Pharmacy Reports

https://doi.org/10.51511/pr.56

RESEARCH ARTICLE

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Exploring the binding affinity of rutin, catechin, and epicatechin to ALK and caspase-3: implications for colorectal cancer treatment

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Abstract: Overexpression of anaplastic lymphoma kinase (ALK) and inhibition of caspase-3 leads to an increase in cell number due to inhibition of the apoptotic mechanism. This research aimed to study the interaction of rutin, catechins, and epicatechins to ALK and caspase-3 by using in silico molecular docking. The experiment was carried out by ALK (PDB ID 5USQ) and caspase-3 (PDB ID: 2XZT) preparation, docking validation, optimization of test compounds, and docking of test compounds. The molecular docking method had been valid with a RMSD value of ≤ 3 Å. The docking results showed that the tested compounds rutin (-8.58 kcal/mol), catechins (-8.41 kcal/mol), and epicatechin (-7.82 kcal/mol) had a weaker affinity than the ALK's native ligand (-10,27 kcal/mol). Meanwhile, the tested compounds rutin (-6.03 kcal/mol), catechins (-5.28 kcal/mol), and epicatechin (-4.95 kcal/mol) had a stronger affinity than caspase 3's native ligand (-2,54 kcal/mol). Based on the docking results, it can be seen that rutin, catechins, and epicatechins had the potential as colorectal anticancer agents against ALK and caspase-3 by molecular docking method.

Keywords: anaplastic lymphoma kinase, caspase-3, epicatechin, catechin, rutin

Introduction

Cancer is characterized by uncontrolled cell growth. Colorectal cancer, in particular, is a significant concern. In 2020, colorectal cancer accounted for approximately 10% of new cancer cases worldwide (19.29 million new cases) and was responsible for 9.4% of all cancer-related deaths (9.96 million cases) [1]. The rise in colorectal cancer incidence is attributed to a combination of genetic and environmental risk factors, including obesity, sedentary lifestyle, poor diet, alcohol consumption, and smoking [2].

A notable molecular feature of colorectal cancer is the overexpression of ALK, observed in 0.4% to 2.5% of exon scans [3–5]. This overexpression leads to the suppression of caspase proteins, thereby inhibiting apoptosis. Caspase proteins, such as caspase-9 and caspase-3, induce morphological changes characteristic of apoptosis. Caspase-9 acts as a pro-apoptotic initiator, activating the effector protein caspase-3, which is crucial for apoptosis [6]. Therefore, targeting ALK inhibition and caspase-3 induction represents a significant strategy in colorectal cancer treatment.

Rutin, catechin, and epicatechin are predominant compounds in the seeds of the avocado (*Persea americana* Mill.). Studies have shown that lipid extracts from avocado seeds exhibit superior anticancer properties compared to those extracted from the fruit, demonstrating effectiveness against HepG2 and HCT11 cancer cell lines [7]. Previous studies have predominantly focused on the lipid content of avocado seeds and their inhibitory effects on cancer cells. However, no research addresses the anticancer potential of the seeds' phenolic chemical compounds.

Molecular docking, a computational technique, is used to predict the interactions between a ligand and a protein, specifically how the ligand fits into the active site [8]. This study aims to explore the effects of rutin, catechin, and epicatechin derived from avocado seeds on inhibiting anaplastic lymphoma kinase (ALK) and activating caspase-3, which are implicated in the pathogenesis of colorectal cancer.

Methods

Protein preparation

The target proteins, anaplastic lymphoma kinase (ALK) (PDB ID: 5USQ) and caspase-3 (PDB ID: 2XZT), were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/). Preparation was conducted using Chimera version 1.11.1, which involved dissociating the protein sequences from their respective native ligands—N-[2-(5-chloro-2-fluorophenyl)pyridin-4-yl]-2-[(piperidine-4-yl) methyl]-2H-pyrazolo[4,3-b]pyridin-7-amine (8LY) in the ALK protein and (4R)-2-Methylpentane-2,4-Diol (MRD) in caspase-3.

