Therapeutic effects of pomegranate (*Punica granatum* L.) pith and carpellary membrane extract on lead-induced toxicity in rats

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Abstract

Pomegranate is an ancient edible fruit with various pharmaceutical bioactivities such as antioxidant, lipid regulation and anti-inflammation. In this study the effectiveness of pomegranate pith and carpellary membrane (PPCM) extract in treatment of experimental lead intoxication was assessed in rats. Female Wistar rats were exposed to 1000ppm lead acetate in drinking water for 35 days and treated thereafter with PPCM extract (100 and 200mg/kg, orally) twice a day for 35 days. The concentration of lead in blood, kidney, liver, bone and brain were measured using atomic absorption spectrophotometry. Treatment with PPCM extract reduced lead retention in blood and tissues. With the highest dose of PPCM extract, the greatest rate of reduction of lead concentrations were observed in brain (61%), blood (53%), and bone (34.5%). No significant changes were observed in copper, zinc and iron concentration of serum and liver, in neither doses of PPCM extract. In conclusion it was demonstrated that PPCM and carpellary membrane had therapeutic effect in the treatment of lead intoxication without any side effects on essential elements in blood and tissues of rats.

Keywords: punica granatum, lead intoxication, microelements, rat

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Introduction

Lead is a soft, malleable non-essential heavy metal with neither beneficial nor desirable nutritional effects on animals and humans. It is widely used in various industrial products, such as storage batteries, automobile radiator, paint pigments, glass, weights, ceramic, plastic and mortar. Organo-lead compounds, such as tetraethyl and tetramethyl lead are also used as additives in certain gasolines particularly in developing countries (Kosnnett 2001). On the other hand, lead is an industrial and environmental cumulative pollutant that affects all major organs inducing a broad range of physiological, biochemical and behavioral dysfunctions in both animals and humans (Patrick 2006a). Over the past few decades, along with lowering lead exposure, there has been growing evidence and concern that prolonged exposure to low levels of lead has proven serious risk factor for neurological, cardiovascular and reproductive diseases (Ahmed and Siddiqui 2007; Needleman 2009). Exposure to low levels of lead has been associated with behavioral abnormalities, learning impairment, decreased hearing, impaired cognitive functions and hypertension in human and experimental animals (Patrick 2006a; Patrick 2006b; Jakubowski 2011). Oxidative stress has been suggested as the primary mechanism for the pathogenesis of lead intoxication. Highly reactive oxygen species (ROS) produced aftermath of lead exposure may result in systemic mobilization and depletion of the cell intrinsic antioxidant defenses. Formation of ROS beyond the scavenging capacity of these antioxidant defense mechanisms results in accumulation of harmful free radicals and likelihood of oxidative damage of critical bio-molecules, such as enzymes, DNA, proteins and membranes lipids (Ahmed and Siddiqui 2007). The current approved treatment for lead intoxication is relied primarily on the administration of chelating agents that forms an insoluble complex with lead and removes the same from lead burdened tissue. However, most of these chelating agents suffer from many disadvantages (Domingo 1998; Kalia and Flora 2005). Administration of antioxidants such as vitamins and herbal drugs during chelation therapy has been found to be beneficial in increasing lead mobilization and providing recoveries in altered biochemical variables (Gurer and Ercal 2000; Hsu and Guo 2002; Patrick 2006b; Antonio-Garcia and Masso-Gonzalez 2008).

Pomegranate (Punica granatum L.), belongs to the family Punicacea and is an ancient edible fruit widely grown in Iran and some tropical and subtropical countries. It is known as a sacred fruit conferring powers of fertility, abundance, and good luck. It also features prominently in the ceremonies, art and mythology (Jurenka 2008). Pomegranate has been used in various regions and folk or traditional medical systems as a food supplement or a medicine because of its enormous compounds with lots of activities and without toxicity (Wang, Ding et al. 2010). Various pharmaceutical bioactivities such as antioxidant, lipid regulation, anti-hypertension, gastroprotection, hepatoprotection, anti-diarrhea, anti-helminthes, antibacterial, antivirus, anti-inflammation, anti-angiogenesis, anti-diabetes and immunomodulation, for pomegranate have been appointed (Li, Zhang et al. 2003; Huang, Peng et al. 2005; Haidari, Ali et al. 2009; Osman, Ahmed et al. 2011; Bhandari 2012; Jasuja, Saxena et al. 2012; Shiban, Al-Otaibi et al. 2012). El-Ashtoukhy (2008) used pomegranate pith, to remove lead from aqueous solution (El-Ashtoukhy, Amin et al. 2008). In the current investigation we studied the protective effect of PPCM and carpellary membrane extract against lead intoxication in rats.

