

Protecting Effects of Rutin and N-Acetyl Cysteine on PON1 Enzyme in Cyclosporine A-Treated Rats: An *In-Vivo*, *In-Vitro*, and *In-Silico* Study

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Abstract

Background: Paraoxonase 1 (PON1) is a potent antioxidant enzyme in serum that protects against the oxidation of low-density lipoprotein. This study investigated the effect of N-acetylcysteine (NAC), a synthetic precursor to the natural antioxidant glutathione, and Rutin, a natural antioxidant, on protecting PON1 enzyme activity in rats treated with cyclosporine A (CsA) using experimental and molecular dynamics simulation studies.

Materials and Methods: In this study, 48 male rats were divided into six groups, each consisting of eight rats. Groups I, II, and III were CsA-free, while groups IV, V, and VI received 25 mg/kg CsA. Groups V and VI received daily NAC and Rutin (100 mg/kg) via intragastric gavage. After 21 days of treatment, all rats were euthanized, and the high-density lipoprotein parameter was measured using an auto analyzer in serum samples. PON1 enzyme activity was determined by its arylesterase property in serum samples. Docking and molecular dynamics simulation studies were performed using AutoDock V.4.2 and GROMACS 2022 software.

Results: CsA decreased the PON1 enzyme arylesterase activity, while this activity increased in the groups that received CsA and Rutin, and/or CsA and NAC. The simulation results showed that all three compounds could bind into the PON1 independently and moderate its activity. Though serum PON1 arylesterase activity decreases in CsA received group, the combination of NAC or Rutin has a synergistic effect in further boosting PON1 aryl esterase enzyme activity when CsA is administered.

Conclusion: The present findings indicate that NAC and Rutin have protective effects on the PON1 enzyme in CsA-treated adult rats.

Keywords: Cyclosporine A, N-acetylcysteine, PON1, Rutin

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INTRODUCTION

Paraoxonase (PON) is an antioxidant enzyme that belongs to a multigene family with three main members: PON1, PON2, and PON3, with paraoxonase, arylesterase, and

lactonase activities.^[1] The PON1 enzyme is synthesized in the liver and transported to the serum for antioxidant activities. PON1 is the most important enzyme of this family

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that is involved in the high-density lipoprotein (HDL) and prevents low-density lipoprotein (LDL) oxidation to hinder the production of LDL-ox.^[2,3] This property of the PON1 enzyme is associated with cardiovascular diseases. PON1 enzyme is also a potent catalyst for degrading some of the organophosphorus compounds *via* its antioxidant activity.^[4,5]

However, some drugs, such as haloperidol and fluoxetine hydrochloride, are known effective inhibitors of the PON1 enzyme activity.^[6] Inhibition of this enzyme can lead to an increase in cardiovascular diseases, especially when these drugs are used alongside others.^[7] Cyclosporine A (CsA), an immunosuppressing compound, was discovered in the late 1970s through isolation from the fungus *Tolypocladium inflatum*.^[8] This immunosuppressing drug has a range of clinical applications, including kidney and liver transplantation, treatment of eye diseases, and other diseases.^[9-11] Some studies have shown that CsA can induce liver toxicity in rats^[12] and can cause a decrease in PON1 activity.^[13] This reduction in PON1 enzyme activity can lead to increase the risk of atherosclerosis and cardiovascular disorders.^[14] This emphasizes the significant side effects associated with the use of drugs that inhibit PON1.

Some antioxidant compounds, such as quercetin and glabridin, can increase and regulate PON1 activity in biological systems.^[15] Rutin, a potent plant antioxidant that is also known as vitamin P, has various pharmacological effects and commonly exists in tea and apples.^[16] Additionally, N-acetylcysteine (NAC) is a synthetic precursor to the natural antioxidant glutathione and acts as a disulfide-breaking agent with various clinical applications as a well-known drug.^[17,18] Current studies on the protective effects of natural products and antioxidant compounds are rapidly growing; however, there are still unexplained aspects. Based on this information, we hypothesize that NAC and rutin may be effective in reducing the adverse

effects of CsA. To that end, this study investigated the effect of NAC and Rutin on PON1 enzyme activity in CsA-treated rats by experimental and molecular dynamics simulation studies [Figure 1].

MATERIALS AND METHODS

Animal studies

In this study, 48 adult male albino Wistar rats (200–250 g) were obtained from the Pasteur Institute of Iran. The animals were housed under uniform standard conditions with a temperature of $22^{\circ}\text{C} \pm 2$ and 12 hours of light and 12 hours of darkness. All rats were randomly divided into six equal groups of eight rats after 1 week. The three compounds used in the treatment, including NAC, Rutin, and CsA, were obtained from Sigma-Aldrich Company, and the doses of NAC, Rutin, and CsA were selected based on the literature.

- The first group received a gavage of distilled water twice within 24 hours, with an interval of 1 hour for 21 days.
- The second group received a gavage of distilled water and then, after 1 hour, received an oral administration of NAC at a dose of 100 mg/kg of body weight for 21 days.^[19]
- The third group received a gavage of distilled water and then, after 1 hour, received an oral administration of Rutin, also at a dose of 100 mg/kg of body weight for 21 days.^[20]
- The fourth group (test without treatment) received a gavage of distilled water and then, after 1 hour, received an oral administration of CsA at a dose of 25 mg/kg of body weight once every 24 hours for 21 days.^[21]
- The fifth group received a gavage of CsA at a dose of 25 mg/kg of body weight and then, after 1 hour, received an oral administration of NAC at a dose of 100 mg/kg of body weight for 21 days.
- The sixth group received a gavage of CsA at a dose of 25 mg/kg of body weight and then, after 1 hour, received

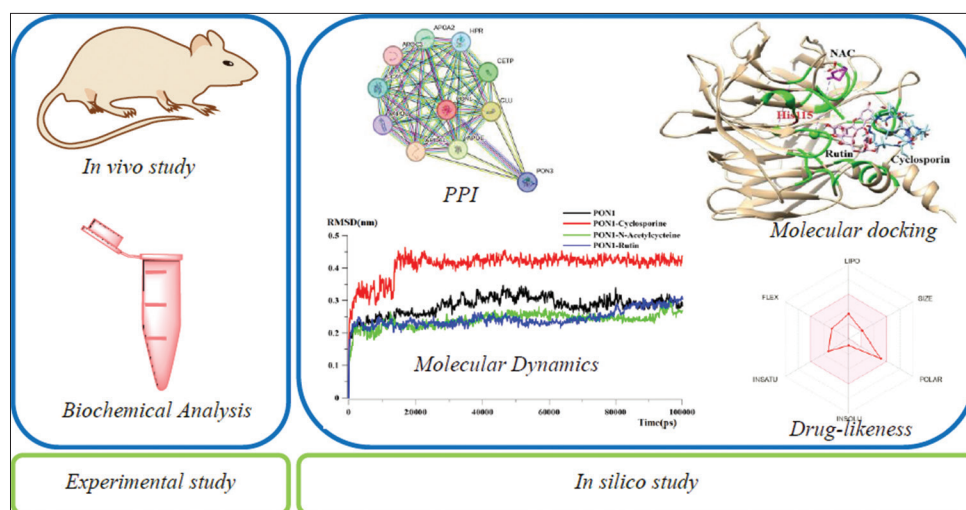


Figure 1: The workflow of this study

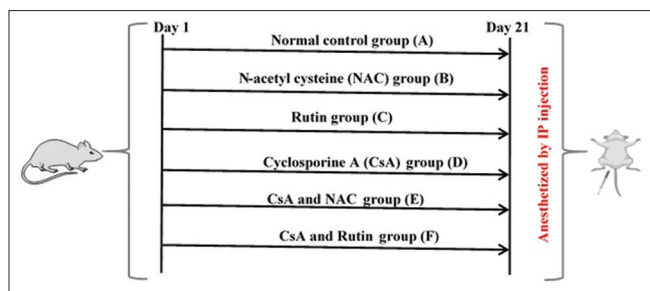


Figure 2: Schematic diagram of animal groups and employed experimental design

an oral administration of Rutin at a dose of 100 mg/kg of body weight for 21 days [Figure 2].

