Effects of oleuropein on lipid peroxidation, lipid profile, atherogenic indices, and paraoxonase 1 status in gentamicin-induced nephrotoxicity in rats

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Abstract

BACKGROUND: Oleuropein is a natural antioxidant and scavenging free radicals. In the present study, we examined effect of oleuropein on paraoxonase 1 (PON1) activity, lipid peroxidation, lipid profile, atherogenic indices, and relationship of PON1 activity by high-density lipoprotein cholesterol (HDL-C) and atherogenic indices in gentamicin (GM)-induced nephrotoxicity in rats.

METHODS: This is a lab trial study in Khorramabad, Lorestan province of Iran (2013). Thirty Sprague-Dawley rats were divided into three groups to receive saline; GM, 100 mg/kg/d; and GM plus oleuropein by 15 mg/kg i.p daily, respectively. After 12 days, animals were anaesthetized, blood samples were also collected before killing to measure the levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), HDL-C, atherogenic index, lipid peroxidation and the activities of PON1 of all groups were analyzed. Data were analyzed, and P < 0.050 was considered significant.

RESULTS: Oleuropein significantly decreased lipid peroxidation, TG, TC, LDL, VLDL, atherogenic index, atherogenic coefficient (AC), and cardiac risk ratio (CRR). HDL-C level was significantly increased when treated with oleuropein. The activity of PON1 in treated animals was (62.64 ± 8.68) that it was significantly higher than untreated animals (47.06 ± 4.10) (P = 0.047). The activity of PON1 in the untreated nephrotoxic rats was significantly lower than that of control animals (77.84 ± 9.43) (P = 0.030). Furthermore, the activity of PON1 correlated positively with HDL-C and negatively with AC, CRR1, and CRR2 in the treated group with oleuropein.

CONCLUSION: This study showed that oleuropein improves PON1 activity, lipid profile, and atherogenic index and can probably decrease the risk of cardiovascular death in nephrotoxic patients.

Keywords: Gentamicin, Paraoxonase 1, Lipid Peroxidation, Nephrtoxicity, Lipid, Rat, Atherogenic Index, Oleuropein

Introduction

Human serum paraoxonase 1 (PON1) is a high-density lipoprotein cholesterol (HDL-C)-bound enzyme and are considered the major determinant of the antioxidant action of HDL-C. Major part of this enzyme in the serum is associated with HDL-C particles, but a low level of PON1 was also observed in very low-density lipoprotein (VLDL) and postprandial chylomicrons. PON1 inhibits LDL oxidation in vitro, and other studies have shown that PON1 prevents the formation of oxLDL, inactivates LDL-derived oxidized phospholipids and protects phospholipids in HDL from oxidation. PON1 has antiatherogenicity properties because PON1 has ability to protect lipoprotein particles from free radical oxidation, and it can hydrolyze oxidized cholesteryl esters, phosphatidylcholine core aldehydes, and degrade hydrogen peroxide.

Gentamicin (GM) is a commonly used aminoglycoside antibiotic that is effective against most of the gram negative microorganisms. Therapeutic doses of GM can cause nephrotoxicity, and it is among the most common causes of acute kidney injury.
kidney injury. The toxicity of GM is believed to be related to the generation of reactive oxygen species (ROS) in the kidney. ROS have been suggested as a cause of death for many cells in different pathological states including various models of liver, renal and cardiac diseases. Antioxidant and antioxidative enzyme activities reduce due to using GM or increase of lipid peroxidation products. Most research studies against GM-induced nephrotoxicity are focused on the use of various anti-oxidants. A number of natural antioxidants such as vitamin E and phenolic compounds are known to have protective effects on liver injury and nephrotoxicity. Chemical drugs as an antioxidant have many side effects; therefore, screening for new natural antioxidants is still attractive because they are safe and good alternative for the prevention of nephrotoxicity induced by GM.

Oleuropein is a secoiridoid derived from olive leaf and olive oil. Several studies have demonstrated that oleuropein has a high antioxidant activity. Previous our study showed that oleuropein has a protective effect in oxidative stress in spinal cord injury. Another study showed that the diets enriched with extra virgin olive oil increase of small, dense HDL enriched with apo A-IV tightly bound to PON.

Therefore, oleuropein as antioxidative supplements is good for the prevention of nephrotoxic complications such as hyperlipidemia. Since the effects of oleuropein on lipid profile, atherogenic indices, PON1 activity and its Association with atherogenic indices in nephrotoxicity induced by GM in rats have not previously been reported; the objectives of this lab trial study were to evaluate effects of oleuropein on lipid peroxidation, lipid profile, atherogenic index, atherogenic coefficient (AC), cardiac risk ratio (CRR), and CRR and serum PON1 status and its Association with atherogenic indices in GM-induced nephrotoxicity in rats in Khorramabad, Lorestan province of Iran (2013).

