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Original Article

Effect of hydroalcoholic extract of Tanacetum parthenium on gene expression and paraoxonase 1 enzyme activity: An experimental and molecular dynamic study

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Abstract

Background and aims: Cardiovascular diseases are among the most common causes of death worldwide. The paraoxonase 1 (PON1) enzyme is a main factor in preventing these diseases. This study investigated the effect of hydroalcoholic extract of *Tanacetum parthenium* plant on the activity and gene expression of PON1.

Methods: Forty male rats were divided into 4 groups: a control group, a hyperlipidemic group, and two hyperlipidemic groups that were treated with 400 and 800 mg/kg of hydroalcoholic extract. Arylesterase activity of PON1 and serum triglyceride and cholesterol levels were measured. Moreover, the gene expression of PON1 in the liver tissue samples was measured using real-time PCR. Finally, molecular dynamics studies of apigenin as a main compound in the plant were performed to investigate the activity of PON1 in the simulated environment.

Results: Hydroalcoholic extract of *T. parthenium* decreased serum triglyceride (from 105.8 ± 10.1 to 60.5 ± 21.5 and 50.9 ± 11.2 mg/dL) and total cholesterol (from 97.5 ± 16.8 to 59.6 ± 8.5 and 52.0 ± 9.6 mg/dL) levels, while it increased the activity of arylesterase paraoxonase1 significantly ($P<0.001$). It also showed a significant ($P<0.001$) effect on PON1 gene expression. However, the dose of 800 mg/kg was more effective. The results of simulation and molecular dynamics showed that apigenin binds to PON1 with a high affinity and induces changes in the molecular dynamics parameters.

Conclusion: The 800 mg/kg of hydroalcoholic extract *of T. parthenium* can increase the activity of PON1 and its gene expression. It seems that apigenin as one of the most important antioxidant compounds of this plant can increase the activity of this enzyme by direct binding to PON1.

Keywords: Paraoxonase1, *Tanacetum parthenium*, Apigenin, Docking, Molecular dynamics

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Introduction

Paraoxonase 1 (PON1) is an enzyme with antioxidant activity that is expressed in the liver and is transferred to and works in the serum (1). Through its antioxidant activity, this enzyme plays an important role in preventing atherosclerosis and arteriosclerosis (2). PON1 is one of the most important serum enzymes related to high-density lipoprotein, which plays a protective role against the oxidation of serum low-density lipoprotein (LDL) (3,4). Therefore, an increase in its activity is inversely correlated with the incidence of atherosclerosis (5). Decreased activity of PON1 and oxidation of LDL lead to cholesterol deposition in arterial walls and the incidence of cardiovascular diseases, which are currently among the leading causes of death across the world (6,7). Inactivity and a high-fat diet, diabetes, aging, hypertension, and smoking are among the most important factors in the process of LDL oxidation and the development of atherosclerosis (8-10). Today, it has been established that many plants with antioxidant effects can play an important role in preventing the incidence of cardiovascular diseases. Antioxidants that exist in most medicinal plants can prevent the deposition of cholesterol in the arteries and consequently the incidence of atherosclerosis (11). In addition, many antioxidant and polyphenolic compounds of plants can potentially increase the activity of PON1, which is the most important factor in preventing LDL oxidation and the incidence of atherosclerosis (12,13). Plant-derived antioxidants can play a role in the efficacy of plants through increasing enzyme gene expression or increasing enzyme activity (13,14). Spontaneously growing in many regions of Iran, *Tanacetum parthenium* is one of the medicinal plants that are rich in antioxidant compounds (15). In traditional medicine, *T. parthenium* is mainly known for its soothing and pain-relieving properties. Accordingly, many studies have shown that the plant can serve as a pain reliever by increasing serotonin levels (16). Studies have also shown that the

compounds extracted from *T. parthenium* can relieve pain in vivo by exhibiting anti-inflammatory activity (17). In addition, some studies showed that doses less than 1000 mg/kg of *T. parthenium* are not toxic in rats (18). However, the plant can produce protective effects against liver and kidney damage and lipid peroxidation (19,20). Apigenin is one of the most important antioxidant compounds of *T. parthenium*, which plays a substantial role in the process of cholesterol metabolism and the prevention of cardiovascular diseases. Additionally, this antioxidant compound potentially contributes to the prevention of lipid peroxidation and increase of serum cholesterol levels (21,22). Given the antioxidant effects of *T. parthenium*, this study was conducted to investigate the role of the hydroalcoholic extract of this plant in reducing triglyceride and cholesterol levels in hyperlipidemic rats, and the effect of this extract on the activity and gene expression of PON1. Besides, the prediction of the interaction of apigenin with the PON1 enzyme was examined using simulation, docking, and molecular dynamics studies.