Validation of docking

The docking methodology was validated by employing the AutoDock Tools suite (AutoDock 4 and AutoGrid). This process included redocking the native ligands 8LY to ALK and MRD to caspase-3. Grid boxes were configured by setting the grid center coordinates for ALK (X = 17.1 Å, Y = 69.362 Å, Z = 4.852 Å) and caspase-3 (X = 27.926 Å, Y = -28.908 Å, Z = -36.737 Å), with identical grid sizes (X = 40 Å, Y = 40 Å, Z = 40 Å). Docking validation was based on the root mean square deviation (RMSD) of the docking results, with RMSD values ≤ 3.0 Å considered valid [9].

Compounds structure optimization

The 3D structures of rutin, catechin, epicatechin, and 5-fluorouracil, selected for their anticancer properties, were optimized using HyperChem version 8. Optimization was executed utilizing the AM1 (Austin Model 1) semi-empirical method, incorporating single-point calculations and geometry optimization steps.

Docking of test compounds to the ALK and caspase-3

Following optimization, rutin, catechin, and epicatechin were docked to the prepared ALK and caspase-3 proteins using AutoDock 4.2. The docking results identified conformations of rutin, catechin, and epicatechin with the lowest binding energy to the target proteins. Interaction analysis highlighted the nature of the bindings, including hydrogen bonds, Van der Waals forces, hydrophobic interactions, and electrostatics, with lower binding energy values indicating stronger and more stable interactions.

Results

Preparation of ALK and caspase-3

Preparation of ALK and caspase-3 provided with 8LY and MRD native ligands (Figure 1).

Docking validation

The validation of the docking protocol was determined by the root mean square deviation (RMSD) values, which were found to be 0.64 Å for ALK and 1.55 Å for caspase-3, confirming the validity of the procedure (RMSD < 3.0 Å). This validation confirmed the RMSD values and elucidated the binding energies between ALK and caspase-3 with their respective native ligands, 8LY and MRD. The selected conformations with the lowest RMSD values exhibited binding energies of -10.27 kcal/mol for ALK and -2.54 kcal/mol for caspase-3 (Table 1).

Optimization of compound test

The three-dimensional (3D) structures of the test compounds were optimized through single-point calculations and geometry optimizations. The single-point energy calculations yielded energies of -7722.77 kcal/mol for rutin, -3850.87 kcal/mol for catechin, -3849.90 kcal/mol for epicatechin, and -1292.80 kcal/mol for 5-fluorouracil. Following geometric optimization, the energies were observed to be -7753.19 kcal/mol for rutin, -3857.82 kcal/mol for catechin, -3857.64 kcal/mol for epicatechin, and -1297.81 kcal/mol for 5-fluorouracil. Illustrations of the 3D structures post-optimization are provided in Figure 2.

Docking of compound test to ALK and caspase-3

Docking experiments involving rutin, catechin, and epicatechin against ALK and caspase-3 resulted in ten binding conformations for each compound. A conformation displaying the lowest binding energy was selected for each, indicating the most stable interaction. The binding energies for the interactions of the native ligand, rutin, catechin, and epicatechin with ALK were -10.27 kcal/mol, -8.58 kcal/mol, -8.41 kcal/mol, and -7.82 kcal/mol, respectively. Similarly, the interactions with caspase-3 yielded binding energies of -2.54 kcal/mol, -6.03 kcal/mol, -5.28 kcal/mol, and -4.95 kcal/mol, respectively (Table 2). These negative values indicate a stable affinity of the test compounds for each target protein.

