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Proceeding Paper

Molecular Docking-Based Evaluation of Phytochemicals Against Key Targets in Paracetamol-Induced Hepatotoxicity †

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Abstract

Paracetamol (acetaminophen) is widely used as an analgesic and antipyretic; however, overdose results in hepatotoxicity primarily mediated through its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). This study aims to explore the hepatoprotective potential of selected phytochemicals—silymarin, piperine, quercetin, and gallic acid—through molecular docking against key proteins implicated in paracetamol-induced liver injury. In silico docking studies were performed using Schrödinger Release 2021-4 on a high-performance Ubuntu workstation. Ligands were sketched in Maestro, processed via LigPrep under OPLS_2005 force field conditions, and docked using Glide in XP mode. Seven protein targets were selected: succinate dehydrogenase (SDH), glutathione reductase (GSHR), cyclooxygenase-2 (Cox2), TNF-α, IL-6, IL-1β, and JNK, representing key pathways including mitochondrial dysfunction, oxidative stress, and inflammation. The results revealed that quercetin exhibited the strongest binding affinities across all targets, with a notable docking score of −9.060 for JNK and –8.027 for IL-1β, suggesting potent anti-inflammatory and mitochondrial protective roles. Gallic acid demonstrated broad-spectrum efficacy, especially against GSHR (−5.288) and JNK (−8.052), implicating its antioxidant potential. Silymarin showed significant binding to Cox2 (−7.073) and SDH (−5.665), supporting its known hepatoprotective effect. Piperine, while moderate in most targets, showed enhanced affinity for Cox2 (−6.608), indicating anti-inflammatory relevance. Overall, the study highlights quercetin and gallic acid as promising phytochemicals that may counteract paracetamol-induced hepatotoxicity by targeting multiple pathophysiological proteins. Further in vitro and in vivo validations are warranted to establish their therapeutic potential.

Keywords: paracetamol toxicity; computational drug discovery; natural hepatoprotective agents; Schrödinger Glide docking; quercetin; reactive oxygen species (ROS)

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1. Introduction

Paracetamol (acetaminophen) remains one of the most widely prescribed analgesic and antipyretic drugs worldwide. However, overdose leads to severe hepatotoxicity, accounting for a major proportion of acute liver failure cases globally. The primary mechanism of toxicity is mediated by the cytochrome P450-driven conversion of paracetamol to

the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is detoxified through conjugation with glutathione (GSH). In overdose situations, GSH reserves become depleted, resulting in excessive covalent binding of NAPQI to mitochondrial proteins, oxidative stress, and activation of pro-apoptotic and inflammatory signaling cascades.

Phytochemicals have emerged as promising hepatoprotective candidates due to their multitarget therapeutic mechanisms, including antioxidant reinforcement, anti-inflammatory activity, and CYP450 inhibition. Compounds such as piperine, quercetin, and gallic acid, alongside the gold standard silymarin, show distinct potential to attenuate paracetamol-induced liver injury by restoring redox balance and preventing mitochondrial dysfunction.

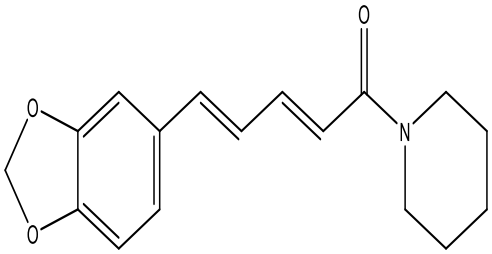


Figure 1. Piperine ($C_{17}H_{19}NO_3$),.

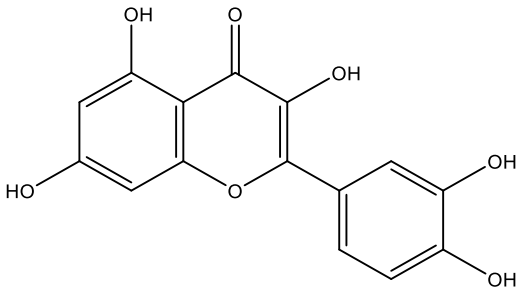


Figure 2. Quercetin ($C_{15}H_0O_7$).

