An *in silico* investigation on the interactions of curcumin and epigallocatechin-3-gallate with NLRP3 Inflammasome complex

Atala B. Jena [a], Umesh C. Dash [b], Asim K. Duttaroy [c],[*]

[a] *Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA*
[b] *Regional Plant Resource Centre, Medicinal & Aromatic Plant Division, Forest & Environment Department, Govt. of Odisha, Nayapalli, Bhubaneswar 751015, India*
[c] *Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, 0317 Oslo, Norway*

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ABSTRACT

Interleukin-1β (IL-1β) and IL-18 are the underlying factors of the inflammatory response and are necessary for the host's reaction and pathogen resistance. The NLRP3 inflammasome involves in the secretion of pro-inflammatory cytokines IL-1β/IL-18 in response to microbial infection and cellular damage. Curcumin and epigallocatechin-3-gallate (EGCG) suppress the activation of the NLRP3 inflammasome; however, the exact mechanisms are not yet well known. In the current study, we investigated the interaction of curcumin and EGCG, the plant-derived compounds, with NLRP3 complex using *in silico* approach. The molecular docking and protein-protein interaction were used to investigate the apparent binding processes and affinities between components of the NLRP3 complex with curcumin and EGCG. Our data showed that NLRP3 had a higher binding affinity for curcumin and EGCG than other complex proteins, with − 8.2 Kcal/mol and − 9.6 Kcal/mol, respectively. Similarly, ASC had a lower binding affinity for curcumin and EGCG, with − 5.0 Kcal/mol and − 7.4 Kcal/mol, respectively. The higher binding affinity of both compounds for the key NLRP3 protein in their complexes as compared to that of MCC950 (a selective inhibitor of NLRP3 complex) suggests that curcumin and EGCG may impact the complex's function. Protein-protein interaction studies also corroborated the efficacy of these two polyphenols in hindering the formation of NLRP3 complex. The therapeutic effect of curcumin and EGCG may be due to the inhibition of inflammasome activation. The molecular and protein-protein interaction data indicated that the therapeutic effects of these two polyphenols are mediated by preventing the development of the NLRP3 complex.

Proposed mechanisms to prevent the development of the NLRP3 complex by antioxidant curcumin and catechin.

1. Introduction

The inflammasome regulates the processing and production of pro-inflammatory cytokines and the development of pyroptosis in innate immune responses. The inflammasome, a cytosolic supramolecular protein complex that serves as a signalling platform and triggers the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18, is activated as a result of this recognition process. Primarily, pathogen recognition receptors (PRRs) have four sub-families, i.e. toll-like receptors (TLRs), nucleotide-binding oligomerisation domain (NOD)-leucine-rich repeats (LRR) containing receptors (NLR), retinoic acid-inducible gene 1 (RIG-1) like receptors (RLR; aka RIG-1 like helicases-RLH), and C-type lectin receptors (CLRs) [1].

Inflammasomes are identified by their sensor protein PRRs, which oligomerise in response to damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) to form a pro-caspase-1 activating platform. The NLR [NOD (Nucleotide-binding oligomerisation domain) like receptors] family consists of NLRP1, NLRP2, NLRP3, NLRP6, NLRP12 and NLRC4. They have a nucleotide-binding domain (NBD), a Leucine-rich repeat (LRR) at the C-terminus, and a variable N-terminal domain containing either pyrin or the caspase activating and recruitment domain (CARD) [1]. The inflammasome is

formed by another NLR family member, NLRP6 or PYPAF5. It triggers the maturation of the pro-inflammatory cytokines IL-1 and IL-18, as well as the activation of pyroptosis, in the same way that NLRP1 does [2].

The NLRP3 inflammasomes comprise a sensor protein that activates caspase-1 via the linker molecule ASC. In mice, non-canonical inflammasomes convert pro-caspase-11 to caspase-11, but in humans, they activate caspases 4 and 5 [3,4]. These caspases (inflammatory) recognize intracellular lipopolysaccharide (LPS) in gram-negative bacteria and digest the pore-forming protein gasdermin-D along with caspase-1 (GSDMD). Further, in non-canonical pathways, the adaptor molecules are not required because Caspase 11, 4, or 5 directly attaches to LPS via a CARD-CARD interaction [5]. When adaptor molecules bind to receptors, they transform into a prion-like form and produce long ASC filaments, which play a crucial role in inflammasome activation. Procaspase-1, the effector molecule, undergoes autoproteolytic maturation due to its interaction with ASC, resulting in active Caspase-1 [6]. The functional subunits p10 and p20 are produced by proteolytic cleavage. These subunits promote the secretion of cytokines such as IL1B and IL-18, as well as pyroptosis, a type of cell death [7].

NLRP3 is the most studied among the other NLRs, and most NLR responses are agonist specific (e.g., NLRP1, anthrax lethal factor, NLRC4, bacterial flagellin) [8]. However, the NLRP3 inflammasome is triggered by structurally and chemically diverse human, microbial, and environmental stimuli [9]. Activating mutations in the NLRP3 gene also cause inherited autoinflammatory disorders contributing components in various inflammatory and autoimmune disorders [10].

Inflammasomes play a role in the onset and progression of numerous diseases, including Alzheimer's disease, Parkinson's disease, type 2 diabetes, nephropathy, cardiovascular diseases, atherosclerosis, obesity, and many more [11]. In addition, several autoinflammatory disorders linked with high IL-1β and IL-18 production are caused by dysregulation of inflammasome activation. The assembly and activation of inflammasomes have all made substantial advances in recent years.

Different inhibitors of the NLRP3 inflammasome pathway, such as polyphenols, carotenoids, terpenoids, phytosterols, tocopherols, alkaloids, and triterpenes have been validated in vitro studies and animal models of NLRP3-related diseases [12]. However, their mechanisms of action are still not well known. In addition, mechanisms of the molecular foundation for inflammasome construction and dissolution are still in their early stages [13]. Therefore, comprehensive structural and biophysical investigations may help in identifying variables that influence inflammasome formation and disassembly and therapeutic targets for developing new anti-inflammatory compounds from natural sources. Understanding the mechanism of inflammasome formation and activation is critical to better handling inflammasome-associated diseases [14].

Aloe-emodin, sulforaphane, resveratrol, quercetin, mangiferin, ginseng, curcumin, genipin, and epigallocatechin gallate are the natural compounds that regulate the NLRP3 inflammasome-mediated inflammatory response [14]. Some of these inhibitors target the NLRP3 protein directly, while others target additional inflammasome components and products. Directly targeting the NLRP3 protein may be preferable because it avoids off-target immunosuppressive effects and limits tissue damage. These natural polyphenols can dramatically lower ROS levels, lowering or downregulating NLRP3 inflammasome activation in the process [14,15].

Curcumin is a polyphenol primarily obtained from turmeric rhizomes (*Curcuma longa*). It has been used to treat several diseases, such as inflammatory bowel disease (IBD), rheumatoid arthritis, Alzheimer's disease (AD), and cancers of the colon, lungs, stomach, skin, and breast. It has a broad spectrum of health benefits and has been proven in several experimental and pharmacologic trials [16–18]. Curcumin reduces inflammation by inhibiting lipopolysaccharide-induced nuclear factor-κB (NF-κB) p65 translocation and mitogen-activated protein kinase activation in dendritic cells. In several studies, curcumin has been shown to inhibit NLRP3 inflammasome activation in various models and

culture mediums [19,20]. TLR4/MyD88/NF signaling and P2X7R expression are dual down regulated by curcumin, inhibiting NLRP3-induced release of IL-1β and Caspase-1 [21]. Curcumin treatment for diabetic nephropathy resulted in lower levels of NLRP3, Caspase-1, and IL-1β and also inactivated the NLRP3 by TXNIP suppression [22,23]. Curcumin therapy resulted in a substantial reduction in levels of IL-1β and glutamate in mice hippocampi to explore the relationship between inflammasome and ischemia damage; which may also suppress NLRP3 activation via modulating AMPK activity and downregulating TXNIP [24].

