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Systematic analysis of Quercetin and its derivatives with special reference to anti-inflammatory property-based on network pharmacology

Sharmistha Dutta[1], H.P. Prashanth Kumar[1], Nijalingappa Ramesh[1] & Sushil Kumar Middha[2*]

[1]Department, Biotechnology, School of Applied Sciences Reva University, Rukmini Knowledge Park, Bengaluru-560 064, India
[2]Department of Biotechnology, Maharani Lakshmi Ammanni College For Women, Bengaluru-560 012, India

*Email: drsushilmiddha@gmail.com

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Abstract

The clinical success of phyto-chemicals and computationally assisted drug discovery-derived agents has stimulated the development of novel compounds for human illnesses. In the field of anticancer drug discovery, rational investigation has sped up the process. Quercetin and its derivatives have a long history of anti-inflammatory efficacy in pomegranate and other natural products, according to scientific evidence. Small structural alterations, for example, can drastically affect cell death behaviour and trigger inflammation, making it difficult to unravel the structure-activity relationship. As a result, our objective is to use a rational drug discovery approach to develop a mechanistic approach for quercetin and its derivatives, which will be compared to market anti-inflammatory medications utilising in-silico tools. Using the software Cytoscape, we created pharmacology networks of inflammatory proteins based on data acquired from several databases. The networks show how bioactives interact with molecular targets and how they relate to illnesses, particularly inflammation. Quercetin's network pharmacology study has shown novel connections between bioactive targets and possible inflammatory aetiology applications. The future prospect is to understand these chemicals *in vitro* and *in vivo*.

Keywords

Anti-inflammatory, CADD, Pomegranate, Quercetin

Introduction

Inflammation is the body's reaction to the invasion of a foreign body such as bacteria, parasites or viruses. It can be brought on by injury or infection. Inflammation research has been a major focus of scientific research around the world. Non-steroidal anti-inflammatory medicines (NSAIDs) can cause gastrointestinal ulcers, bleeding and renal damage due to their non-selective inhibition of both COX-1 and COX-2 (1, 2). Reactive oxygen species (ROS) cause oxidative damage and may speed up the progression of diseases such as ageing, arthritis, cancer and heart disease (3, 4). Prostaglandin plays a key role in the generation of the inflammatory response. Prostaglandin production depends on the activity of prostaglandin G/H synthases, colloquially known as COXs, bifunctional enzymes that contain both cyclooxygenase and peroxidase activity and which exists as district isoforms referred to as COX-1 and COX-2 (5). COX-2 induced by inflammatory stimuli, hormones and growth factors is the more important source of prostanoid formation in inflammation and in proliferative diseases such as cancer (6).

Medicinal plants have been and continue to be a valuable source of novel medicine development. A great number of critical components have been isolated from *Punica granatum* and examined for their potential therapeutic effects as part of novel drug research. (7). *Punica granatum* L. is a large-fruited shrub belonging to the Punicaceae family (New family Lythraceae). Though it is a native to Iran, it is grown all across the Middle East, South Asia and the Mediterranean. The pomegranate is a popular fruit that is well-known for its nutritional and therapeutic properties (1). The fruit is also separated into several compartments, including the leathery peel, fleshy aril and seed, each with its own set of toxicological and pharmacological properties. It has a number of beneficial phytoconstituents with antibacterial, antifungal, antiulcer, anticancer and anti-diarrheal properties (8). Natural flavonoids derived from different plant sources or medicinal plants including *Punica granatum* possess antioxidant and anti-inflammatory activities (8, 9). Quercetin is a flavonoid that belongs to the flavonols subclass and is one of the most common flavonoids in the human diet (10-12). Inflammatory markers (IL-6, IL-8, IFN-, COX-2 and TNF-) and oxidative stress (LOX-1 and ROS) mediators are inhibited by quercetin (13). Data strongly suggest that quercetin will be a useful method for suppressing inflammatory activity (14), based on previous research findings. Computational biology aids in accelerating and reducing the time and expense of drug development (15). The goal of the study was to use an *in-silico* method to discover a mechanistic approach and network pharmacology approach for quercetin and its derivatives against COX-2.

Materials and Methods

Molecular descriptor analysis

The prominent features of a drug which influences its activity are its lipophilicity/hydrophobicity, electronic potential within the molecule, molecular volume, polar surface area of the molecule and number of rotatable bonds. The different parameters are calculated using the Molinspiration software. (https://www.molinspiration.com/cgi-bin/properties).

