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Potential Of Curcumin As Antitumor In Retinoblastoma

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ABSTRACT

Background: Retinoblastoma occurs due to gene mutations that have the potential to cause death. Retinoblastoma occurs due to mutations in the allele of the Retinoblastoma gene (RB1) which acts as a tumor suppressor gene, causing retinal cells to undergo uncontrolled proliferation. Mutations in the RB1 gene due to changes in the coding of this gene indirectly cause overexpression of the CDK4 and CDK6 proteins. curcumin can inhibit the proliferation of cancer cells, inhibit angiogenesis, inhibit metastasis, trigger apoptosis and increase the sensitivity of chemotherapy and radiotherapy. Curcumin induces Y79 cell apoptosis through activation of the JNK and p38 MAPK pathways. Purpose: analyzed the potential of curcumin in the treatment of retinoblastoma by examining its binding to CDK4, CDK6 and pRB proteins in In Silico with molecular docking techniques. Methods: The structure of the CDK4, CDK6, pRb receptors in the form of *.pdb was converted into *.pdbqt format through the AutoDock Tools 1.5.6 program. The docking method is done by tethering the ligand to the CDK4, CDK6, pRb receptors. The mooring coordinates are CDK4 (Grid Center) x = 13.0, y = 8.836, z = 43.2 and Grid Box size x= 70, y= 64, z= 62 . The mooring coordinates are CDK6 (Grid Center) x = 11,533, y = 25,223, z = 0.104 and Grid Box size x= 72, y= 66, z= 68 . The mooring coordinates are pRb (Grid Center) x = 33,576, y = 13.979, z = 21,171 and Grid Box size x= 84, y= 58, z= 64. Result: Curcumin has a binding affinity value for CDK4, CDK6, pRb proteins of -7.7, -7.8, -7.1. Conclusion: Curcumin has potential as a drug candidate for retinoblastoma.

Keyword: Curcumin, CDK4, CDK6, pRb, Retinoblastoma.

INTRODUCTION

Retinoblastoma is a malignancy of the retinal cells of the eye due to gene mutations that have the potential to cause death. Retinoblastoma occurs in children around the world with an increase in new cases of about 7000 - 8000 cases per year(Yu et al., 2016; Azlia et al., 2018). Retinoblastoma occurs due to mutations in the allele of the Retinoblastoma gene (RB1) which acts as a tumor suppressor gene, causing retinal cells to undergo uncontrolled proliferation (Dimaras et al., 2015). In the cell cycle, the RB1 gene that encodes Retinoblastoma protein (pRb) which is a key protein in the pathophysiology of Retinoblastoma which plays an important role in the G1/S transition phase and controls most cellular processes related to cell cycle checkpoints, replication, genome stability, proliferation and cell apoptosis. Mutations in the RB1 gene cause inactivation of the Rb protein so that it can induce the formation of retinoblastoma. Rb inactivation can be caused by the phosphorylation process by CDK4/6-Cyclin D which acts as tumor promoting. The presence of phosphorylation of Rb causes the release of Rb bonds with E2F which acts as a transcription factor that facilitates the development of the G1 phase of the cell cycle (Sivashanmugamet al., 2013; Engelet al., 2014).

The current treatment for retinoblastoma patients includes enucleation, cryotherapy, thermotherapy, radiotherapy, chemotherapy, and so on depending on the stage of the tumor. This drug belongs to a class of

cancer drugs known as alkylating agents that work by slowing or stopping the growth of cancer cells in the body (Hsiao et al., 2012; Eagle, 2013; Dimaras et al., 2015). This treatment is a race against time if not done immediately, there is a risk of tumor spread both intraocularly and extraocularly. Several innovations are being developed at this time, many targeted molecular therapies have been found that can be used to treat various diseases, including cancer. The therapy can maximize its effect towards the target and minimize its systemic effect so as to increase the effectiveness and tolerability and reduce the level of toxicity. Until now, no specific therapy for Retinoblastoma has been found that can completely treat Retinoblastoma, has minimal side effects, and is easily accessible to the public.(Mendoza and Grossniklaus, 2016). Natural compounds in plants that have anticancer or anti-proliferative potential are flavonoid compounds such as curcumin(Yu et al., 2016; Liu and Zhou, 2017).

