Molecular Mechanism of Caffeine-Aspirin Interaction in Kopi Balur 1 as Anti-Inflammatory Agent: A Computational Study

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Abstract

Balur treatment (BT) is a method that works to remove toxins in the form of free radicals released via the skin. One of the remedies used is Kopi Balur 1 (KB1) which contains aspirin and caffeine used during the BT, so that KB1 is believed to have the potential to reduce or inhibit the occurrence of inflammatory processes in the body. Therefore, we investigate the interactions that occur between ligands and proteins, predict the action mode of caffeine-aspirin contained in KB1 as an anti-inflammatory agent, and modeling the 3D structure of pro-inflammatory proteins in the human body. The target compound, aspirin-caffein, was obtained from a database, then used to identify the pathway on the STITCH webserver, then the target protein was obtained and modeled in 3D using the SWISS-MODEL webserver, and the structures obtained were represented in PyMol software. In sum, the aspirin-caffeine ligand complex contained in KB1 has the potential as an anti-inflammatory agent in the human body via the molecular mechanism of binding and inhibiting the biological activity of proinflammatory proteins, i.e. PTGS1, PTGS2, and ADOR2A.

***Keywords**: Anti-inflammatory agent, computational study, kopi balur 1, molecular mechanism.*

Introduction

Balur treatment (BT) is a method used to remove toxins in the form of free radicals contained in the human body. Balur is a treatment method from the Javanese society that has been developed to improve the quality of life. BT uses natural ingredients as free radical scavenging and components of aromatic chemical compounds such as amino acids and other aromatic compounds. The body of a person undergoing BT must lie on a copper plate and this therapy is believed to have the potential to reduce or inhibit the effects of inflammation in the body[1].

Caffeine is a type of heterocyclic alkaloid in the methylxanthine group, and by definition means organic compounds containing nirogens with a two-ring structure. This molecule naturally occurs in many types of plants as secondary metabolic. Caffeine is a compound belonging to the xanthine derivative alkaloids that contain a methyl group. Caffeine has long been obtained from plant extracts such as coffee beans, tea, and chocolate[2]. In addition, Indonesia possesses a vast biodiversity with a high number of potential natural medicinal sources[3,4].

On the other hand, aspirin or acetylsalicylic acid (acetosal) is a type of drug that is often used as an analgesic, antipyretic, and anti-inflammatory. Aspirin can also be used in low doses for a long period of time to prevent heart attacks. Aspirin is made by esterification, in which the active ingredient of aspirin is reacted with anhydrous acetic acid and glacial acetic acid. In the process of making this esterification reaction, it is assisted by an acid catalyst to speed up the reaction[5].

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Inflammation is an immune response to the local tissue caused by injury or damage caused by certain factors, which function to destroy, reduce infectious agents that damage the tissue or play a role in the regeneration process of the injured tissue[6,7]. Therefore, we investigate the interactions that occur between ligands and proteins, predict the action mode of caffeine-aspirin contained in KB1 as an anti-inflammatory agent, and modeling the 3D structure of proinflammatory proteins in the human body.

Materials and Methods

Sample Preparation

This study conducted at Department of Biology, Faculty of Mathematic and Natural Sciences, Universitas Brawijaya, Indonesia. This study used aspirin (ID 2244) and caffeine (ID 2519) in the form of 3D structures (structure data format or sdf) and canonical SMILES obtained from the PubChem database (pubchem.ncbi.nlm.nih.gov). Pubchem is a specific database that stores information related to organic chemical compounds, synthesis, and certain substances and is a branch of NCBI. A sample of chemical compounds originating from PubChem are in the form of 3D and 2D structures[8].

Drug-Likeness Prediction

Samples of target compounds were tested for their potential as drug molecules by checking their accuracy according to Lipinski's rules[9,10]. The structure of 3D compounds in the sdf format was analyzed on the Lipinski webserver (scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) and the results were identified which consisted of molecular weight, hydrogen acceptor donors, lipophilicity, and molar refractivity.

