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

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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. These cytokines also promote damage during chronic inflammation along with acute tissue injury. However, little is known about how these proteins are made and secreted from cells. Inflammasomes are multi-protein complexes which are required for the canonical synthesis of IL-1β. The NLRP3 inflammasome complex is one of the most studied inflammasome complexes. Its activation is dependent on two signals, i.e. one "primes" the cells by inducing the production of NLRP3 and pro-IL-1β, while the other causes the complex to assemble and activate. Lysosomal rupture, reactive oxygen species, and cytosolic ion perturbation function as the second signal. Despite extensive research, the exact role and regulation of the NLRP3 inflammasome are still unknown. In the current study, we investigated the inhibitory effect of plant-derived compounds such as curcumin and epigallocatechin-3-gallate (EGCG) on NLRP3-mediated IL-1β and IL-18 production using in silico approach. Our data suggest that 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.

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, PRRs have four sub-families, i.e., toll-like receptors (TLRs), nucleotide-binding oligomerization 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]. The activation of NLRP3 (inflammasome) is triggered by multiple molecular and cellular processes, including ionic flux, mitochondrial malfunction, reactive oxygen species generation, and lysosomal damage.

Inflammasomes are identified by their sensor protein, pathogen recognition receptors (PRRs), which oligomerizes 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 oligomerization 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]. NLRP 12 has a pyrin domain at the N-terminus, a nucleotide-binding domain in the middle, and a leucine-rich repeat at the C-terminus. It has
been demonstrated to have a role in forming an inflammasome response to pathogen infections and can act as an inflammatory signalling regulator.

The canonical inflammasomes, like NLRP3, 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. Procaspsase-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 ranging from mild familial cold autoinflammatory syndrome (FCAS) to severe neonatal-onset multisystem inflammatory disease (NOMID) [10]. Furthermore, NLRP3 inflammasome responses that are dysregulated are a contributing component in various inflammatory and autoimmune disorders [10].

Reactive oxygen species (ROS) also prompted mitochondrial sepsis-related oxidative stress. ROS produced by NLRP3 inflammasome activators caused various responses to exogenous stimuli and endogenously produced or secreted molecules from damaged cells, such as DAMPs. ROS/NLRP3 signalling pathway activated by the sepsis-related immune response via activating PAMPs/DAMPs, which triggers P2X7 activation and promotes Ca^{2+} influx and K^{+} efflux.

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]. The activation of NLRP3 inflammasome promotes various inflammatory disorders such as Alzheimer’s, atherosclerosis, and diabetes [1]. 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 (natural antioxidants), including polyphenols, carotenoids, terpenoids, phytosterols, tocopherols, alkaloids, and triterpenes of the NLRP3 inflammasome pathway, have been discovered recently, and they have been validated in vitro studies and animal models of NLRP3-related diseases [12]. However, the mechanism of their action is still not well known. In addition, mechanisms of the molecular foundation for inflammasome construction and dissolution are still in their early stages [13].
Comprehensive structural and biophysical investigations will aid in identifying variables that influence inflammasome production and disassembly, as well as identifying therapeutic targets for developing new anti-inflammatory medicines from natural sources. Therefore, 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. In addition, 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, lung, stomach, skin, and breast (REFs). 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 signalling and P2X7R expression are dual downregulated by curcumin, and this inhibits 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 it also inactivates 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]. In addition to anti-hypertensive, antibacterial, anti-inflammatory, and antioxidative activities, as well as a preventive effect against atherosclerosis, catechins have an anti-carcinogenic activity such in the cancers of mouth, oesophagus, stomach, small intestine, colon, lung, liver, pancreas, skin, prostate, mammary gland, and bladder. By reducing the activation of proangiogenic factors, including vascular endothelial growth factor (VEGF), catechins have reduced carcinogenesis, tumour growth, cancer cell invasion, and angiogenesis [26]. Epigallocatechin-3-gallate (EGCG), a major catechin in green tea, is a powerful antioxidant that can reduce oxidation and inflammation [14].

Although catechin 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.

Material And Methods

Canonical SMILES ids of Curcumin and Epigallocatechin-3-gallate 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.

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 of curcumin and EGCG towards the NLRP3 were compared, and the respective binding energies were reported. To find out the specific ligand binding sites of the NLRP3 complex, we investigated the binding affinity, receptor-ligand interaction site, atomic contact energy (ACE), and side amino acid residues. 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 visualization and analysis of the data.

Protein-Protein Interaction

The NLRP3 complex (NLRP3, ASC, Pro Caspase-1) protein docking study was done with clusPro2.0, an automated rigid body docking tool, in the presence and absence of curcumin and EGCG. This programme enables the screening of docked conformations, including 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 being an FFT-based rigid docking tool, serves the ClusPro clustering program for detecting native sites by providing 1000 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.
Molecular visualization

Biovia Discovery Studio Visualizer 16.1.0 tools were used to visualize 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 modelling programme that allows us to screening, share, and analyze protein and small molecule data. Molecular interaction and its outcomes are readily and efficiently handled, with no loss of time or scientific data.