All experimental animal procedures were approved by the Animal Care and Use Ethics Committee at Shahrekord University of Medical Sciences (approval No. IR.SKUMS.AEC.1401.045) and complied with the Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines. All methods used in this study were performed under relevant institutional guidelines and regulations.^[22]

Experimental studies

At the end of 21-day period, the rats were anesthetized through intraperitoneal (IP) injection of Ketamine/Xylazine (100 mg/10 mg/kg), and blood samples were collected from their hearts. Then, the serum samples were separated by centrifugation at 1200 RPM for 10 min and stored at -70 °C. The biochemical HDL parameter was measured using the BT-3000 device, and the PON1 enzyme activity was assessed based on aryl esterase property using a spectrophotometer (Shimadzu UV-1800) at a wavelength of 270 nm.^[23]

Simulation studies

Bioinformatics analysis

The function or feature of the PON1 protein was explained using several bioinformatics tools, including Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway online databases. The protein–protein interaction (PPI) network was analyzed using the STRING server (<http://string-db.org/>). In addition, the biological process (BP), cellular component (CC), and molecular function (MF) were explored through searching the STRING database, and the obtained results were plotted by the Weishengxin online drawing website (www.bioinformatics.com.cn).

Protein and ligand preparation

To conduct studies related to the simulation and molecular dynamics of the PON1 enzyme, the molecular structure of the PON1 protein (PDB ID: 1V04) was obtained from the Protein Data Bank (www.rcsb.org) website. All water and other ligand molecules were removed, and then the final PDB file was optimized using Chimera software. Additionally, the three-dimensional structures of Rutin (CID: 5280805),

CsA (CID: 5284373), and NAC (CID: 12035) were obtained from the PubChem database. The energy minimization and optimization of these molecules were performed using Avogadro software, and finally, they were converted to PDB files for the docking study.

Molecular dynamics simulation of PON1 enzyme in water

The studies related to the molecular dynamic simulation of the PON1 enzyme's protein structure were initially carried out in a water environment so that the relevant structure is subjected to changes in temperature, pressure, and concentration of 140 mM to reach the equilibrium state. In this study, the Gromacs 2022 software with the G43A1 force field and the SPC 216 model was used. The concentration of the system was created by adding the required amounts of Na⁺ and Cl⁻ until the concentration of 140 mM was reached and then replacing the mentioned number of ions with a solvent. The simulation system was set to run for 100 nanoseconds (ns). All the data obtained from the simulation were used as controls to analyze the structural parameters of the PON1 enzyme in the presence of ligands.

Molecular docking

Autodock 4.1 software was utilized for docking the Rutin, NAC, and CsA as ligands into the PON1 enzyme as the receptor. The Kollman charges were applied to the PON1 protein, while the Gasteger charge was employed for the ligands. A special grid was created to define the docking area, including the entire volume of the protein for each docking, to perform the blind dockings. A genetic algorithm was implemented for the docking process, with a maximum of 200 runs. The output files were investigated by the Discovery Studio V.16, San Diego, CA, USA (2013) software to identify the amino acid residues at the binding sites.

Molecular dynamics simulation of the protein–ligand complex in water

At the last step, the molecular dynamics simulation of the PON1 enzyme in complex with the Rutin, NAC, and CsA in the water and salt environment was performed according to the above method (simulation of the molecular dynamics of the PON1 in water).

ADMET and drug-likeness evaluation of ligands

A good drug is not necessarily the one that has the best binding interactions to its target. The ligand's ADME (absorption, distribution, metabolism, excretion, and toxicity) properties must be evaluated to determine its activity within the human body. Therefore, all possible drug-likeness features, pharmacokinetic parameters, and toxicity of the selected molecules were predicted using SwissADME (<https://www.swissadme.ch/>) and ProTox-II (http://tox.charite.de/protox_II/). These predictive models use physicochemical properties, water solubility, and drug likeness to determine the potential of the compounds to serve as effective drug molecules. Additionally, the toxicity levels of the compounds are evaluated, with the expectation that they fall within the low toxicity category, specifically classes IV and V.

Biological activity prediction of NAC and rutin

The Prediction of Activity Spectra for Substance (PASS) server was utilized to predict the potential activity of NAC and rutin based on their structure–activity relationships.^[24] PASS gives a biological spectrum for the compounds on the probable activity (Pa) and probable inactivity (Pi) ($Pa > Pi$). A Pa value higher than Pi indicates a higher probability of activity for the compound.

Statistical analysis

The experimental data are presented as mean $\pm$ SD. All statistical analyses were performed using one-way analysis of variance (ANOVA) with Statistical Package for the Social Sciences (SPSS) V.22 software. The mean values of the groups were compared using the Tukey *post hoc* test. All experimental values were considered statistically significant at $P < 0.05$.

RESULTS

Biochemistry parameters

The serum HDL levels for all groups are demonstrated in Figure 3. The serum HDL parameter increased nonsignificantly in all treated groups in comparison with the control group (A group). However, this parameter increased significantly in the group receiving NAC and CsA (E group).

PON1 enzyme activity

The aryl esterase activity of the PON1 enzyme showed that groups receiving NAC (B), Rutin (C), and CsA (D) have a significant ($P < 0.05$) decrease in enzymatic activity compared to the control group (A). Additionally, PON1 activity in group D was significantly lower than that in both the control and NAC (A and B) groups ($P < 0.05$). However, the serum PON1 enzyme activity significantly increased ($P < 0.05$) in the group that received NAC+CsA (E) and Rutin + CsA (F) in comparison with the control group. Furthermore, there is a significant ($P < 0.05$) increase in the (F) group compared to the (B, C, D) groups [Figure 4].

SIMULATION RESULTS

Protein–protein interaction network analysis

The PON1 protein–protein interaction (PPI) network was constructed using the STRING database, which consists of 11 nodes and 50 edges with an interaction score > 0.4 ($P < 1.11 \times 10^{-16}$) [Table S1]. The PON protein interacts with several other proteins, including APOA2, HRP, CETP, CLU, APOE, APOA1, APOB, LCAT, APOC3, and PON3 [Figure 5].