Pathway Analysis

The interaction between aspirin-caffeine and the target protein in the human body can be identified on STITCH (stitch.embl.de). STITCH is a webserver whose role is to predict the interaction of a chemical compound in the target organism, which will get stronger association scores based on a database or evidence from experiments that have been carried out[11].

Protein Modeling and Validation

Protein sequences contributing to the inflammatory response were obtained from the Uniprot database (uniprot.org), then modeling via the SWISS MODEL webserver (swissmodel.expasy.org), and the results of the modeling were identified by Ramachandran plot. The modeling used in this research is homology and works based on the availability of templates in the database to produce a target protein model[8,12]. The quality of the model is identified via a plot that works based on the position of specific amino acids that will determine the favored score on the model[13].

Result and Discussion

Molecular Interaction of Caffein-Aspirin with Proteins in Human Body

Based on the results of *in silico* analysis of the interaction between ligand-protein on the STITCH webserver, caffeine (ID 2519) have a canonical SMILES CN1C=NC2=C1C(=O)N(C(=O)N2C)C and aspirin (ID 2244) have a canonical SMILES CC(=O)OC1=CC= CC=C1C(=O)O, most likely interact with 10 proteins inside the body of *Homo sapiens*. The results of identifying the interaction of aspirin-caffeine with 10 proteins in the body of *Homo sapiens* showed that all of them are the highest evidence because they have a score of above 0.900 or 90.0%. In STITCH, there are a number of edge convention scores starting from low (0.150), medium (0.400), high (0.700), and highest confidence (0.900)[11]. So, the greater the score, the stronger of the suspicion of interaction occurs when the caffeine aspirin ligand complex is in the body of *Homo sapiens*.

Based on the identification of the interaction between the aspirin-caffeine ligand complexes with the target protein, there are also target proteins that contribute to the inflammatory mechanism in the human body, such as PTGS1 and PTGS2 (interact with aspirin) and ADORA2A (interact with caffeine). Carrol *et al*. have explained that PTGS1 can increase the production of mucus layers on the inside of the stomach, making other contributions such as reducing stomach acid levels[14]. PTGS1 is often found in areas where there are inflammatory mechanisms, although digestive tract organs are also found[15]. Consequently, most likely when aspirin is in the human body, it will interact with the PTGS1 receptor.

ADORA2A is a type of adenosine receptor[16,17]. A2A receptors can be found throughout the body and are present on the surface of immunocompetence cells. These receptors have an important role in the inflammatory process (anti-inflammatory agent)[6]. Thus, when caffeine is in the human body, it is likely that caffeine will interact with the ADORA2A receptor. The results of the STITCH analysis in the form of a complex interaction of aspirin-caffeine molecules with target proteins in the human body, are displayed in the form of shells which have several lines connecting between one another. Proteins with colored boxes are proinflammatory agents in the human body (Figure 1).

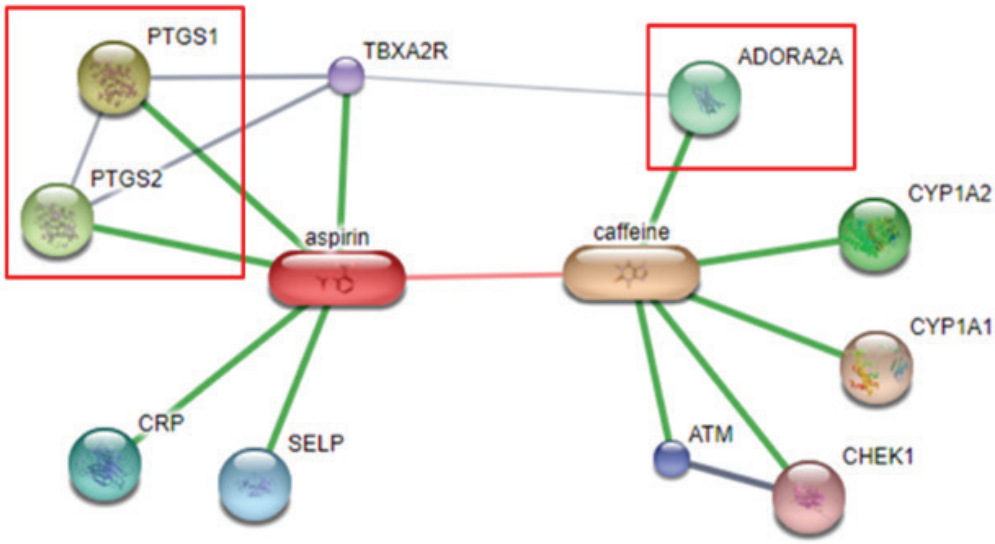

Figure 1. The molecular pathway of the aspirin-caffeine complex with proteins in the human body. The lines show the interactions of proteins (gray), ligands (green), and ligands (red).

