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Original Article

Investigation of the effects of *Terminalia chebula* seed hydroalcoholic extract on Paraoxonase1 enzyme activity in rats

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Abstract

Background and aims: Paraoxonase 1 (PON1) is related to high-density lipoprotein (HDL) in serum and protects against low-density lipoprotein (LDL) oxidation. This study aimed to investigate the effects of *Terminalia chebula* on PON1 in hyperlipidemic rats and the molecular docking effects of some compounds of this medical plant on PON1 activity.

Methods: Overall, 40 male rats (200–250 gr) were randomly divided into four groups, including the control group, the hyperlipid group, the hyperlipid group receiving 400 mg/kg of the hydroalcoholic extract of *T. chebula* seeds, and the hyperlipid group receiving 800 mg/kg of the hydroalcoholic extract of *T. chebula* seeds. The PON1 arylesterase activity in serum and the *PON1* gene expression in the liver tissue underwent investigation. Then, the molecular docking effects of its compounds were studied on PON1 through *in-silico* studies using the AutoDock software (version 4.2.0).

Results: *T. chebula* decreased ($P<0.001$) the serum triglycerides from 105.88±10.15 mg/dL in the hyperlipidemic group to 66.88±14.90 and 74.25±9.51 mg/dL in hyperlipidemic groups receiving 400 and 800 mg/kg of the hydroalcoholic extract of *T. chebula* seeds, respectively. In addition, the PON1 serum aryl esterase activity increased from 202.12±6058 in hyperlipidemic rats to 224.34±58.74 ($P=0.83$) and 235.80±37.05 ($P=0.6$) in hyperlipidemic groups receiving 400 mg/kg and 800 mg/kg of the hydroalcoholic extract of *T. chebula* seeds, respectively. It also demonstrated a significant effect on *PON1* gene expression ($P<0.001$). In addition, the *in-silico* and docking results revealed that the main antioxidant compounds of *T. chebula*, such as catechin, kaempferol, and quercetin, could bind to the PON1 enzyme directly and influence the enzyme activity.

Conclusion: *T. chebula* increased PON1 activity and *PON1* gene expression. However, among the plant's compounds, catechin, kaempferol, and quercetin played the most substantial role in the PON1 activity. It seems that these compounds can be useful as co-treatments in hyperlipidemia therapies.

Keywords: *Terminalia chebula*, Hyperlipidemia, PON1 Enzyme, Molecular docking

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Introduction

Paraoxonase 1 (PON1) is an enzyme with a high antioxidant capacity that is expressed in liver tissue and works in serum (1). This enzyme is responsible for a wide range of physiological activities, including the metabolism of drugs and the catalysis of organophosphorus compounds such as paraoxon, sarin, and other toxic compounds (2–4). The protective role of the PON1 enzyme against the oxidation of low-density lipoprotein (LDL) is one of its most important physiological activities (5). The increase in oxidized LDL is directly related to the occurrence of cardiovascular diseases and atherosclerosis, especially in hyperlipidemic people (6).

Nowadays, the use of medicinal plants in the treatment of many diseases has drawn much attention due to the presence of antioxidant-active ingredients. *Terminalia chebula* is a traditional plant known for its medicinal properties, including antioxidant, antiproliferative, antimicrobial, antidiabetic, anti-aging, hepatoprotective, and anti-inflammatory properties. This medicinal plant also plays a modulating role in the metabolism of glucose and lipids (7). Ellagic and gallic acids are the most abundant among the antioxidant compounds found in the seeds of *T. chebula* (8). Studies have shown that ellagic acid, with its high antioxidant capacity, protects cells against oxidative stress, lipid peroxidation, and free radical damage (9). Its anti-inflammatory and anti-atherosclerosis effects have been confirmed as well (10).

Gallic acid is also a potent antioxidant compound that is known to exist in many medicinal plants, and its anti-inflammatory and anti-atherosclerosis effects have been demonstrated (11). Furthermore, *T. chebula* contains various antioxidant and flavonoid compounds such as catechin, chebulagic acid, chebulinic acid, chebulic acid, methyl neochebulinate, corilagin, quercetin, kaempferol, and shikimic acid, each of which potentially has anti-inflammatory capacity (12). The studies showed that quercetin and catechin with antioxidant activity could protect against increasing the oxidation of serum LDL via increasing the PON1 enzyme activity (12).