PPIs present a crucial understanding for identifying novel therapeutic targets. Furthermore, Figure S1 demonstrates the functional enrichment of all relevant PON1 protein pathways. We also investigated the functional annotation and enrichment analysis to explain the biological roles of differentially expressed proteins. A total of 85, 12, and 18 GO terms were significantly enriched in biological process (BP), cellular component (CC), and molecular function (MF), respectively. Within the BP category, observations included

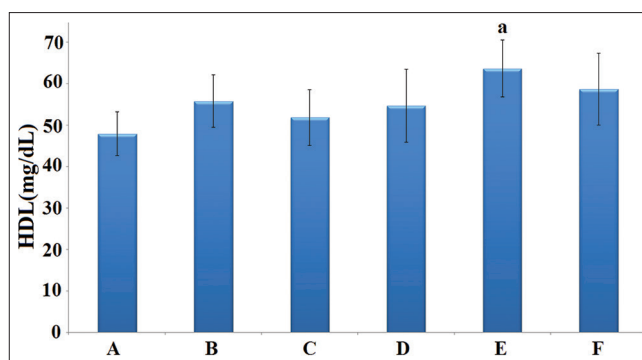


Figure 3: The serum HDL parameter: A; control group, B; N-Acetyl Cysteine, C; Rutin, D; Cyclosporine A, E: N-acetylcysteine + Cyclosporine A, F; Rutin + Cyclosporine A. Data are reported as mean $\pm$ standard deviation (SD). The mean difference is significant compared to the control group ($P < 0.05$). a) Compared with group A

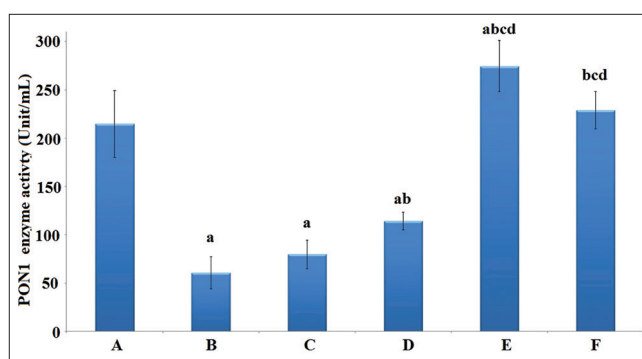


Figure 4: The serum PON1 enzyme activity. A; control group, B; N-Acetyl Cysteine, C; Rutin, D; Cyclosporine A, E: N-acetylcysteine + Cyclosporine A, F; Rutin + Cyclosporine A. Data are reported as mean $\pm$ standard deviation (SD). The mean difference is significant with the control group ($P < 0.05$). a) Compared with group A; b) compared with group B; c) compared with group C; d) compared with group D

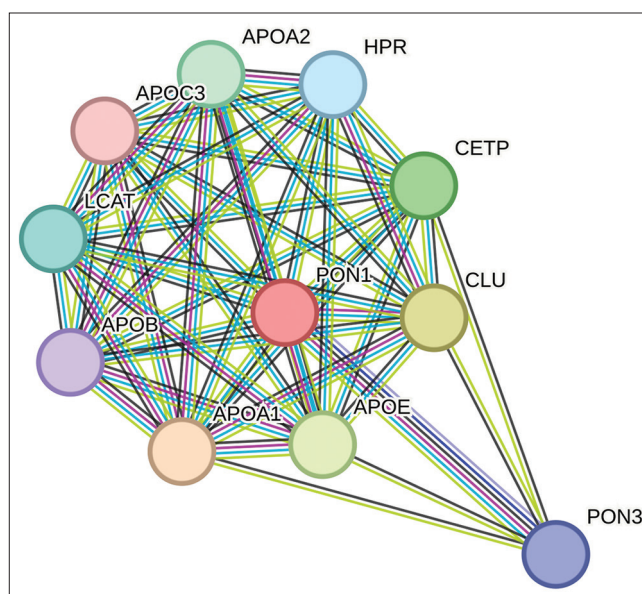


Figure 5: The protein–protein interaction (PPI) network of PON1 was determined using the STRING web server

reverse cholesterol transport, plasma lipoprotein particle remodeling, high-density lipoprotein particle remodeling, cholesterol transport, and triglyceride-rich lipoprotein particle remodeling. Consistent with BP GO term retrieval, proteins were enriched in CC, such as high-density lipoprotein particle, spherical high-density lipoprotein particle, Chylomicron, very-low-density lipoprotein particle, blood microparticle, and intermediate-density lipoprotein particle. In terms of MF, most were closely associated with lipoprotein particle receptor binding, cholesterol transfer activity, high-density lipoprotein particle receptor binding, phosphatidylcholine-sterol O-acyltransferase activator, cholesterol binding, and low-density lipoprotein particle receptor binding [Figure 6].

Docking results

All the docking results of binding the CsA, NAC, and Rutin, as ligands, into the PON1 enzyme, as a receptor, are presented in Table 1. The binding energies (BE) of CsA, NAC, and Rutin into the PON1 enzyme were -4.30, -4.62, and -5.27 (kcal/mol), respectively. Additionally, the inhibition constants (Ki) for the binding of the CsA, NAC, and Rutin into the PON1 enzyme were 701.09, 409.73, and 137.80 μ M, respectively.

The best binding site of ligands into the PON1 enzyme has the lowest amount of BE and Ki. The amino acid residues of the binding site of CsA into the PON1 enzyme are Tyr71, Phe347, Pro189, Tyr294, Tyr190, Phe293, His197, Ser193, Met196, and Phe292. The amino acid residues of the binding site of NAC into the PON1 enzyme are Leu44, Lys349, Asp49, Val45, Gly47, Ile48, and Lys46. In addition, the amino acid residues of the binding site of CsA into the PON1 enzyme are Pro189, Tyr71, Lys192, His184, Leu69, His115, Thr332, Phe222, Glu53, Asp269, Asn168, Asn224, Leu240, Val346, Phe347, Pro300, Tyr294, Ile291, Phe292, and Ser193 [Table 1 and Figure 7].

Figure 8 illustrates the binding sites of NAC, Rutin, and CsA in the PON1 enzyme. The active site pocket of the PON1 enzyme is shown in green. All three compounds bind to the active site of the PON1 enzyme, but only Rutin can interact with His115, which is the most active amino acid residue at the binding site.

Molecular dynamics simulation results

The molecular dynamics parameters, including RMSD (root mean square deviation), RMSF (root mean square fluctuation), Rg (radius of gyration), and variations in the secondary