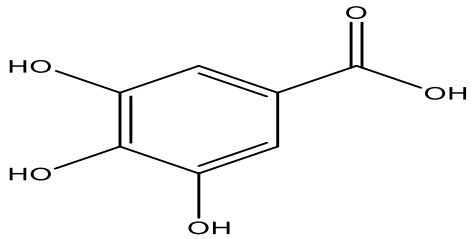


Figure 3. Gallic acid (3,4,5-trihydroxybenzoic acid; $C_7H_6O_5$).

The present study applied an in silico molecular docking approach to evaluate these phytochemicals against seven critical protein targets involved in paracetamol hepatotoxicity: succinate dehydrogenase (SDH), glutathione reductase (GSHR), cyclooxygenase-2 (Cox2), TNF-α, IL-6, IL-1β, and JNK. The objectives were (i) to assess their binding affinities, (ii) to identify key ligand–target interactions, and (iii) to highlight lead compounds for further preclinical exploration.

2. Materials and Methods

2.1. Computational Setup

All molecular docking studies were performed using the Schrödinger Suite (Release 2021-4) installed on an Ubuntu 22.04 LTS workstation equipped with an Intel® Core™ i5-12400 CPU, 16 GB RAM, and an Nvidia GeForce RTX 3050 GPU.

2.2. Target Proteins

Seven proteins implicated in paracetamol hepatotoxicity were retrieved from the RCSB Protein Data Bank (PDB): SDH (1ZOY), GSHR (2LV3), Cox2 (5JVY), TNF-α (2AZ5), IL-6 (1P9M), IL-1β (1ITB), and JNK (1UKI). Protein preparation was conducted using the Protein Preparation Wizard, with optimization of hydrogen bonds and energy minimization under the OPLS_2005 force field.

2.3. Ligand Preparation

Ligands included paracetamol, NAPQI, silymarin, piperine, quercetin, and gallic acid. Structures were generated using Maestro's 2D sketcher and refined via the LigPrep module (OPLS_2005 force field, pH 7.0 ± 2.0).

2.4. Molecular Docking

In Glide (Maestro v2020-4) all the docking process was conducted. The active site coordinates containing grid file was uploaded, and ligands in *out.maegz*format were designated. Docking was accomplished with default settings except XP (extra precision) mode. The resultant output file, glide dock_XP.*csv*, was examined for 2Dvisualization. The analysis focused on hydrophobic and hydrogen-bond-related interactions.

The protein selected were known molecular targets implicated in paracetamol induced hepatotoxicity. The receptors selected were as follows-

- **Succinate Dehydrogenase (SDH)|PDB: 1ZOY**

SDH (mitochondrial complex II) converts succinate to fumarate in the TCA cycle and electron transport chain. NAPQI binds SDH, disrupting ATP synthesis and increasing ROS, directly linking it to mitochondrial dysfunction in paracetamol toxicity.

- **Glutathione Reductase (GSHR)|PDB: 2LV3**

GSHR regenerates reduced glutathione (GSH) from GSSG. Paracetamol overdose depletes GSH, and NAPQI inhibits GSHR, exacerbating oxidative stress.

- **Cyclooxygenase-2 (Cox2)|PDB: 5JVY**

Cox2 drives prostaglandin synthesis during inflammation. Paracetamol-induced necrosis activates Cox2, amplifying inflammatory damage in hepatocytes.

- **TNF-α|PDB: 2AZ5**

TNF-α promotes apoptosis and inflammation. It amplifies oxidative damage in paracetamol toxicity by recruiting immune cells and activating JNK.

- **IL-6|PDB: 1P9M**

IL-6 regulates acute-phase inflammation and hepatocyte regeneration. Chronic IL-6 signaling worsens liver injury by sustaining oxidative stress and fibrosis.

- **IL-1β|PDB: 1ITB**

IL-1β triggers pro-inflammatory cascades. It synergizes with TNF-α to promote neutrophil infiltration and mitochondrial permeability transition in hepatocytes.