Materials and methods

Pomegranate Pith and Carpellary
Membrane Extract preparation

Fresh pomegranates (Feizabad cultivar) were obtained from local market. PPCM and carpellary membrane were manually separated, dried in room temperature and powdered in a grinder. The powder was extracted using distilled water. The extract was filtered through a mesh for removal of particles. Fine particles were also separated by centrifugation at 4000g for 15min at 4°C.

Animals and experimental design

Thirty two female Wistar rats weighing 170-200g were obtained from Razi Institute of Mashhad (Mashhad, Iran). The animals were kept in polypropylene cages and fed a standard pelleted laboratory animal diet (Javaneh Khorasan-Iran). The rats were allowed free access to food and water. The animals were allowed to acclimatize for 14 days before the experiment. Then, the rats were randomly divided into 4 groups (n=8). Group 1 was received no lead acetate in drinking water during the experiment. Group 2-4 was received 1000ppm lead acetate (Merck, Darmstadt, Germany) in drinking water for 35 days (Aslani, Najarnezhad et al. 2009). Group 2 was received no PPCM extract. Groups 3 and 4 were received 100 and 200 mg of PPCM extract, respectively, twice daily by stomach gavage for 35 days. At the end of the experiment the rats were anesthetized with ether, bled by cardiac puncture. Liver, kidney, brain and femur were removed. Liver was also used for measurement of essential elements, copper, zinc and iron. All the procedures concerning the use of animals were approved by the Animal Welfare Committee of Ferdowsi University of Mashhad.

Determination of lead in blood

Blood samples were dissolved in 65% nitric acid (Merck, Darmstadt, Germany) and ammonium vanadate (Merck, Darmstadt, Germany) and then centrifuged (2,500 rpm for 5 min) (Najar-Nezhad, Aslani et al. 2008). Lead concentrations were determined by atomic absorption spectrophotometer (Perkin-Elmer AS800, Massachusetts, U.S.A.) at 283.3 nm wave-lengths using graphite furnace.

Determination of lead, copper, zinc and iron in tissues

Tissue samples were digested in a 1:1 mixture of 95-97% sulfuric acid (Merck, Darmstadt, Germany) and 65% nitric acid (Merck, Darmstadt, Germany), with a slight modification of the wet-ashing technique (Najar-Nezhad, Aslani et al. 2008). Lead, copper, zinc and iron concentrations were determined by atomic absorption spectrophotometer (Perkin-Elmer AS800, Massachusetts, U.S.A.) at 283.3, 324.8, 248.3 and 213.9 nm wave-lengths, respectively. Lead was measured through graphite furnace atomic absorption spectrometry (Table 1) and Flame atomic absorption spectrometry was used for measurement of liver copper, zinc and iron (Table 2). The certified reference material NIST-1577C (bovine liver) was used for validation purposes. Detection limits were defined as 3 times the standard deviation of an average of procedural blanks. The corresponding values for lead, copper, zinc and iron were obtained equal to 0.03, 0.1 0.3 and 0.1ppb. The repeatability of the measurement of the analytical signals usually was not worse than 1% (Table 3). To control the stability of the analytical signals the measurements for the standard solutions were repeated before and after measurements of the samples.

Table 1. Instrumental conditions for determination of lead in liver.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Temperature (°C)</th>
<th>Cleaning volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>0.7</td>
<td>120-400</td>
<td>2450</td>
</tr>
</tbody>
</table>
Table 2. Instrumental conditions for determination of copper, zinc and iron in liver.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Wavelength, nm</th>
<th>Slit width, nm</th>
<th>Flame type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>324.6</td>
<td>0.7</td>
<td>Air–acetylene, oxidizing</td>
</tr>
<tr>
<td>Zn</td>
<td>248.3</td>
<td>0.2</td>
<td>Air–acetylene, oxidizing</td>
</tr>
<tr>
<td>Fe</td>
<td>213.9</td>
<td>0.7</td>
<td>Air–acetylene, oxidizing</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the found and certified values of lead, copper, zinc and iron in the certified reference material.

<table>
<thead>
<tr>
<th>Certified material</th>
<th>Bovine liver NIST-1577C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certified value ppb</td>
</tr>
<tr>
<td>Pb</td>
<td>62.8</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td></td>
</tr>
</tbody>
</table>

Determination of copper, zinc and iron in serum

Serum concentration of copper, zinc and iron were determined by an automated chemistry analyzer (Biotecnica Targa 3000, Rome, Italy) using commercial kits (Shimazmoon, Tehran, Iran for copper; Giesse Diagnostic, Rome, Italy for zinc; and Parsazmoon, Tehran, Iran for iron). The data were checked for errors and compared with written reports. Out layers were rechecked to ensure that values were accurate.