Research on curcumin on CDK4, CDK6 and pRB proteins has never been done before. However, Mimeault and Batra in 2011 conducted in vivo and in vitro studies and found that curcumin can inhibit the proliferation of cancer cells, inhibit angiogenesis, inhibit metastasis, trigger apoptosis and increase the sensitivity of chemotherapy and radiotherapy. This can be proven to have an effect on several types of cancer including, leukemia, lymphoma, prostate cancer, cervical cancer, breast cancer, ovarian cancer, colon cancer, and others.(Mimeault and Batra, 2011). Another study conducted by (Xiaoming Yu et al., 2016) said curcumin induces Y79 cell apoptosis through activation of the JNK and p38 MAPK pathways. These findings provide a novel treatment strategy for human RB. (Shigenobu Suzuki et al., 2015) said the risk of extraocular tumor spread after intravitreal injection was low, and other side effects were rare. Sixty-eight percent of the treated eyes achieved complete vitreous seed remission, and about half of them maintained a practical level of vision. For this reason, the researchers intend to analyze the potential of curcumin as antitumor in retinoblastoma.

METHODS

Receptor and Ligand Preparation

The macromolecules used were the CDK4 receptor, CDK6, pRb obtained from the NCBI (National Center of Biotechnology Information) (www.ncbi.nlm.nih.gov/) with the appropriate keywords pRb, CDK4, CDK6 protein, through PDB (Protein Data Bank) with search on the site's search fieldhttp://www.rscb.org/. Protein Rb with code 3N5U, CDK4 with code 2W96 and CDK6 with code 3NUP. Search for ligand Curcumin obtained from the sitehttps://www.rcsb.org/. Curcumin enzyme with code 3OV2.

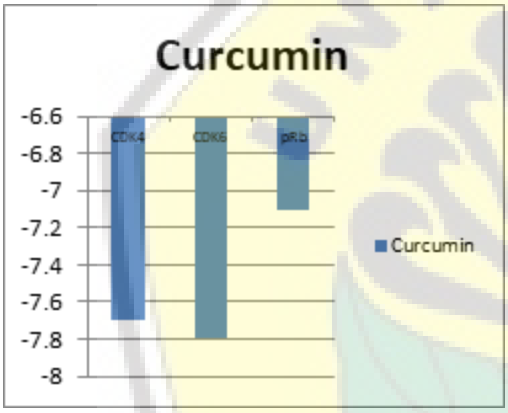
Docking Simulation

The structure of the CDK4, CDK6, pRb receptors *.pdb was converted into *.pdbqt format through the AutoDock Tools 1.5.6 program. The docking method is done by tethering the ligand to the CDK4, CDK6, pRb receptors. The mooring coordinates are CDK4 (Grid Center) x = 13.0, y = 8.836, z = 43.2 and Grid Box size x= 70, y= 64, z= 62 . The mooring coordinates are CDK6 (Grid Center) x = 11,533, y = 25,223, z = 0.104 and Grid Box size x= 72, y= 66, z= 68 . The mooring coordinates are pRb (Grid Center) x = 33,576, y = 13.979, z = 21,171 and Grid Box size x= 84, y= 58, z= 64. Each ligand is in a flexible condition that will interact with biomacromolecules in a rigid condition. AutoDock Vina was used in the simulation of the docking of the test ligand against the CDK4, CDK6, pRb receptors. The docking results were scored and the best value (most negative G) was observed in the ligand binding area to the CDK4, CDK6, pRb receptors. The binding area of each ligand to the CDK4, CDK6, pRb receptors in *.pdbqt format was converted into *.pdb format through the AutoDock Tools 1.5.6 program. Interactions in the form of hydrogen bonds, hydrophobic interactions, and bond distances can be visualized using LigPLot+ 1.5.4 (2D) and PyMOL 3.1 (3D) with interaction radius < 5Å from the position of the anchored ligand.