Catechins are naturally occurring polyphenols in certain foods and plants, teas, buckwheat, grapes, cocoa beans, onion, litchis, and apples [25]. Antioxidant catechins mediate their anti-inflammatory and anti-cancer effects via several mechanisms, including reduced activation of proangiogenic vascular endothelial growth factor (VEGF) [26]. Epigallocatechin-3-gallate (EGCG), a major catechin in green tea, is a powerful antioxidant that can reduce oxidation and inflammation [14]. Although EGCG and curcumin bind to a wide range of viral and human proteins, no evidence is available regarding how these compounds interact with NLRP3 inflammasome complexes [27]. The current study investigated the interaction of EGCG and curcumin with NLRP3 inflammasome complexes using computational methods. The computational approaches (Molecular docking and protein-protein interaction) investigate the apparent binding processes and affinities of ligands for macromolecules before undertaking costly and time-consuming experimental investigations. Furthermore, for the development in speed, reliability, and accuracy, computational docking approaches have been undertaken to make it a feasible alternative for developing structure-based medications in recent years. This paper describes the results of EGCG and curcumin molecular docking with the NLRP3 inflammasome complexes. The binding affinities of EGCG and curcumin and protein-protein interactions with NLRP3 inflammasome complexes indicated that both polyphenols considerably altered the structure of the NLRP3 inflammasome complex.

2. Material and methods

2.1. Structure preparation

Canonical SMILES ids of curcumin and Epigallocatechin-3-gallate and MCC950, a selective inhibitor of NLRP3 complex (as a positive control), were acquired from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and transformed into 3D structures in Chimera 1.11.2 [28]. Before studying molecular and protein-protein interactions, the 3D structure of both antioxidants is well energy minimized in the Chimera 1.11.2 program. The crystal structures of NLRP3 complexes were obtained from the Protein Data Bank (https://www.rcsb.org/) with an appropriate resolution. The software Discovery studio 2017 R2 Client was used to remove the water and ligand molecules, and only the pure protein structure was taken for the *in silico* analysis.

2.2. Molecular interaction study

The interaction between components of the NLRP3 complex and antioxidants curcumin and EGCG was evaluated by using the molecular docking software Auto Dock 4.2 [29]. The binding sites and energy of curcumin and EGCG towards the NLRP3 were compared with MCC950, the selective inhibitor of the NLRP3 complex, and the respective binding energies were reported. To find out the specific ligand binding sites of NLRP3, various characteristics such as binding affinity, receptor-ligand interaction site, atomic contact energy (ACE), and side amino acid residues were investigated. The grid box was set up using 96, 76, and 92, directing in x, y, and z orientations, respectively, with a grid point spacing of 0.375 Å. The centre grid box is of − 0.279 Å, − 0.15 Å and − 0.403 Å around the selected amino acids of ASC. And the grid box was set up for Caspase-1 using 102, 94, and 76, pointing in x, y, and z

Table 1

The binding energy, Interaction type, and amino acids involved in the interaction of NLRP3 complex proteins with curcumin and EGCG.

Protein	Ligand	Binding Affinity (Kcal/mol)	Interaction	AA: Name; Chain Name; AA: No.
ASC	MCC950	-5.3	van der Waals	SER157, ARG160
			Conventional Hydrogen Bond	GLU152, LYS161, SER164
			Pi-Cation	ARG119
			Pi-Anion	ASP116
			Pi-Sigma	LYS161
			Alkyl	ILE115, LYS109
ASC	Curcumin	-5.0	van der Waals	ILE115, GLN117, SER195, LEU192, ARG194, ALA121
			Conventional Hydrogen Bond	HIS118, ARG125
			Pi-Anion	ASP191
			Pi-Pi stacked	PHE114
ASC	EGCG	-7.4	van der Waals	GLU80, GLY83, ALA98, GLN101, PRO103, ALA87, PRO89, THR89, SER93
			Conventional Hydrogen Bond	GLN91, GLY92, GLN86, HIS90, PRO97, ALA96
			Carbon-Hydrogen Bond	ARG74
			Unfavorable Donor-Donor	GLY94
			Pi-Sigma	ALA95
			Pi-Alkyl	ILE100, ALA102
Caspase-1	MCC950	-6.2	van der Waals	ARG396, TYR153, ALA150, MET156, LYS158, ASP157, HIS404
			Conventional Hydrogen Bond	ILE152, ILE155
			Pi-Sigma	ILE155
			Alkyl	PRO154, TRP145, ALA141, PHE401
Caspase-1	Curcumin	-6.9	van der Waals	TRP340, PHE337, GLN379, SER376, GLU355, MET345, SER347, GLY346
			Conventional Hydrogen Bond	HIS342, ARG383
			Carbon-Hydrogen Bond	GLY351
			Pi-Anion	ARG352, ASP381
			Pi-Alkyl	VAL348
Caspase-1	EGCG	-7.6	van der Waals	ARG383, HIS342, GLY346, ALA384, PRO380, GLN385, GLU378, HIS356, GLN379, GLY351
			Conventional Hydrogen Bond	SER347, PHE377, SER376
			Unfavorable acceptor-acceptor	MET345
			Pi-Anion	ARG352, ASP381
			Pi-Alkyl	VAL348
NLRP3	MCC950	-8.2	van der Waals	PHE640, VAL641, ASP639, GLN642, GLU637, SER264, THR299, HIS262, ASP261, GLY696, GLN636, ALA644
			Conventional Hydrogen Bond	ARG697
			Pi-Anion	GLU135
			Pi-Sulfur	MET645
			Amide-Pi Stacked	GLU638

Table 1 (*continued*)

Protein	Ligand	Binding Affinity (Kcal/mol)	Interaction	AA: Name; Chain Name; AA: No.
NLRP3	Curcumin	-8.2	Pi-Alkyl	ARG136, HIS698
			van der Waals	ALA644, GLN636, GLU693, GLY696, THR299, ASP261, HIS262, PRO134
			Conventional Hydrogen Bond	HIS698, LYS694, ARG136, GLU135
			Carbon-Hydrogen Bond	GLU638, GLU695
			Unfavorable Donor-Donor	GLU637
			Pi-Cation	ARG697
NLRP3	EGCG	-9.6	van der Waals	ALA165, SER120, GLU228, LYS163, TYR202, GLU1005, ILE123, SER234, GLN233, TYR237, VAL1029, LEU1001, GLY1002, SER973, LEU1003, ARG918, LEU974, GLU945, GLY975, TYR201, SER1033
			Conventional Hydrogen Bond	VAL1028, ASN1000, SER1004, PRO1032, ASP947
			Carbon-Hydrogen Bond	PRO164
			Pi-Pi T-shaped	PHE1030
			Pi-Alkyl	LYS124, LYS127

directions, respectively, with a grid point spacing of 0.375 Å. The centre grid box is 46.113 Å, 62.287 Å and − 6.162 Å. Similarly, the grid box for NLRP3 was 106, 122, and 126, pointing in x, y, and z directions, respectively, with a grid point spacing of 0.375 Å. The centre grid box was 105.815 Å, 104.664 Å and 104.867 Å. The Computed Atlas of Surface Topography of Proteins (CASTp) was used to estimate the pocket amino acids of ASC, Caspase-1, and NLRP3. The grid box parameters for all compounds (catechin, curcumin and positive control) are constant. The conformer with the lowest energy was chosen for analysis after the AutoDock 4.2 interaction. The analysis above was performed by Discovery Studio 2017 R2 Client handled the visualisation and analysis of the data.