This study aimed to investigate the effects of the hydroalcoholic extract of *T. chebula* seeds on lipid profile and PON1 in hyperlipidemic rats *in vitro* and the effects of molecular docking of the most important active ingredients of the plant on PON1 *in silico*.

Materials and Methods

Preparation of the extract of Terminalia chebula

In this study, extraction was conducted through maceration. To this end, following the purchase of the studied plant samples from a local grocery and authentication of their quality by a pharmacognosy specialist, it was registered as 27 in the Herbarium of the Medical Plant Research Center of Shahrekord University of Medical Sciences. Then, the samples were pulverized by an electric mill, and an appropriate amount of the obtained powder was introduced to a mixture of 97% ethanol (70%) and water (30%). After 72 hours, the obtained mixture was filtered by means of a Buchner funnel and extracted in a rotary evaporator. After the incubation of the extract to dry, it was shaved, and the concentrations of interest were prepared from it.

In vivo Investigations

In this study, according to *E = Total number of animals – Total number of groups* formula (13), 40 male rats (200–250 g) in four groups of 10 each were divided into four groups. Thus, the mean weight of each group was 200–250 g. Group 1 consisted of 10 rats as a control group given a routine diet for 90 days. Group 2 included 10 rats, as the hyperlipidemic group was given a fat-enriched diet with Persintra-M intralipid serum (14) for 90 days. Group 3 contained 10 rats receiving a fat-enriched diet with Persintra-M intralipid serum and hydroalcoholic *T. chebula* seed extract at 400 mg/kg for 90 days. Group 4 consisted of 10 rats receiving a fat-enriched diet with Persintra-M intralipid serum and hydroalcoholic *T. chebula* seed extract at 800 mg/kg for 90 days.

In vitro studies

After the sacrifice of the animals, the blood serum samples of all groups were obtained, and the levels of high-density lipoprotein (HDL), LDL, triglycerides, and total cholesterol were calculated using BT 3000. Moreover, the specific activity of PON1 was measured based on its arylesterase activity using a spectrophotometer at 270 nm wavelength. To measure the gene expression of PON1, RNA was first extracted from liver tissue samples by means of the RNX kit (CinnaGen Company). Additionally, cDNA synthesis was conducted using the Thermo Fisher kit. Real-time polymerase chain reaction (RT-PCR) was performed using PON1-specific primers (forward primer sequence: 5'-TTG AAT GAG AAG GAG CCA GC-3' and reverse primer sequence: 5'-CAC GGT GGA CGA GGA GTC-3') in the presence of the beta-actin reference gene (forward primer sequence: 5'-CTT CTA CAA TGA GCT GCG TGT GGC C-3' and reverse primer sequence: 5'-GGA GCA ATG ATC TTG ATC TTC ATG G -3'). RT-PCR was performed using a three-minute cycle at 95 ˚C, followed by 40 three-step cycles of 10 seconds at 95 ˚C, 20 seconds at 62 ˚C, and 20 seconds at 72 ˚C. Finally, RT-PCR was completed at 72 ˚C for 5 minutes. The results were analyzed by the 2-ΔΔCt method (15).

In silico studies

To conduct *in silico* and molecular docking studies, the PDB file of PON1 was drawn from the uniprot.org database. In addition, the structure of the main antioxidant ingredients of *T. chebula*, namely, catechin, chebulagic acid, chebulinic acid, chebulic acid, methyl neochebuligate, corilagin, ellagic acid, gallic acid, quercetin, kaempferol, and shikimic acid, was obtained from the PubChem database and converted to a PDB file using Avogadro software. The energy minimization of all molecules was conducted using Swiss PDB Viewer software, version 4.1.

In this study, *in silico* molecular docking was performed by AutoDock 4.2 software. Catechin, chebulagic acid, chebulinic acid, chebulic acid, methyl neochebuligate, corilagin, ellagic acid, gallic acid, quercetin, kaempferol, and shikimic acid as ligands were docked to PON1. The genetic algorithm was applied for docking. For this purpose, new configurations of the ligand in the binding sites of the protein were detected by displacing the ligand and rotating it around the protein (16). To this end, 200 docking steps were accomplished. The lowest energy level of the interaction between the ligands and the enzyme was considered the most appropriate site for docking. The number of hydrophobic and hydrogen bonds between PON1 and the ligands in question was drawn after docking by means of LigPlot+.