Materials and Methods

Preparation of the hydroalcoholic extract of Tanacetum parthenium

In this study, extraction was done using maceration. For this purpose, after procurement of the studied plant samples from a local grocery and confirmation of their quality and authenticity by a pharmacognosy specialist, it was registered with herbarium code 111 in the Medical Plant Research Center of Shahrekord University of Medical Sciences. Then, they were pulverized using an electric mill and a suitable amount of the resulting powder was added to a mixture of water (30%) and ethanol 97% (70%). After 72 hours, the resulting mixture was filtered using a Buchner funnel and extraction was done using a rotary evaporator. After drying the extract in the incubator, the desired concentrations were prepared from it.

In vivo studies

In the present study, 40 rats were studied in 4 groups of 10. Group 1 consisted of 10 rats as a control group receiving a conventional diet for 90 days. Group 2 consisted of 10 rats as a hyperlipidemic group receiving a fat-enriched diet with Persintra-M for 90 days. Group 3 consisted of 10 rats given a fat-enriched diet with Persintra-M and hydroalcoholic extract of *T. parthenium* at 400 mg/kg for 90 days. Group 4 consisted of 10 rats given a fat-enriched diet with Persintra-M and hydroalcoholic extract of *T. parthenium* at 800 mg/kg for 90 days.

In vitro studies

After sacrificing the animals, the blood serum samples of the different groups were separated and triglycerides and total cholesterol levels were measured using BT 3000. Additionally, the specific activity of PON1 was measured based on its arylesterase activity using a spectrophotometer at 270 nm wavelength (23). In order to measure the gene expression of PON1, RNA was first extracted from liver tissue samples using the RNX kit (CinnaGen Co.). In addition, cDNA synthesis was done using the Thermo Fisher kit. Real-time polymerase chain reaction (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 β-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'). Real-time PCR consisted of 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, real-time PCR was completed at 72 °C for 5 minutes. The results were calculated using the $2^{-\Delta\Delta Ct}$ formula (24).

In silico studies

To perform simulation and molecular dynamics studies, the PDB file of PON1 was obtained from the UniProt database and the structure of the most important antioxidant compound of *T. parthenium* (i.e., apigenin) was obtained from the PubChem database and converted to a PDB file using Avogadro software.

The simulation and molecular dynamics studies of the protein structure of the enzyme were first carried out in the water environment. To do so, the relevant structure was subjected to changes in temperature, pressure, and concentration of 140 mM and reached equilibrium. For this purpose, GROMACS software and G43a1 force field were used. SPC216 model was used in this study and the simulation time was 10 ns. Based on the current standards, the concentration of ions in the system was increased to 140 mM by adding the required amounts of Na^+ and Cl^- ions, and a number of the mentioned ions were replaced with water solvent. The paths saved in the simulation were used as controls to analyze the structural parameters of the PON1 protein in the presence of ligands. Next, molecular docking was performed on the output PDB file of the simulated structure in water according to the following method (25).

In this study, to dock small molecules in macromolecules, AutoDock 4.2 software was used and apigenin was docked to PON1. The genetic algorithm was used for docking. In this method, new configurations of the ligand were identified in the binding sites of the protein by moving the ligand and rotating it around the protein. For this purpose, 200 docking steps were considered. The lowest energy level of the interaction between the ligand and the enzyme was considered the most suitable position for docking. The position of the best ligand-binding site was used from the Prodrug site to generate the necessary files for the ligand-protein simulation process. The number of hydrogen and hydrophobic bonds between PON1 and the studied ligand was obtained after docking using LigPlot.

Finally, the complex of PON1 and the studied ligands was created in the water environment according to the above-mentioned method (molecular dynamics simulation of PON1 protein in water) and as mentioned before, the paths saved in the simulation were used to analyze the structural parameters of the complex (25). In molecular dynamics studies, the root-mean-square deviation (RMSD) and radius of gyration (Rg) values and the changes in the secondary structure of the protein as well as the types of hydrogen and hydrophobic bonds related to the studied structures in the steps of the molecular dynamics simulation of PON1 protein in water and the molecular dynamics simulation of the protein-ligand complex in water were determined using GROMACS software for Linux and the results were analyzed.

Data analysis

Data analysis was done using descriptive statistics (mean ± standard deviation), ANOVA, and Tukey's test. $P < 0.05$ was considered the significant level. Molecular dynamics studies were done using simulation software for Linux.