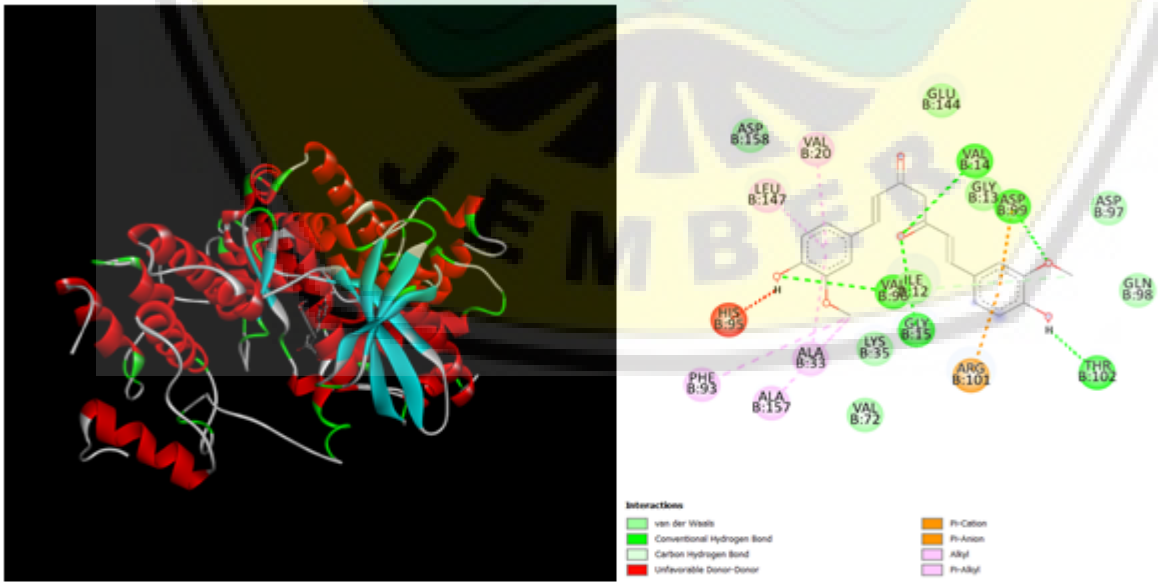
Result

Table 1. binding affinity

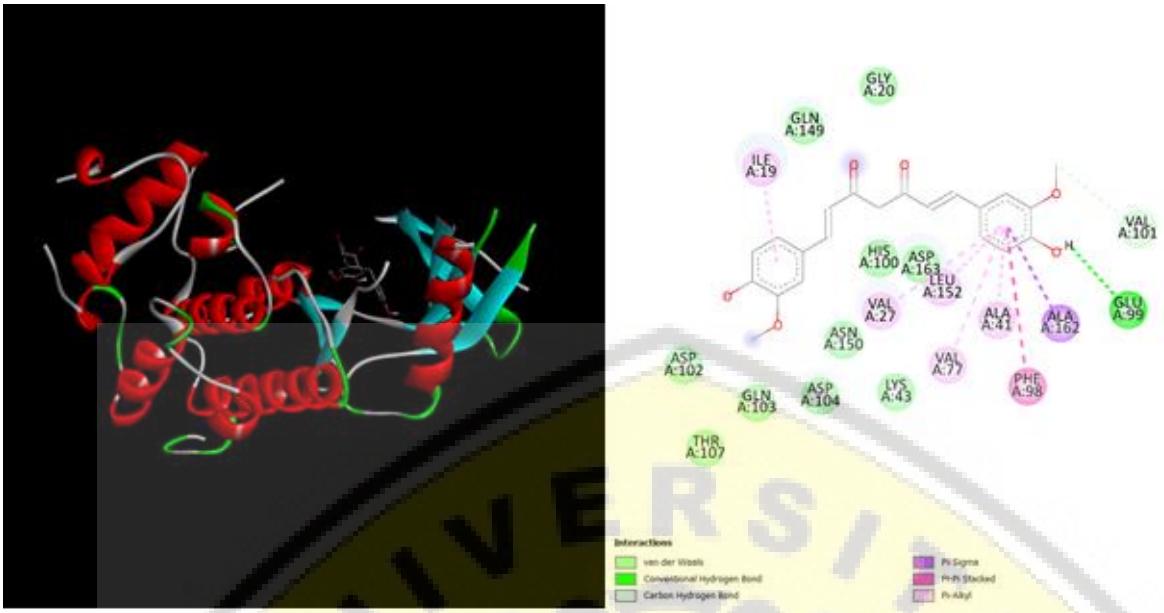
Mode	CDK4	CDK6	pRb
1	**-7.7**	**-7.8**	**-7.1**
2	-7.3	-7.7	-6.7
3	-6.7	-7.6	-6.4
4	-6.7	-7.5	-6.4
5	-6.6	-7.5	-6.4
6	-6.5	-7.3	-6.3
7	-6.5	-7.0	-6.3
8	-6.4	-6.9	-6.3
9	-6.2	-6.6	-6.3