Data analysis

The data were analyzed using descriptive statistics (Means ± standard deviations), analysis of variance, and Tukey's test. The significance level (*P* value) was considered to be < 0.05.

Results

Serum triglyceride, total cholesterol, HDL, and LDL levels are illustrated in Figure 1. The results showed that being on a high-fat diet for 90 days led to a significant increase

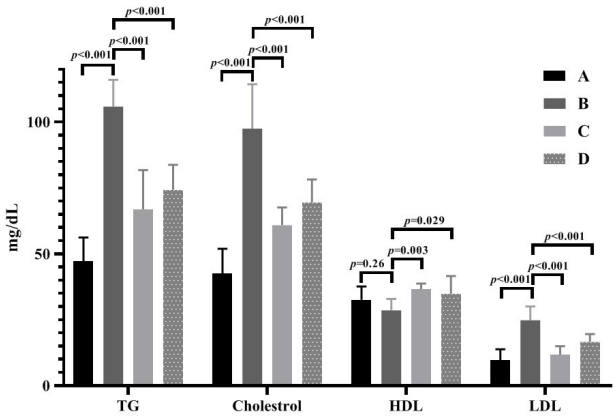

Figure 1. Serum total cholesterol and triglyceride levels: (A) Controls, (B) Hyperlipidemic group, (C) Hyperlipidemic group given 400 mg/kg of *T. chebula* extract, and (D) Hyperlipidemic group receiving 800 mg/kg of *T. chebula* extract.

in serum cholesterol, LDL, and triglyceride levels in rats. Further, treatment with the *T. chebula* extract (400 and 800 mg/kg body weight) caused a significant decrease in serum total cholesterol, triglyceride, and LDL levels ($P < 0.001$).

Figure 2 displays the arylesterase activity of PON1. The mean ± SD of PON1 activity was 281.65 ± 39.12, 202.12 ± 6058, 224.34 ± 58.74, and 235.80 ± 37.05 unit/mL for the control group, hyperlipidemic group, hyperlipidemic group receiving 400, and hyperlipidemic group receiving 800 mg/kg of the *T. chebula* extract, respectively. As shown, the activity of PON1 decreased significantly in the hyperlipidemic group (B) compared to the control group (A) ($P = 0.044$). In addition, the activity of the enzyme in the groups given *T. chebula* extract (C and D) increased in comparison to the hyperlipidemic group, but not significantly.

The gene expression of PON1 is shown in Figure 2. A high-fat diet caused an insignificant increase (1.11 ± 0.18, 1.79 ± 0.35, and 1.60 ± 0.31 for the hyperlipidemic group and hyperlipidemic groups receiving 400 and 800 mg/kg of the *T. chebula* extract, respectively) in enzyme gene expression compared to the control group ($P = 0.85$). The treatment groups (receiving the *T. chebula* extract) exhibited higher *PON1* gene expression in comparison to the control group; thus, *PON1* gene expression was significantly different in the group given 400 and 800 mg/kg of the plant extract compared to the hyperlipidemic and control groups ($P < 0.05$).

Table 1 presents the results regarding the molecular docking of binding the ligands into PON1. Numerous hydrogen and hydrophobic interactions were observed between PON1 and ligands. Some ligands (e.g., catechin and chebulinic acid) create the most hydrophobic bonds, and some ligands (e.g., corilagin, kaempferol, methyl neochebuligate, quercetin, and shikimic acid) produce more hydrogen bonds than other ligands at the binding sites. The amount of energy that was released after docking the ligands into the PON1 enzyme was widely different among the ligands. The most binding energy