Materials and Methods

Thirty male Sprague-Dawley rats (180-200 g) were prepared from Pasteur Institute of Tehran, and they were allowed to adapt themselves with the new location for 1 week. They were kept at a room temperature of 22 °C and a humidity of 50 ± 10% with 12-hour light/dark cycles. This study was approved by the Animal Ethics Committee of Lorestan University of Medical Sciences and was in accordance with the National Health and Medical Research Council guidelines. The animals were divided into 3 equal groups randomly including 10 rats each as follows: group 1 (control group), intraperitoneal saline injection, 0.25 ml/d for 12 days; group 2, GM injection for 12 days; group 3, GM and intraperitoneal oleuropein, 15 mg/kg/d injection. 1 h before GM injection. GM, 100 mg/kg/d, was injected intraperitoneally for 12 days. After the last injection of GM, all the animals were immediately kept in individual, metabolic cages in order to collect 24-h urine. Blood samples were obtained from animals’ hearts under anesthesia (nedsonal, 50 mg/kg, intraperitoneal), were allowed to clot for 20 min in laboratory temperature, and were centrifuged at 3000 rpm for 15 min for serum separation. Then, the kidneys and livers were excised and used for homogenization.

The serum levels of triglyceride (TG), total cholesterol (TC), LDL, VLDL, HDL-C, and atherogenic index of all groups were analyzed. TC and TG concentrations were measured by biochemical analyzer using commercial kits (Olympus AU-600, Tokyo, Japan). HDL-C was analyzed by a Pars Azmoon kit from Iran. LDL and VLDL were determined by calculation using the Friedewald et al. equation. The Atherogenic index [units] [log (TG/HDL-C)], AC [TC-HDL-C]/HDL-C], CRR: (TC/HDL-C) and CRR: (LDL/HDL-C) were determined by calculation using the Ikewuchi and Ikewuchi equation.

Serum levels of lipid peroxidation were measured by the thiobarbituric acid assay. Liver and kidney contents of lipid peroxidation were also analyzed. Absorbances were measured spectrophotometrically at 532 nm and the concentrations were expressed as nmol lipid peroxidation/mg-pr.

PON activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412 nm due to the formation of 4-nitrophenol as already described. The activity was measured at 25°C by adding 50 µl of serum to 1 ml Tris-HCl buffer (100 mM at PH 8.0) containing 2 mM CaCl2 and 5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using molar extinction coefficient 17 100 M/cm.

All values are expressed as mean ± SD. Data between groups were first tested Kruskal-Wallis one-way and then between two groups were analyzed by Mann–Whitney U-test. The Spearman’s correlation analysis was used for statistical calculations. Statistical analyses were performed...
The treatment of a nephrotoxic animal with oleuropein could not significantly (26.32%) inhibit the increase of FBG, TG and TC in comparison with the untreated nephrotoxic animals (P = 0.001, P = 0.006, P = 0.001). The level of TG and TC in the untreated nephrotoxic rats were significantly higher than that of control animals (P = 0.002, P = 0.006, P = 0.001) (Table 1). The level of HDL in the untreated nephrotoxic rats was not significantly (0.91-fold) lower than that of control animals (P = 0.615). The treatment of a nephrotoxic animal with oleuropein could significantly inhibit the increase (2.02-fold) higher than that of control animals (P = 0.002, P = 0.006, P = 0.001). The level of TG and TC in the untreated nephrotoxic rats were higher than that of control animals (P = 0.020, P = 0.006). The treatment of a nephrotoxic animal with oleuropein could significantly inhibit the increase of LDL and in comparison with the untreated nephrotoxic animals (P = 0.010).

The level of atherogenic index (units) (log [TG/HDL-C]) and AC (log (TC-HDL-C)/HDL-C) in the untreated nephrotoxic rats was significantly higher than that of control animals (P = 0.044, P = 0.003). The treatment of a nephrotoxic animal with oleuropein could significantly inhibit the increase of AC (1.55-fold) higher than that of control animals (P = 0.032). The treatment of a nephrotoxic animal with oleuropein could significantly (25.48%) inhibit the increase of atherogenic index and AC in comparison with the untreated nephrotoxic animals (P = 0.03). The level of AC (TC-HDL-C)/HDL-C in the untreated nephrotoxic rats was significantly (2.02-fold) higher than that of control animals (P = 0.003, P = 0.002) (Table 1). The level of CRR (TC/HDL-C) and CRR (TC/HDL-C) in the untreated nephrotoxic rats were significantly higher than that of control animals (P = 0.003, P = 0.003). The treatment of nephrotoxic animal with oleuropein could significantly (43.71%) inhibit the increase of CRR (TC/HDL-C) and CRR (TC/HDL-C) in comparison with the untreated nephrotoxic animals (P = 0.001, P = 0.001) (Table 1).