- **JNK|PDB: 1UKI**

JNK phosphorylates mitochondrial proteins, inducing apoptosis. Paracetamol activates JNK via oxidative stress, driving mitochondrial dysfunction and necrosis.

These receptors represent key nodes in paracetamol toxicity: mitochondrial dysfunction (SDH, JNK), oxidative stress (GSHR), and inflammation (Cox2, TNF-α, IL-6, IL-1β). Targeting them may restore redox balance, block necrosis, and mitigate inflammation.

3. Results and Discussion

3.1. Docking Score Analysis

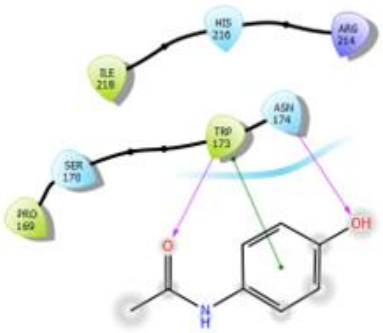
The docking score matrix (Table 1) revealed clear differences in phytochemical binding across targets. Among the compounds tested, quercetin and gallic acid consistently exhibited superior binding affinities relative to paracetamol, NAPQI, and in many cases, silymarin.

Table 1. Docking Score.

Compound Name & PubChem CID	Succinate Dehydrogenase (PDB ID:1ZOY)	GSH Reductase (PDB ID:2LV3)	Cox2 (PDB ID:5JVY)	TNF-a (PDB ID:2AZ5)	IL-6 (PDB ID:1P9M)	IL-1b (PDB ID:1ITB)	JNK (PDB ID:1UKI)
Paracetamol CID-1983	−2.891	−3.848	−4.575	−3.573	−4.940	−4.015	−5.547
NAPQI CID-39763	−3,422	−3.227	−4.318	−2.207	−3.583	−3.760	−5.769
Silymarin CID-5213	−5.665	−5.550	−7.073	−5.634	−4.009	−3.873	−8.204
Piperine CID-638024	−2.764	−3.372	−6.608	−3.532	−2.634	−2.531	−7.705
Quercetin CID-5280343	−4.932	−4.811	−8.174	−3.526	−5.636	−8.027	−9.060
Gallic Acid CID-370	−4.836	−5.288	−6.720	−5.330	−5.599	−5.360	−8.052

- Quercetin demonstrated exceptional affinity toward JNK (−9.060) and IL-1β (−8.027), suggesting strong inhibitory potential against apoptotic and inflammatory pathways.
- Gallic acid displayed broad-spectrum efficacy, with strong binding to GSH reductase (−5.288) and JNK (−8.052), reinforcing its role in redox balance.
- Piperine showed moderate docking scores but preferential binding to Cox2 (−6.608), aligning with its reported anti-inflammatory properties.
- Silymarin, considered the gold standard hepatoprotectant, showed consistent activity but was outperformed by quercetin in several targets, notably JNK and IL-1β.

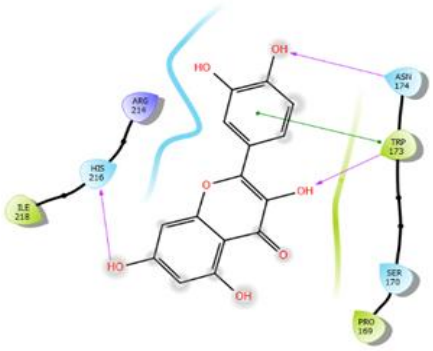
2D-Intraction with 1ZOY



Pi-pi stacking (4.72) TRP173

1 H-bonds (2.06 & 2.10) TRP173& ASN174

Figure 4. 2D view interaction of paracetamol with 1ZOY (Docking score: −2.891 kcal/mol).