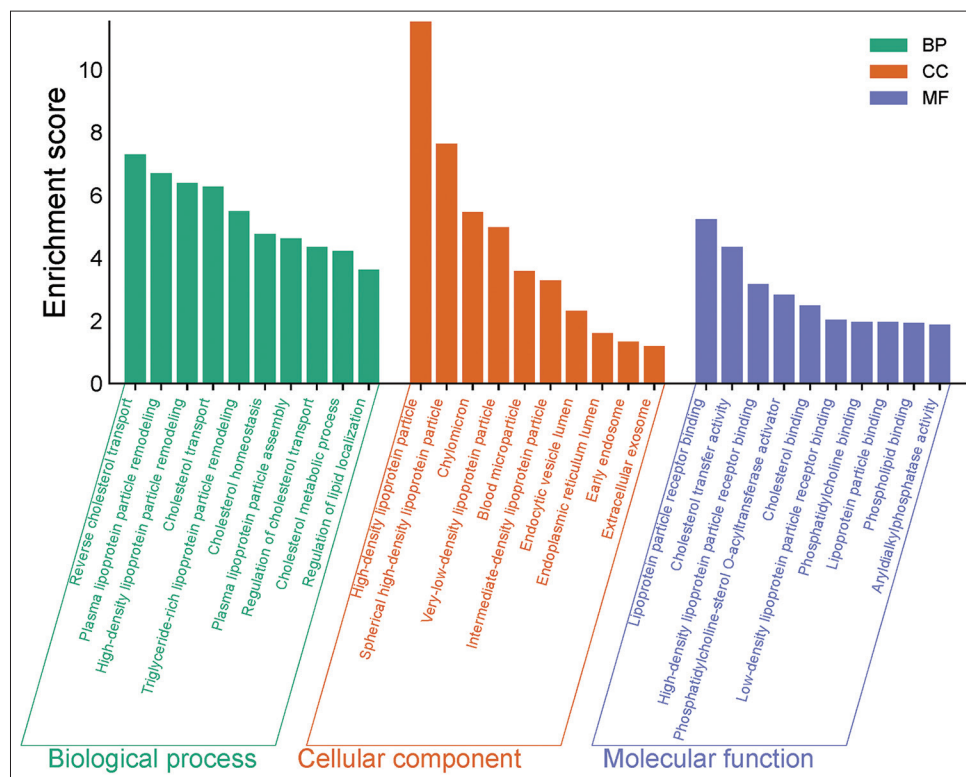


Figure 6: The top 10 GO terms on biological process (BP), cellular component (CC), and molecular functions (MF)

Table 1: The molecular docking of Cyclosporine A, N-acetylcysteine, and Rutin into the PON1 enzyme			
PON1-Ligand	Ki (μ M)	BE (kcal/mol)	Amino acid residues in the Binding site
PON1-Cyclosporine A	701.09	-4.30	Tyr71, Phe347, Pro189, Tyr294, Tyr190, Phe293, His197, Ser193, Met196, Phe292
PON1-N-Acetylcysteine	409.73	-4.62	Leu44, Lys349, Asp49, Val45, Gly47, Ile48, Lys46
PON1-Rutin	137.80	-5.27	Pro189, Tyr71, Lys192, His184, Leu69, His115, Thr332, Phe222, Glu53, Asp269, Asn168, Asn224, Leu240, Val346, Phe347, Pro300, Tyr294, Ile291, Phe292, Ser193

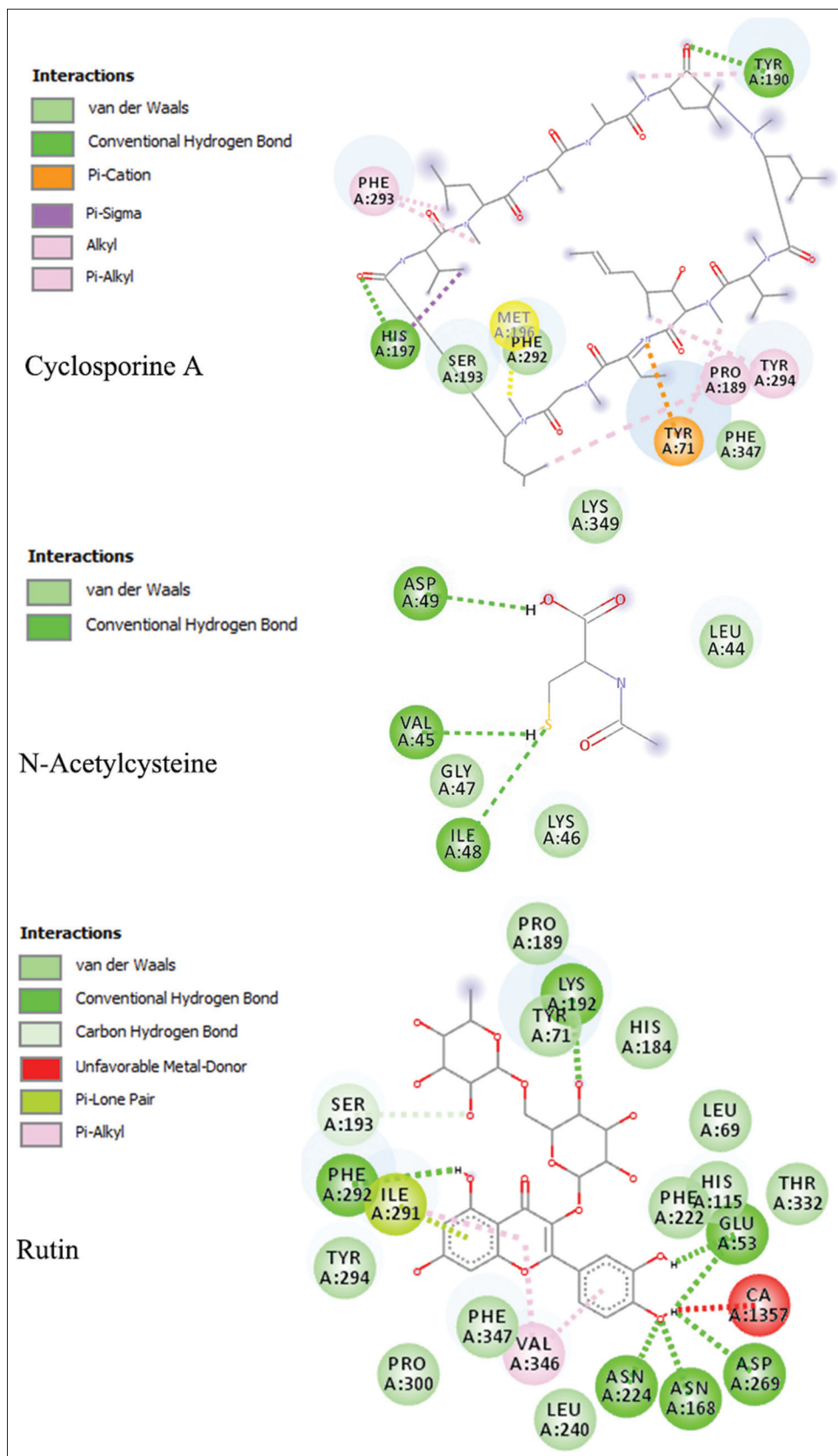


Figure 7: The molecular docking of ligands into the PON1 enzyme

structures, changed after docking the CsA, NAC, and Rutin into the PON1 enzyme. Figure 9 illustrates the RMSD parameter

during the 100 ns of simulation time for both PON1 and PON1-ligand complexes. All molecules exhibited stability

after 20 ns of simulation. Also, the mean RMSD of PON1-CsA increased significantly in comparison with other molecules after 100 ns of simulation time.

