Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X

Volume 30 | No. 2 | **August** | 2024

Original article

In silico study of Hydrogen Peroxide (H_2O_2) binding effect to Extracellular Signal-Regulated Kinases (ERK)

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Abstract

H_2O_2 can provide a physiological response by activating mitogen-activated potein kinase (MAPK) signaling components; it can phosphorylate and activate several regulatory proteins closely related to gene expression and growth promotion. H_2O_2 has neuroprotective abilities and the potential to be a complementary therapy. It can be implemented in Parkinson's Disease (PD) therapy by activating; Extracellular signal-regulated kinases (ERK/1/2/5), playing a central role in the control of endothelial cell proliferation, and triggering apoptosis signal-regulating kinase 1 (ASK1), critical modulators of the apoptosis cell or death balance. The primary pathology of PD is amyloid fibrils composed of αSyn (α-Synuclein). Aggregate of αSyn causes mitochondrial dysfunction, dysregulation, and apoptosis in brain endothelial cells (BECs); it leads to blood brain barrier (BBB) leakage and increases BBB permeability. Therefore, treatments that prevent vascular degeneration may be new targets for PD. This research used the in-silico method. The 3D ligand and protein target structures were retrieved from PubChem and the protein data bank (PDB). Ligand was converted using the Open Babel, and the molecular docking program used AutoDock Vina as a plug-in in PyRx. Results were analyzed using PyMOL and Discovery Studio. Molecular dynamics simulation used YASARA. From this study, H_2O_2 lowered the RMSD and RMSF values of ERK 1/2/5 and ASK1. It is known that H_2O_2 stabilize ERK 1/2/5 and ASK1.

Keywords: ASK1, ERK, Hydrogen peroxide, *In Silico*

Received: May 10, 2024 Revised: June 27, 2024 Accepted: July 17, 2024

Introduction

Mitogen-activated protein kinase (MAPKs) can phosphorylate and activate several regulatory proteins closely related to gene expression and growth promotion; activation of these signaling components is a physiological response of H_2O_2 (Blanc et al. 2003). Low-dose H_2O_2 has neuroprotective potential (Widyarti et al. 2023) and potential to be a complementary therapy, by activating Extracellular signal-regulated kinases (ERK/1/2/5), p38 and JNK (Bretón-Romero & Lamas 2014) which belong to the MAPK family (Olea-Flores et al. 2019). ERK1/2 phosphorylation plays a central role in the control of endothelial cell proliferation (Srinivasan et al. 2009) by inducing the expression of serum response factor (SRF), MAP kinase-interacting serine/threonine-protein kinase 1 and 2 (MNK1/2), and RSK2 to activate c-fos and cAMP-response element-binding protein (CREB) transcription factors; thus regulating cell transcription and translation; promoting cell proliferation (Cargnello & Roux, 2011; Olea-Flores et al. 2019; Song et al. 2023). ERK5 is expressed in high levels in the brain. It is proposed to regulate vascular development, neuronal activity, survival (Olea-Flores et al. 2019), and inhibit apoptosis of endothelial cells (Bretón-Romero & Lamas 2014). Phosphorylates ERK5 increase the activity of nuclear receptor subfamily 4 group A member 1 (Nur77) transcription factor and promote cell proliferation (Song et al. 2023). However, a significant increase in the number of brain endothelial cells (BECs) it can also give side effects (Bradaric et al. 2012). Thus, homeostasis regulation is needed to ensure that the growth of BECs remains normal. The regulation is done by activating apoptosis signal-regulating kinase 1 (ASK1) by H_2O_2. ASK1 activates stress-regulated protein kinases p38-MAPK (mitogen-activated protein kinases) and c-Jun N-terminal kinases (JNKs) cascades, which are key modulators of the cell survival versus apoptosis/death balance (Meijles et al. 2020).

H_2O_2 has the potential to be a complementary therapy; by activating ERK/1/2/5 and ASK1, it can be implemented in Parkinson's Disease (PD) therapy. The main pathological of PD is amyloid fibrils composed of α-synuclein (αsyn) (Hijaz & Volpicelli-Daley 2020). Toxic αSyn accumulation on BECs (Jeong et al. 2023), caused mitochondrial dysfunction, dysregulation, and apoptosis in BECs (Hourfar et al. 2023; Thanvi & Lo 2004), it leads to BBB leakage (Alkhalifa et al. 2023), increase BBB permeability and BBB disruption (Burré 2015; Hourfar et al. 2023; Yuan et al. 2023). BBB disruption causes neuroinflammation, which can lead to neuronal dysfunction and neurodegeneration (Profaci et al. 2020) that eventually leads to neurodegenerative diseases, including PD. L-DOPA is a commonly used symptomatic treatment for PD (Thanvi & Lo 2004), until recently. L-DOPA is used because it can cross BBB, easily transported to the central nervous system (CNS), and then converted to dopamine by the enzyme DOPA decarboxylase (Muthuraman et al. 2018). L-type amino Acid Transporter 1 (LAT-1) is a transmembrane protein

