Research Paper: Renal Protective Effects of L-Carnitine on Lead-Induced Nephropathy in Wistar Rats

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Background: Lead is a heavy metal used in industries in developing countries. Lead exposure remains a widespread problem. Lead may induce molecular damage in the kidney as a consequence of Reactive Oxygen Species (ROS) formation, induction of caspase-3, and apoptosis.

Methods: Thirty male Wistar rats (Mean±SD weight: 300±20 g) were randomly divided into 3 equal groups: control (normal saline, oral), lead group (lead 100 mg/kg/d, oral) and lead+L-Carnitine (lead 100 mg/kg+L-Carnitine 200 mg/kg, intraperitoneal injection) for one week. At the end of the experiment, plasma creatine kinase activity, plasma creatinine and urea concentrations and plasma Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), catalase and nitric oxide levels were determined. Glutathione and malondialdehyde levels in renal tissue were also measured.

Results: Creatine kinase, creatinine and urea levels increased significantly, in the group treated with lead (P<0.05), compared to the control group. Administration of L-Carnitine in (Lead+carnitine treated group) significantly (P<0.05) decreased creatine kinase activity and plasma urea and creatinine contents. Enzymatic activity (SOD, GPx, and CAT) decreased significantly in the lead group, in comparison with the control group (P<0.05). Treatment with L-Carnitine significantly retrieved the depletion in enzyme activity (P<0.05). However, there were no significant differences in the GPx parameter between the Lead+carnitine group, in comparison with the control group.

Conclusion: L-Carnitine administration in rats with lead-induced nephropathy led to improved kidney protection, due to the reduction of Lipid Peroxidation (LPO). Furthermore, L-Carnitine prevents the adverse effects of Reactive Oxygen Species (ROS), which is an important biomolecules mechanism.

Keywords: Lipid Peroxidation (LPO), L-Carnitine, Reactive Oxygen Species (ROS), kidney, Lead

ABSTRACT

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1. Introduction

Lead is a heavy metal used in industries in developing countries. Lead exposure remains a widespread problem [1]. Research has revealed that encounter with this heavy metal at any level is unsafe in children and many studies have demonstrated the harmful impact of lead exposure in the development of the body systems. Some studies suggest a relationship between renal problems and exposure to lead [2]. Exposure to lead occurs due to contamination of water and food resources and air pollution. Lead has a lot of toxic effects on various organs of the body, including the kidney [3-5].

Lead exposure can affect the development of the kidney in the fetal or the neonatal period, or result in renal impairment in adults [2, 6]. Early lead exposure may alter glomerular development that may cause renal disease, even after lead has already disappeared from the body. This may explain the etiology of some idiopathic renal diseases, like poorly developed renal system and eventually renal impairment [7, 8]. One study showed that the deposition of iron in proximal tubular cells of the kidney, together with histopathological lesions are associated with remained lead in the kidney, after lead administration in rats through drinking water. The role of erythrocytes in lead-induced nephrotoxicity in vitro was investigated in a co-culture system and in vivo in rats. The erythropagocytosis of lead exposure in renal tubular cells contributes to lead-related renal damage [9].

L-Carnitine is an amino acid derivative, mainly obtained from food. It is also synthesized endogenously from the essential amino acids like methionine and lysine [10, 11]. Carnitine is an essential cofactor for translocation of active fatty acid. This transport is essential to allow β-mitochondrial oxidation of long-chain fatty acids and provide energy to cells [12]. L-Carnitine is an antioxidant that plays a preventive role in accumulation of lipid peroxidation end-products [13, 14].

L-Carnitine has successfully been applied in the treatment of different diseases. Beneficial roles of L-Carnitine have been demonstrated in several studies, such as cisplatin-induced injury of renal and small bowel, gentamicin nephrotoxicity, kidney ischemia-reperfusion, and chronic renal failure [15, 16]. L-Carnitine plays a preventive role in Reactive Oxygen Species (ROS) formation and decreases biomolecule damage. Furthermore, L-Carnitine has a sequestering role on ROS and a preventive role against renal toxicity of cisplatin, cyclosporine A, gentamicin, and doxorubicin [17-20]. The present study investigated the protective role of L-Carnitine against lead-induced nephrotoxicity in rats.