2.3. Protein-protein interaction

The NLRP3 complex (NLRP3, ASC, and Pro-caspase-1) protein docking study was done with clusPro 2.0, an automated rigid body docking tool, in the presence and absence of curcumin and EGCG. This programme enables the screening of docked conformations, including the cluster of characteristics, while considering various protein properties, and the filtered conformations were selected using empirical free energy calculations. To calculate free energy, the lowest desolvation and electrostatic energies were used. ClusPro is accessible at https://cluspro.bu.edu/publications.php. Piper, an FFT-based rigid docking tool, serves the ClusPro clustering program for detecting native sites by providing 10^3 low-energy outcomes. NLRP3 complex protein-protein interaction was conducted in the presence and absence of curcumin and EGCG to study its impact on the formation of the NLRP3 complex.

2.4. Molecular visualisation

Biovia Discovery Studio Visualizer 16.1.0 tools were used to visualise the molecular structures. (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/ visualization-download.php). The BIOVIA Discovery Studio Visualizer is a free molecular

Table 2

Protein-Protein interaction depicting 5 lowest binding energy for NLRP3 complex proteins in the presence or absence of curcumin and EGCG.

Macromolecules	1	2	3	4	5	Average lowest energy (kJ/mol)
Caspase 1 – NLRP3	-1101.9	-947	-1122.7	-896	-975.9	-1008.7
NLRP3-ASC	-1365.8	-1118.5	-1196.2	-1179.2	-1240.3	-1220
Caspase 1- NLRP3-ASC	-1141.1	-1183.5	-1202.3	-1172.3	-1053.4	-1150.52
Caspase 1 With NLRP3-EGCG	-1027.3	-942.3	-904.8	-1017.3	-963.7	-971.08
Caspase 1 With NLRP3-Curcumin	-1027.3	-942.3	-904.8	-1015.3	-963.7	-970.68
Caspase 1-EGCG With NLRP3	-970	-1001.7	-1000.8	-1007.1	-950.1	-985.94
Caspase 1-Curcumin With NLRP3	-945.3	-960.7	-976.4	-1007.1	-970.1	-971.92
Caspase 1-EGCG With NLRP3-EGCG	-914.3	-953.2	-909.1	-1005	-873.9	-931.1
Caspase 1-Curcumin With NLRP3-Curcumin	-934.3	-963.2	-918.1	-1000.5	-878.9	-939
NLRP3 With ASC-EGCG	-1164.8	-1180.5	-1092.4	-1117.1	-1045.6	-1120.08
NLRP3 With ASC-Curcumin	-1264.8	-1080.5	-1192.4	-1017.1	-1141.6	-1139.28
NLRP3-EGCG With ASC	-1250.7	-1177.8	-1072.2	-1071.3	-1236.9	-1161.78
NLRP3-Curcumin With ASC	-1250.7	-1077.8	-1192.2	-1111.3	-1136.9	-1153.78
NLRP3-EGCG With ASC-EGCG	-1050.1	-1179.1	-1069.7	-1009.1	-1138.2	-1089.24
NLRP3-Curcumin With ASC-Curcumin	-1110.1	-1079.1	-1089.7	-1110.1	-1145.2	-1106.84
Caspase 1-EGCG With NLRP3-EGCG With ASC-EGCG	-1060.1	-1000.3	-959.5	-953.2	-909.8	-976.58
Caspase 1-Curcumin With NLRP3-Curcumin With ASC-Curcumin	-960.1	-992.5	-1159.7	-1053.8	-987.3	-1030.68

modelling programme that allows us to screening, share, and analyses protein and small molecule data. Molecular interaction and its outcomes are readily and efficiently handled, with no loss of time or scientific data.

3. Results

3.1. Molecular docking

Autodock Vina 1.1.2 [29] investigated the binding interactions of curcumin, EGCG and MCC950 with the NLRP3 complex proteins. When curcumin interacts with ASC, Caspase-1, and NLRP3, the binding energies were discovered to be − 5.0 Kcal/mol, − 6.9 Kcal/mol, and − 8.2 Kcal/mol, respectively (Table 1). Similarly, the binding energies of EGCG with the aforementioned complex proteins of NLRP3 complex are − 7.4 Kcal/mol, − 7.6 Kcal/mol, and − 9.6 Kcal/mol, respectively. MCC950 interacted with ASC, Caspase-1, and NLRP3, with binding energies − 5.3 Kcal/mol, − 6.2 Kcal/mol, and − 8.2 Kcal/mol, respectively. The outcome of our findings depicted that the EGCGhas a higher affinity toward all complex proteins than curcumin and MCC950. The binding energies of curcumin and MCC950 are almost identical.

The interaction of curcumin with NLRP3, Caspase-1 and ASC resulted in forming 6, 3, and 2 hydrogen bonds, respectively Table 1, Sup. Fig. S1-S3. Similarly, when EGCG interacted with the aforementioned complex proteins, 6, 3 and 7 hydrogen bonds were generated, respectively Sup. Fig. S4-S6. When MCC950 interacted with the aforementioned complex proteins, 1, 2 and 3 hydrogen bonds were formed (Table 1).

Curcumin was also bound to amino acids in this region of ASC protein of NLRP3 complex through van der Waals interactions (ILE115, GLN117, SER195, LEU192, ARG194, ALA121), Conventional hydrogen bond (HIS118, ARG125), Pi-anion (ASP191), Pi-Pi stacked (PHE114) Sup. Fig. S3. Similarly, curcumin bind with amino acid residues of Caspase-1 through van der Waals interactions (TRP340, PHE337, GLN379, SER376, GLU355, MET345, SER347, GLY346), Conventional hydrogen bond (HIS342, ARG383), Carbon-hydrogen bond (GLY351), Pi-Anion (ARG352, ASP381), Pi-Alkyl (VAL348) Sup. Fig. S2. In contrast, amino acids of NLRP3 interacted with curcumin through van der Waals interactions (ALA644, GLN636, GLU693, GLY696, THR299, ASP261, HIS262, PRO134), Conventional hydrogen bond (HIS698, LYS694, ARG136, GLU135), Carbon-hydrogen bond (GLU638, GLU695), Unfavorable donor-donor (GLU637), Pi-cation (ARG697) Sup. Fig. S1. Curcumin binds to amino acids in this region of ASC protein of NLRP3 complex through van der Waals interactions (ILE115, GLN117, SER195, LEU192, ARG194, ALA121), Conventional hydrogen bond (HIS118, ARG125), Pi-anion (ASP191), Pi-Pi stacked (PHE114). Similarly, Caspase-1 curcumin bind with amino acid residues through van der Waals interactions (TRP340, PHE337, GLN379, SER376, GLU355, MET345, SER347, GLY346), Conventional hydrogen bond (HIS342, ARG383), Carbon-hydrogen bond (GLY351), Pi-anion (ARG352, ASP381), Pi-alkyl (VAL348). In contrast, amino acids of NLRP3 interact with curcumin through van der Waals interactions (ALA644, GLN636, GLU693, GLY696, THR299, ASP261, HIS262, PRO134), Conventional hydrogen bond (HIS698, LYS694, ARG136, GLU135), Carbon-hydrogen bond (GLU638, GLU695), Unfavorable donor-donor (GLU637), Pi-Cation (ARG697).

