Cytotoxic activity of andrographolide in colon cancer through inhibition cox-2 by in silico study

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Abstract. Colon cancer is a cancer with characteristics of cyclooxygenase (COX-2) protein overexpression. Increased COX-2 expression may inhibit regulation of apoptotic mechanism. Andrographolide was known has activity as an anticancer. The aimed of this study was determine the mechanism and affinity of andrographolide with COX-2 protein as anti-colon cancer using molecular docking. In silico assay used molecular docking method with Chimera 1.10.1 (use for protein preparation COX-2), Hyperchem 8 (use for optimization 3D structure of andrographolide), and Autodock 4.2 programs (use for molecular docking). The results in molecular docking was a binding energy value of and hydrogen bonds. Data were compared with native ligand, celecoxib, and meloxicam. The binding energy values between COX-2 protein with andrographolide, native ligand, celecoxib, and meloxicam were respectively -8.66; -9.94; -10.12; and -7.68 kcal/mol. This indicate that andrographolide had a stronger affinity and more stable than meloxicam for COX-2 protein with hydrogen bonds on Arg120 amino acid. Based on the results obtained, andrographolide had an anti-colon cancer activity caused it can bind to the protein COX-2. The mechanism of andrographolide as anti-colon cancer by inhibited the overexpression of COX-2 in order to increase apoptotic regulation.

1. Introduction
Cancer is a disease characterized by the unchecked division and survival of abnormal cells. One of the other cancer’s types that often cause of death was colon cancer. Colon cancer is a disease that includes the deadly disease cause not known until a more severe level [4]. Colon cancer is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) worldwide [3]. Cancer Country Profiles (2014) reported colon cancer was the third cancer in men (10,516 cases, 10.2% of the total) and the second in women (7,837 cases, 8.5% of the total) in Indonesia.

Cyclooxygenase (COX)-2 is an 80% overexpression in colon adenocarcinomas. COX-2 is normally absent in most cells, this immediate-early response gene is rapidly induced by a variety of pro-inflammatory and growth associated stimuli, resulting in increased PGE2 synthesis. The overexpression of COX-2 can inhibit apoptotic, stimulate adhesion, invasion, and induce angiogenesis [9]. Based on this, it is necessary to develop a new anti-colon cancer’s drug from natural product.

Andrographolide has several pharmacological activities, for instance anticancer [8]. Rajagopal et al. [6] studied the cellular processes and targets modulated by andrographolide treatment in human cancer and immune cells. Andrographolide treatment inhibited the in vitro proliferation of different tumor cell lines, which representing the various types of cancers. The compound exerts...
directly in anticancer activity in cancer cells by arresting cell-cycle in G0/G1 phase. Furthermore, through the induction of cell-cycle inhibitory protein p27 and the inhibition expression of Cyclin-dependent Kinase 4 (CDK4).

Generally, by discovering and developing the anti-colon cancer’s drug, it needs very long times progress, costs, and the possibility of the results are not appropriate yet. In silico molecular docking have benefit, for example safe, free from chemical waste, cost-effective and the time progress is short [5]. The aim the molecular docking is to achieve the optimized conformation and relative orientation for both the protein and ligand. Therefore, we want to know about the affinity and mechanism between andrographolide with COX-2 protein through in silico as anti-colon cancer.

2. Method
The three dimensional of COX-2 (6COX) were obtained from Protein Data Bank (http://www.rcsb.org). The 3D structure of andrographolide (5318517) downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov) which provides information on the biological activities of small molecules. The initial preparation of the pdb files to select the need chains, remove water, add polar hydrogen, delete multiple ligands and non-protein parts in using Chimera 1.10.1 program. Open Babel GUI version 2.3.2 program was used to convert the pdb file format. The three-dimensional structure of andrographolide was optimized by using HyperChem 8 semi-empirical AM1 (Austin Model 1) method and calculating with a single point and geometry optimization. Autodock 4.2 program used for validation of molecular docking method with Root Mean Square Deviation (RMSD) parameter value ≤ 3 Å. Next running docking protein target and ligand in file Autodock 4.2 configuration then the ligands binding energy to target protein score was compared to native ligand.

3. Result and Discussion
COX-2 database (6COX) downloaded from http://www.rcsb.org/pdb/home/home.do. Preparation of COX-2 protein aimed to separate protein with native ligand. Therefore, it is available pocket/cavity during docking progress with andrographolide.

Figure 1. COX-2 Protein Structure without Native Ligand

Figure 2. S58 Native Ligand
The protein preparation is performed by removing water molecules (H₂O), so the only one that interact is compound test with amino acids. Those preparations were done by using Chimera 1.10.1 program that obtained protein (without native ligand) and S58 native ligand.

The optimization of 3D structure andrographolide was done by using semi-empirical AM1 on Hyperchem 8. The first step was by calculating single point that used to determine the total molecular energy of the structure. The energy obtained at this single point calculation is -5471.48 kcal/mol. Moreover, to employ the energy minimization algorithms that locate the stable structure was using geometric optimization calculation. The energy obtained in here is -5509.50 kcal/mol.