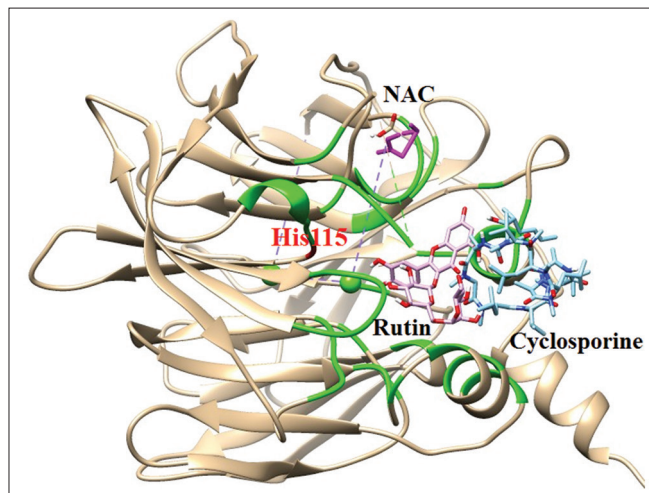


Figure 8: The binding sites of NAC, Rutin, and Cyclosporine A into the PON1 enzyme

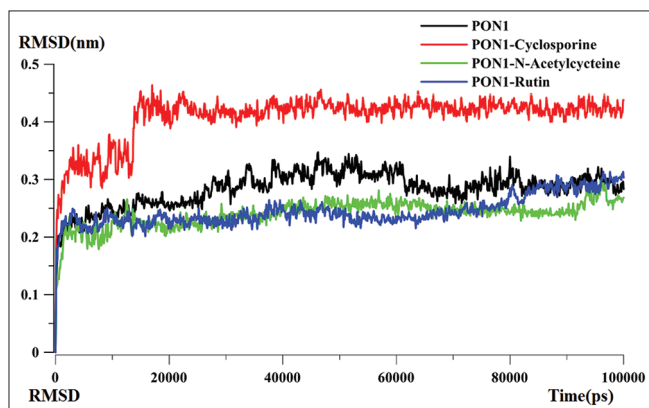


Figure 9: The RMSD results of PON1 (Black) and the complex of PON1-Cyclosporine A (Red), PON1-NAC (Green), and PON1-Rutin (Blue) in the 100 ns of simulation time. The simulation system has remained stable throughout the simulation for all molecules

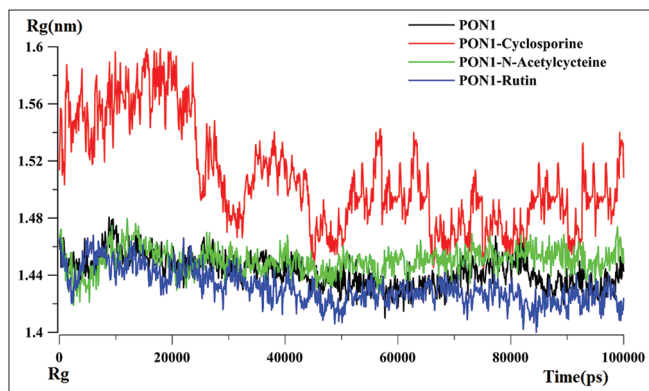


Figure 10: The Rg results of PON1 (Black) and the complex of PON1-Cyclosporine A (Red), PON1-NAC (Green), and PON1-Rutin (Blue) in the 100 ns of simulation time

The molecular dynamic parameter known as the radius of gyration (Rg) [Figure 10] confirms the stability of the simulation system throughout the 100 ns of simulation time, especially the PON1 enzyme and its complex with Rutin and NAC. The Rg parameter was changed after docking the ligands into the PON1 enzyme. Docking the Rutin into the PON1 enzyme decreased the Rg, while docking the NAC into the PON1 enzyme increased the Rg. Furthermore, docking the CsA into the PON1 enzyme increased the Rg sharply compared to docking the NAC into the PON1 enzyme after 100 ns of simulation time.

Figure 11 shows the results of root mean square fluctuation (RMSF) and solvent-accessible surface area (ASA) molecular dynamic parameters for the PON1 enzyme and its complexes with Rutin, NAC, and CsA. The variation of some amino acid residues increased, and some other amino acid residues decreased after docking the ligands into the PON1 enzyme. CsA enhanced the fluctuation of 30–50, 75–80, and 180–200 amino acid residues, while reducing the fluctuation of 320–325 amino acid residues after docking into the PON1 enzyme. In the case of NAC, the fluctuations of amino acid residues 50–54 and 152–155 increased, while the amino acid residue fluctuation of 80–85, 140–145, and 292–298 decreased. Moreover, following the docking of Rutin into the PON1 enzyme, the fluctuation of 75–85, 115–150, and 251–259 amino acid residues increased, and the fluctuation of 52–55 and 195–197 amino acid residues decreased.

The percentage of Coil, β -Sheet, Bend, Turn, and α -helix secondary structural changes of PON1 after docking of the CsA, NAC, and Rutin is shown in Figure 12. There are many conformational changes in the PON1 enzyme after the ligands are docked. After docking the CsA into the PON1 enzyme, the Coil and α -helix secondary structures increased, while the β -Sheet, Bend, and Turn secondary structures decreased. In addition, the Coil, Bend, Turn, and α -helix secondary structures decreased after docking the NAC into the PON1, while the β -Sheet secondary structures increased. After docking the Rutin into the PON1 enzyme, the Coil and Turn secondary structures increased, while the β -Sheet secondary structure decreased.

ADMET properties of NAC and rutin

The SWISSADME server analyzed the drug-likeness of NAC and rutin. The physicochemical properties of NAC indicate no violations of the Lipinski rule of five, while rutin has three violations ($MN > 500$, $HBA > 10$, and $HBD > 5$). The molecular weight of NAC is very low, whereas the molecular weight of rutin slightly exceeds 500 g/mol. Phytochemicals are frequently utilized in therapeutics, even though their molecular weight surpasses 500. Furthermore, NAC exhibits high gastrointestinal (GI) absorption, whereas rutin displays low GI absorption. Neither NAC nor rutin can cross the blood–brain barrier, and they are not inhibitors of cytochrome P450 enzymes CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Therefore, neither compound exhibits hepatotoxicity [Table 2].

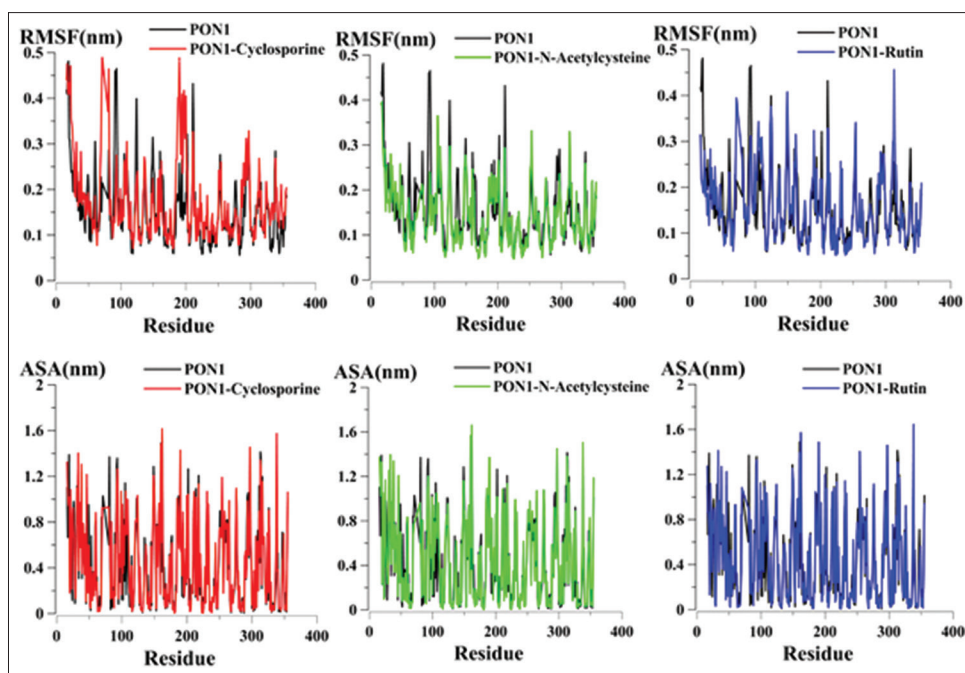


Figure 11: The RMSF and ASA results of PON1 (Black) and the complex of PON1-Cyclosporine A (Red), PON1-N-Acetylcysteine (Green), and PON1-Rutin (Blue) in the 100 ns of simulation time