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http://dx.doi.org/10.23869/bphjbr.30.2.20245
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in the BECs transports L-DOPA across the BBB to the brain to reach its target location (Gynther et al. 2019; Puris et al. 2020). Only L-DOPA therapy cannot overcome apoptosis of BEC and BBB disruption, it is making L-DOPA ineffective. (Hijaz & Volpicelli-Daley 2020). Therefore, treatments that prevent vascular degeneration may be new targets for PD. This research aims to determine the potential of H_2O_2 in ERK and ASK1 stabilization using in-silico methods. It is known that H_2O_2 is a candidate for complement therapy with L-DOPA for PD.

Methods

Protein target preparation

The protein target three-dimensional (3D) crystal structure of *Homo sapiens* was retrieved from Protein Data Bank (PDB) (https://www.rcsb.org/), ERK1 (PDB ID: 8AOJ), ERK2 (PDB ID: 4QTB), ERK5 (PDB ID: 5BYZ) and ASK1 (PDB ID: 2CLQ), was retrieved in pdb format and prepared by removing water molecules (Lestari et al., 2023) using Discovery Studio 2021 Client v21.1.0.20298, operated under Microsoft Windows 11.

Molecular docking

Hydrogen peroxide (H_2O_2) (CID: 784) as a ligand was converted using the Open Babel into pdbqt (Price et al., 2014). Docking between H_2O_2 molecules and ERK proteins to form complexes of ERK1-H_2O_2, ERK2-H_2O_2, ERK5-H_2O_2, and ASK1-H_2O_2 was performed using AutoDock Vina integrated in PyRx v.0.9.8. This study used rigid docking method, with 50 exhausted, nine modes parameters and dimensions X; Y; Z: 10. Visualization used PyMOL v.2.5.5 (Schrodinger, LLC) and Discovery Studio 2021. Then, analyzed the binding affinity and binding site of each complex.

Molecular dynamic

Molecular dynamics (MD) simulation using YASARA Structure v.23.9.29, with forcefield AMBER 14. The variables for simulation include the water solvent with a density of 0.997 g/ml; ion concentration of NaCl 0.9%; pH 7.4; temperature of 310K; (Lestari et al., 2023), save interval in 25000 fs and duration of simulation in 20 ns. The analysis parameter includes total & Cα Root mean square deviation (RMSD) and the root-mean-square-fluctuation (RMSF).

Results

Docking was performed with targeted docking by adjusting the phosphorylation sites of each ERK and ASK1 protein. ERK is a class of MAPK, activated by double phosphorylation on tyrosine and threonine residues (Blanc et al. 2003; Olea-Flores et al. 2019). ERK1 with phosphorylation site at residue Thr202 & Tyr204, ERK2 at residue Thr185 & Tyr187 and ERK5 at residue Thr219 & Tyr221 (Olea-Flores et al. 2019). Analysis of docking results from discovery studio in "Table. 1" show that, the H_2O_2 molecule binds to site phosphorylation on ERK1 only at Thr202. In ERK2, the H_2O_2 molecule binds to both site phosphorylation at Thr185 and Tyr187. In ERK5, the H_2O_2 molecule binds to site phosphorylation only at Thr219. Site phosphorylation of ASK1 at the Thr-838 residue (Hayakawa et al. 2012; Shiizaki et al. 2013; Betanzos et al. 2016), while in the docking results between ASK1 and H_2O_2 at the Thr-840 residue becomes the phosphorylation activation site. All complex of docking results will be shown in "Figure. 1".