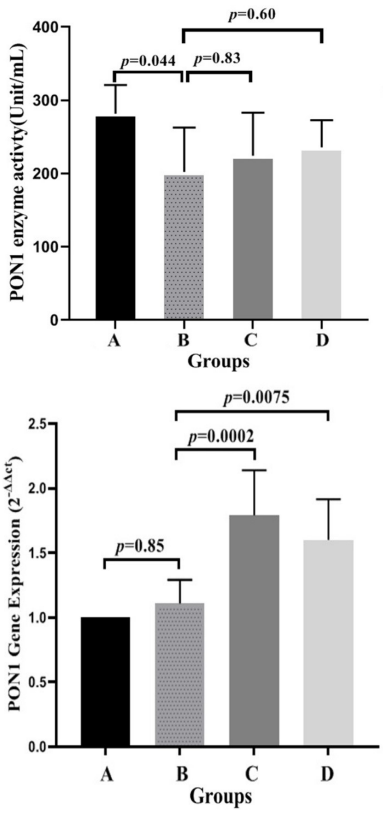

Figure 2. The activity and gene expression of PON1 in the studied groups: (A) Controls, (B) Hyperlipidemic group, (C) Hyperlipidemic group receiving 400 mg/kg of the *T. chebula* extract, and (D) Hyperlipidemic group receiving 800 mg/kg of the *T. chebula* extract.

(BE) is released from docking catechin, kaempferol, and quercetin with -8.12, -7.80, and -7.62 kcal/mol, respectively. Furthermore, the lowest amount of inhibition constant (Ki) was obtained for catechin, kaempferol, and quercetin (1.11, 1.93, and 2,59 µM, respectively, Table 1).

Moreover, Figure 3 depicts the docking of catechin, kaempferol, and quercetin into the PON1 binding site that interacted with amino acid residues through hydrogen and hydrophobic bonds in binding sites.

Discussion

Today, numerous drug treatments are used to control lipid levels and lose weight, but they have few effects and often cause many side effects. Given that the use of traditional medicine and medicinal plants in the treatment of many diseases is currently common, using medicinal plants can be a suitable alternative to combination drugs. The use of medicinal plants and their active ingredients in terms of fewer side effects and the use of natural substances have become widespread for the prevention or treatment of metabolic diseases such as hyperlipidemia and hypercholesterolemia (17,18). *T. chebula* is known as a plant rich in tannins and flavonoids and can produce a variety of medicinal effects (19). In this study, the hydroalcoholic extract of *T. chebula* at 400 and 800 mg/kg could decrease the lipid profile level but did not exhibit a significant effect on the level of HDL. In agreement with our study, the research of Dusi et al showed that

Table 1. Molecular docking parameters

PON1-Ligand	BE (kcal/mol)	Ki (μM)	Hydrogen Bond	Hydrophobic Bond
PON1-Catechin	-8.12	1.11	Ile57, Leu58, and Lys338	Pro59, Ser272, Val273, Asp274, Pro275, Trp281, Ala335, Val336, and Tyr337
PON1-Chebulagic acid	-4.78	314.85	Lys316, Val317, and Ser263	Leu262, Asp265, Leu305, Glu314, Pro315, Thr318, and Val319,
PON1-Chebulic acid	-5.08	207.08	Asn50, Lys70, and Lys81	Gly68, Leu69, and Asn80
PON1-Chebulinic acid	-1.78	51480.00	Ser193 and His197	Tyr71, His184, Tyr190, Lys192, Met196, Phe222, Phe292, Phe293, Tyr294, and Phe347
PON1-Corilagin	-6.98	7.70	Glu53, Asn224, Asn168, Asp183, Lys192, Ile291, and Phe292	Tyr71, His15, His184, Phe222, Asp269, Thr332, Phe347, and Val346
PON1-Ellagic acid	-7.61	2.62	Asn227, Val273, and Val336	Glu56, Ile57, Leu58, Pro59, Ser272, Pro275, Ala335, and Tyr337
PON1-Gallic acid	-5.67	69.46	-	Glu56, Ile57, Ser272, Val273, Pro275, Ala335, and Val336
PON1-Kaempferol	-7.80	1.93	Leu55, Glu56, Leu58, Asp274, and Val336	Ile57, Pro59, Asn227, Ser272, Val273, Pro275, Ala335, and Tyr337
PON1-Methyl neochebuligate	-3.24	4.22	Tyr71, Asn224, Asp269, Phe292, and Tyr294	Phe222, His285, Ile291, and Phe293
PON1-Quercetin	-7.62	2.59	Leu55, Glu56, Leu58, Asn227, and Asp274	Ile57, Pro59, and Ser272 Val273, Pro275, Ala335, val336, and Tyr337
PON1-Shikimic acid	-5.23	147.64	Asn43, Lys46, Glu94, Pro95, and Tyr352	Leu44 and Val45