![Figure 3. The 3D Structure Andrographolide after Single Point Calculation](image1)

Molecular docking was performed to elucidate the molecular mechanism underlying andrographolide inhibits COX-2 receptor by seeing the drug-receptor binding energy. Score docking shows the bond strength between the active compound and the target protein. Lower score docking shows stronger and more stable bond. When the bond grows stronger, the affinity between the compounds and the target protein increases. Higher its affinity will be caused higher its biological activity [5].

Validation method aims to prove the parameters on the method are appropriate to use for the requirement where it’s used. The RMSD value is a parameter used to indicate that the molecular docking method is valid. RMSD value is a measurement media to compare between two poses atomic position, those are the experimental and the predicted structure. Smaller the RMSD value that indicates the predicted ligand poses better as it approaches the native conformation [1]. Validation result from COX-2 protein showed molecular docking protocol for that protein could be accepted with RMSD value < 3 Å (table 1) and molecular docking andrographolide to COX-2 could be continued.

![Figure 4. The 3D Structure Andrographolide after Geometry Optimization Calculation](image2)
Table 1. Docking results between COX-2 Protein and S58 Native Ligand

| Protein | Conformation | RSMD (Å) | Energy Binding (Kcal/mol) | Hydrogen Bond |
|---------|--------------|----------|---------------------------|---------------|
| COX-2   | 1            | 0.83     | -10.55                    | Phe518        |
|         | 2            | 0.88     | -10.39                    | Arg120        |
|         | 3            | 0.83     | -10.23                    | His90         |
|         | 4            | 0.85     | -10.09                    | -             |
|         | 5            | 1.01     | -9.69                     | -             |
|         | 6            | 0.64     | -9.94                     | Arg120        |
|         | 7            | 0.96     | -9.44                     | His90         |
|         | 8            | 0.94     | -8.92                     | -             |
|         | 9            | 1.42     | -9.29                     | Phe518        |
|         | 10           | 2.91     | -9.74                     | -             |

Based on the results of re-docking in table 1 show, the value of RMSD is below 3 Å. Those results that indicate the method are valid. Binding energy values obtained from the docking of COX-2 protein with S58 native ligand, were compared to determine the andrographolide potential of anti-colon cancer by inhibiting mechanism of COX-2 protein.

In silico molecular docking between andrographolide and COX-2 protein are performed by using the Autodock 4.2 program. The docking result showed in the table 2. Conformation with the lowest binding energy value was selected from the conformation which has the most stable conformation. The potential of andrographolide as an anti-colon cancer which has an inhibition mechanism COX-2 can be determined by comparing the value of binding energy for drugs that have the same mechanism, for example celecoxib and meloxicam. Based on the comparison binding energy value between native ligand and andrographolide with COX-2 protein in table 3, it shows that andrographolide has greater binding energy value than S58 native ligand. However, in the binding energy value obtained in here, it got negative value that indicates the formation of bond between COX-2 and andrographolide.

Table 2. Docking results between COX-2 Protein and Andrographolide

| Protein | Conformation | Energy Binding (Kcal/mol) | Hydrogen Bond |
|---------|--------------|---------------------------|---------------|
| COX-2   | 1            | -8.66                     | Arg120        |
|         | 2            | -8.27                     | Ser530        |
|         | 3            | -7.92                     | Un1           |
|         | 4            | -8.09                     | Arg120        |
|         | 5            | -7.8                      | Arg120        |
|         | 6            | -7.31                     | Arg120        |
|         | 7            | -6.48                     | Arg120        |
|         | 8            | -5.64                     | Arg120        |
|         | 9            | -4.54                     | -             |
|         | 10           | -4.26                     | Arg120        |
Table 3. Docking results between COX-2 protein with Native Ligand, Andrographolide, Celecoxib, and Meloxicam

| Ligand                | Energy Binding (Kcal/mol) | Hydrogen Bond |
|-----------------------|---------------------------|---------------|
| Native Ligand (S58)   | -9.94                     | Phe518        |
| Andrographolide       | -8.66                     | Arg120        |
| Celecoxib             | -10.12                    | His90         |
| Meloxicam             | -7.68                     | Arg120        |

Figure 5. Interaction between Andrographolide and COX-2 Protein

The result that occur in docking of andrographolide compound against COX-2 receptor are energy binding and affinity. Based on the table 3, andrographolide has greater binding energy value than celecoxib, but it is lower than meloxicam. This outcome indicates that andrographolide has stronger affinity and more stable bond than meloxicam. The interaction between proteins and ligands are indicated the form of hydrogen bonds. The hydrogen bonds showed figure 5. Hydrogen bonds are electronegativity that can affect the bond strength of a compound. The hydrogen bond is formed in the same active side between the andrographolide and meloxicam in the COX-2 protein, which it is in the Arg120 amino acid. Those indicates that andrographolide has potential as an anti-colon cancer by inhibiting the expression of COX-2 with a stronger affinity and a more stable bond than meloxicam. Higher its affinity will be caused higher its biological activity.

4. Conclusion
The in silico test with molecular docking showed that andrographolide is potentially as anti-colon cancer through the increasing apoptotic mechanism by inhibiting the activity of the COX-2 protein. The molecular docking demonstrate high affinity interaction between andrographolide and COX-2.

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