EGCG shows higher binding affinity due to its free functional OH group on its surface and interacted with amino acid residues of ASC by van der Waals interactions (GLU80, GLY83, ALA98, GLN101, PRO103, ALA87, PRO89, THR89, SER93), Conventional hydrogen bond (GLN91, GLY92, GLN86, HIS90, PRO97, ALA96), Carbon-hydrogen bond (ARG74), Unfavorable donor-donor (GLY94), Pi-sigma (ALA95), Pi-alkyl (ILE100, ALA102) Sup. Fig. S6. It is similarly, interacting amino acid residues of Caspase-1 for EGCG by van der Waals interactions (ARG383, HIS342, GLY346, ALA384, PRO380, GLN385, GLU378, HIS356, GLN379, GLY351), Conventional hydrogen bond (SER347, PHE377, SER376), Unfavorable acceptor-acceptor (MET345), Pi-anion (ARG352, ASP381), Pi-alkyl (VAL348) Sup. Fig. S5. In contrast, EGCG interacted with NLRP3 of the NLRP3 complex through van der Waals interactions (ALA165, SER120, GLU228, LYS163, TYR202, GLU1005, ILE123, SER234, GLN233, TYR237, VAL1029, LEU1001, GLY1002, SER973, LEU1003, ARG918, LEU974, GLU945, GLY975, TYR201, SER1033), Conventional hydrogenbond (VAL1028, ASN1000, SER1004, PRO1032, ASP947), Carbon-hydrogen bond (PRO164), Pi-Pi T-shaped (PHE1030), Pi-alkyl (LYS124, LYS127) Sup. Fig. S4.

While, MCC950 bound with the ASC through van der Waals interactions (SER157, ARG160), Conventional hydrogen bond (GLU152, LYS161, SER164), Pi-cation (ARG119), Pi-anion (ASP116), Pi- sigma (LYS161), Alkyl (ILE115, LYS109). MCC950 bind with amino acid residues of Caspase-1 through van der Waals interactions (ARG396, TYR153, ALA150, MET156, LYS158, ASP157, HIS404), Conventional hydrogen bond (ILE152, ILE155), Pi- sigma (ILE155), Alkyl (PRO154, TRP145, ALA141, PHE401). Similarly, MCC950 bound with amino acid residues of NLRP3 through van der Waals interactions (PHE640, VAL641, ASP639, GLN642, GLU637, SER264, THR299, HIS262, ASP261, GLY696, GLN636, ALA644), Conventional hydrogen bond (ARG697), Pi-anion (GLU135), Pi-sulfur (MET645), Amide-Pi stacked (GLU638), Pi-alkyl (ARG136, HIS698).

NLRP3 had a higher binding affinity for curcumin and EGCG than other complex proteins, with − 8.2 Kcal/mol and − 9.6 Kcal/mol, respectively (Table 1). Similarly, ASC had a lower binding affinity for curcumin and EGCG, with − 5.0 Kcal/mol and − 7.4 Kcal/mol, respectively. The higher binding affinity of both compounds for the key
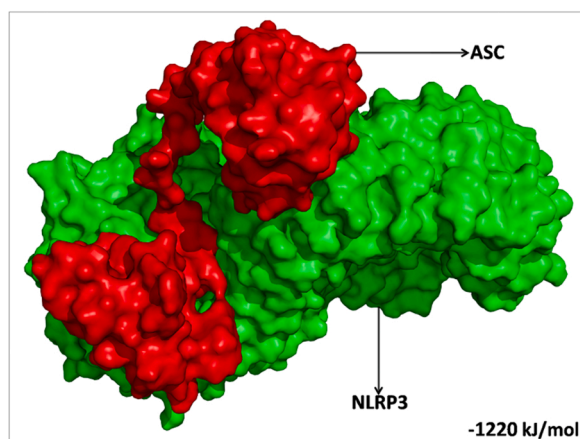
Fig. 1. Docked model depicting the interaction of NLRP3 with ASC in the. absence of polyphenol.

NLRP3 protein in their complexes suggests that curcumin and EGCG may impact the complex's function. EGCG showed stronger binding affinity towards each protein in the complex structures than curcumin because it has a greater number of functional OH groups that are more likely to establish a hydrogen bond.

3.2. Protein-protein interaction

The top 10 docking models with varying free energies were obtained from the ClusPro database, and the total RMSD value was applied as a grouping criterion [30–33]. We investigated 5 ClusPro docking models that were chosen based on the likelihood of NLRP3 complex proteins,

such as ASC, Caspase-1, and NLRP3, interacting with curcumin and EGCG conceivable interactions (Table 2), as well as the lowest binding energy during such interactions. For the Caspase 1–NLRP3, NLRP3-ASC, and Caspase 1-NLRP3-ASC interactions, the average binding energy of all five binding sites is − 1008.7 kJ/mol, − 1220 kJ/mol, and − 1150.52 kJ/mol, respectively (Table 2). In the presence of curcumin and EGCG, the formation of NLRP3 complexes was hindered by a reduction in its binding affinity. The NLRP3-ASC interaction had a higher binding affinity than the Caspase 1–NLRP3 and Caspase 1-NLRP3-ASC complexes in the NLRP3 complex.

Protein-protein interaction studies on the NLRP3 complex show that the NLRP3-ASC complex has high binding energy. Like the Caspase 1-NLRP3 complex, curcumin and EGCG also hinder the formation of the NLRP3-ASC complex by reducing its binding affinity with the NLRP3 inflammasome complex. When EGCG binds with ASC, NLRP3, or both ASC–NLRP3, the average binding energy falls from − 1220 kJ/mol to − 1120.08 kJ/mol, − 1161.78 kJ/mol, and − 1089.24 kJ/mol in the presence of EGCG in the NLRP3-ASC complex Figs. 1 and 2; Table 2; Sup. Fig. S11, S13. Similarly, in NLRP3-ASC complex binding energy decreases by − 1139.28 kJ/mol, − 1153.78 kJ/mol and − 1106.84 kJ/mol when curcumin bind with ASC, NLRP3 and both ASC – NLRP3 Fig. 3; Table 2; Sup. Fig. S12, S14. During the interaction of ASC-NLRP3 in the presence of EGCG (both ASC and NLRP3), a substantial decrease in the binding energy of 130.76 kJ/mol was found compared to their direct binding. Likewise, in the ASC–NLRP3 complex, the average decrease in binding energy for EGCG was 96.3 kJ/mol. Similarly, during the interaction of ASC-NLRP3 in the presence of curcumin (in both ASC and NLRP3), a substantial decrease in the binding energy of 113.16 kJ/mol was found in comparison to their direct binding. Curcumin has an average decreased binding energy of 86.7 kJ/mol in the ASC–NLRP3 complex.
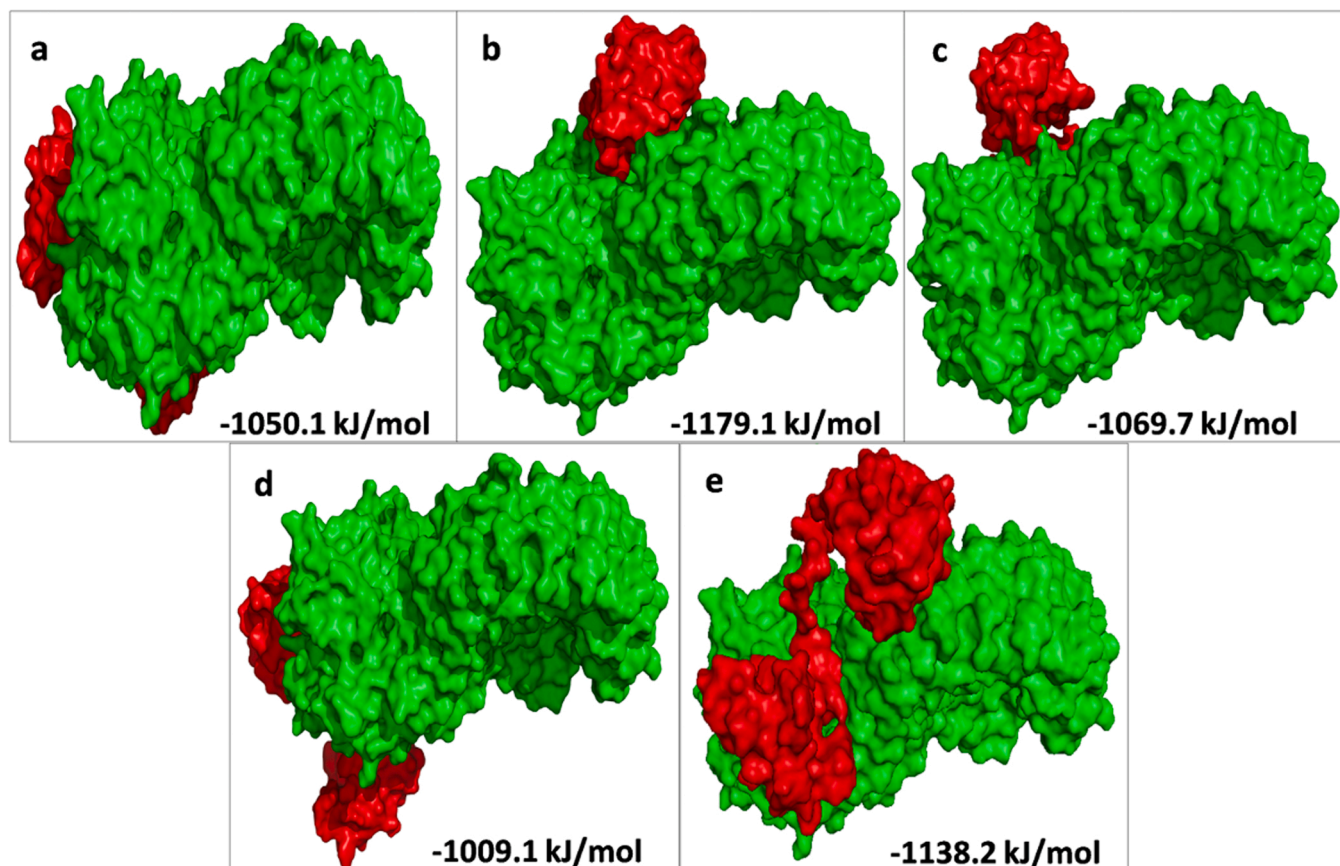