Note. BE: Binding energy; Ki: Estimated inhibition constant.

the oral administration of an ethanolic extract from *T. chebula* fruits led to a significant reduction in serum cholesterol and triglyceride levels in hyperlipidemic rats (20). Reddy et al investigated the protective effect of the *T. chebula* extract in induced hyperlipidemic rats and found that the plant extract could have the potential to reduce hyperlipidemia-induced complications (21). It has also been shown that the flavonoid and antioxidant compounds in plants have a significant effect on preventing the occurrence of hyperlipidemia and hypercholesterolemia. These compounds play an active role by influencing the expression of factors involved in this process or increasing or decreasing the activity of enzymes that affect the metabolism of these compounds (22).

According to our results, the level of arylesterase activity of PON1 significantly decreased in the hyperlipidemic group. However, in the groups given 400 and 800 mg/kg of the hydroalcoholic extract of *T. chebula*, there was a slight increase in enzyme activity, although this increase was not statistically significant. On the other hand, in the groups receiving 400 and 800 mg/kg of the hydroalcoholic *T. chebula* extract, a significant increase was observed in gene expression; nonetheless, this change was absolutely insignificant in the hyperlipidemic group. This increase in gene expression was more evident for the concentration of 800 mg than for the concentration of 400 mg. Various studies have been conducted on the effect of different plants that contain flavonoids and other active ingredients on PON1 activity. Aviram et al concluded that the oral administration of pomegranate juice (containing flavonoids) to rats with APO E deficiency caused a 43% increase in PON1 activity (23). Huda et al also showed that consuming kefir can significantly increase the activity of the PON1 enzyme (24). Exogenous compound administration strategies can increase PON1 activity. The anti-atherogenic property of PON1 is related to oxidized esters' hydrolyzation of cholesterol and oxidized phospholipids, so it can protect against the oxidation of LDL (25). Various studies have demonstrated that antioxidant compounds can consistently lead to an increase in PON1 enzyme gene expression (26, 27). While an increase in gene expression does not necessarily indicate an increase in enzyme activity, based on the results of this study, although the expression level of the *PON1* gene increased significantly in the groups receiving the *T. chebula* extract, we noticed a slight increase in PON1 enzyme activity, indicating the influence of hyperlipidemic conditions on the enzyme activity. Feingold et al found that the expression of the *PON1* gene decreases *in vitro* by creating inflammatory conditions (28). However, in the present study, *PON1* gene expression slightly increased, but its activity decreased significantly in hyperlipidemic conditions. Feingold et al noticed that serum PON1 activity relied on several factors, including physiological and pathological conditions, so that a decrease in serum PON1 activity was observed in kidney disease, diabetes mellitus, HDL deficiency, and liver cirrhosis. Additionally, high-fat diets reduce PON1 activity (28). In our study, PON1 activity in the serum decreased in hyperlipidemic conditions.

In an *in silico* study, Hu et al observed that different regions of PON1 exhibited higher flexibility than other regions. These active regions affect the enzyme's catalytic site, and changes in the amino acid residues of these regions affect the enzyme's activity (29). The results of the present study revealed that the active ingredients in *T. chebula* could bind to different sites of PON1 with a high affinity. Among the active ingredients of *T. chebula*,

