M. Design and synthesis of benzo[d]thiazol-2-yl-methyl-4-(substituted)-piperazine-1-carbothioamide as novel neuronal nitric oxide inhibitors and evaluation of their neuroprotecting effect in 6-OHDA-induced unilateral lesioned rat model of Parkinson's disease. *Biomed Pharmacother.* **156**, 113838. <https://doi.org/10.1016/j.biopha.2022.113838> (2022).

## Author contributions

OA: Investigation, Conceptualization, Data curation, Formal Analysis, Software, Writing – review & editing. AH: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Writing – original draft, Writing – review & editing. IFO: Data curation, Formal Analysis, Software, Conceptualization. FY: Investigation, Data curation, Data analysis. MAY: Investigation, Data analysis, Formal Analysis. MO: Data curation: Data curation, Formal Analysis.

## Funding

This work was supported by Research Fund of the Ataturk University. (Project Number: TYL-2022-10746)

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to O.A., A.H. or I.F.O.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025