Table 1. Amino acid residue from docking results of ERK and ASK1 protein with H_2O_2

No	Protein target	Binding Affinity	Amino acid residue	Interaction
1	ERK1	-2.8	LEU201 THR202* ASN218 ILE215 TYR250 SER219	Conventional Hydrogen Bond
		-2.3	LEU167 ARG165	Conventional Hydrogen Bond
2	ERK2	-1.9	ILE198 SER202	Conventional Hydrogen Bond
			THR185* LYS203	Carbon Hydrogen Bond
		-2.2	GLU186 TYR187*	Conventional Hydrogen Bond
3	ERK5	-2.8	GLU220 SER235 THR219*	Conventional Hydrogen Bond
		-3.0	GLU231 GLU238	Convention Hydrogen Bond
			THR240	Unfavorable
4	ASK1	-1.8	THR840	Unfavorable

*) site phosphosrylation that relevant to native region

The result of the MD analysis consists of total RMSD, Cα RMSD and RMSF. "Figure 2" Total RMSD analysis of ERK1 and ERK2 protein has a value of >3Å, increase in total RMSD value in ERK1 complex is more significant than ERK2, even reaches 5 Å. While in the ERK1-H_2O_2 and ERK2-H_2O_2 complex, the total RMSD value becomes <3Å. In the ERK5-H_2O_2 and ASK1-H_2O_2 complex, there is an increase in the total RMSD value when compared to ERK5 and ASK1, but the increase in total RMSD value that occurs is in the range of 3Å.

Cα RMSD results in "Figure 3". Cα RMSD has almost the same value as the total RMSD. The ERK1-H_2O_2 and ERK2-H_2O_2 complexes have lower values (<3 Å), when compared to the ERK1/2 complex without H_2O_2 molecule (Cα RMSD values >3 Å). While the ERK5 and ASK1 complexes undergo a slight increase in the Cα RMSD value, they are still <3Å. "Figure 4" shows the RMSF of ERK1-H_2O_2, ERK2- H_2O_2, ERK5-H_2O_2, and ASK1-H_2O_2 complexes, all of the complexes have a lower RMSF value (RMSF < 3Å), compared to ERK1, ERK2, ERK5, and ASK1. Several amino acid residues in the ERK1 complex undergo significant fluctuation, at residue numbers 80 and 273, which have RMSF values of more than >10 Å. In the ERK2 complex, residue numbers 28 and 157 undergo significant fluctuations (RMSF value is > 8 Å).

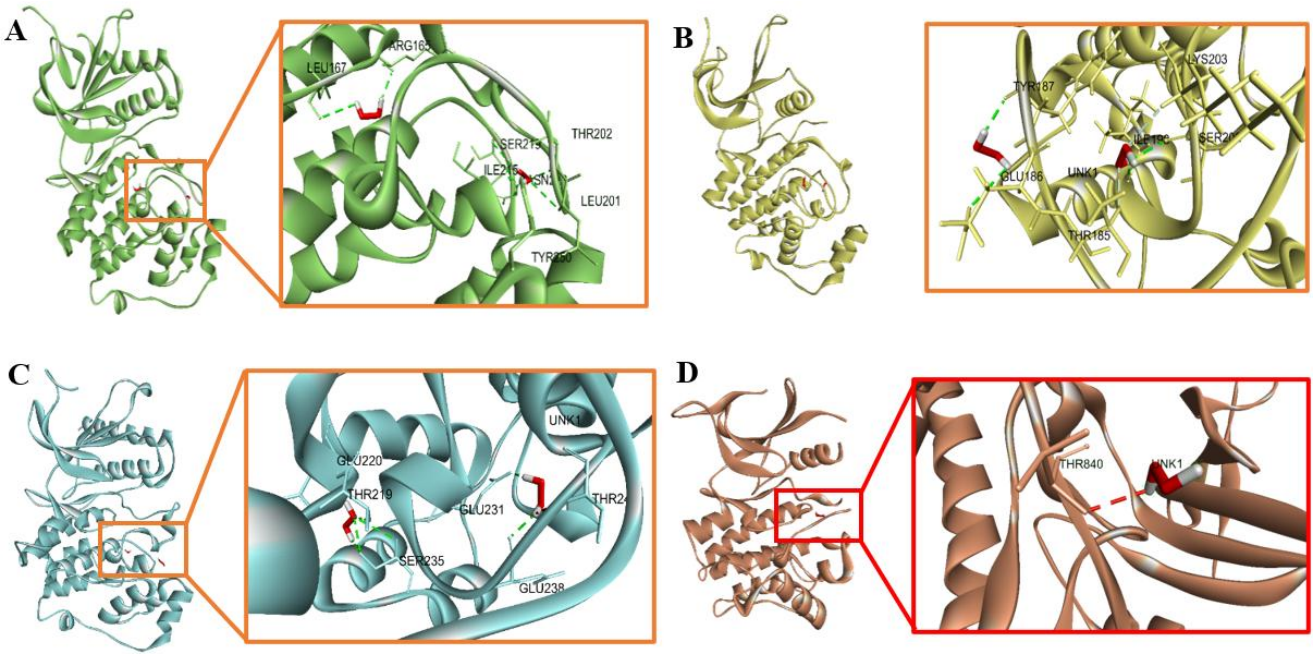

Figure 1. Docking results of each protein ERK with H_2O_2. (A) ERK1- H_2O_2, (B) ERK2- H_2O_2, (C) ERK5- H_2O_2, (D) ASK1- H_2O_2