The level of lipid peroxidation in the untreated nephrotoxic rats was significantly (1.58-fold) higher than that of control animals (P = 0.03). The treatment of a nephrotoxic animal with oleuropein could significantly (25.48%) inhibit the increase of activity of lipid peroxidation in comparison with the untreated nephrotoxic animals (P = 0.05). The activity of PON1 in the untreated nephrotoxic rats was significantly (1.58-fold) lower than that of control animals (P = 0.03). The treatment of a nephrotoxic animal with oleuropein could significantly (33.11%) elevate the decrease of activity of PON1 in comparison with the untreated nephrotoxic animals (Table 1) (P = 0.047).

The activity of PON1 correlated positively with HDL-C (r = 0.291, P = 0.006, Figure 1). The activity of PON1 correlated negatively with AC (r = −0.207, P = 0.001, Figure 2) and CRR1 (r = −0.273, P = 0.009, Figure 3) and CRR2 (r = −0.228, P = 0.018, Figure 4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Nephrotoxic</th>
<th>Nephrotoxic+OLE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>111.17 ± 18.90</td>
<td>143.00 ± 21.27</td>
<td>110.00 ± 13.91</td>
<td>0.009</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>62.00 ± 14.38</td>
<td>79.71 ± 10.70</td>
<td>83.60 ± 8.08</td>
<td>0.013</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>108.50 ± 13.08</td>
<td>159.00 ± 39.16</td>
<td>116.00 ± 13.08</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>47.47 ± 18.48</td>
<td>42.97 ± 12.56</td>
<td>54.28 ± 14.05</td>
<td>0.463</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>48.63 ± 19.78</td>
<td>100.08 ± 44.85</td>
<td>45.00 ± 25.64</td>
<td>0.017</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>12.40 ± 2.87</td>
<td>15.94 ± 2.14</td>
<td>16.72 ± 1.62</td>
<td>0.036</td>
</tr>
<tr>
<td>Atherogenic index (units)</td>
<td>0.13 ± 0.05</td>
<td>0.29 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.021</td>
</tr>
<tr>
<td>AC (TC-HDL-C)/HDL-C</td>
<td>1.51 ± 0.75</td>
<td>3.06 ± 1.66</td>
<td>1.28 ± 0.68</td>
<td>0.036</td>
</tr>
<tr>
<td>CRR1 (TC/HDL-C)</td>
<td>2.51 ± 0.75</td>
<td>4.05 ± 1.66</td>
<td>2.28 ± 0.69</td>
<td>0.360</td>
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<tr>
<td>CRR2 (LDL/HDL-C)</td>
<td>1.23 ± 0.68</td>
<td>2.65 ± 1.55</td>
<td>2.28 ± 0.69</td>
<td>0.033</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol/mg protein)</td>
<td>82.48 ± 20.40</td>
<td>128.18 ± 7.36</td>
<td>95.52 ± 38.39</td>
<td>0.029</td>
</tr>
<tr>
<td>PON1 activity (nmol/min/ml)</td>
<td>77.84 ± 9.43</td>
<td>47.06 ± 4.10</td>
<td>62.64 ± 8.68</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD; * Significant change in comparison with nephropathy without treatment at P < 0.050; ** Significant change in comparison with control at P < 0.050; OLE: Oleuropein; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; PON1: Paraoxonase 1; AC: Atherogenic coefficient; CRR: Cardiac risk ratio
Effect of oleuropein on paraoxonase activity

**Figure 1.** Correlation between maternal serum paraoxonase 1 activity and levels of high-density lipoprotein (HDL) cholesterol in nephrotoxic rats treated with oleuropein \((r = 0.291, P = 0.006)\).

**Figure 2.** Correlation between maternal serum paraoxonase 1 activity and levels of Atherogenic coefficient (AC) \([TC (TC-HDL-C)/HDL-C]\) in nephrotoxic rats treated with oleuropein \((r = -0.404, P = 0.001)\)

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol

**Figure 3.** Correlation between maternal serum paraoxonase 1 activity and levels of CRR1 \([CRR (TC/HDL-C)]\) in nephrotoxic rats treated with oleuropein \((r = -0.273, P = 0.009)\)

CRR1: Cardiac risk ratio 1; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol

**Figure 4.** Correlation between maternal serum paraoxonase 1 activity and levels of cardiac risk ratio 2 (CRR2) \([CRR (LDL/HDL-C)]\) in nephrotoxic rats treated with oleuropein \((r = -0.228, P = 0.018)\)