2. Materials and Methods

Animal treatment

Thirty male Wistar rats (Mean±SD weight:300±20 g) were housed in a standard controlled room, temperature (22°C±2°C), lighting (12:12 h light:dark cycle), humidity (40%-50%) and were fed with standard pallet diet. All animals had access to food and water ad libitum. The study was approved by University of Tabriz in accordance with the protocol of animal care and use, as well as the protocol provided by the Research Ethics Committee of the Medical Education in Iran (adapted April 17, 2006). Rats were randomly divided into 3 equal groups: 1. Control group: rats that received a single oral administration of normal saline for 7 days; 2. Lead group: rats that were treated with a single oral dose of lead 100 mg/kg/d for 7 days; and 3. Lead+L-Carnitine group: rats that were treated with a single oral dose of lead 100 mg/kg/d+L-Carnitine (200 mg/kg, intraperitoneal), for 7 days. All groups received the same volume of administration.

Biochemical and Hematological assay

The rats were anesthetized with chloroform on day 8 and blood samples were taken by decapitation. Blood samples were immediately divided as per following: 1. EDTA-containing anticoagulant tube for hematologic tests; and 2. A sample for the biochemical test. Blood serum was separated using centrifugation at 3000 rpm for 10 minutes and was stored at -20°C until use. The kidney tissues were stored at -80°C until use.

Urea, creatinine, and creatine kinase activity assays

Plasma levels of creatinine and urea were evaluated with standard diagnostic kits (Pars Amazon, Iran). The activity of plasma Creatine Kinase (CK), an indicator of myopathy, was measured spectrophotometrically, using a standard diagnostic kit (Pishtaz Teb, Iran).

Nitrate and nitrite assay

Nitrate and nitrite are the main oxidation products of Nitric Oxide (NO) which are used as an indicator of nitric oxide synthesis. The NO content of plasma was determined spectrophotometrically at 545 nm, based on the Griess reaction, using a mixture of naphthyl enylene diamine and sulfanilamide [21]. The samples were deproteinized. Nitrate was reduced to copper-coated cadmium...
nitrite in glycine buffer (pH 9.7). The sum of nitrite and nitrate contents (mmol/L) was measured using a standard sodium nitrate solution.

**Determination of glutathione and malondialdehyde levels**

The samples of kidney tissue were homogenized in iced KCl for the measurement of glutathione and Malondialdehyde (MDA) levels. The homogenates were centrifuged at 3000 rpm for 15 minutes. MDA concentrations and lipid peroxidation indicator were measured spectrophotometrically at 532 nm and expressed as nmol MDA/mg in kidney tissue [22]. The Glutathione level (GSH) was determined spectrophotometrically at 412 nm, according to the Ellman’s method and was expressed in mmol/g [23].

**Antioxidant enzyme measurement**

The activity of Superoxide Dismutase (SOD) was determined with commercial kit for SOD activity measurement (Randox Co.) SOD activity is expressed in U/mg proteins [24]. Catalase activity based on decomposition of H2O2 was measured spectrophotometrically, at 230 nm. The results are expressed as a speed/mg constant of homogenized protein [25]. The activity of Glutathione Peroxidase (GPx) was assayed by Randox commercial kit at 340 nm [26]. The results are reported as U/mg proteins. The protein content of the plasma samples was determined [27].

### 3. Results

As shown in Table 1, concentrations of CK, creatinine and urea significantly increased (P<0.05) in the lead group, compared to the control group. L-carnitine in (Lead+Carnitine group) significantly (P<0.05) decreased CK, urea and creatinine content in plasma. The plasma concentrations of NO significantly decreased in the lead group, compared to the controls. The nitric oxide levels were significantly elevated in the lead+carnitine group, compared to the lead group.

### Table 1. Plasma biochemical test results in the control and treated rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>CK (U/L)</th>
<th>NO (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>42.6</td>
<td>0.67</td>
<td>350.21</td>
<td>27.34</td>
</tr>
<tr>
<td>Lead (n=10)</td>
<td>302.53</td>
<td>4.23</td>
<td>839.21</td>
<td>15.84</td>
</tr>
<tr>
<td>Lead+Carnitine (n=10)</td>
<td>185.28</td>
<td>2.04</td>
<td>651.58</td>
<td>21.21</td>
</tr>
<tr>
<td>SEM</td>
<td>1.77</td>
<td>0.02</td>
<td>27.48</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Data are presented as the Mean±SEM. CK: Creatine Kinase; NO: Nitric Oxide; a: Significant difference between controls, lead, Lead+Carnitine group, P<0.05; b: Significant difference between controls and the lead group, P<0.05; c: Significant difference between lead group and lead+Carnitine group, P<0.05.