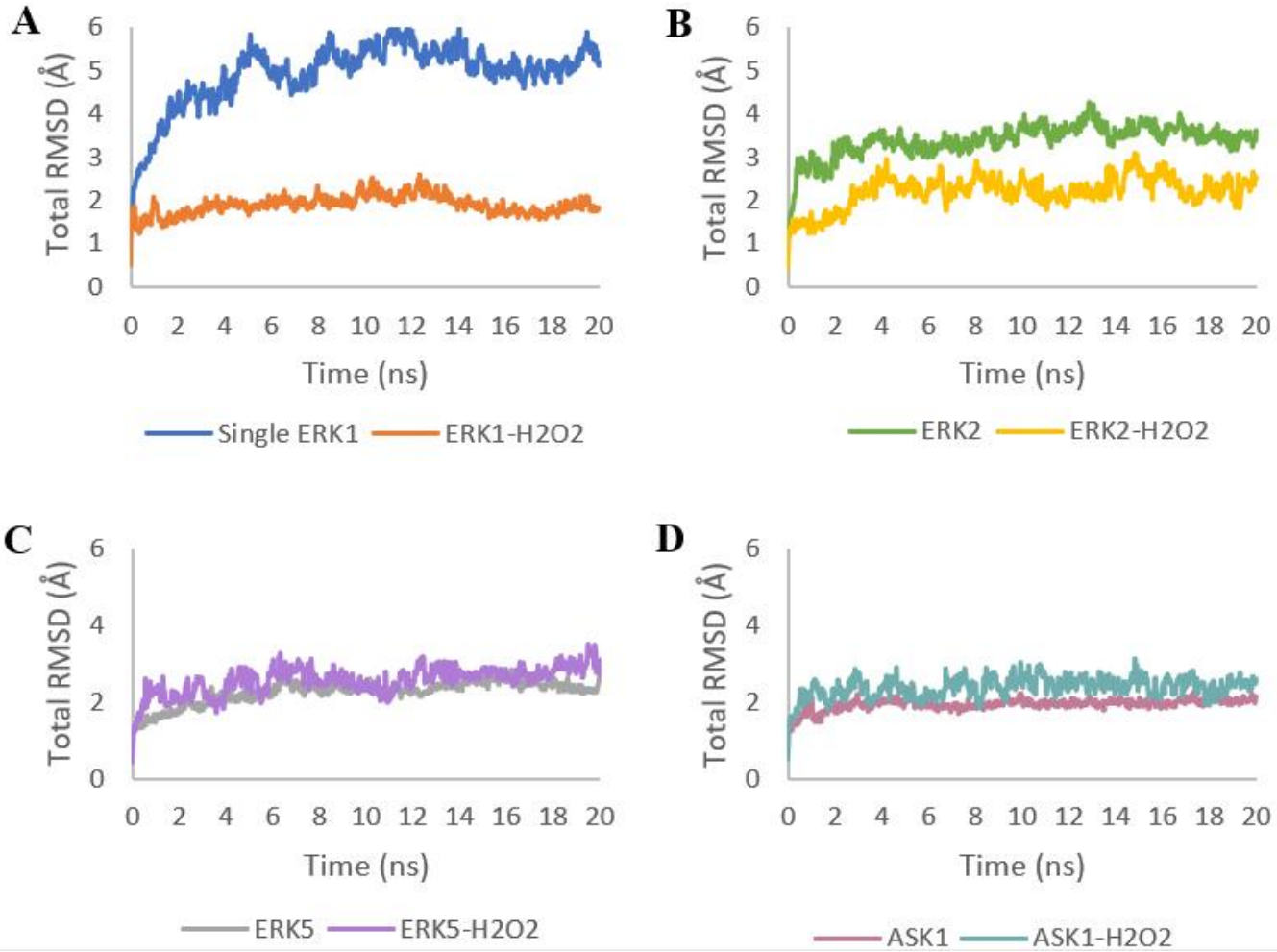

Figure 2. Total RMSD of all complexes (A) ERK1 and ERK1-H_2O_2, (B) ERK2 and ERK2-H_2O_2, (C) ERK5 and ERK5-H_2O_2, (D) ASK1 and ASK1-H_2O_2