Fig. 2. The top 5 docked models display the interaction of NLRP3 with ASC in the presence of EGCG.
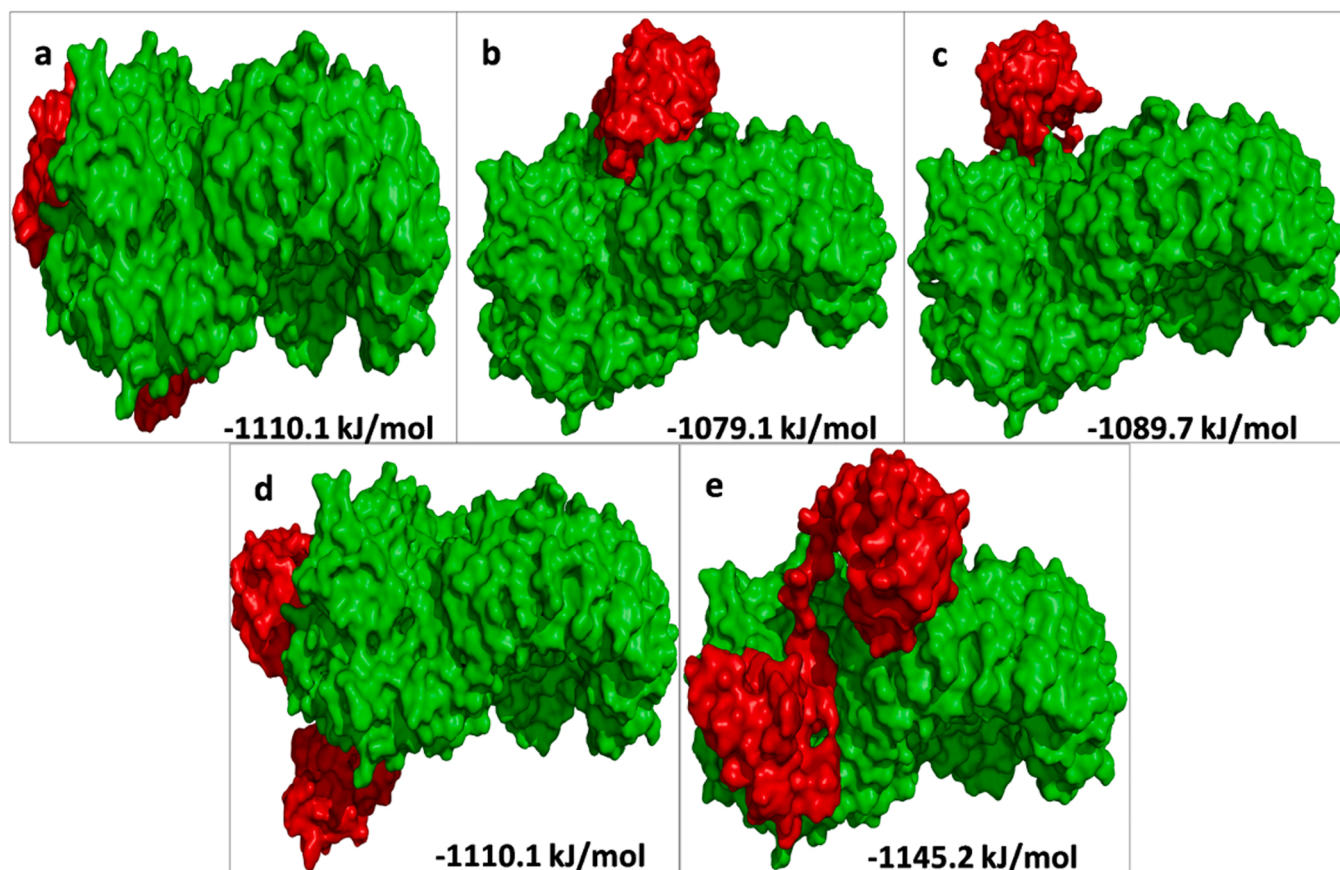
Fig. 3. The top 5 docked models display the interaction of NLRP3 with ASC in the presence of curcumin.
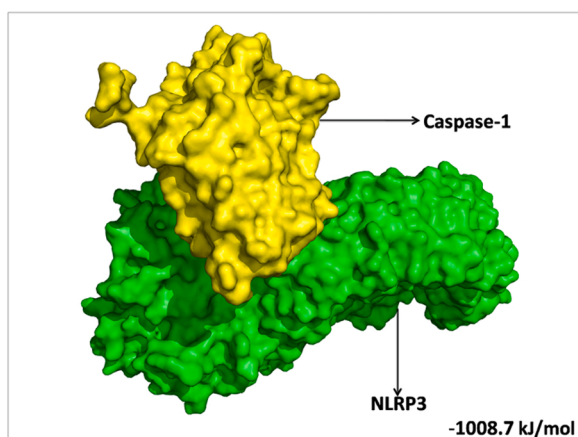

Fig. 4. Docked model depicting the interaction of NLRP3 with Caspase-1 in the absence of polyphenol.