Preparation of ligands

The PubChem database (https://pubchem. ncbi.nlm.nih.gov/) was used to determine the structure of Quercetin derivatives identified in *Punica granatum* L. (Pomegranate) peel. The properties of ligands were predicted, including hydrogen donors, acceptors, refractivity, Log P value, pH and molecular weight. Lipinski's rule of five was used to test these compounds for drug-likeness (15). Using Marvin Sketch, a variety of conformers for each ligand were constructed (energy reduction), and the lowest energy conformer was picked for further study.

Preparing the receptors

COX-2 was chosen as the target receptor due to their vital role in anti-inflammation. The Protein Data Bank (PDB) (http://www.pdb.org/) was used to find the structure of human COX-2 in association with the inhibitor (pdb id: 5IKT). A PDB structure file is often made up of heavy atoms, metal ions and water molecules, with no information about bond ordering, topologies or formal atomic charges. Considering all these parameters, the 3D structures of COX-2 was prepared by removing the heteroatoms and cleaned (clean geometry module) using Discovery Studio 3.5 (DS) software (16).

ADMET Predictions

ADMET prediction and significant descriptors of drug likeliness such as mutagenicity, toxicological dosage level for different organs and pharmaceutically relevant properties of the compounds were predicted using PreADMET server (https://preadmet.bmdrc.kr/). The 3D structures were subjected to absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis that predicted solubility, intestinal absorption, hepatotoxicity, blood-brain barrier (BBB) penetration of ligands;CYP2D6 inhibition using swiss ADME. ADMET describes the amount of pharmaceutical compound deposited within an organism (17, 18).

Protein Networking

Through a literature review, proteins that are important in the inflammation process were identified, and a dataset was created (19, 20). Then, using the String database, protein networking was carried out to analyse the relationships between proteins in order to find the important influence factor of different proteins in the network (21).

Results

Lipinski's rule of five

Using Lipinski's rule of five, the drug likeness score of the ligands and Tolfenamic acid (TLF) was assessed for non-steroidal anti-inflammatory medicine (NSAID).

TLF is also used to alleviate migraine headaches (22). In Table 1, the physicochemical parameters of the ligands are listed. Compounds that showed drug like properties or the compounds that accept Lipinski's rule are quercetin, isorhamnetin, rhamnetin, tamarixetin and quercetin 3-O-sulfate. Previously, there are reports on the evaluation of anti-cancerous properties of quercetin (23).

PDBsum interactions

The ligand and receptor interactions were studied using a 3D structure of COX-2 (5IKT) linked to a ligand molecule Tolfenamic acid (TLF). The interactions of the ligand with residues such as GLY526 (A), VAL349 (A), LEU352 (A), SER530 (A), TYR385 (A) and ALA527 (A) are clearly seen in Fig. 1. After docking tests, the majority of these amino acid residues were discovered to be bound to the quercetin derivatives.

The binding energies of the ligands were verified using the Auto Dock tool, as shown in Table 2. The docking data revealed that the ligands occupied the same COX-2 active region as TLF. quercetin, isorhamnetin, rhamnetin, tamarixetin and quercetin 3-O-sulfate all followed Lipinski's rule, with binding energies of -6.02, -6.31, -6.93, -6.05 and -6.82 respectively. The binding mode of the ligands within the active site of COX-2 was also analysed

Table 1. Lipinski's rule of five showing physicochemical properties of the selected compounds

Sl. No.	Name of the Compound	H-Bond donors	H-Bond Acceptors	Molecular weight	LogP
1	Quercetin	5	7	302.24	1.68
2	Quercetrin	7	11	448.38	0.64
3	Isoquercetin	8	12	464.38	-0.36
4	Rutin	10	16	610.52	-1.06
5	Quercetin 3-O- galactoside	8	12	464.38	-0.36
6	Quercetin 7-O- glucoside	8	12	464.38	-0.10
7	Isorhamnetin	4	7	316.26	1.99
8	Rhamnetin	4	7	316.26	2.22
9	Tamarixetin	4	7	316.26	1.99
10	Quercetin 3-O-Sulphate	5	10	382.30	-0.75
11	Quercetin 3-Glucuronide 7-Glucoside	11	18	640.50	-2.27
12	Tolfenamic acid (TLF)	2	3	261.71	5.00