Action Mode of Aspirin-Caffein as Antiinflammatory Agent

Aspirin and caffeine are identified in relation to drug-likeness to determine whether the compound can be categorized as a drug molecule that refers to the five Lipinski rules. We predicted that the aspirin compound has a molecular weight of 180.000 D, high lipophilicity 1.310, one hydrogen bond donor, four hydrogen bond acceptors, molar refractivity 44.710, whereas for caffeine it has a molecular weight of 194.000 D, high lipophilicity 0.061, 0 hydrogen bond donor, five hydrogen bond acceptor, and molar refractivity 49.100. Lipinski's rules explain that a compound can be categorized as a drug molecule because it fulfills at least two rules, here are the rules of molecular weight ≤500 D, high lipophilicity ≤5, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, and molar refractivity 40-130[9,10]. Thus, aspirin and caffeine are categorized as drugs because they meet all five Lipinski's rules.

Table 1. Drug-likeness test of aspirin and caffeine.

Compound	Lipinski Rules				
	MM (D)	LOGP	HBD	HBA	MR
Aspirin	180.000	1.310	1	4	44.710
Caffein	194.000	0.061	0	5	49.100

Note: MM: Molecular mass; LOGP: High lipophilicity; HBD: Hydrogen bond donors; HBA: Hydrogen bond acceptors; MR: Molar refractivity.

After obtaining Lipinski results, aspirin and caffeine compounds were re-identified, the prediction of the action or the complex interaction of the ligand when inside the human body via STITCH webserver. This can produce information related to the nature of the molecular interactions that occur in the query ligand with the target protein, usually in the form of information on binding, activation, expression, and inhibition[11,18]. Thus, it can be seen the type of action mode of the two query compounds, with the detected properties marked using the sign (√). There are five target proteins whose activity can be inhibited by the aspirin-caffeine ligand complex using direct binding, these proteins include PTGS1, PTGS2, CYPA2, ADORA2A, and ATM (Table 2). In this study, we revealed that the aspirin-caffeine ligand complex has an action mode to be an anti-inflammatory agent by binding directly to the target proteins such as PTGS1, PTGS2, and PTGS3.

Table 2. The interaction of aspirin-caffeine ligand complex in the target protein.

Compound	Target Protein	Binding	Inhibition
Aspirin-Caffeine	PTGS1	√	√
	PTGS2	√	√
	CYP1A2	√	√
	ADORA2A	√	√
	CRP	-	√
	SELP	-	√
	ATM	√	√
	TBXA2R	-	√
	CHEK1	-	√
	CYP1A1	√	-

The 3D Model of Pro-Inflammatory Agent

Based on the analysis of previous stages, it is known that there are three proteins that play an important role in the occurrence of inflammatory mechanisms in the human body, PTGS1, PTGS2, and ADORA2A. The structure of three target proteins is modeled on the SWISS-MODEL webserver using a homology modeling method that has the principle of modeling based on query sequence alignment with a template in the database[19]. If there are more templates available in the database, the resulting model is increasingly homologous, the modeling results also show a score, which must be above 20% in order to be called identical to the template[12,20]. After obtaining the protein structure of the model, the quality of the model is identified again, which refers to a minimum favored region score of 80%, so that the model can be said to be valid[13]. Before doing the modeling, the sequences of the three target proteins in the FASTA format that were successfully obtained from the Uniprot database can be seen in Figure 2.