When EGCG bound to NLRP3, Caspase 1, and both Caspase 1-NLRP3, the average binding energy falls from − 1008.7 kJ/mol to − 971.08 kJ/mol, − 985.94 kJ/mol, and − 931.1 kJ/mol, respectively Figs. 4 and 5; Table 2; Sup. Fig. S7, S9. Similarly, when curcumin bound to NLRP3, Caspase 1, and both Caspase 1-NLRP3 complexes, binding energy reduces by − 970.68 kJ/mol, − 971.92 kJ/mol, and − 939 kJ/mol Fig. 6; Table 2; Sup. Fig. S8, S10. In the presence of EGCG in Caspase 1 and NLRP3, a substantial decrease in 77.6 kJ/mol binding energy was found during the interaction of Caspase 1-NLRP3 compared to the direct binding. The average decrease in binding energy for EGCG is 45.99 kJ/mol in the Caspase 1-NLRP3 complex. In contrast, during the interaction

of Caspase 1-NLRP3 in the presence of curcumin, a substantial decrease in 69.7 kJ/mol binding energy was found in both Caspase 1 and NLRP3 compared to their direct binding. Curcumin has an average decreased binding energy of 48.16 kJ/mol in the Caspase 1-NLRP3 complex.

4. Discussion

NLRP3 inflammasome is involved in various pathological conditions and diseases as an intracellular innate immune sensor [11]. The disorder and diseases linked to inflammasomes almost always include an inflammatory component. Different inflammasome activators play an essential role in the development of diseases [11]. Therefore, an association between dysregulated inflammasome activation, IL-1β production, and disease pathogenesis are suggested. Pathogenesis of the disease is often connected to disease-related stressors that cause mutations in genes associated with the inflammasome, its associated pathways, and inflammasome-dependent IL-1β production [34].

Disease-related mutations trigger caspase-1 activation and IL-1β secretion in NLRP3-related component genes, misfolded protein aggregation, and abnormal metabolite accumulation, resulting in NLRP3 constitutive activation [35,36]. Dysregulation of NLRP3 is linked to a variety of diseases, including neurodegenerative diseases [37] such as Alzheimer's disease [38] and Parkinson's disease [37], Psoriasis [39], renal pathologies [5], carcinogenesis [40,41], and cephalic [42].

Treatment of numerous disorders involving inflammasome complex can be improved by better understanding the pathways and mechanism of NLRP3 inflammasome activation. Using natural compounds is advantageous as they have a strong binding affinity with high efficacy and minimal side effects. Both natural and synthetic therapies have several benefits, but the effectiveness of treatments depends largely on the disease status. Natural bioactive compounds may have more help with
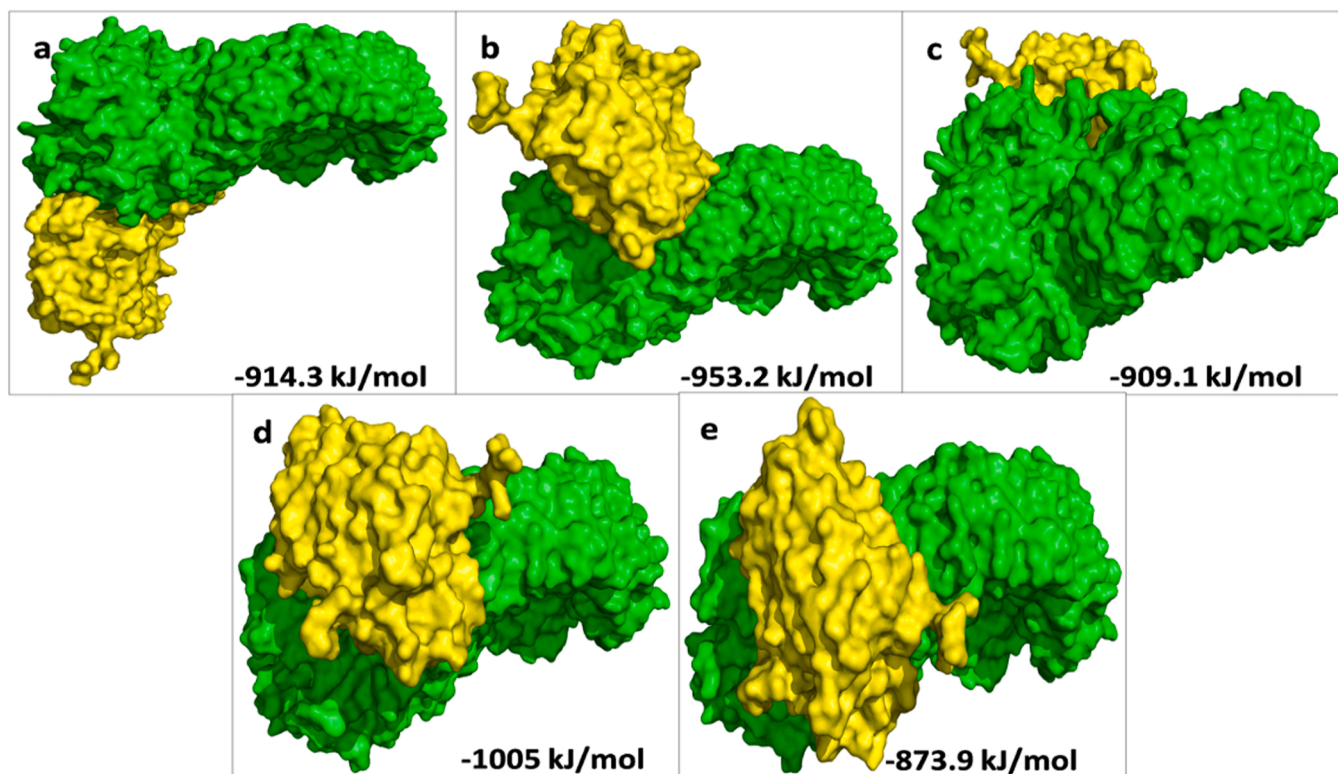
Fig. 5. The top 5 docked models display the interaction of NLRP3 with Caspase-1 in the presence of EGCG.
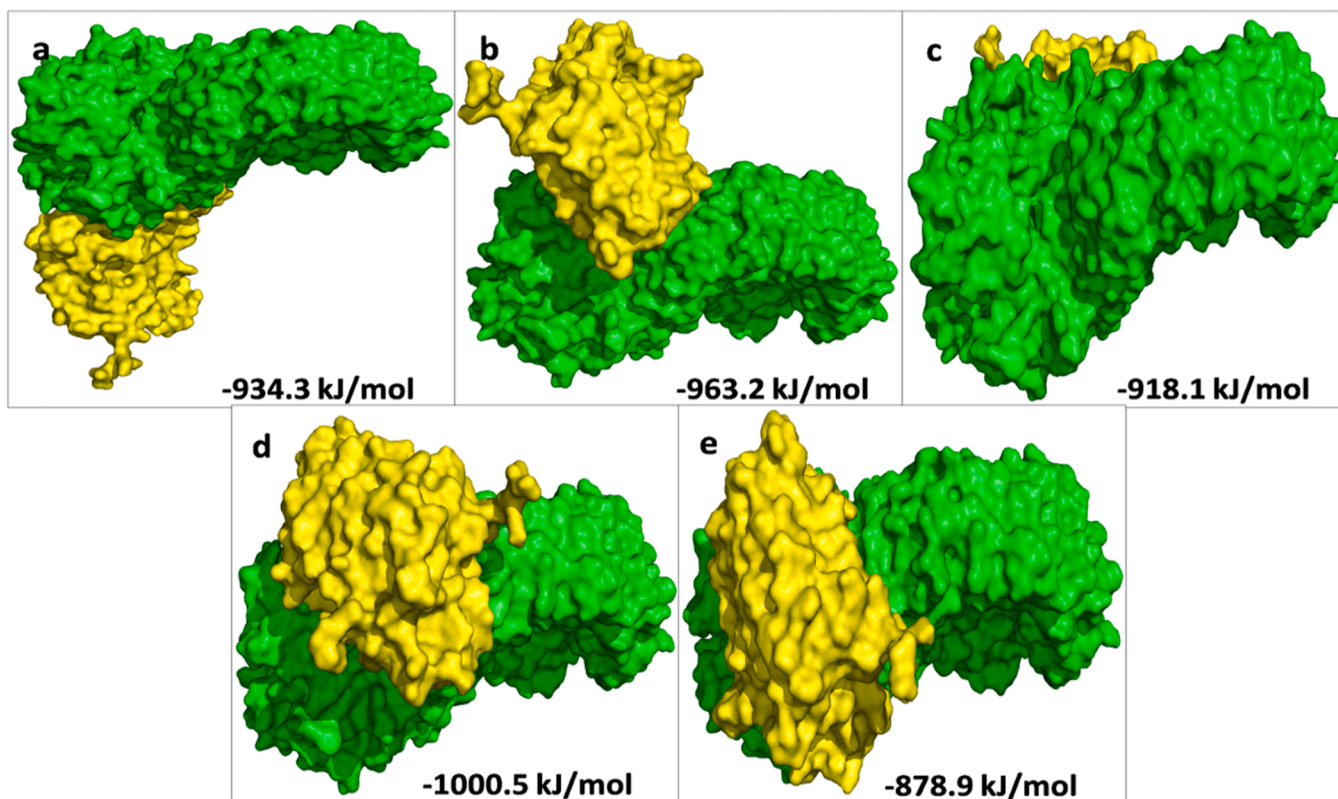