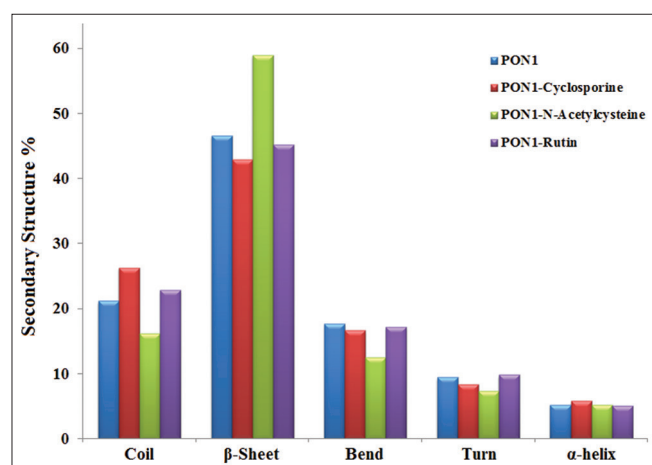


Figure 12: The percentage of changes in the secondary structures (Coil, β -Sheet, Bend, Turn, and α -helix) of PON1 and the complex of PON1-ligands during 100 ns simulation time

PROTOX II toxicity prediction categorizes NAC and rutin as toxicity class 5 with LD50 values of 4400 and 5000 mg/kg, respectively. The findings also showed that NAC has a mutagenicity risk, while rutin demonstrates potential immune toxicity. Furthermore, both NAC and rutin show no hepatotoxicity and cytotoxicity risks [Table 3].

PASS analysis: biological activity predictions

This study has considered the activity of NAC and rutin compounds on the PON1 enzyme that belongs to the family of paraoxonases, as a potent antioxidant enzyme. The PASS spectra for both the NAC and rutin exhibited high predicted scores for antioxidant properties, ability to scavenge free

radicals, and Caspase 8-stimulating activity. Both NAC and rutin were found to be effective inhibitors of lipid peroxidation. The activity score of rutin is remarkable for its anti-inflammatory activity and is a strong antioxidant [Table 4].

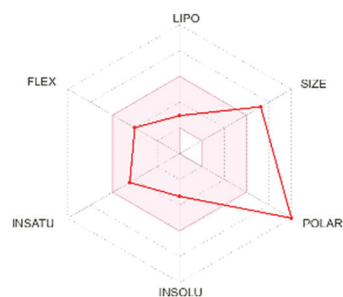
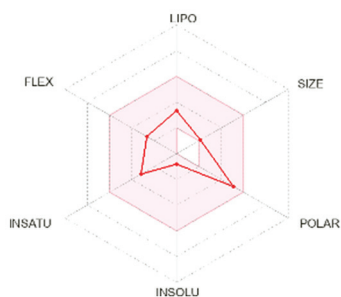
DISCUSSION

CsA is one of the main methods of drug therapies for organ transplantation and some autoimmune disorders. However, the studies show that this compound can induce severe injuries in both the liver and kidneys.^[25,26] PON1 is a vital enzyme that protects substrates against oxidation, expressed in the liver and kidneys. Damage to liver and kidney tissues can affect the activity of this enzyme, making serum levels of this enzyme useful as a biomarker for liver injury.^[27] On the other hand, discovering new methods and compounds to increase PON1 enzyme activity or protect against decreasing its activity is one of the most significant approaches in medicine.^[28] Additionally, a decrease in PON1 activity, along with a lack of correlation with HDL and APOA1, may increase the potential risk of diseases related to atherosclerosis by causing HDL dysfunction.^[29] Some studies have shown that PON1 activity may depend on the content of HDL particles.^[30,31] Fourteen different apolipoproteins, such as APOA1, APOA2, APOB, and APOC3, have been identified within the HDL structure and regulate cholesterol transport and metabolism.^[32]

The PON1 enzyme exists in the HDL, so any increase or decrease of this lipoprotein can affect the overall activity of the PON1 enzyme in the serum. The results of this study indicated that neither NAC, Rutin, CsA, nor the combination

Table 2: Lipinski's properties of NAC and rutin from the SWISSADME server

Properties	NAC	Rutin
Physicochemical properties		
Formula	C ₅ H ₉ NO ₃ S	C ₂₇ H ₃₀ O ₁₆
Molecular weight (g/mol)	163.19	610.52
Num. rotatable bonds	4	6
Num. H-bond acceptors	3	16
Num. H-bond donors	23	10
Molar Refractivity	38.85	141.38
TPSA (Å ²)	105.20	269.43
Lipophilicity		
Log Po/w (iLOGP)	0.67	0.46
Water Solubility		
Log S (ESOL)	-0.81	-3.30
Class	Very soluble	Soluble
Pharmacokinetics		
GI absorption	High	Low
BBB permeant	No	No
P-gp substrate	No	Yes
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Druglikeness		
Lipinski	Yes: 0 violation	No; 3 violations: MW>500, NorO>10, NHorOH>5
Bioavailability Score	0.56	0.17
Medicinal Chemistry		
PAINS	0 alert	1 alert: catechol_A
Brenk	1 alert: thio_2	1 alert: catechol
Leadlikeness	No; 1 violation: MW < 250	No; 1 violation: MW>350
Synthetic accessibility	2.08	6.52
Radar		
LIPO: Lipophilicity		
FLEX: Flexibility		
POLAR: Polarity		
INSAT: Insaturation		
INSOLU: Insolubility		
SIZE: Size		


Table 3: Toxicity properties of NAC and rutin

Compound	LD50 (mg/kg)	Toxicity class	Hepatotoxicity	Immune toxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
NAC	4400	5	No	No	No	Yes	No
Rutin	5000	5	No	Yes	No	No	No

of Rutin and CsA significantly increased serum HDL levels. However, only the group of rats that received the NAC+CsA showed a significant increase in serum HDL. These results are going to lead to the assumption that enzyme activity will also increase. In this study, PON1 enzyme activity decreased significantly in the group with liver and kidney injury that

received CsA. In addition, Rutin and NAC were able to enhance PON1 activity.

These results are inconsistent with some other previous studies that indicated an increasing effect of some antioxidants, such as quercetin, ellagic acid, and gallic acid, on PON1

Table 4: PASS predicted activities of the NAC and Rutin

Compound	Activity	Pa	Pi
NAC	Antioxidant	0.174	0.074
	Lipid peroxidase inhibitor	0.197	0.136
	Free-radical scavenger	0.400	0.017
	Caspase 8 stimulant	0.342	0.094
	NADPH peroxidase inhibitor	0.948	0.012
Rutin	Antioxidant	0.923	0.003
	Lipid peroxidase inhibitor	0.987	0.001
	Free-radical scavenger	0.988	0.001
	Caspase 8 stimulant	0.691	0.004
	NADPH oxidase inhibitor	0.850	0.010
	Anti-inflammatory	0.728	0.009

activity.^[33,34] However, some studies have shown that some flavonoids do not affect PON1 activity. Martini *et al.*^[35] found that some polyphenols and flavonoids containing hydroxyl, glucuronic, or sulfhydryl groups could regulate the PON1 activity.