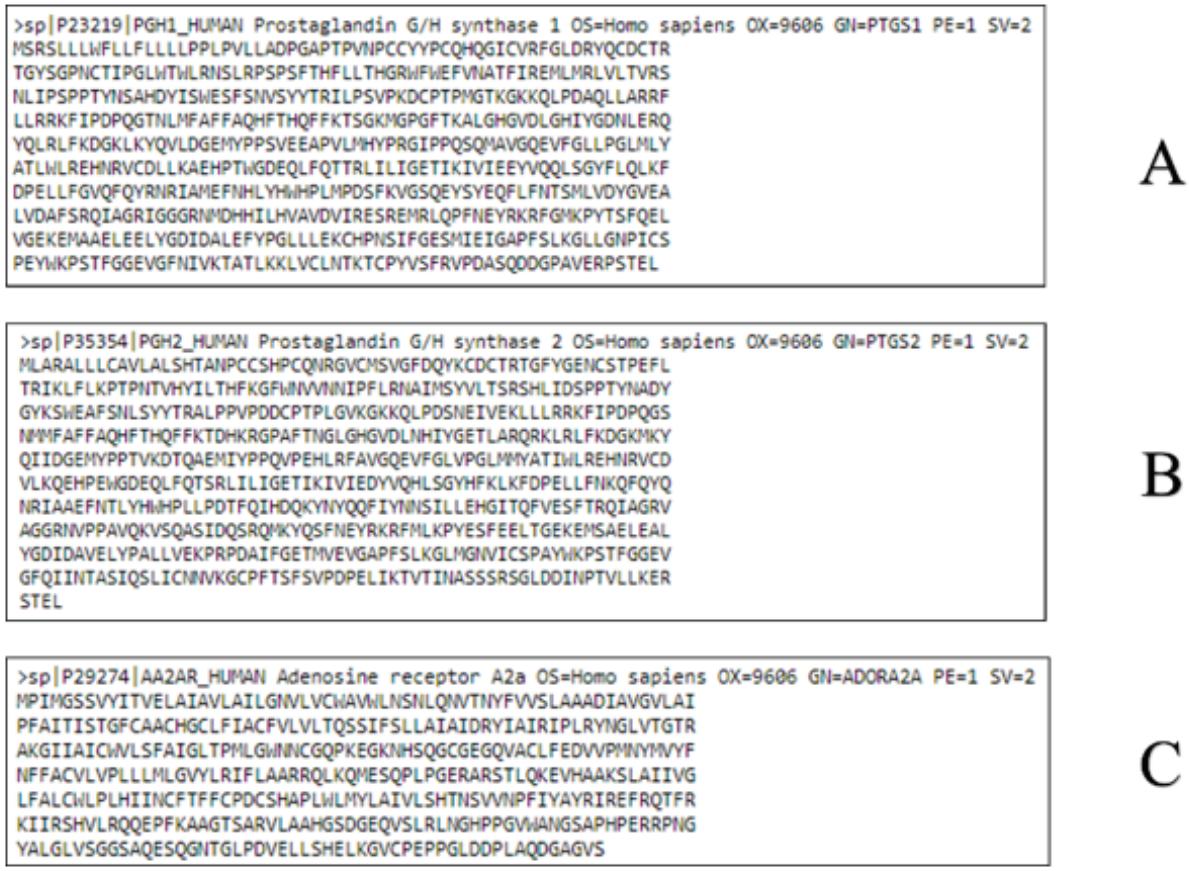

Figure 2. The target protein sequences were obtained from the Uniprot database in FASTA format. (A) PTGS1, (B) PTGS2, and (C) ADORA2A.

PTGS1 protein sequence sample (ID P23219) has a length of around 599-mer, 92.88% identified as similar to the template, and 96.64% favored region, PTGS2 protein sequence (ID P35354) has a length of around 604-mer, 100% identified as similar with a template, and 97.09% favored region, the ADORA2A protein sequence has a sequence length of around 412-mer, 98.42% identified as similar to the template, and 98.39% favored region. So the results of modeling the protein structure of the three target proteins produce a structure that is identical to the template because everything is similar to the template and the results of the quality test show that the favored region score of the three target proteins is above 80% so that the protein structure produced in this study is valid. The structure of the modeling results is displayed in the form of transparent surface, cartoon, and coloring selection based on the chain of constituent proteins in the PyMol8 software[8]. The target protein that has two chains namely A (green) and B (blue) are PTGS1 and PTGS2, while ADORA2A has only one chain, visualizing the results of modeling also displays the results of the Ramachandran plot (Figure 3).