### Table 2. Oxidative stress parameters in the control and treated rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>MDA (nmol/mg Protein)</th>
<th>GSH (µmol/g Tissue)</th>
<th>SOD (U/mg Protein)</th>
<th>CAT (µ/mg Protein)</th>
<th>GPx(U/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>0.43</td>
<td>3.46</td>
<td>9.48</td>
<td>0.56</td>
<td>2.06</td>
</tr>
<tr>
<td>Lead (n=10)</td>
<td>0.79</td>
<td>0.93</td>
<td>7.25</td>
<td>0.28</td>
<td>1.14</td>
</tr>
<tr>
<td>Lead+Carnitine (n=10)</td>
<td>0.61</td>
<td>1.93</td>
<td>7.92</td>
<td>0.42</td>
<td>1.70</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
<td>0.09</td>
<td>0.14</td>
<td>0.02</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Data are presented as the Mean±SEM. MDA: Malondialdehyde; GSH: Glutathione; SOD: Superoxide Dismutase; CAT: Catalase; and GPx: Glutathione Peroxidase. a: Significant difference between controls, lead, Lead+Carnitine group, P<0.05; b: Significant difference between controls and lead group, P<0.05; c: Significant difference between the lead group and lead+Carnitine group, P<0.05.
According to Table 2, the levels of MDA in the renal tissue significantly increased (P<0.05) in the lead group, compared to the controls. The lead+Carnitine group showed a significant decrease in MDA levels, compared to the lead group. The lead group showed a significantly decreased antioxidant enzymatic activity (SOD, GPx and CAT), compared to the control group. L-Carnitine significantly improved enzyme activity. However, there were no significant differences between the lead+Carnitine group and the controls with regard to the GPx parameter. The levels of GSH decreased in the lead group, compared to the controls. Administration of L-carnitine increased GSH levels significantly (P<0.05).

4. Discussion

The results showed that treatment with L-Carnitine reduced CK activity, BUN levels, creatinine and alleviated MDA production, and the reduction of GSH. L-Carnitine has antioxidant effects and plays protective roles against oxidative stress in various tissues, including the kidney [28, 29]. Administration of L-Carnitine has been shown to inhibit the formation of MDA in serum and renal tissues, in response to renal impairment, due to ischemia-reperfusion [30].

Carnitine improves the antioxidant enzymes activities including SOD, GPx and CAT and increases GSH levels, and reduces the MDA concentration in rat renal tissues [31]. One study showed that L-Carnitine prevents the formation of MDA and improves GSH levels in the heart, kidney, aorta and cavernous bodies, and decreases plasma levels of creatinine and urea in rats afflicted with chronic renal failure [32]. We demonstrated that treatment with L-Carnitine, restored the activity of SOD, CAT and GSH and decreased the levels of MDA in the lead+Carnitine group.

Nitric oxide plays a crucial role in moderating tissue lesions and normal blood circulation in the healthy kidney, as well as various pathological renal diseases. In the present study, treatment with L-Camitine significantly increased nitric oxide levels. Plasma or serum CK activity is a marker for muscle damage [33, 34]. The results showed a protective effect of L-Carnitine against muscle damage. Although L-Carnitine is known to protect renal tissue from chemotherapy agents, no studies investigated its effect on radiation-induced nephrotoxicity. Carnitine protects tissues through the elimination of oxygen radicals and other mechanisms [35-37].

This evidence may provide a preventive/therapeutic strategy for the lead-induced nephropathy. Previous studies reported antioxidant and protective effects of L-Carnitine against oxidative damage in various tissues, including the kidney [32, 38, 39]. Administration of L-Carnitine inhibited the MDA formation in the serum and renal tissue, in renal ischemia-reperfusion injury [13, 40, 41].

Although the exact mechanism of renal toxicity caused by lead is illustrated, there is evidence that lead can cause the formation of reactive oxygen metabolites and diminish the renal antioxidant enzyme activities [5, 42]. Studies showed that lead could induce DNA damage in renal tissue and that these effects are associated with free radicals formation, resulting in caspase-3-dependent apoptosis [42, 43].

5. Conclusion

The present study indicate that ROS formation, lipid peroxidation, the exhaustion of the antioxidant state and decrease of NO levels may be involved in the pathophysiological mechanisms of lead-induced nephrotoxicity. Although our data do not define the precise mechanism of action of L-Carnitine in preventing lipid peroxidation, the reduction of ROS and decrease in antioxidant depletion and the improvement of NO levels may be the putative mechanisms in this model. Eventually, we hypothesized that L-Carnitine improves tissue health by decreasing oxidative parameters in lead-induced nephropathy.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by University of Tabriz in accordance with the protocol of animal care and use, as well as the protocol provided by the Research Ethics Committee of the Medical Education in Iran (adapted April 17, 2006).

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Conflict of interest

The authors certify that they have no affiliation with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials dismissed in this manuscript.
References