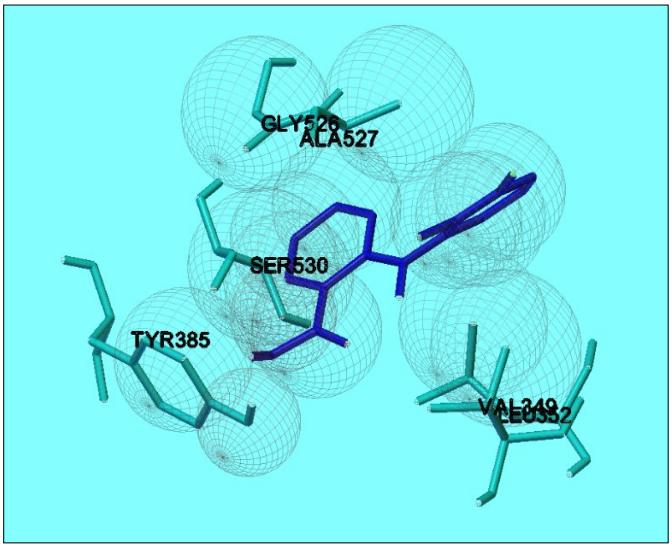
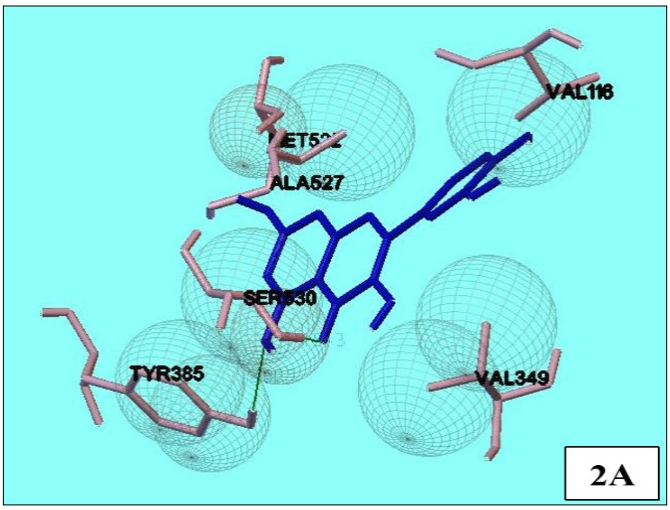


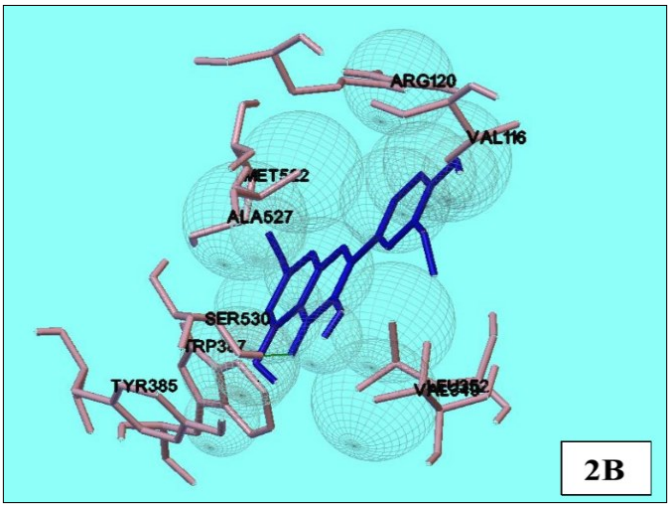
Fig. 1. Interaction of Tolfenamic acid (TLF) with the residues of COX-2 receptor. The ligand is depicted in dark blue sticks and the light blue sticks represented the interacted amino acids in the receptor.

Table 2. Binding energy of docked ligands using AutoDock

Sl. No.	Compound Name	Binding energy (kcal/mol)
1	Quercetin	-6.02
2	Quercetrin	-3.83
3	Isoquercetin	-5.33
4	Rutin	25.71
5	Quercetin 3-O- galactoside	0.96
6	Quercetin 7-O- glucoside	-2.22
7	Isorhamnetin	-6.31
8	Rhamnetin	-6.93
9	Tamarixetin	-6.05
10	Quercetin 3-O-Sulphate	-6.82
11	Quercetin 3-Glucuronide 7-Glucoside	66.82
12	Tolfenamic acid (TLF)	-7.59

using AutoDock tool (Fig. 2). The binding mode interactions of quercetin (Fig. 2A) with COX-2 were *ALA527 (A), SER530 (A), TYR385 (A), VAL116 (A), MET522 (A).* The binding sitefor Isorhamnetin (Fig. 2B) were found to bind with