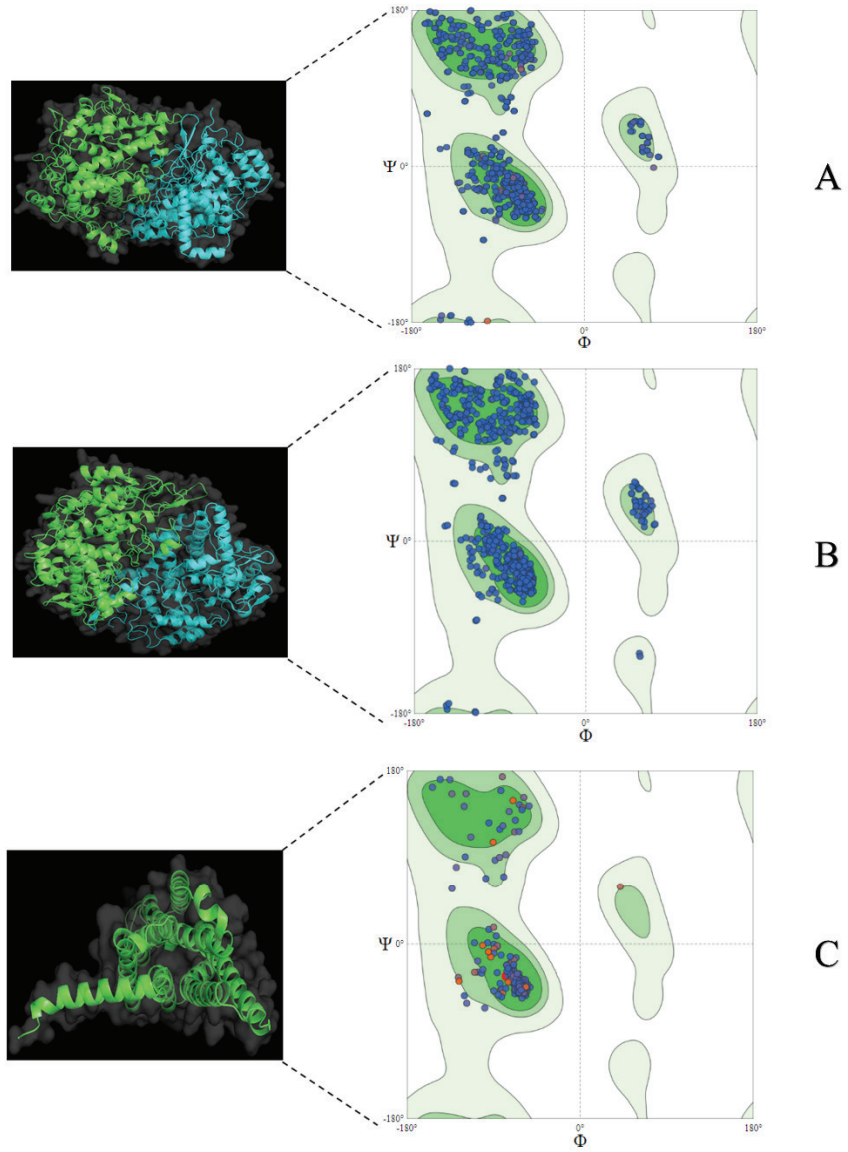

Figure 3. Results of protein modeling and Ramachandran plots. (A) PTGS1, (B) PTGS2, and (C) ADORA2A.

Conclusion

In sum, the aspirin-caffeine ligand complex contained in KB1 has the potential as an antiinflamatory agent in the human body via the molecular mechanism of binding and inhibiting the biological activity of proinflammatory proteins, i.e. PTGS1, PTGS2, and ADOR2A.

Conflict of Interest : The authors declare that they have no conflict of interest.

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