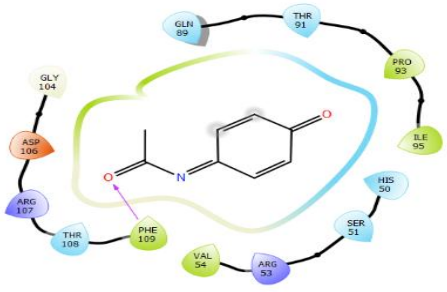


1 Pi-pi stacking (4.66) TRP173

3 H-bonds (2.13,2.20,2.45) with TRP173,ASN174 & HIS216

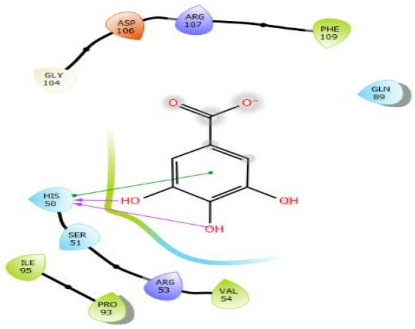
Figure 5. 2D view interaction of quercetin with 1ZOY(Docking score: −4.932 kcal/mol).

2D-Intraction with 2LV3



1 H-bond(2.41) PHE109

Figure 6. 2D view interaction of NAPQI with 2LV3 (Docking score: −3.227 kcal/mol).

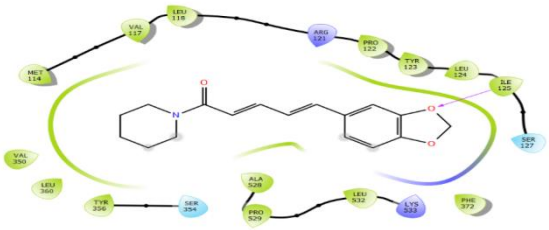


1 Pi-pi stacking (4.66) HIS50

2 H-bonds (1.72,1.94) HIS50

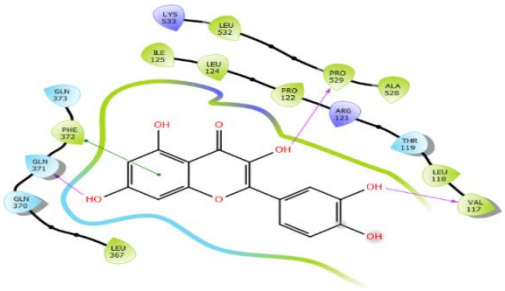
Figure 7. 2D view interaction of Gallic acid with 2LV3 (Docking score: −5.288 kcal/mol).

2D-Intraction with 5JVY



1 H-bond(2.16) ILE125

Figure 8. 2D view interaction of piperine with 5JVY (Docking score: −6.608 kcal/mol).

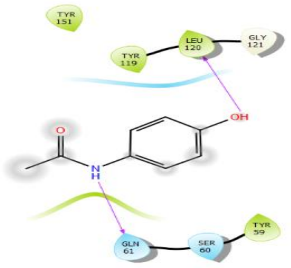


1 Pi-pi stacking (4.74) PHE372

3 H-bonds (1.60,2.34,1.85) GLN371,PRO529,VAL117

Figure 9. 2D view interaction of quercetin with 5JVY (Docking score: −8.174 kcal/mol).

2D-Intraction with 2AZ5



2 H-bonds (2.09,1.82) GLN61&LEU120

Figure 10. 2D view interaction of paracetamol with 2AZ5 (Docking score: −3.573 kcal/mol).

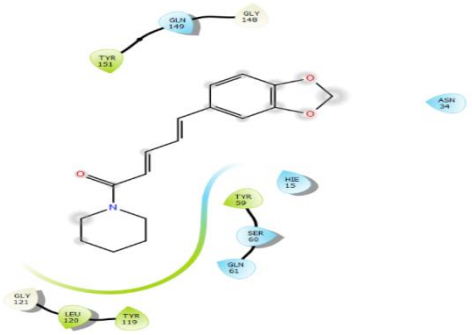
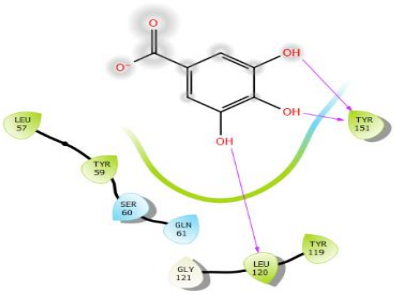