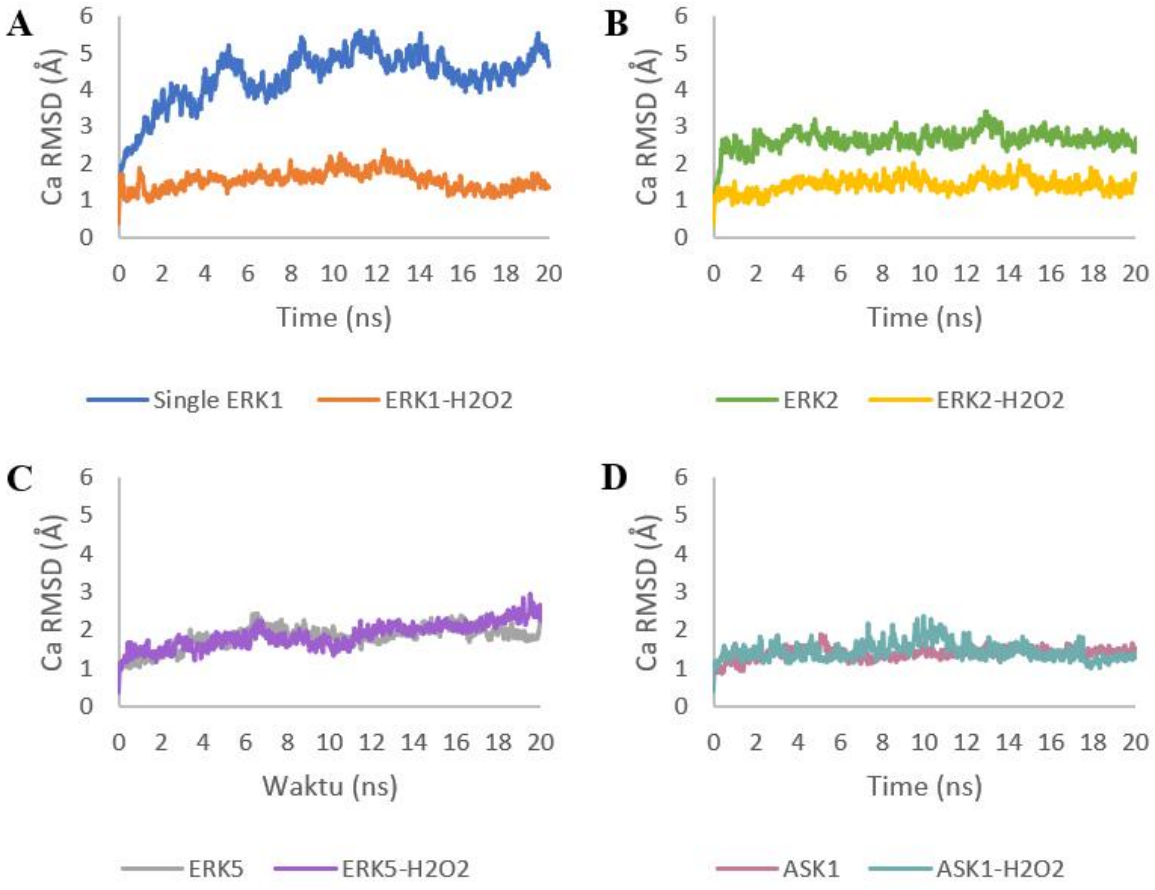

Figure 3. Ca RMSD of all complexes (A) ERK1 and ERK1-H_2O_2, (B) ERK2 and ERK2-H_2O_2, (C) ERK5 and ERK5-H_2O_2, (D) ASK1 and ASK1-H_2O_2