Picture 1. Binding affinity



Picture 2. Curcumin CDK4



Picture 3. Curcumin CDK6

Picture 4. Curcumin pRb

Curcumin has low binding affinity against CDK4, CDK6 and pRb, so it has the potential for antitumor in retinoblastoma.

DISCUSSION

Retinoblastoma associated protein (pRb) is a tumor suppressor that plays an important role in the cell cycle. This protein limits normal cell development in the G1 phase (Datta et al., 2021). However, in the majority of cancers, the Rb protein is often inactivated (Dick and Rubin, 2013). The Rb protein works by binding to the E2F transcription factor which is a gene promoter that plays a role in cell development. The existence of these interactions can block the entry of transcriptional coactivators in E2F, so that RB1 gene expression can be suppressed and the G1/S transition phase in the cell cycle can be stopped. During the cell cycle, CDK activity will decrease and protein phosphatase 1 (PP1) activity will increase. PP1 protein functions to dephosphorylate Rb (Vélez-Cruz and Johnson, 2017). This dephosphorylation process occurs during the S and G2 phases in response to hypoxia and DNA damage. This process is very important in regulating Rb activity because dephosphorylation

can activate Rb which acts as a tumor suppressor (Hirschi et al., 2010). In the hypophosphorylated Rb state, a complex with the transcription factor E2F will be formed which can result in inhibition of the transcription process and end in cell cycle termination. If there is mitogen stimulation, then cyclin-CDK will be active and phosphorylate Rb. Phosphorylation of Rb allows E2F to enter transcriptional coactivators which results in inhibition of transcriptional repression of the gene so that cell development can continue. Deregulation in Rb protein is caused by mutations in the RB1 gene itself or mutations in other Rb protein families such as p107 and p130. Both mutations cause hyperphosphorylation of Rb and an increase in viral oncoproteins that inhibit Rb activation. Rb has interactions with more than 200 proteins, which are important in various cell cycle processes (Vélez-Cruz and Johnson, 2017). The structure of pRb that binds to PP1 (PDB ID: 3N5U) can be seen in the following figure 5.

Figure 5. Crystal structure of pRb associated with PP1 (Source: PDB, 2021)

Cyclin Dependent Kinase4 (CDK4) is a protein that plays an important role in cell cycle regulation, especially to control cell proliferation(Topacio et al., 2019). Cyclin Dependent Kinases (CDKs) are involved in controlling gene transcription processes that integrate extracellular and intracellular signals in the cell cycle by coordinating the response to environmental changes and apoptosis. Activation of CDKs occurs through the phosphorylation of threonine residues specifically by CDK activating kinases and binds to cyclin proteins.(Shafiqet al., 2012). CDK4 activity is regulated by its interaction with cyclin D (D1, D2 and D3). The presence of these bonds can lead to phosphorylation and inactivation of the Retinoblastoma protein family (pRb, p130, and p107). The phosphorylation of pRb by CDK4 causes the activation of the transcription factor E2F which initiates development in the G1 phase of the cell cycle. If the transcription process occurs continuously, it will induce the formation of retinoblastoma(Sivashanmugam et al., 2013). The structure of CDK4 that binds to Cyclin-D (PDB ID: 2W96) can be seen in the following figure 6.