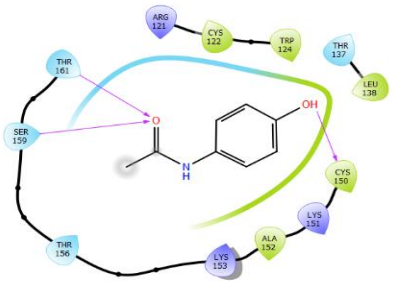
Figure 11. 2D view interaction of piperine with 2AZ5 (Docking score: −3.532 kcal/mol).



3 H-bonds (1.82,1.94,1.94) TRY151,TRY151&LEU120

Figure 12. 2D view interaction of Gallic acid with 2AZ5 (Docking score: −5.330 kcal/mol).

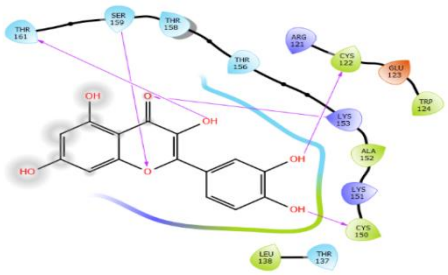
2D-Intraction with 1P9M



3 H-Bonds(1.91,1.85,1.98) SER159,

THR161&CYS150

Figure 13. 2D view interaction of paracetamol with 1P9M (Docking score: −4.940 kcal/mol).



5 H-bonds(2.31, 2.61, 2.24, 2.25 & 1.88)

THR161,SER159,LYS153,CYS122&CYS150

Figure 14. 2D view interaction of quercetin with 1P9M (Docking score: −5.636 kcal/mol).

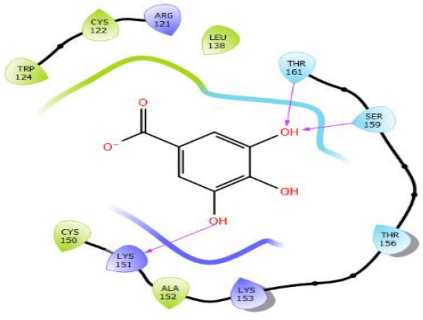
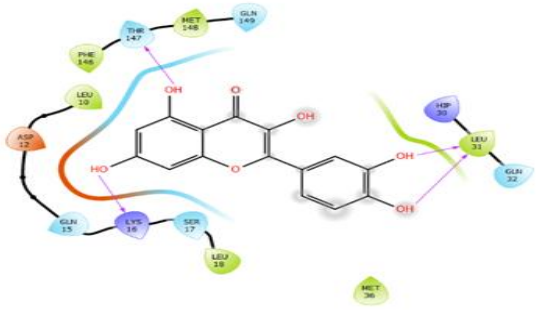


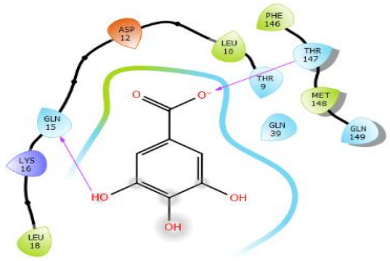
Figure 15. 2D view interaction of Gallic acidwith 1P9M (Docking score: −5.599 kcal/mol).

2D-Intraction with 1ITB



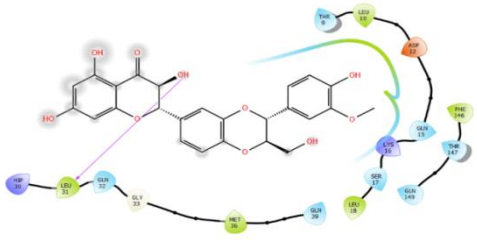
3 H-bonds(1.91, 1.96& 1.82)LEU31,LEU31& LYS16

Figure 16. 2D view interaction of quercetin with 1ITB (Docking score: −8.027 kcal/mol).