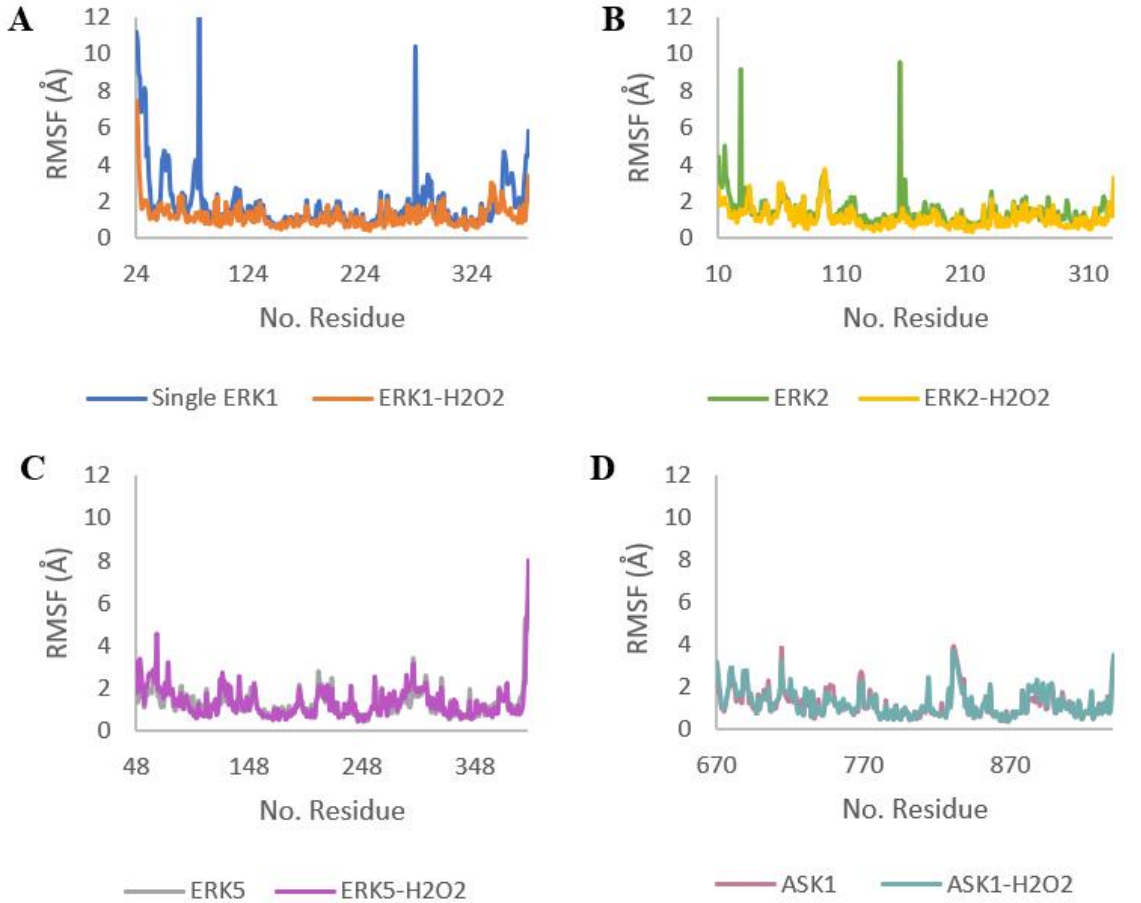

Figure 4. RMSF of all complexes (A) ERK1 and ERK1-H_2O_2, (B) ERK2 and ERK2-H_2O_2, (C) ERK5 and ERK5-H_2O_2, (D) ASK1 and ASK1-H_2O_2

Discussion

Molecular docking result in "Table 1", show that H_2O_2 with ERK1/2/5 and ASK1 has a binding affinity with a negative value, indicating that the receptor-ligand interaction spontaneously (Du et al. 2016). H_2O_2 molecule in the ERK1-H_2O_2, ERK2-H_2O_2, and ERK5-H_2O_2 complex binds to the phosphorylation site according to its native region. In the ASK1-H_2O_2 complex, the H_2O_2 molecule binds differently from its native place (THR-838), binding to the novel phosphosite at residue THR-840. There may be a change in the phosphorylation site. According to Carrasco-Navarro & Aguirre (2021), H_2O_2-induced phosphorylation changes in several proteins, and a large part of H_2O_2-induced changes in MAPK. Protein phosphorylation (serine/threonine) changes some of these components in response to H_2O_2. Their research using phospho-proteomic analysis mentioned that phospho-proteomic results confirmed this by showing that while SakA was dephosphorylated in the absence of H_2O_2, it became phosphorylated at the T171 and Y173 residues, and a novel phosphosite was detected at T176 in the presence of H_2O_2. Identifying differentially phosphorylated proteins through phospho-proteomic analysis provides insight into the signal transduction pathways activated in response to growth factor stimulation or toxic-induced apoptosis. Thus, in this study, phospho-proteomics techniques can be combined to investigate dynamic changes in protein phosphorylation quantitatively (de Graauw et al. 2006).