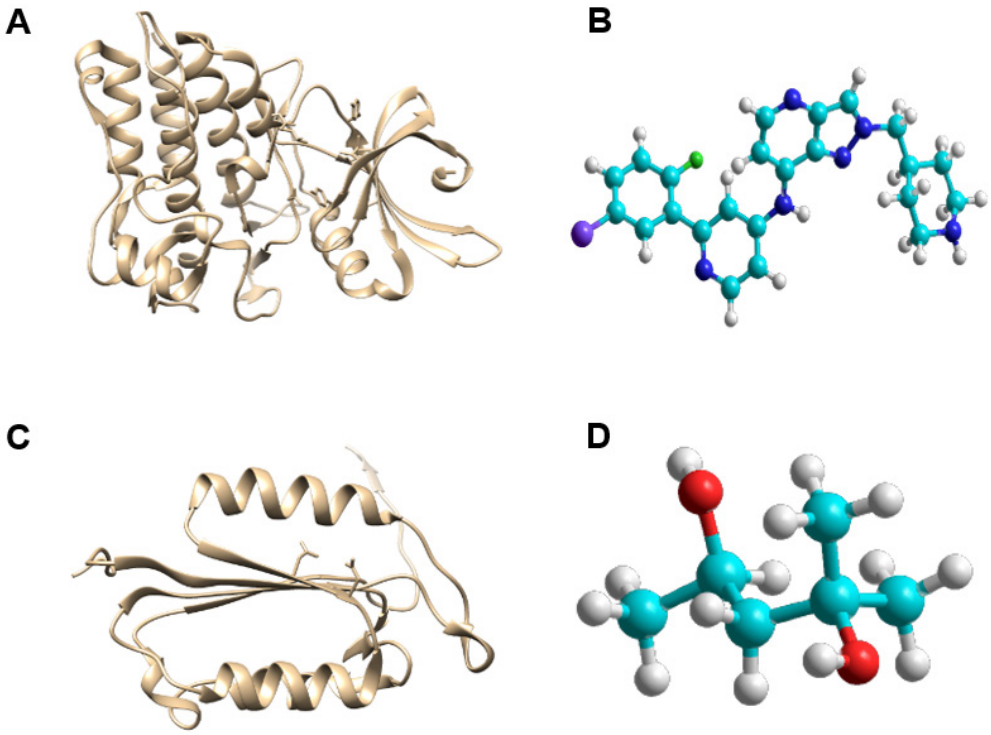

Figure 1. The result of protein preparation. (A) Structure of prepared ALK protein target, (B) 8LY native ligand, (C) Structure of prepared caspase-3, (D) MRD native ligand

Table 1. Validation parameters of the target protein and native ligand

Protein target	Ligand	Conformation	RMSD (Å)	Binding energy (kcal/mol)
ALK	8LY	1	0.81	-10.07
		2	3.68	-7.13
		3	3.42	-8.58
		4	2.67	-8.67
		5	1.85	-8.64
		6	3.50	-7.82
		7	2.62	-8.17
		8*	0.64	-10.27
		9	2.28	-8.58
		10	0.68	-10.16
Caspase-3	MRD	1	6.00	-2.84
		2	5.91	-2.81
		3*	1.55	-2.54
		4	5.74	-2.95
		5	5.78	-2.93
		6	5.79	-2.93
		7	5.84	-2.88
		8	5.97	-2.82
		9	5.93	-2.88
		10	5.71	-2.95

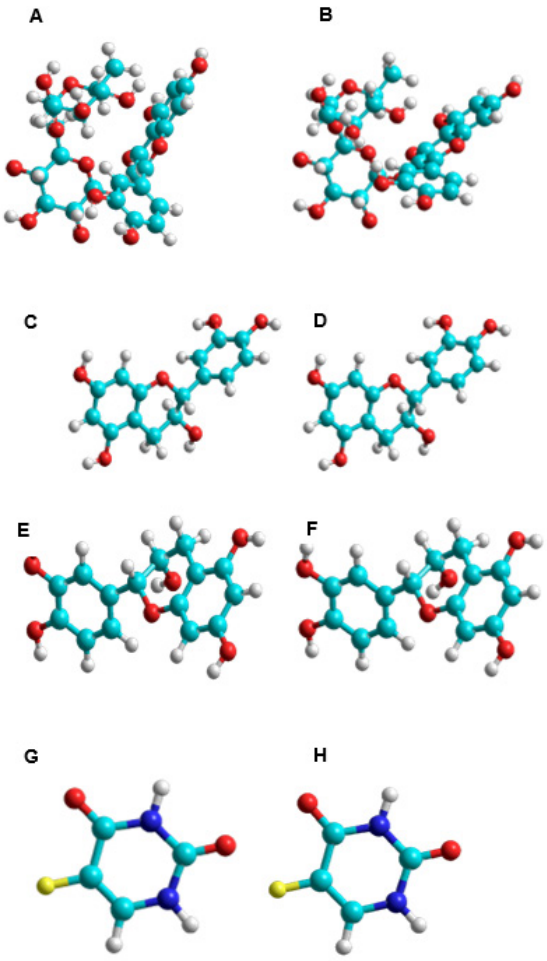

Figure 2. 3D structure of optimization results. (A) rutin single point calculation (B) rutin's geometry optimization, (C) cathecin's single point calculation, (D) cathecin's geometry optimization, (E) epicatechin's single point calculation, (F) epicathecin's geometry optimization, (G) 5-fluorouracil's single point calculation, (G) 5-fluorouracil's geometry optimization

Specifically, rutin was found to form hydrogen bonds with the histidine residue in ALK, while catechin interacted through hydrogen bonds with a serine residue. In contrast, epicatechin and 5-fluorouracil did not form hydrogen bonds with ALK. Regarding caspase-3, all test compounds, including rutin, catechin, epicatechin, and 5-fluorouracil, were observed to form hydrogen bonds with asparagine residues, mirroring the interactions of the native ligand.