In the present study, Rutin and NAC could not increase serum PON enzyme activity, while the combination of Rutin and CsA, as well as NAC and CsA, significantly increased the PON1 activity, and they can protect from decreasing the PON1 enzyme activity. The findings of this study provide valuable insights into the potential benefits of NAC and Rutin in preventing kidney and liver injury induced by CsA, as well as modulating the PON1 activity. This suggests that Rutin and NAC may be more effective in preventing organ injury caused by oxidative stress by increasing the PON1 activity. Naderi *et al.*^[36] demonstrated that curcumin can protect against CsA-induced cardiotoxicity in rats by reducing oxidative activity, apoptosis, and regulating autophagy. In another study, the natural compound of lutein showed protective effects against cyclosporin-induced reproductive impairment by upregulation of Nrf2/HO-1/Cx-43 signaling and downregulation of NOX-1 signaling.^[37] Also, hesperidin or sitagliptin administration comforted the kidney damage induced by CsA through reducing oxidative stress, apoptosis, and inflammation.^[26] Rutin and NAC demonstrated protective effects on both renal and liver function in several previous studies. Ma *et al.*^[38] found that Rutin treatment significantly mitigated fluoride-induced renal dysfunction. It was found that administration of rutin led to a remarkable decrease in urea, creatinine, ALT, and AST levels in the liver and kidney affected by Malathion.^[39] Bozali *et al.* showed that Rutin/cyclodextrin administration reduced the toxic effects of diisononyl phthalate.^[40] NAC and tadalafil treatment significantly decrease the cisplatin-induced testicular toxicity.^[41] Additionally, the administration of lycopene and NAC, either alone or in combination, alleviated hepatorenal toxicity and apoptosis induced by cisplatin.^[42]

The simulation studies sections, including molecular docking and molecular dynamics studies, show the mechanism of action of the Rutin, NAC, and CsA compounds and their interaction with the PON1 enzyme. The docking results showed that all

three compounds exhibit a high affinity for binding to the PON1 enzyme. However, Rutin has the most affinity to interact with PON1, with a binding energy of -5.27 kcal/mol.

The docking results showed that Rutin could bind to the His 115 residue of the PON1 enzyme, which is the most important residue in its active site.^[43] This binding can inhibit the activity of the PON1 enzyme. Additionally, both NAC and CsA can also bind into the active site pocket, thereby inhibiting PON1 activity, although they do not have any interaction with the His115 amino acid residue. It appears that binding of all three compounds to the PON1 binding site can inhibit its activity. However, binding CsA and Rutin or NAC together protects against the inhibition caused by His115 by inducing the conformational changes on the PON1 3D structure.

Docking Rutin, NAC, and CsA into the PON1 enzyme induces a conformational change in this enzyme that can influence its activity. The RMSD parameter shows that the simulation system, including PON1 and its complexes with Rutin, NAC, and CsA, is stable after 100 ns of simulation time. The average RMSD did not change after docking Rutin and NAC into the PON1 enzyme, while the mean of RMSD significantly increased after docking CsA into the PON1 enzyme. This variation in the RMSD parameter leads to an increase in the variation of the PON1 structure and can affect its enzymatic activity.^[44] Additionally, decreasing the Rg parameter after docking the Rutin into the PON1 enzyme indicates the compactness of PON1 after docking. An increase in the Rg parameter predicts enhanced enzymatic activity for greater exposure of active sites and catalytic clefts for enzymatic activity.^[45] It appears that Rutin, by docking into the PON1 enzyme, can induce the compactness, which may inhibit its activity. RMSF molecular dynamic parameter reflects the variation of amino acid residues during the simulation time.^[46]

In this study, the fluctuations of some amino acid residues of the PON1 enzyme changed after docking the Rutin, NAC, and CsA [Figure 7]. These fluctuations are different from each other, so docking the Rutin to the PON1 enzyme induces different fluctuations in amino acid residues than docking NAC and CsA to the PON1 enzyme. Sargolzaei in 2021 showed that the fluctuation of some coronavirus main protease residues is related to the fluctuation in turn and coil structures of this enzyme.^[47] The significant changes in the secondary structure of the PON1 enzyme indicate the influence of Rutin, NAC, and CsA on its 3D structure after docking. According to Figure 4, these three ligands bind to different sites of the PON1 enzyme, and each of them has a different effect on the enzyme's structure [Figure 8]. These variations in the secondary structure of PON1 may induce the conformational changes that could lead to changes in enzyme activity.

One of the main limitations of this study is that it is based on an animal model, which may not fully represent the direct application of the findings to humans. Additionally, the use of only a single dose of NAC and Rutin limits a complete understanding of the dose-response relationship.

Future studies should explore a broader range of doses to determine the optimal therapeutic dose. Furthermore, future studies should broaden their scope to include these organs to provide a more comprehensive understanding of the protective effects of NAC and Rutin. The study did not investigate molecular markers of inflammation, such as proinflammatory cytokines (e.g., TNF- α , IL-6, IL-1 β), signaling pathways (e.g., NF- κ B, iNOS), or changes in gene and protein expression, despite assessments of the protective effects of Rutin and NAC on PON1 enzyme. Future research should investigate these mechanisms at both the genetic and protein levels of the Rutin and NAC. Nevertheless, these findings indicate that NAC and rutin have protective effects on the PON1 enzyme against cyclosporine toxicity.

CONCLUSION

The experimental and simulation results revealed significant changes in the structure and activity of PON1 in the presence of CsA. Although both Rutin and NAC inhibited the PON1 enzyme activity, the administration of either NAC or Rutin compounds with CsA significantly increased PON1 enzyme activity. This indicates that NAC and Rutin have the potential to enhance the body's antioxidant defense system and protect against oxidative stress-induced kidney and liver injuries. This study suggests that the combination of NAC or Rutin could have synergistic effects in further boosting PON1 aryl esterase enzyme activity when CsA is administered.

Ethics approval and consent to participate

This study was approved by the research and ethics committee of Shahrekord University of Medical Sciences (approval No. IR.SKUMS.AEC.1401.045).

Availability of data and material

Data generated or analyzed during this study are provided in full within the published article.

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Conflicts of interest

There are no conflicts of interest.