ALA527 (A), SER530 (A), TRP387 (A), TYR385 (A), VAL116 (A), VAL349 (A), LEU352 (A), MET522 (A). For Rhamnetin (Fig. 2C), *ALA527 (A), MET522 (A), VAL523 (A), TYR355 (A), VAL349 (A), TYR385 (A).* For quercetin 3-O-sulfate (Fig. 2D) the

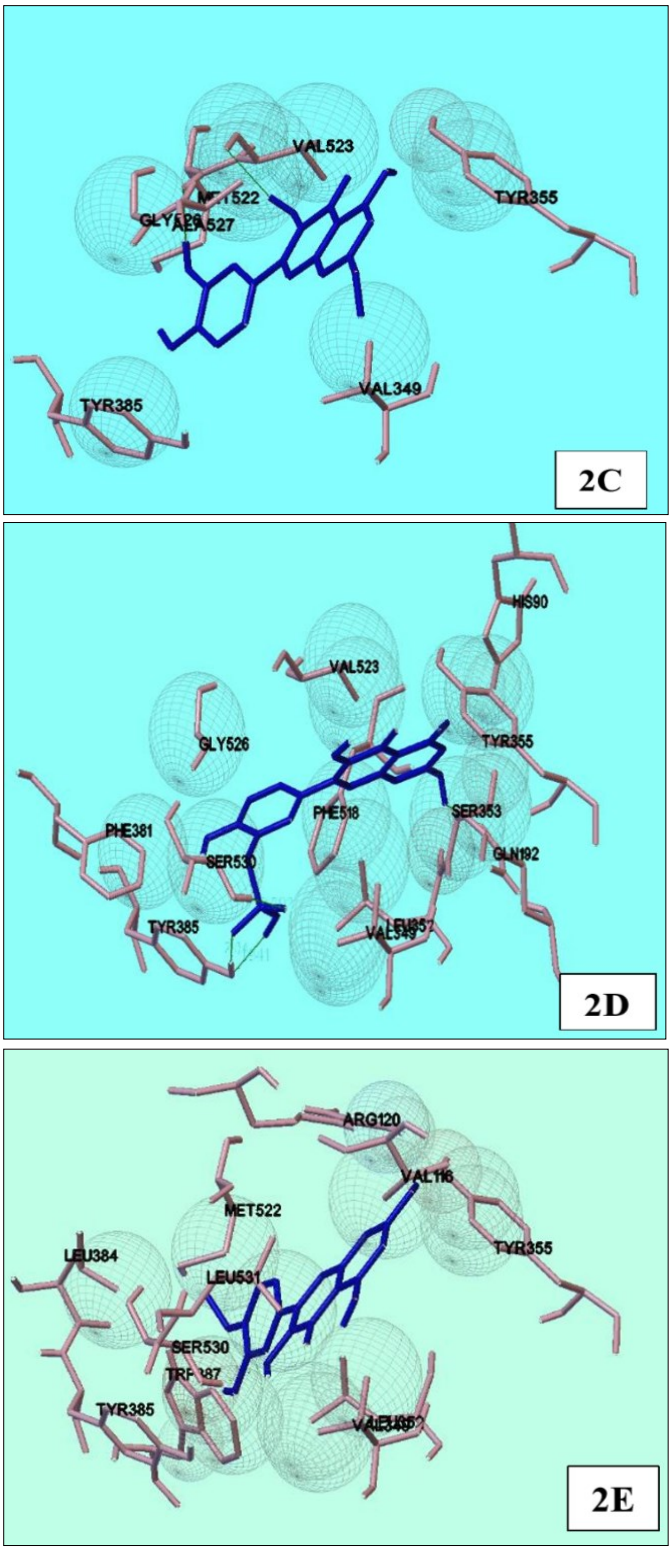


Fig. 2. Interaction of the ligands (**A**: Quercetin; **B**: Isorhamnetin; **C**: Rhamnetin, **D**: Quercetin 3-O-Sulphate; **E**: Tamarixetin) with the COX-2 receptor.

binding sites were *VAL523 (A), TYR355 (A), SER353 (A), SER539 (A), LEU352 (A), PHE518 (A).* For Tamarixetin (Fig. 2E) the amino acids associated were *ARG120 (A), LEU531 (A), SER530 (A), VAL349 (A), TYR355 (A), TRP387 (A).* These amino acids play a crucial role in binding of inhibitors within the active site of COX-2 (Table 3).

Toxicity Prediction

PreADMET was used to predict ADME characteristics for the ligands. The expected ADME features are summarised in Table 4. All of the compounds in Table 4 have restricted intestinal absorption and are CYP2D6 inhibitors, with the exception of quercetin, isorhamnetin, rhamnetin and tamarixetin.