Fig. 6. The top 5 docked models display the interaction of NLRP3 with Caspase-1 in the presence of curcumin.

fewer adverse effects.

The current study investigated the interaction of curcumin and EGCG with NLRP3 complex, Caspase-1, and ASC. The amino acid residue of a protein complex (ASC, Caspase-1, and NLRP3) and the OH group of polyphenols (curcumin and EGCG) form H-bonds during the interaction. Due to several functional OH groups, EGCG has a high binding affinity
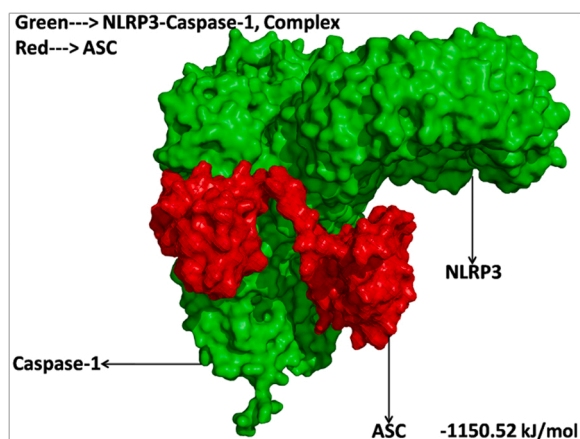
Fig. 7. Docked model depicting the interaction of NLRP3-Caspase-1 complex with ASC in the absence of polyphenol.

for the receptor. Furthermore, it has a higher affinity for all NLRP3 complex proteins due to their chemical characteristics, which include a large amount of OH groups on their surface [32,43]. The higher binding affinity of both antioxidants for the essential NLRP3 protein in their complexes suggests that curcumin and EGCG may impact the complex's function. MCC950 is a known inhibitor of the NLRP3 complex, which blocks the ATPase domain of NLRP3, resulting in the inhibition of canonical and non-canonical NLRP3 inflammasome activation [44]. MCC950 is a highly potent small molecule which inhibits NLRP3 and also prevents both canonical and non-canonical NLRP3 activation. It not only prevents NLRP3 activation but also decreases the severity of experimental autoimmune encephalomyelitis (EAE), a disease, by

inhibiting Interleukin-1β (IL-1β) production. Additionally, MCC950 therapy is effective in ex vivo samples from people with Muckle-Wells syndrome; thus, it can potentially treat NLRP3-related disorders, such as autoimmune and autoinflammatory diseases [45]. MCC950 blocks the release of IL-1β, which inhibits NLRP3 inflammasome. Furthermore, it specifically targets the NLRP3 NATCH domain and interferes with the Walker B motif function, thus preventing the conformational change and oligomerization of NLRP3 [46–48]. In contrast, molecular interaction studies suggest that the binding energies of curcumin and MCC950 are almost comparable, whereas EGCG has greater binding energies than curcumin and MCC950. In addition, EGCG suppressed the activation of the NLRP3 inflammasome by suppressing the expression of NLRP3, Caspase1, and IL-1and also suppressed the Nrf2 pathway's activity. The effects of EGCG on a rat model of contrast-induced nephropathy (CIN), a condition marked by inflammation and oxidative stress, were also studied. EGCG lowered oxidative stress and decreased IL-1 and NLRP3 gene output [11].

According to the above molecular interaction data, EGCG interacts with more amino acid residues of all proteins in the NLRP3 complex, which accounts for its higher binding affinity. Similarly, curcumin shows a good binding relationship towards all the above proteins of the complex according to its molecular weight in comparison to EGCG. Furthermore, it was also revealed that the keto groups of curcumin promote the formation of hydrogen bonds, whereas the hydroxyl groups of EGCG endorse hydrogen bond formation.

Protein-protein interaction study predicted that the binding energy score of − 1150.52 kJ/mol when the Caspase 1-NLRP3 complex interacts with ASC Fig. 7. In the presence of EGCG and curcumin, this interaction binding energy was reduced to − 976.58 kJ/mol and − 1030.68 kJ/mol, respectively Figs. 8 and 9; Table 2. Furthermore, it was observed that when EGCG and curcumin interact with this multi-complex protein, the binding affinity drops significantly to 173.94 kJ/
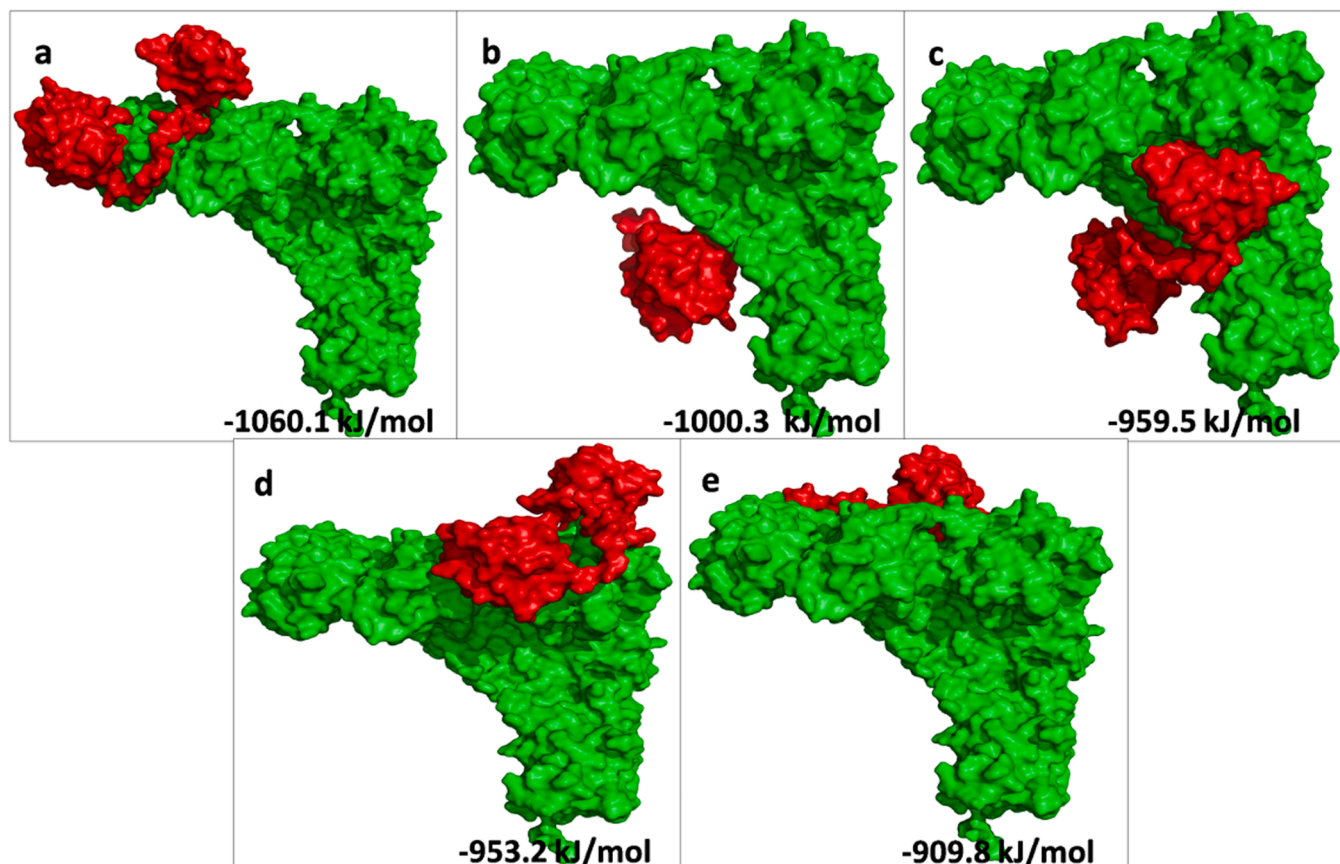