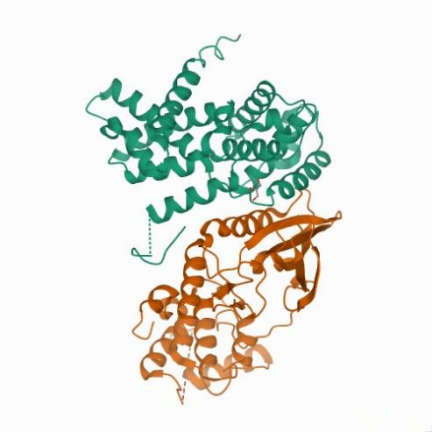


Figure 6. Crystal structure of the CDK4 complex with cyclin D (Source: PDB, 2021)

Cyclin Dependent Kinase6 (CDK6) has the same role as CDK4 which works by phosphorylating pRb and synergizing with cyclin D(Topacio et al., 2019). Similar to CDK4, CDK6 activity will increase if there are several mechanisms such as cyclin D overexpression, mutation or amplification in CDK 4/6, and loss of negative regulators such as p16INK4A.(Hamilton and Infante, 2016). In cancer conditions, activation of CDK6 occurs permanently which will make pRb and other retinoblastoma protein families hyperphosphorylated and inactive resulting in the release of transcription factor E2F, then protein synthesis for DNA replication will begin and make the cell cycle uncontrollable.(Engel et al., 2014; Yousuf et al., 2020). Not only controlling the cell cycle, CDK6 also plays a role in changes in cancer cell metabolism. CDK6 regulates the production of Reactive Oxygen Species (ROS) by inhibiting the activity of phosphofructokinase (PFK) and other protein kinases of the glycolytic pathway. Changes in the glycolytic pathway can prevent cell apoptosis so that it can induce cancer formation(Yousuf et al., 2020). The structure of the CDK6 complex that binds to the inhibitor (PDB ID: 3NUP) can be seen in the following figure 7.

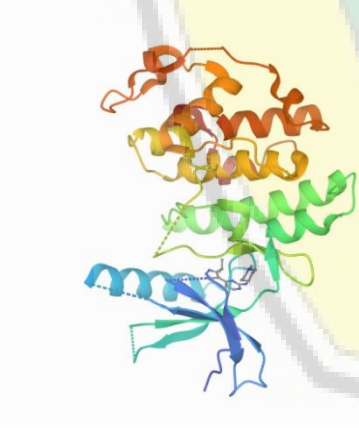


Figure 7. The structure of the CDK6 complex and its inhibitors (Source: PDB, 2021)

Molecular docking is one of the computational methods used to identify and optimize drug candidates. This is done by observing and modeling the interaction and binding capacity of a ligand with macromolecules (target proteins) to form a stable complex (Wadood et al., 2013; Faridah et al., 2019). The existence of this interaction can be known by looking at the binding site on the target protein's active site (Faridah et al., 2019). The docking process involves 2 processes, namely prediction of ligand conformation and assessment of bond affinity (Meng

et al., 2012). Some of the software used for molecular docking include ArgusDock, DOCK, FRED, eHITS, Cluspro, AutoDock and FTDock (Wadood et al., 2013).

CONCLUSION

Based on the results of molecular docking, curcumin has a small binding affinity value for CDK4, CDK6, pRb proteins so that curcumin has the potential for antitumor in retinoblastoma. Future research is expected to be carried out in vivo or in vitro using curcumin against CDK4, CDK6 and pRb.

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