Statistical analysis

The statistical analyses were carried out using the SPSS 11.5 software (SPSS Inc., Chicago, IL, U.S.A.). The significance of difference was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni procedure to compare means among different groups. Results are expressed as mean ± standard deviation and significance was defined as values of $p<0.05$.

Results

Lead concentration in blood and tissues

Lead concentration in various tissues of rats exposed to lead is given in Table 4. For lead exposed untreated group (group II), the distribution of lead was in the following order: bone > kidney > liver > blood > brain. Lead treated animals showed significantly ($p < 0.05$) higher levels of lead in blood, liver, kidney, bone and brain compared to those of the control animals. Groups treated with PPCM and carpellary membrane extract (groups III and IV) had entire body tissues lead burden (2.57 and 2.41µg/kg respectively) significantly ($p < 0.05$) lower than that of lead exposed untreated group (3.30µg/kg).

Table 4. Lead concentration in blood (µg/l) and tissues (µg/gr) of various groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.043±0.030$^a$</td>
<td>0.192±0.031$^a$</td>
<td>0.257±0.025$^a$</td>
<td>0.002±0.000$^{ad}$</td>
<td>1.932±0.198$^a$</td>
</tr>
<tr>
<td>II</td>
<td>64.43±10.792$^{bc}$</td>
<td>0.309±0.128$^a$</td>
<td>2.474±0.197$^b$</td>
<td>0.044±0.004$^b$</td>
<td>10.527±0.609$^b$</td>
</tr>
<tr>
<td>III</td>
<td>50.50±5.961$^{cd}$</td>
<td>0.160±0.040$^d$</td>
<td>1.390±0.230$^{cd}$</td>
<td>0.025±0.008$^{bc}$</td>
<td>8.729±0.778$^{bc}$</td>
</tr>
<tr>
<td>IV</td>
<td>30.44±19.152$^{abcd}$</td>
<td>0.373±0.127$^{cd}$</td>
<td>2.215±0.424$^{bd}$</td>
<td>0.017±0.006$^{cd}$</td>
<td>6.898±1.575$^{cd}$</td>
</tr>
</tbody>
</table>

Mean ± S.D. (eight values). In each column, a, b, c and d was significantly differed ($p<0.05$)
Moreover, kidney lead concentration of group treated with 100mg/kg PPCM and carpellary membrane extract (group III) was significantly ($p < 0.05$) lower than that of lead exposed untreated group (group II); and blood, brain and bone lead concentration of group treated with 200 mg/kg PPCM and carpellary membrane extract (group IV) was significantly ($p < 0.05$) lower than those of lead exposed untreated group (group II).

**Liver and serum concentration of copper, zinc and iron**

Copper, zinc and iron concentration in liver and serum of various groups are given in Table 5 and Table 6, respectively. Groups treated with PPCM and carpellary membrane extract (groups III and IV) had liver copper, zinc and iron burden lower than those of lead exposed untreated group (group II), but this reduction was not significant ($p > 0.05$). Group treated with 100mg/kg pomegranate pith and carpellary membrane extract (group III) had serum copper, zinc and iron burden lower than those of lead exposed untreated group (group II), but this reduction was not significant ($p > 0.05$). Group treated with 200 mg/kg capillary and carpellary membrane extract (group IV) had lower serum copper and more serum zinc and iron burden than those of lead exposed untreated group (group II), but these changes were not significant (carpellary $>0.05$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cu (µg/gr)</th>
<th>Zn (µg/gr)</th>
<th>Fe (µg/gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.880±0.596</td>
<td>25.750±0.590</td>
<td>341.18±36.915</td>
</tr>
<tr>
<td>II</td>
<td>4.185±0.291</td>
<td>25.250±0.750</td>
<td>324.35±20.113</td>
</tr>
<tr>
<td>III</td>
<td>3.228±0.481</td>
<td>23.925±2.303</td>
<td>283.25±30.539</td>
</tr>
<tr>
<td>IV</td>
<td>3.093±0.852</td>
<td>21.433±3.404</td>
<td>309.70±62.328</td>
</tr>
</tbody>
</table>

Mean ± S.D. (eight values).