The medication absorption prediction for numerous substances utilised in the study is shown in Fig. 3. The chemicals that are projected to pass through the blood-brain barrier passively are found in the yolk of a boiled egg, as shown in Fig. 3. The white of a boiled egg contains molecules that are expected to be passively absorbed by the gastrointestinal tract. The Central Nervous System (CNS) is represented by blue dots and P-glycoprotein is predicted to expel a molecule from there.

Table 3. Represents the selected compounds interacting with the aminoacid residues in the active site of COX-2 receptor (The residues in bold are the common amino acids in ligand and standard)

Compound name	Interacted residues in the COX-2 receptor
Quercetin	*VAL116 (A), TYR385 (A), MET522 (A), ALA527 (A), SER530 (A)*
Isorhamnetin	*VAL116 (A), VAL349 (A), LEU352 (A), TYR385 (A), TRP387 (A), MET522 (A), ALA527 (A), SER530 (A)*
Rhamnetin	*VAL349 (A), TYR355 (A), TYR385 (A), MET522 (A), VAL523 (A), ALA527 (A)*
Quercetin 3-O-sulfate	*LEU352 (A), SER353 (A),TYR355 (A), PHE518 (A), VAL523 (A), SER539 (A),*
Tamarixetin	*ARG120 (A), VAL349 (A), TYR355 (A), TRP387 (A), SER530 (A), LEU531 (A)*
Tolfenamic acid (TLF) Standard drug	VAL349 (A), LEU352 (A), TYR385 (A), GLY526 (A), ALA527 (A), SER530 (A)

Table 4. ADMET characteristics of Quercetin and their derivatives

Compound name	Solubility	BBB Permeant	CYP2D6 inhibitor	GI Absorption	Leadlikeness
Quercetin	Soluble	-	+	High	+
Quercetrin	Soluble	-	-	Low	-
Isoquercetin	Soluble	-	-	Low	-
Rutin	Soluble	-	-	Low	-
Quercetin 3-O- galacto-side	Soluble	-	-	Low	-
Quercetin 7-O- gluco-side	Soluble	-	-	Low	-
Isorhamnetin	Soluble	-	+	High	+
Rhamnetin	Soluble	-	+	High	+
Tamarixetin	Moderately soluble	-	+	High	+
Quercetin 3-O-Sulphate	Soluble	-	-	Low	-
Quercetin 3-Glucuronide 7-Glucoside	Soluble	-	-	Low	-
TLF	Moderately soluble	+	-	High	-

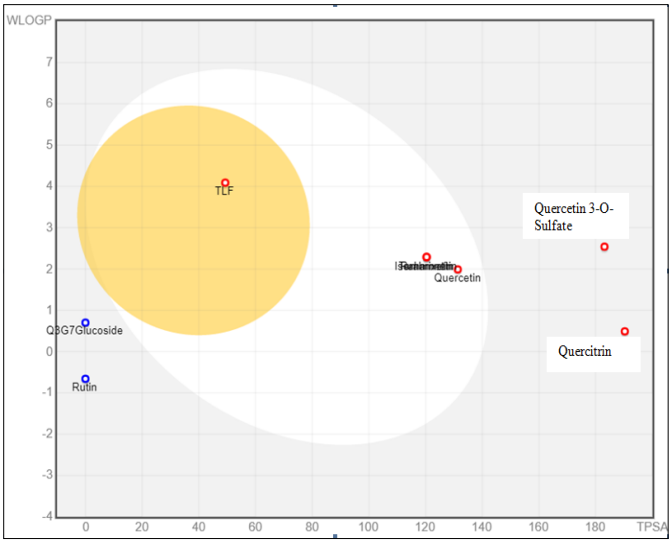


Fig. 3. Drug absorption prediction for several compounds: (a) Molecules predicted to pass through the blood-brain barrier passively are located in the yolk of a boiled egg. (b) Molecules projected to be passively absorbed by the gastrointestinal tract are located in the boiled egg's white. (c) *P*-glycoprotein is expected to effluent a molecule from the Central Nervous System, which is shown by blue dots.

Protein network

A protein collection of around nineteen proteins was identified as being significant in numerous inflammation pathways based on the literature review. Then, in order to better comprehend the interactions between the proteins, a string database was employed to create a protein network (Fig 4). The network has a total of 12 nodes (proteins) and 39 interacting edges (lines linking the nodes).