Fig. 8. The top 5 docked models display the interaction of NLRP3-Caspase-1 complex with ASC in the presence of EGCG.
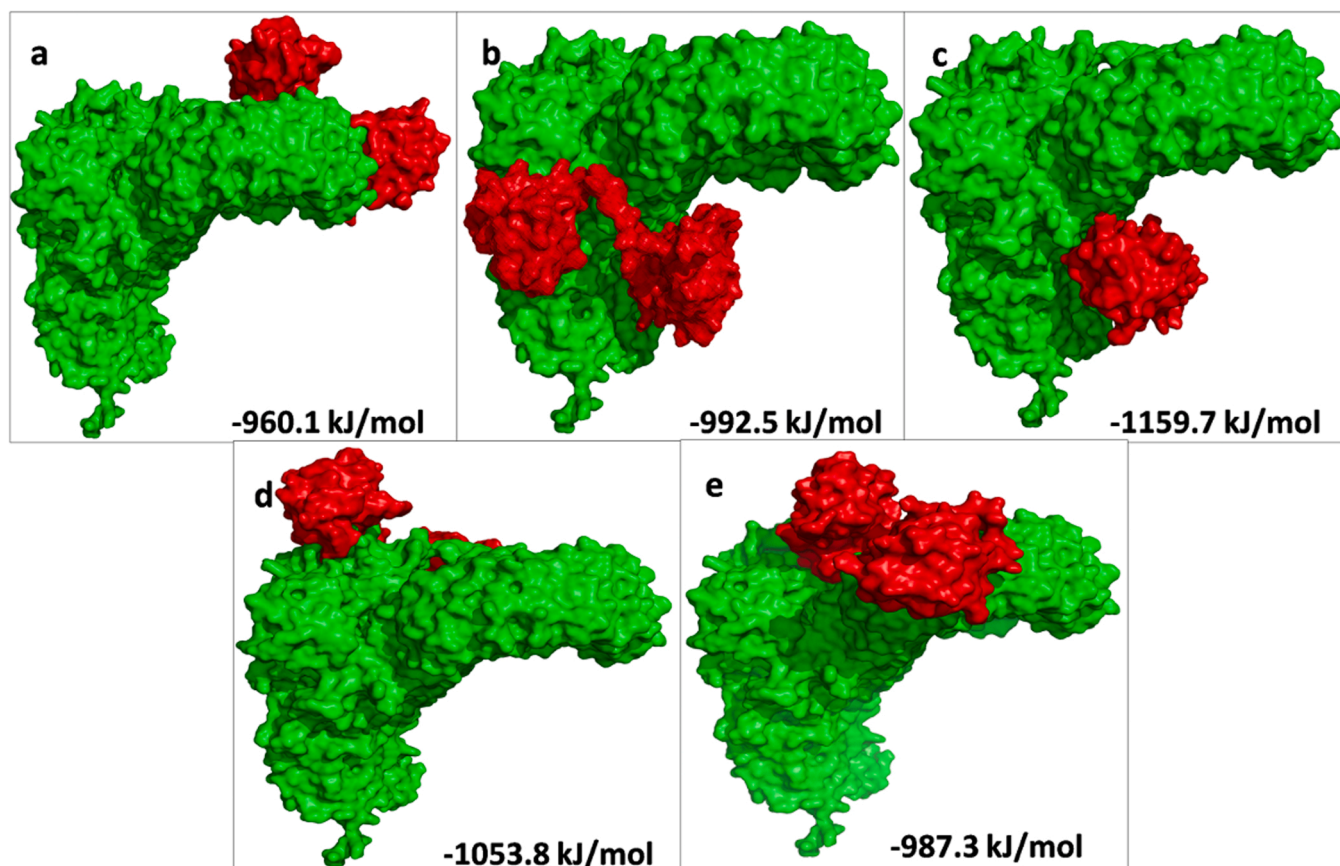
Fig. 9. The top 5 docked models display the interaction of NLRP3-Caspase-1complex with ASC in the presence of curcumin.

mol and 119.84 kJ/mol, respectively.

Data from molecular interaction studies suggest that EGCG has a greater binding affinity for all proteins in the NLRP3 inflammasome complex. Furthermore, a protein-protein interaction analysis found that EGCG inhibits the formation of the NLRP3 complex the most. Protein-protein interaction experiments in the presence of curcumin or EGCG support the findings above (molecular interaction research), suggesting that these two polyphenols effectively prevent the development of the NLRP3 complex Fig. 10.

5. Conclusion

Excessive inflammation is involved in many diseases, including cardiovascular disease and cancer. The inappropriate activation of the NLRP3 inflammasome complex leads to increased production of IL-1β and IL-18. It thus contributes to the development of various inflammatory diseases, including autoimmune, autoinflammatory, chronic inflammatory and metabolic disorders. Several inhibitors of NLRP3 inflammasome are developed using in vitro studies and animal models of NLRP3 inflammasome-related diseases; however, some have proven ineffective in clinical settings. This highlights the need for further in-depth research into activation pathways of the NLRP3 inflammasome for developing potent inhibitors. Since there is no FDA-approved medication to regulate or inhibit the inflammasome's excessive activation, natural compound-based therapies are being used in curing inflammasome-linked diseases. Both curcumin and EGCG are also inhibitors of NLRP3 inflammasome activation. However, the exact mechanism of their anti-inflammatory actions remains unclear. Therefore, the computational approach of curcumin and EGCG is investigated in this study and evaluates the potency towards the inhibition of excessive inflammasome activation. According to molecular interaction studies, EGCG has the most significant binding affinity towards

inflammasome due to more interacting OH groups in its structure. In contrast, curcumin has a decent binding relationship with the compounds mentioned above but less than EGCG.

Similarly, protein-protein interactions suggest that both antioxidants inhibit the development of inflammasome complexes by lowering their binding energy. To precisely define the function of curcumin and EGCG for the treatment of immune-related diseases, randomized clinical studies are required the determination of the efficacy of curcumin and EGCG as therapeutic agents. The bioavailability and toxicity of these polyphenols must be assessed before they are used as complementary therapeutic agents in treating human disease.

Credit authorship contribution statement

Conceptualization, ABJ, and AKDR; writing-original draft preparation, methodology, ABJ, and UCD; supervision, writing review and editing, AKDR.

Conflict of interest statement

We declare no conflict of interest. All co-authors have agreed to transfer of copyright to the publisher if it is accepted for publication.

Consent for publication

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113890.

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