Discussion

This study validated the docking protocol, with RMSD values of 0.64 Å for ALK and 1.55 Å for caspase-3, indicating the reliability of the simulation results (≤ 3.0 Å). The docking outcomes revealed that the binding energies of rutin, catechin, epicatechin, and 5-fluorouracil with the ALK target protein were -8.58 kcal/mol, -8.41 kcal/mol, -7.82 kcal/mol, and -3.63 kcal/mol, respectively, compared to -10.27 kcal/mol for the native ligand. These results suggest that, while these compounds have a lower affinity for ALK than the native ligand, they still exhibit potential as anticancer agents by targeting ALK protein.

In contrast, the binding energies for the same compounds with caspase-3 were more negative than that of the native ligand (-2.54 kcal/mol), with values of -6.03 kcal/mol, -5.28 kcal/mol, -4.95 kcal/mol, and -3.40 kcal/mol, respectively. This indicates a potentially stronger interaction with caspase-3, suggesting that these compounds may act as effective inducers of caspase-3, a key component in apoptosis.

Table 2. Compound test docking result on ALK and caspase-3

Compound test	Protein target	Binding energy (kcal/mol)	Amino acid residue	Groups in hydrogen bonds (protein-ligands)
Native ligand	ALK	-10.27	His84	HN-N16
	Caspase-3	-2.54	Asn56	O-H
Rutin	ALK	-8.58	His84	O-H
	Caspase-3	-6.03	Asn19	HD21-O
			Asp35	ODI
			Arg42	HE-O
Catechin	ALK	-8.41	Ser81	HG-O
	Caspase-3	-5.28	Asn18	HD21-O
Epicatechin	ALK	-7.82	-	-
	Caspase-3	-4.95	Asn18	HD21-O
5-fluorouracil	ALK	-3.63	-	-
	Caspase-3	-3.40	Arg42	HE-N
			Asn54	HN-O

Regarding the specific interactions, rutin and catechin were observed to form hydrogen bonds with histidine and serine residues on ALK, respectively, indicating a potential to inhibit ALK similar to the native ligand. However, epicatechin and 5-fluorouracil did not form hydrogen bonds with ALK. For caspase-3, all test compounds formed hydrogen bonds with asparagine residues, similar to the native ligand. However, according to Schroeder et al. (2013), asparagine does not participate in the active site caspase-3 [10], suggesting the need for further investigation into the binding mechanisms.

This research primarily visualized hydrogen bonds between the test compounds and the target proteins, ALK and caspase-3. It indicates that other types of interactions, such as van der Waals, hydrophobic, and electrostatic bonds, were not explored, which could further elucidate the compounds' mechanisms of action. Therefore, a comprehensive analysis encompassing these additional bonding interactions is essential to understand the potential of rutin, catechin, and epicatechin as anticancer agents.

Conclusion

In summary, the test compounds tend to induce caspase-3 rather than inhibit ALK, as evidenced by their negative binding energies. However, to fully understand the compounds' mechanisms and their potential to replace the function of native ligands in target proteins, further investigation into a broader spectrum of bonding interactions is warranted.

Acknowledgment

We express our gratitude to the Department of Pharmacy, Udayana University for providing the necessary facilities to complete this work.

Declaration of interest

The authors declare no conflict of interest.

Author contributions

INMPA, NPLL conceptualized the study design; NLPCP, NMPS investigated the data; INMPA, NLPCP wrote the original draft; INMPA, NLPCP, NMPS, NPLL reviewed and edited the final version, NPLL supervised all experiment. All authors have read the final manuscript.

Submitted: June 26, 2023
Revised: September 23, 2023
Accepted: December 4, 2023
Published online: December 31, 2023

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