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> Biological Process (Gene Ontology)					
GO-term	description	count in network	strength	signal	false discovery rate
GO:0043691	Reverse cholesterol transport	7 of 18	2.84	7.31	1.08e-14
GO:0034369	Plasma lipoprotein particle remodeling	7 of 29	2.64	6.7	6.26e-14
GO:0034375	High-density lipoprotein particle remodeling	6 of 16	2.83	6.39	7.36e-13
GO:0030301	Cholesterol transport	8 of 65	2.34	6.28	3.06e-14
GO:0034370	Triglyceride-rich lipoprotein particle remodeling	5 of 10	2.95	5.49	6.26e-11
(more ...)					
> Molecular Function (Gene Ontology)					
GO-term	description	count in network	strength	signal	false discovery rate
GO:0070325	Lipoprotein particle receptor binding	6 of 30	2.55	5.24	5.42e-11
GO:0120020	Cholesterol transfer activity	5 of 23	2.59	4.35	4.49e-09
GO:0070653	High-density lipoprotein particle receptor binding	3 of 3	3.25	3.17	1.82e-05
GO:0060228	Phosphatidylcholine-sterol O-acyltransferase activator activity	3 of 6	2.95	2.83	6.68e-06
GO:0015485	Cholesterol binding	4 of 52	2.14	2.49	1.03e-05
(more ...)					
> Cellular Component (Gene Ontology)					
GO-term	description	count in network	strength	signal	false discovery rate
GO:0034364	High-density lipoprotein particle	10 of 29	2.79	11.54	5.88e-24
GO:0034366	Spherical high-density lipoprotein particle	6 of 8	7.64	6.97e-15	
GO:0042627	Chylomicron	5 of 13	2.84	5.46	5.43e-11
GO:0034361	Very-low-density lipoprotein particle	5 of 20	2.65	4.98	2.88e-10
GO:0072562	Blood microparticle	6 of 118	1.96	3.58	4.72e-09
(more ...)					
> Reference Publications (PubMed)					
publication	(year) title	count in network	strength	signal	false discovery rate
PMID:22339300	(2012) Proteomics investigations of HDL: challenges and promise.	10 of 22	2.91	10.73	1.47e-21
PMID:25894274	(2015) A comparison of the mouse and human lipoproteome: suite...	10 of 25	2.86	10.54	2.09e-21
PMID:30571168	(2018) Distinct Proteomic Signatures in 16 HDL (High-Density Lipo...	10 of 26	2.84	10.51	2.09e-21
PMID:20625533	(2010) High-Density Lipoprotein Proteomics: Identifying New Drug...	10 of 26	2.84	10.51	2.09e-21
PMID:36901834	(2023) Lipids at the Nexus between Cerebrovascular Disease and V...	10 of 27	2.82	10.47	2.09e-21
(more ...)					
> Local Network Cluster (STRING)					
cluster	description	count in network	strength	signal	false discovery rate
CL18962	High-density lipoprotein particle	8 of 21	2.83	9.03	2.86e-18
CL18970	Triglyceride-rich lipoprotein particle remodeling, and Spherical high...	7 of 11	3.06	8.68	5.23e-17
CL18956	Lipoprotein particle, and Assembly of active LPL and LIPC lipase c...	9 of 46	2.54	8.55	1.29e-18
CL18973	Regulation of very-low-density lipoprotein particle clearance, and N...	2 of 5	2.86	1.44	0.0025
> KEGG Pathways					
pathway	description	count in network	strength	signal	false discovery rate
hsa04979	Cholesterol metabolism	7 of 48	2.42	6.24	9.74e-14
hsa03320	PPAR signaling pathway	3 of 75	1.86	1.38	0.0016
hsa04977	Vitamin digestion and absorption	2 of 24	2.17	1.06	0.0102
hsa05143	African trypanosomiasis	2 of 36	2.0	0.94	0.0165
hsa04975	Fat digestion and absorption	2 of 41	1.94	0.93	0.0170
> Reactome Pathways					
pathway	description	count in network	strength	signal	false discovery rate
HSA-8963899	Plasma lipoprotein remodeling	7 of 34	2.57	6.56	7.35e-14
HSA-8964058	HDL remodeling	5 of 10	2.95	5.58	4.25e-11
HSA-8963901	Chylomicron remodeling	5 of 10	2.95	5.58	4.25e-11
HSA-8963888	Chylomicron assembly	5 of 10	2.95	5.58	4.25e-11
HSA-975634	Retinoid metabolism and transport	5 of 44	2.31	3.93	1.15e-08
(more ...)					
> WikiPathways					
pathway	description	count in network	strength	signal	false discovery rate
WP3601	Lipid particles composition	7 of 10	3.1	8.91	2.18e-17
WP430	Statin inhibition of cholesterol production	7 of 29	2.64	7.21	4.67e-15
WP4522	Metabolic pathway of LDL, HDL and TG, including diseases	6 of 17	2.8	6.71	1.49e-13
WP5110	Familial hyperlipidemia type 3	5 of 13	2.84	5.63	2.49e-11
WP5304	Cholesterol metabolism	7 of 72	2.24	5.5	8.05e-13
(more ...)					
> Subcellular Localization (COMPARTMENTS)					
compartment	description	count in network	strength	signal	false discovery rate
GOCC:0034364	High-density lipoprotein particle	10 of 30	2.78	11.42	8.81e-24
GOCC:0034366	Spherical high-density lipoprotein particle	7 of 11	3.06	8.88	2.11e-17
GOCC:0034361	Very-low-density lipoprotein particle	6 of 20	2.73	6.39	4.99e-13
GOCC:0042627	Chylomicron	5 of 14	2.81	5.44	5.52e-11
GOCC:0034363	Intermediate-density lipoprotein particle	4 of 8	2.95	4.49	5.17e-09
(more ...)					
> Human Phenotype (Monarch)					
phenotype	description	count in network	strength	signal	false discovery rate
HP:0010979	Abnormality of lipoprotein cholesterol concentration	6 of 47	2.36	4.36	1.57e-09
HP:0031888	Abnormal HDL cholesterol concentration	5 of 21	2.63	4.27	7.44e-09
HP:0010981	Hypolipoproteinemia	5 of 27	2.52	4.05	1.52e-08
HP:0010980	Hyperlipoproteinemia	5 of 33	2.43	3.88	2.57e-08
HP:0031886	Abnormal LDL cholesterol concentration	4 of 36	2.3	2.67	6.44e-06
(more ...)					
> Annotated Keywords (UniProt)					
keyword	description	count in network	strength	signal	false discovery rate
KW-0345	HDL	4 of 16	2.65	3.87	4.97e-08
KW-0445	Lipid transport	6 of 110	1.99	3.61	5.53e-09
KW-0153	Cholesterol metabolism	5 of 65	2.14	3.47	4.97e-08
KW-0065	Atherosclerosis	3 of 8	2.83	3.12	1.79e-06
KW-0162	Chylomicron	3 of 9	2.78	3.07	2.12e-06
(more ...)					
> Protein Domains (Pfam)					
domain	description	count in network	strength	signal	false discovery rate
PF01731	Arylesterase	2 of 3	3.08	1.21	0.0065
PF01442	Apolipoprotein A1/A4/E domain	2 of 4	2.95	1.21	0.0065
> Protein Domains and Features (InterPro)					
domain	description	count in network	strength	signal	false discovery rate
IPR002640	Arylesterase	2 of 3	3.08	0.94	0.0203
IPR000074	Apolipoprotein A/E	2 of 4	2.95	0.94	0.0203

Figure S1: Functional amelioration of paraoxonase 1 (PON1) protein network

Table S1: The description of protein-protein interaction (PPI) and associated parameters

Network Stats	Values
Number of nodes	11
Number of edges	50
Average node degree	9.09
Avg. local clustering coefficient	0.949
Expected number of edges	12
PPI enrichment p-value	1.11e-16