Results

Serum total cholesterol and triglyceride levels are shown in Figure 1. The results showed that receiving a high-fat diet for 90 days caused a significant increase in serum cholesterol and triglyceride levels in rats. Meanwhile, receiving *T. parthenium* extract at 400 and 800 mg/kg caused a dose-dependent decrease in serum total cholesterol and triglyceride levels in the animals.

Figure 2 illustrates the arylesterase activity of PON1 in the studied groups. As can be seen, the activity of PON1 in the hyperlipidemic group (B) decreased significantly compared to the control group (A) ($P = 0.003$). Besides, in the intervention groups receiving *T. parthenium* extract (C and D), the activity of this enzyme increased significantly compared to the hyperlipidemic group ($P < 0.001$).

The gene expression of PON1 is also illustrated in Figure 2. Receiving a high-fat diet led to an increase in the enzyme gene expression, but the increase was not statistically significant compared to the control group. The intervention groups (given *T. parthenium* extract) showed higher levels of PON1 gene expression compared to the control group. Additionally, PON1 gene expression in the group receiving 800 mg/kg of the plant extract was significantly different from that in the hyperlipidemic group ($P < 0.001$).

The binding site of apigenin to PON1 and the hydrogen and hydrophobic interactions between apigenin and the amino acids in the binding site are shown in Figure 3. The amount of binding energy released due to the binding of apigenin to PON1 was calculated to be -7.73 kJ/mol.

The results of molecular dynamics studies including the RMSD and Rg values at 10 ns of the simulation time are depicted in Figure 4. The RMSD variations related to PON1 in the non-binding state to apigenin and were docked with apigenin stabilized from 5 ns of the simulation time onwards. The mean RMSD values of PON1 in the state of binding to apigenin decreased compared to the mean RMSD values of the enzyme in the state of non-binding to apigenin at 10 ns of the simulation time, while the Rg of PON1 in the state of binding to apigenin increased compared to the mean Rg of the enzyme in the state of non-binding to apigenin at 10 ns of the simulation time.

Figure 5 illustrates the changes in the secondary structures of PON1 in the states of non-binding to the ligand and binding to apigenin. An increase in the bend and coil structures and a decrease in the turn, alpha-helix, and beta-sheet structures occurred due to the binding of the ligand to PON1 after the completion of the simulation time. These changes in the secondary structures of the enzyme indicate the influence of these compounds on the structure of the PON1. This impact on the structure can be directly related to the way the enzyme acts.

Discussion

The results of this study showed that hydroalcoholic extract of *T. parthenium* could significantly reduce serum cholesterol and triglyceride levels in hyperlipidemic and hypercholesterolemic rats (Figure 1). Most of the studies conducted on the plant have addressed its soothing and

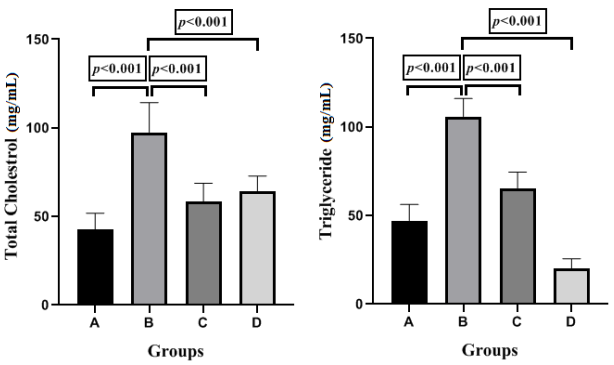

Figure 1. Serum total cholesterol and triglyceride levels; A: Control goup, B: Hyperlipidemic group, C: Hyperlipidemic group receiving 400 mg/kg of *T. parthenium* extract, D: Hyperlipidemic group receiving 800 mg/kg of *T. parthenium* extract

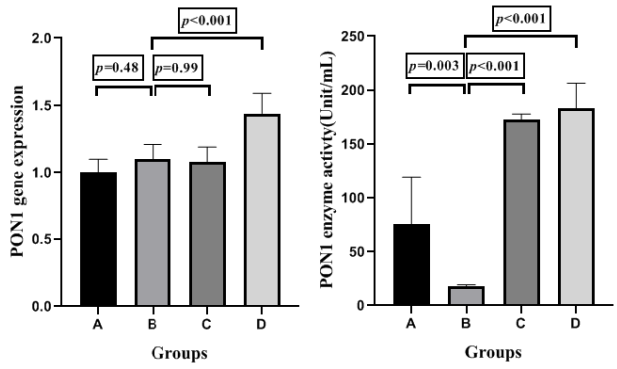

Figure 2. The activity and gene expression of paraoxonase 1 in the studied groups. A: Control group, B: Hyperlipidemic group, C: Hyperlipidemic group receiving 400 mg/kg of *T. parthenium* extract, D: Hyperlipidemic group receiving 800 mg/kg of *T. parthenium* extract