2 H-bonds (2.13 & 1.71) GLN15& THR147

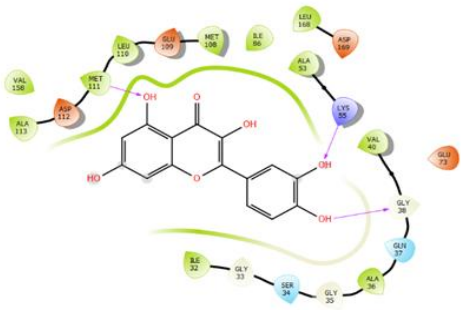
Figure 17. 2D view interaction of Gallic acid with 1ITB (Docking score: −5.360 kcal/mol).



1 H-bond(1.98)LEU31

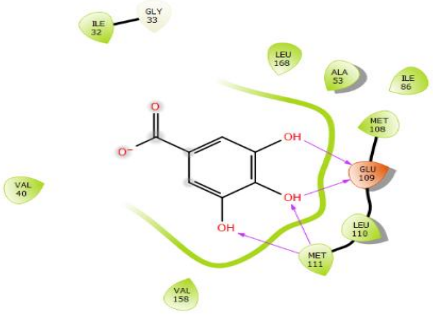
Figure 18. 2D view interaction of silymarin with 1ITB (Docking score: −3.873 kcal/mol).

2D-Intraction with 1UKI



3 H-bonds(2.35, 1.99 & 1.88) GLY38, LYS55 & MET111

Figure 19. 2D view interaction of quercetin with 1UKI (Docking score: −9.060 kcal/mol).



4 H-bonds(2.76, 1.92, 1.99 & 2.52) with MET111,MET111,GLU109 & GLU109

Figure 20. 2D view interaction of Gallic acid with 1UKI (Docking score: −8.052 kcal/mol).

3.2. Lead Compounds

3.2.1. Quercetin

Quercetin emerged as the most potent multitarget compound. Its strong binding to JNK suggests an ability to suppress mitochondrial apoptosis pathways, while high affinity for IL-1β and IL-6 indicates powerful anti-inflammatory potential. The observed inhibition of TNF-α/NF-κB pathways further supports its hepatoprotective role.

3.2.2. Gallic Acid

Gallic acid's capacity to interact favorably with GSH reductase highlights its role in restoring GSH levels, a key defense against NAPQI-induced oxidative stress. Strong JNK inhibition further positions it as a candidate for mitigating mitochondrial-driven necrosis.

3.2.3. Silymarin and Piperine

Silymarin demonstrated balanced efficacy across all targets, reaffirming its use as a benchmark hepatoprotectant. Piperine's selectivity for Cox2 and potential to improve the bioavailability of co-administered compounds suggest its role may be complementary within a poly-phytochemical formulation.

3.3. Molecular Interactions

Analysis of docking poses revealed that hydrogen bonding, π–π stacking, and hydrophobic interactions contributed to high binding affinities. Quercetin and gallic acid formed stable hydrogen bonds with active-site residues of JNK and GSHR, respectively, consistent with their predicted therapeutic actions. Piperine's benzodioxole moiety facilitated hydrophobic interactions within Cox2's binding pocket.

3.4. Structure–Activity Relationships

The catechol moiety in quercetin and gallic acid played a decisive role in ROS scavenging and enzyme binding, while piperine's lipophilic scaffold favored Cox2 selectivity. Silymarin's flavonolignan framework provided moderate yet broad efficacy. Collectively, these structural determinants explain their differential target affinities and biological effects.

4. Conclusions

This in silico study highlights the **therapeutic superiority of quercetin and gallic acid** in mitigating paracetamol-induced hepatotoxicity. Both compounds displayed strong, multitarget binding affinities, particularly toward **JNK, IL-1β, and GSH reductase,** suggesting potent antioxidant, anti-apoptotic, and anti-inflammatory effects. Piperine showed selective Cox2 inhibition, complementing the broader efficacy of quercetin and gallic acid, while silymarin served as a valuable benchmark.

Author Contributions:

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Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

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