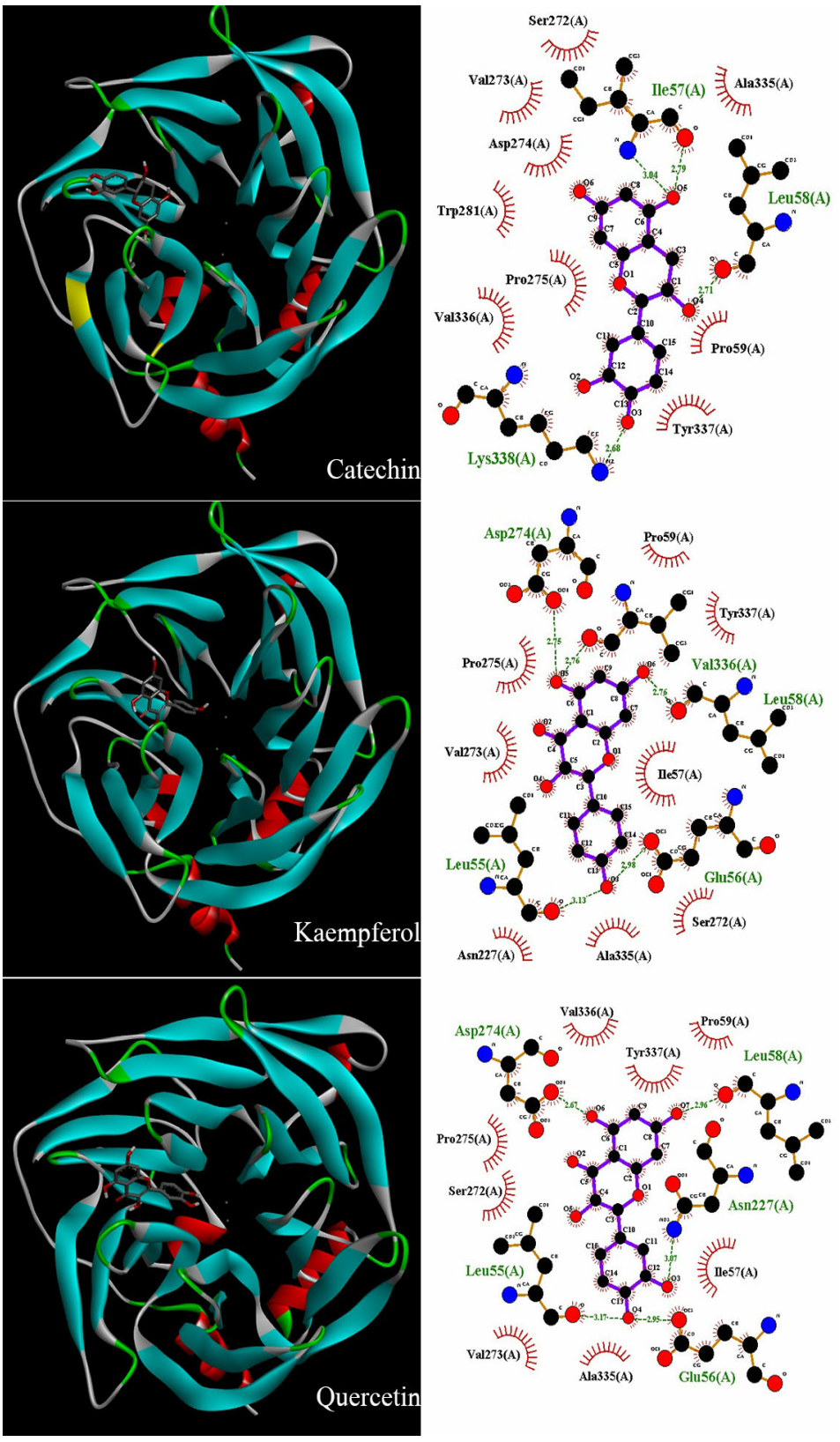

Figure 3. Docking of catechin, kaempferol, and quercetin into the PON 1 binding site

catechin, kaempferol, and quercetin released the highest amount of binding energy by interacting with the enzyme, so it seems that they have the greatest tendency to bind to the enzyme. In an *in-vivo*/*in-vitro* study, Jaiswal et al concluded that quercetin and catechin could increase the activity of PON1 and reduce the oxidation rate of LDL (30). Based on the results of molecular docking studies in our study, it seems that the direct binding of these compounds to PON1 can affect its activity and function.

Conclusion

The results of this study revealed that the hydroalcoholic

T. chebula extract significantly reduced the serum lipid profile in hyperlipidemic rats. In addition, the extract of this plant could slightly increase the activity of PON1, yet it substantially increased the expression of the *PON1* gene. Although hyperlipidemia can affect the level of PON1 activity, the direct interactions of each of the active ingredients in *T. chebula*, especially catechin, kaempferol, and quercetin, can affect its activity by directly influencing the structure of the enzyme. It seems that these three compounds, as the most important compounds of *T. chebula*, can be useful as co-treatments in hyperlipidemia therapies.

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Competing Interests
The authors declare no conflict of interests.

Ethical Approval
This study was performed at the Shahrekord University of Medical Sciences after its approval by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.AEC.1401.021).

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