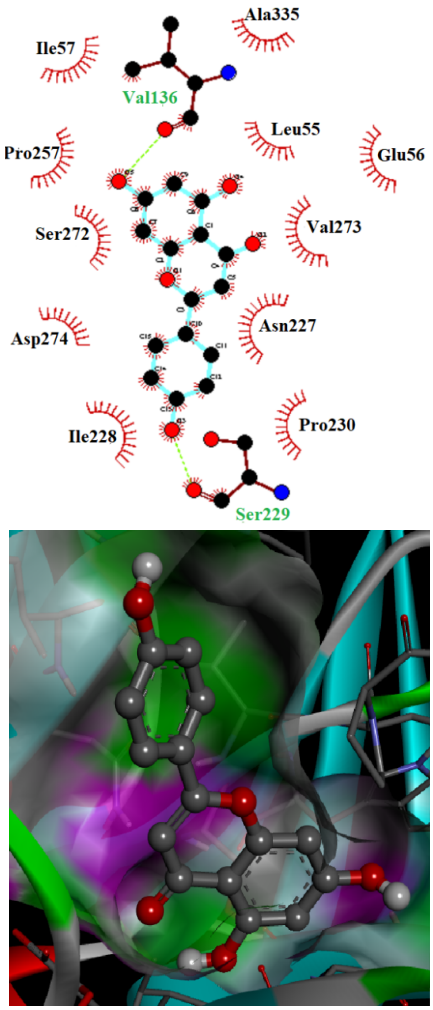

Figure 3. The binding site of apigenin to paraoxonase 1 and the hydrogen and hydrophobic interactions between apigenin and amino acids in the binding site

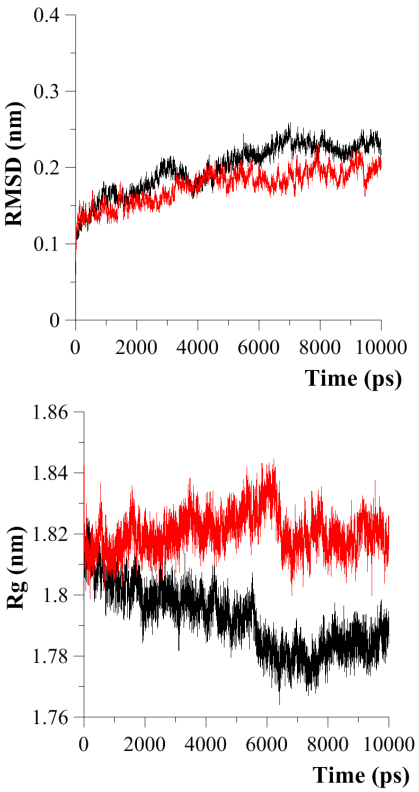

Figure 4. The root-mean-square deviation (RMSD) and radius of gyration (Rg) of paraoxonase 1 at 10 ns of the simulation time (Black: Paraoxonase 1 alone; Red: Paraoxonase 1 docked with apigenin)

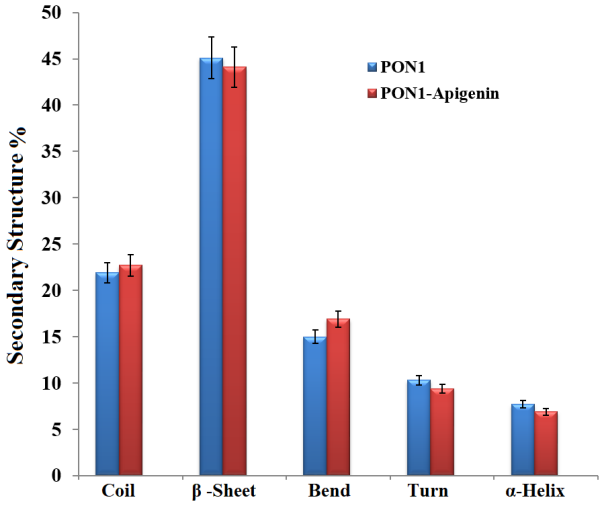

Figure 5. Changes in the secondary structures of paraoxonase 1 in the states of non-binding to the ligand and binding to apigenin

pain-relieving properties (17). However, some studies that show that the extract of *T. parthenium* can prevent the incidence of many cardiovascular diseases, especially the process of lipid oxidation (20). It seems that the antioxidant compounds in *T. parthenium*, by exerting anti-inflammatory effects, can somehow reduce the serum triglyceride and cholesterol levels in hyperlipidemic rats.