MD analysis consists of the total RMSD, Cα RMSD, and RMSF. The total RMSD ensures that the system is in equilibrium, as an indicator of the accuracy of the sample protein complex (Arnittali et al. 2019), and adequately represents the stability of the sample under simulated conditions. Lower values of RMSD indicate more stable molecules (Biswas et al. 2023). Total RMSD analysis was used to confirm the stable nature of the protein-ligand complex under simulated conditions (Alom et al. 2023). Cα RMSD as the main factor that contributes to stabilizing the protein and indicating a conformational change in the target protein (Siraj et al. 2021). "Figure 2", Total RMSD analysis of ERK1 and ERK2 protein has a value of >3Å, while in the ERK1-H_2O_2 and ERK2- H_2O_2 complex, the RMSD value becomes <3Å. This shows that the presence of H_2O_2 molecules lowers the total RMSD value so that proteins and ligands are stable in their complexes during simulation (Tran et al. 2022). The increase of total RMSD value in the ERK5-H_2O_2 and ASK1-H_2O_2 complex occurs in the range of 3Å, which indicates that the complex is still in a stable state (Biswas et al. 2023).

Cα RMSD in complexes with H_2O_2, especially on ERK1-H_2O_2 and ERK2-H_2O_2, have lower values (<3 Å) "Figure 3". ERK5-H_2O_2 and ASK1-H_2O_2 complexes undergo a slight increase in the Cα RMSD value. However, the stability of the complex is still maintained because the Ca RMSD value is still <3Å. RMSF value of ERK1-H_2O_2, ERK2-H_2O_2, ERK5-H_2O_2, and ASK1-H_2O_2 complex in "Figure 4". The RMSF parameter is a standard measure used to quantify fluctuations in the interactions or positions of ligands with amino acid residues on target proteins that occur during simulations (Farmer et al. 2017; Barazorda-Ccahuana et al. 2018; Rampogu et al. 2022). All complexes with H_2O_2 molecules have a lower value (RMSF < 3Å). This suggests that the presence of H_2O_2 molecules makes the complex more stable and compact. The low RMSF value of binding site residues indicates the stability of ligand binding to proteins (Soumia et al. 2022). Therefore, H_2O_2 has the potential to be a complementary therapy because of its ability to stabilize MAPK protein.

Hydrogen peroxide acts as a signaling second messenger in the vasculature. Its targets in the cardiovascular system are diverse and convey various effects on the endothelium (Blanc et al. 2003). However, this discussion focuses on BECs in BBB. Low concentrations of H_2O_2 play a key role in vascular function and homeostasis (Jiang et al. 2017). Erk1/2/5 reported as targets of H_2O_2. The ERK cascade is principally involved in proliferation, differentiation, growth, and cell survival (Bretón-Romero & Lamas 2014). In some studies, low-dose H_2O_2 promotes the proliferation of endothelial cells by enhancing the phosphorylation of ERK1/2/5 proteins. Phosphorylation of transcription factors by MAPKs leads to activation of several genes involved in growth and differentiation; activation of this signaling component has been implicated in mediating some of the physiological responses of H_2O_2 (Blanc et al. 2003).

Studies from Blanc et al. (2003) demonstrated that as low as 100 μM H_2O_2 enhanced the phosphorylation of ERK 1/2 in vascular smooth muscle cells (VSMC) from rat embryonal thoracic aorta. H_2O_2-induced ERK 1/2 activation was associated with enhanced tyrosine phosphorylation of EGF receptor (Rao 1996). According to Jiang et al. (2017) low concentrations of H_2O_2 promote the proliferation of human umbilical vein endothelial cells (HUVECs), migration, and tubule formation. H_2O_2 significantly activated ERK5 in HUVECs. Enhanced ERK5 activity significantly amplified the proangiogenic effects of H_2O_2. Therefore, ERK5 may be a potential therapeutic target for promoting angiogenesis and improving BEC survival (Jiang et al., 2017).