LDL: Low-density lipoprotein; HDL-C: High-density lipoprotein cholesterol

**Discussion**

**Effect of oleuropein on serum level of MDA and PON1 activity and correlation of PON1 activity with HDL and atherogenic index**

Nephrotoxicity significantly increased serum lipid peroxidation concentrations and decreased PON1 activity in comparison with the control group. Treatment of nephrotoxic animals with oleuropein significantly inhibited the increase of serum lipid peroxidation concentrations. Furthermore, treatment of nephrotoxic animals with oleuropein significantly inhibited decrease of serum PON1 activity in comparison with the untreated nephrotoxic animals.

The most relevant finding of this study is that activity of PON1 correlated positively with HDL and negatively with AC CRR1 and CRR2 in treated nephrotoxic animals. Researchers showed that PON1 as an antioxidant enzyme inhibit the oxidative modification of LDL and contribute to most of the antioxidative activity that has been attributed to HDL.\(^{22}\) PON1 activity was positively correlated with HDL-C level.\(^{23}\) In contrast, PON1 activity was inversely correlated with atherogenic index; thus, it can be suggested that decreased PON1 activity may be, in part, due to consumption of PON1 for the prevention of oxidation.\(^{24,25}\) It strongly suggests that decreased PON1 activity may be at least partially related to the consumption of PON1 caused by oxidative stress process.\(^{26}\) PON1 is an ester hydrolase that has both arylesterase and...
PON activities. Recently, roles for PON1 in a number of processes have been studied, including lipid and lipoprotein metabolism, as well as for their antiatherogenic and antioxidant properties.

Results of our study are in accordance with others researchers’ study that showed oleuropein could increase PON1 activity, and increase of PON1 activity has a positive correlation with HDL and positive correlation with the atherogenic index. Furthermore, researchers indicated that oleuropein is found to possess a novel and good antioxidant activity. Furthermore, researchers reported the role of oxidative stress as a central factor in onset and progression of nephrototoxic complications such as hyperlipidemia and hepatic damage. Therefore, numerous reports and our results that showed the efficacy of antioxidative supplements administration in the prevention of nephrototoxic complications. Since antioxidant therapy is as one of the most important treatment strategies for nephrototoxic patients for the prevention and slowing of nephrototoxic complications progression such as hyperlipidemia, hepatic damage.

Effect of oleuropein on serum lipid profile and atherogenic index

Nephrotoxicity significantly increased serum FBG, TG, TC, VLDL, and LDL concentrations in comparison with the control group. Treatment of nephrototoxic animals with oleuropein significantly inhibited the increase of serum FBG, TG, TC, VLDL, and LDL concentrations, atherogenic index, AC and CRR in comparison with the untreated nephrotoxic animals. Also treatment of nephrotoxic animals with oleuropein significantly inhibited decrease of serum HDL-C concentrations in comparison with the untreated nephrotoxic animals. There are reports that natural antioxidant such as alpha lipoic acid, vitamin C, vitamin E, coenzyme Q10, selenium and natural phenolic compounds have hypolipidemic effects. Furthermore, Andreadou et al. showed oleuropein could reduce serum levels of TC and TG in hypercholesterolemic rabbits.

Results of our study are in accordance with others researchers’ study that showed oleuropein could reduce serum lipid and lipoprotein level. Therefore, a natural antioxidant with hypolipidemic and antiatherogenic action of natural antioxidant may be due to the inhibition of dietary lipid absorption in the intestine or its production by liver or stimulation of the biliary secretion of C and C excretion in the faces. Furthermore, the mechanism of hypolipidemic and antiatherogenic action of natural antioxidant may be due to the inhibition of glycation lipoproteins, enzymes and proteins that involve lipid and lipoprotein metabolism. Although the detailed molecular protective mechanisms of oleuropein cannot be fully explained by our results, our results are satisfactory. Oleuropein as a natural antioxidant with multi beneficial properties can be introduced for inhibition of extress oxidative in patients.

Conclusion

This study showed that oleuropein has beneficial effects, in increasing the reduced serum PON1 activity and HDL level of nephrotoxic rats. Furthermore, this study showed that PON1 activity was correlated positively with HDL and negatively with atherogenic index. Furthermore, this study showed that oleuropein has beneficial effects, in reducing the elevated serum lipid peroxidation, lipid profile, and atherogenic index of nephrotoxic rats. Hence, attenuation of PON1 activity, lipid profile, and atherogenic index can decrease the risk of cardiovascular death in nephrotoxic patients.

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Conflict of Interests

Authors have no conflict of interests.

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