The present study showed that different concentrations of hydroalcoholic extract of *T. parthenium* could directly increase the aryl-esterase activity of PON1 in hyperlipidemic rats, while its activity significantly decreased in the hyperlipidemic group compared to the control group (Figure 2). Lack of a significant increase in the expression of the PON1 gene in the hyperlipidemic group receiving 400 mg/kg of hydroalcoholic extract of *T. parthenium* and a significant increase in the enzyme activity in the hyperlipidemic groups given 400 and 800 mg/kg of the extract compared to the control group indicated that the active compounds of *T. parthenium* caused an increase in its arylesterase activity probably by affecting its activity and, to a lesser extent, increasing its gene expression (Figure 2). Bamdad et al also reported that the methanolic extract of *T. parthenium* could increase the arylesterase activity of PON1 in rats damaged by carbon tetrachloride concentrations (26). However, many other medicinal plants can have similar effects on the activity of this enzyme. Khouya et al reported that polyphenol extract of Thymus atlanticus potentially increased the activity of PON1 enzyme in hyperlipidemic hamesters (13). It has been reported that high concentrations of *T. parthenium* can be toxic; however, Subha et al showed that lower concentrations of *T. parthenium* (1000 mg/kg) have no toxic effect (18).

Tanacetum parthenium contains many flavonoids and

antioxidant compounds, of which apigenin is one of the most important compounds that seem to have the greatest effect on reducing serum cholesterol and triglyceride levels and increasing the activity of PON1 (21). Singh et al observed that apigenin played an important role in preventing lipid oxidation (27). Ren et al also reported that apigenin could modulate the process of lipid metabolism in rats with type 2 diabetes (28). Moreover, the study by Zhang et al showed that apigenin played a substantial role in cholesterol metabolism and the prevention of cardiovascular diseases (29).

The results of molecular docking studies in the present study showed that apigenin had a very high affinity for binding to PON1 (Figure 3). The direct binding of apigenin to the amino acids in the binding site of the enzyme through two hydrogen bonds and eleven hydrophobic bonds with a binding energy release of -7.73 kJ/mol facilitated this binding (Figure 2). Studies have shown that the spatial structure of apigenin and especially the hydroxyl groups present in the molecular structure increase its affinity for binding to PON1 (29). In the study of Moradi et al, it was shown that apigenin could significantly increase the activity of PON1 in rats (30). The results of the molecular dynamics studies in the present study showed that the binding of apigenin to PON1 could result in an increase in the activity of the enzyme through the direct effects it exerts on the spatial structure of the enzyme. The simulation system became stable before 10 ns and the RMSD variations were stable during the simulation time (Figure 4). The increase in the Rg of PON1 after binding to apigenin (Figure 4) increases the possibility of making the active sites of the enzyme available for arylesterase activity, which can lead to an increase in enzyme activity (31). Changes in the secondary structure of PON1 after docking the apigenin (Figure 5) illustrate that this compound can increase the flexible sites in the enzyme by increasing the amounts of coil and bend structures and conversely reducing the secondary structures of α-helix and β-sheet, thus increasing the possibility of making the active site of the enzyme available.

Conclusion

This study, which included in vivo, in vitro, and in silico investigations, showed that *T. parthenium* especially in the concentration of 800 mg/kg could decrease the serum cholesterol and triglyceride levels in hyperlipidemic rats. Additionally, it increased the arylesterase activity of PON1 in hyperlipidemic rats. *Apigenin*, as the most effective compound of the *T. parthenium*, could also play an important role in activating PON1 by directly affecting it.

Authors' Contribution

Conceptualization: Keyhan Ghatreh-Samani.
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Formal Analysis: Javad Saffari-Chaleshtori.
Funding acquisition: Keyhan Ghatreh-Samani.
Investigation: Fatemeh Maghsoudi.
Methodology: Javad Saffari-Chaleshtori.
Project administration: Javad Saffari-Chaleshtori.
Resources: Hojatollah Rohi-Brojeni.
Software: Fatemeh Maghsoudi.
Supervision: Javad Saffari-Chaleshtori.
Validation: Keyhan Ghatreh-Samani.
Visualization: Javad Saffari-Chaleshtori.
Writing – original draft: Javad Saffari-Chaleshtori.
Writing – review & editing: All authors.

Competing Interests

The authors have no conflict of interests regarding this study to disclose.

Ethical Approval

This study was performed at the Shahrekord University of Medical Sciences after the ethics committee approved of it (SKUMS. REC.1399.035).

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