Results

Molecular docking

AutodockVina 1.1.2 [29] investigated the binding interactions of curcumin and EGCG with the NLRP3 complex proteins. When curcumin interacts with ASC, Caspase-1, and NLRP3, the binding energies were discovered to be Gb-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 Gb -7.4 Kcal/mol, -7.6 Kcal/mol, and -9.6 Kcal/mol, respectively. The outcome of our findings depicted that the EGCG has a higher affinity toward all complex proteins than curcumin.

The interaction of curcumin with NLRP3, Caspase-1 and ASC resulted in forming 2, 3, and 6 hydrogen bonds, respectively (Table 1, Figure 1, Figure 2 and Figure 3). Similarly, when EGCG interacted with the aforementioned complex proteins, 7, 3 and 6 hydrogen bonds were generated, respectively (Table 1, Figure 4, Figure 5 and Figure 6).

NLRP3 has a higher binding affinity for curcumin and EGCG than other complex proteins, with Gb -8.2 Kcal/mol and -9.6 Kcal/mol, respectively (Table 1). Similarly, ASC has a lower binding affinity for curcumin and EGCG, with Gb-5.0 Kcal/mol and -7.4 Kcal/mol, respectively (Table 1). The higher binding affinity of both antioxidants for the key NLRP3 protein in their complexes suggests that curcumin and EGCG may impact the complex's function.

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) (Figure 3). 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) (Figure 2). 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) (Figure 1). 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).

It is similarly, to interacting amino acid residues of Caspase-1 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) (Figure 5). 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 Hydrogen Bond (VAL1028, ASN1000, SER1004, PRO1032, ASP947), Carbon-Hydrogen Bond (PRO164), Pi-Pi T-shaped (PHE1030), Pi-Alkyl (LYS124, LYS127) (Figure 4).

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 at 5 ClusPro docking models 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 (Figure 7, 8; Table 2; Supply Fig S5, S7). 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 (Figure 9; Table 2; Supply Fig S6, S8). 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 catechin 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.

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 (Figure 10, 11; Table 2; Supply Fig S1, S73). 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 (Figure 12; Table 2; Supply Fig S2, S4). In the presence of catechin in both 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 binding energy for catechin 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.

Discussion

NLRP3 inflammasome is involved in various pathological conditions and diseases as intracellular innate immune sensors [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], cephal [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 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 catechin) form H-bonds during the interaction. Due to the presence of several functional OH groups, catechin has a high binding affinity for the receptor. 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.

In addition, EGCG suppressed the activation of the NLRP3 inflammasome. By suppressing the expression of NLRP3, Caspase1, and IL-1, EGCG suppresses 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]. Catechin 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) (Figure 6).

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 good binding relationship towards all the above proteins of the complex according to its molecular weight in comparison to EGCG. It was also revealed that the keto groups of curcumin promote the formation of hydrogen bonds, whereas the hydroxyl groups of catechin are endorsing 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 (Figure 13). In the presence of catechin and curcumin, this interaction binding energy was reduced to -976.58 kJ/mol and -1030.68 kJ/mol, respectively (Figure 14,15; Table 2). It was observed that when catechin and curcumin interact with this multi-complex protein, the binding affinity drops significantly to 173.94 kJ/mol and 119.84 kJ/mol, respectively.

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

Conclusion

Mainly the inflammatory response is mediated by the NLRP3 inflammasome complex and thus involves several inflammatory diseases, including autoimmune, autoinflammatory, chronic inflammatory and metabolic disorders, which affect dysregulated NLRP3 inflammasome activation. Inflammasome-mediated IL-1 production or caspase-mediated pyroptosis is often advantageous for the host because they protect it from infections. But prolonged cytokine response, particularly in sterile inflammation, triggers endogenous signals leading to the onset of the diseases. Since there is no FDA-approved medication to regulate or inhibit the inflammasome's excessive activation, natural compound-based
therapies focus on curing inflammasome-linked diseases; however, the underlying mechanism is still not well known. The molecular docking approach of curcumin and catechin are investigated in this study and evaluated the potency towards the inhibition of excessive inflammasome activation. According to molecular interaction studies, catechin 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 aforementioned compounds but less than catechin.

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 for the future efficacy of curcumin and EGCG as therapeutic agents. Before the use of curcumin and catechin as complementary therapeutic agents in treating human disease, they first pass the safety assessment and be prescribed with an effective formulated dosage to maximize their bioavailability.