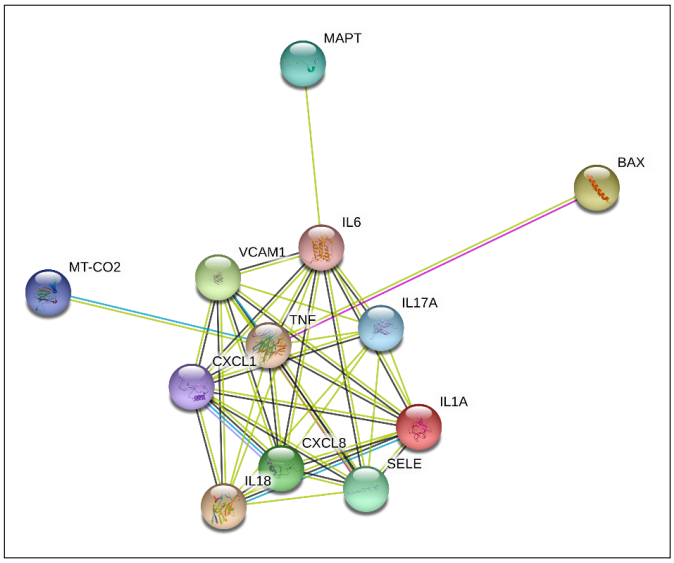


Fig. 4. Cytoscape tool showing the relationship between COX-2 and other proteins in the network.

Discussion

Molecular docking is a type of bioinformatics modelling that involves 2 or more molecules interacting to generate a stable adduct. The three-dimensional structure of any complex is predicted, depending upon binding properties of ligand and target receptor (23). Currently, using a computer to comprehend drug bimolecular interactions is an appealing platform for rational drug design and discovery. It also aids in mechanistic studies by inserting a molecule (ligand) into the target's preferred binding site (24, 25).

Lipinski's rule of 5 predicts that poor absorption or permeation is more likely when there are more than 5 Hydrogen bond donors, hydrogen bond acceptor > 10, molecular weight > 500 and calculated LopP > 5. As a result, compounds with these qualities cannot be pursued further as therapeutic candidates since they lack the features required in ADME (18).

The substances quercetin, isorhamnetin, rhamnetin, quercetin 3-O-sulfate and tamarixetin have drug-like effects. These chemicals had a high affinity for binding to the COX-2 receptor. The maximum binding affinity was found with rhamnetin and quercetin 3-O-sulfate (-6.93 and -6.82, respectively), while the conventional medication TLF had a binding energy of -7.59 with COX-2. Toxicity prediction of isolated phytochemicals or selected compounds is crucial for detecting their negative effects on people or animals. None of the compounds showed BBB penetration except TLF, the reference medication. cytochrome P4502D6 was suppressed by quercetin, isorhamnetin, rhamnetin, and tamarixetin (CYP2D6). CYP2D6 is a liver enzyme that is involved for the metabolism and removal of around a quarter of all clinically used medicines (26). Toxicity of the chemicals may also be related to low levels of intestinal absorption. Except for Quercetin 3-O-sulfate, all of the compounds had drug-like characteristics and significant gastrointestinal absorption.

The relevance of important proteins in the inflammation process was determined via network protein prediction using a string database (19). COX-2 (MT-CO2) is one of the most important proteins that is impacted by the presence of Tumour Necrosis Factor (TNF), which is regulated or controlled by a group of interleukins called IL1A, IL6, IL17A and IL18 (27).

Previously, the BOILED-Egg model has proven to be a valuable tool for lead optimization (28). Our result demonstrates how property-based lead optimization can be used to improve pharmacokinetics.

Conclusion

To conclude, when medications are generated from plant sources, the risk of adverse effects is reduced to some amount. Pharmacological studies show that *Quercetin* displays anti-inflammatory effects in cellular and animal models[28]. This study shows that *Punica granatum* L. contains a few compounds like quercetin that can bind and inhibit the COX-2 enzyme. However, the only approach to anticipate the pharmacological action of the molecules involved is to conduct a molecular docking research. Our investigation provides an important information to study the pathogenesis of diabetes and the potential means of quercetin in the management of diabetes. To confirm anti-inflammatory effectiveness of these drugs, *in vitro* and *in vivo* researches on animal models are required.

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Authors contributions

SD carried out the study, participated in the experimental analysis and drafted the manuscript. HPP carried out the docking studies and also wrote a part of the manuscript. NR participated in the design of the study and approved the manuscript. SKM conceived the idea and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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