Several studies conducted above show it can be concluded that H_2O_2 can activate MAPK proteins by phosphorylation. In this study, H_2O_2 shows its potential in stabilizing ERK and ASK proteins because it lowers the complex's total RMSD and RMSF value during simulation. To assess the stability of the protein structure, it is necessary to calculate the RMSD plot of the Cα backbone of the protein. Unphosphorylated proteins undergo drastic conformational changes, have high RMSD values, and phosphorylated proteins have low RMSD values, undergo minimal conformational changes, and are more stable. Flexibility in the phosphorylation position of the protein (SER, THR, TYR) will be attenuated upon phosphorylation. This can be attributed to the low RMSD and RSMF, indicating that phosphorylation reduces conformational changes and thus stabilizes the protein structure (Mal et al. 2020). Therefore, low concentrations of H_2O_2 stimulate

angiogenesis, suggesting that pharmacologically regulating cellular H_2O_2 levels rationally may be an angiogenic strategy (Jiang et al. 2017); if implemented in the PD as a complementary therapy, it may be comprehensively illustrated in Figure 5.

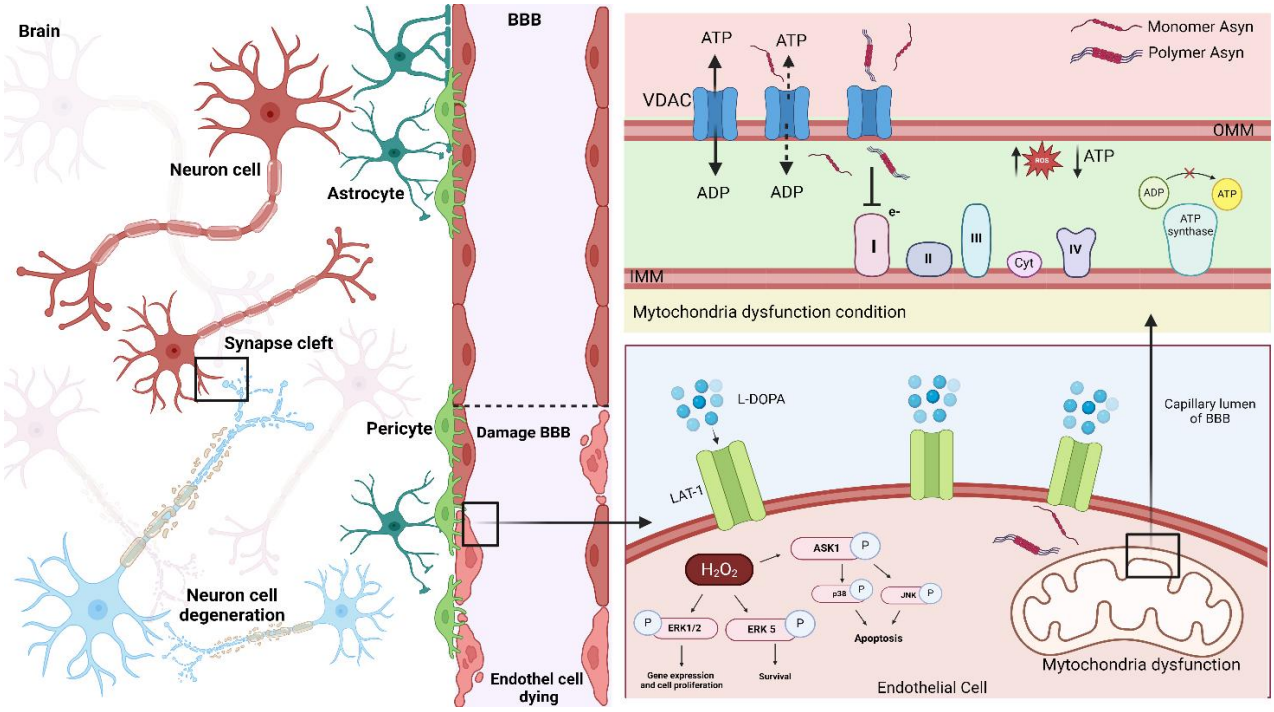

Figure 5. A comphrehensive illustration about the potential of H_2O_2 as complementary therapy in PD. This figure developed from Hourfar et al., (2023). Created with BioRender.com

Conclusion

Docking results show that H_2O_2 binds the site phosphorylation residue, except ASK1 binds the novel phosphorylation site. The MD result is that H_2O_2 can stabilize ERK 1/2/5 and ASK1 because of the reduction in total and Ca RMSD values. In addition, H_2O_2 lowers the RMSF value.

Acknowledgement

This study was supported by a grant from LPDP (the Indonesian Endowment Fund for Education). Thanks to BRC (Bioinformatics Research Centre) for providing access to PyRx 0.9.8 and Prof. Widodo, S.Si., M.Si., PhD.Med.Sc. is used to provide access to YASARA Molecular dynamic.

Disclosure of Interest

The authors report no conflict of interest.

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