Declarations

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Author Contributions:

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

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Tables

Table 1
| Protein  | Ligand | Binding Affinity (Kcal/mol) | Interaction          | AA: Name; Chain Name; AA: No. |
|----------|--------|----------------------------|----------------------|-----------------------------|
| ASC      | Curcumin | -5.0                      | Vanderwaals:         | ILE115, GLN117, SER195, LEU192, ARG194, ALA121 |
|          |        |                            | Conventional Hydrogen Bond: | HIS118, ARG125 |
|          |        |                            | Pi-Anion:            | ASP191                      |
|          |        |                            | Pi-Pi stacked:       | PHE114                      |
| ASC      | Catechin | -7.4                      | Vanderwaals:         | 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| Curcumin | -6.9                      | Vanderwaals:         | TRP340, PHE337, GLN379, SER376, GLU355, MET345, SER347, GLY346 |
|          |        |                            | Conventional Hydrogen Bond: | HIS342, ARG383 |
|          |        |                            | Carbon-Hydrogen Bond: | GLY351                      |
|          |        |                            | Pi-Anion:            | ARG352, ASP381              |
| Protein   | Ligand       | Kd (nM) | Vanderwaals:                                                                 | Conventional Hydrogen Bond:                      | Unfavorable acceptor-acceptor | Pi-Anion:                        | Pi-Alkyl:               |
|-----------|--------------|---------|------------------------------------------------------------------------------|--------------------------------------------------|-------------------------------|----------------------------------|------------------------|
| Caspase-1 | Catechin     | -7.6    | ARG383, HIS342, GLY346, ALA384, PRO380, GLN385, GLU378, HIS356, GLN379, GLY351 | SER347, PHE377, SER376                           |                                | ARG352, ASP381                  | VAL348                 |
|           |              |         |                                                                               |                                                  | MET345                         |                                   |                        |
| NLRP3     | Curcumin     | -8.2    | ALA644, GLN636, GLU693, GLY696, THR299, ASP261, HIS262, PRO134              | HIS698, LYS694, ARG136, GLU135                   |                                |                                   |                        |
|           |              |         |                                                                               |                                                  | GLU638, GLU695                 |                                   |                        |
| NLRP3     | Catechin     | -9.6    | ALA165, SER120, GLU228, LYS163, TYR202, GLU1005, ILE123, SER234, GLN233, TYR237, VAL1029, LEU1001, GLY1002, SER973, LEU1003, ARG918, LEU974, GLU945, GLY975, TYR201, SER1033 | VAL1028, ASN1000, SER1004, PRO1032, ASP947       |                                |                                   |                        |
|           |              |         |                                                                               |                                                  | PRO164                         |                                   |                        |
|           |              |         |                                                                               |                                                  | PHE1030                        |                                   |                        |
|           |              |         |                                                                               |                                                  | LYS124, LYS127                 |                                   |                        |
## Table 2

| 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-Catechin                        | -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-Catechin 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-Catechin With Nlrp3-Catechin               | -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-Catechin                              | -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-Catechin 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-Catechin With Asc-Catechin                     | -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-Catechin With Nlrp3-Catechin With Asc-Catechin | -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                      |

## Figures
Figure 1
Docked pose of curcumin in the binding pocket of NLRP3.

(A) Participating amino acids in the interaction of curcumin and NLRP3, (B) Position of curcumin in Binding pocket of NLRP3. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).

Figure 2
Docked pose of curcumin in the binding pocket of Caspase-1.
(A) Participating amino acids in the interaction of curcumin and Caspase-1, (B) Position of curcumin in Binding pocket of Caspase-1. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).

Figure 3

Docked pose of curcumin in the binding pocket of ASC.

(A) Participating amino acids in the interaction of curcumin and ASC, (B) Position of curcumin in Binding pocket of ASC. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).
Figure 4

Docked pose of EGCG in the binding pocket of NLRP3.

(A) Participating amino acids in the interaction of EGCG and NLRP3, (B) Position of EGCG in Binding pocket of NLRP3. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).

Figure 5

Docked pose of EGCG in the binding pocket of Caspase-1.

(A) Participating amino acids in the interaction of EGCG and Caspase-1, (B) Position of EGCG in Binding pocket of Caspase-1. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).
Figure 6

Docked pose of EGCG in the binding pocket of ASC.

(A) Participating amino acids in the interaction of EGCG and ASC, (B) Position of EGCG in Binding pocket of ASC. This Fig has been developed using Discovery Studio Visualizer (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php).
Figure 7

Docked model depicting the interaction of NLRP3 with ASC in the absence of polyphenol.

![Docked models](image)

- a: -1050.1 kJ/mol
- b: -1179.1 kJ/mol
- c: -1069.7 kJ/mol
- d: -1009.1 kJ/mol
- e: -1138.2 kJ/mol

Figure 8

The top 5 docked models display the interaction of NLRP3 with ASC in the presence of EGCG.
Figure 9

The top 5 docked models display the interaction of NLRP3 with ASC in the presence of curcumin.
Figure 10

Docked model depicting the interaction of NLRP3 with Caspase-1 in the absence of polyphenol.
Figure 11

The top 5 docked models display the interaction of NLRP3 with Caspase-1 in the presence of EGCG.
Figure 12

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

Figure 13

Docked model depicting the interaction of NLRP3-Caspase-1 complex with ASC in the absence of polyphenol.
Figure 14

The top 5 docked models display the interaction of NLRP3-Caspase-1 complex with ASC in the presence of EGCG.
Figure 15

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

Supplementary Files

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