**Discussion**

Many compounds have been experimented in animal models for treatment of lead intoxication (Khan, Mostofa et al. 2008; Aslani, Najarnezhad et al. 2009; Alcaraz-Contreras, Garza-Ocañas et al. 2011; Aslani, Najarnezhad et al. 2011). Pomegranate (Punica granatum L.) is an ancient edible fruit with various pharmaceutical bioactivities (Wang, Ding et al. 2010). In this study the administration of different doses of PPCM and carpellary membrane extract to lead exposed rats significantly lowered tissue lead levels. The present study is the first report that showed pomegranate could reduce lead in the body. PPCM and carpellary membrane extract at a dose of 200 mg/kg was more effective in removing lead from bone and brain. Several studies have revealed that at least some portion of lead in the bone is accessible to chelators, as evidenced by the fact that EDTA and DMSA can reduce lead concentrations in the bone (Cory-Slechta, Weiss et al. 1987; Tendon, Singh et al. 1994; Jones, Singh et al. 1997). However, PPCM and carpellary membrane extract has several advantages over those chelators. First of all, PPCM extract is a natural product and there is no report for its side effect, while several adverse effects have been reported for conventional lead chelators (Gwaltney-Beant 2003; Meldrum and Kok 2003; Casteel 2006). Furthermore, PPCM and carpellary membrane extract has antioxidant activity (Shiban, Al-Otaibi et al. 2012) and may depress pathogenesis of lead intoxication. There are convincing data showing that lead toxicity is associated with oxidative stress and an increase in production of reactive oxygen species (Patrick 2006b; Anderson and Adenuga 2007). It has been shown that
Phenolic compounds have antioxidant activity and can reduce blood lead levels (Aksu, Didin et al. 2012). Pomegranate is a fruit rich of various polyphenolic compounds such as tannins, flavonoids, alkaloids, organic acids (Wang, Ding et al. 2010), so it can decrease the blood and tissues lead levels by reducing the absorption of lead from gastrointestinal tract and/or by limiting the retention of lead in metabolism.

### Table 6. Copper, zinc and iron concentration in serum (µg/dl) of various groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>299.88±10.246</td>
<td>134.12±8.711</td>
<td>319.62±19.998</td>
</tr>
<tr>
<td>II</td>
<td>330.50±11.712</td>
<td>199.00±18.139</td>
<td>328.00±15.552</td>
</tr>
<tr>
<td>III</td>
<td>269.14±13.702</td>
<td>133.71±9.551</td>
<td>298.29±13.525</td>
</tr>
<tr>
<td>IV</td>
<td>263.76±39.388</td>
<td>228.80±99.344</td>
<td>392.80±44.903</td>
</tr>
</tbody>
</table>

Mean ± S.D. (eight values).

Some degrees of mineral depletion, copper and zinc in particulars, are an adverse effect associated with administration of most of current heavy metal chelators (Anderson 1999; Kalia and Flora 2005). Although the clinical importance of such effects is not fully understood, it is suggested that teratogenic potential of most chelators is, at least in part, due to induced trace element deficiencies (Taubeneck, Domingo et al. 1992; Domingo 1998). In the present study no adverse effect on copper, zinc or iron concentration of serum and liver was seen following PPCM and carpellary membrane extract administration.

Different doses of lead have been used to induce lead intoxication in rats and to study therapeutic efficacy of different compounds (Patra and Swarup 2004; Alabbassi, Hussain et al. 2008; Arrak 2010; Kilikdar, Mukherjee et al. 2011). A dose of 1000 ppm lead through drinking water for 35 days has significantly increased blood and tissues lead values. The highest accumulation of lead was detected in bone, followed by kidney, liver, blood and brain. This could be due to different biokinetic pattern of lead distribution in various tissues (Thuppil and Kaushik 2012). The bone is recognized as a tissue to store a considerable portion of the body burden of lead (Zmudzki, Bratton et al. 1983) so the highest lead concentration in the bone observed in this study was not unexpected. The lowest concentration of lead was observed in the brain. The brain has low lead uptake capacity, but it has high tenacious lead retention capacity. Moreover, lead is distributed unevenly in the brain, which depends on the amount of lead exposure. At low levels, the lead concentration in different regions of the brain is significantly correlated with the potassium concentrations, indicating that lead is mostly accumulated in the cell rich parts of the brain like the hippocampus (Grandjean 1978; Petit, Alfano et al. 1983). Only at very high concentrations of lead, the blood-brain barrier is broken and lead enters neuronal tissue (Thuppil and Kaushik 2012).

Alonso et al. (2004) stated metal binding regions of metallothionines, which is increased in lead intoxication, have also specific area for copper and zinc, so lead can disrupt trace element metabolism (Klaassen, Liu et al. 1999; Alonso, Montana et al. 2004). In this study copper, zinc and iron levels in serum and liver of lead exposed rats (group 2) did not differ compared to those of negative control rats (group 1).

The present study is the first report that showed pomegranate could reduce lead in the body without any significant \(p<0.05\) depletion of copper, zinc or iron concentration of serum and liver.

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References


Najar-Nezhad, V., Aslani, M.R. and Balal-


بررسی اثرات درمانی عصاره پوسته سفید اتار در مسمومیت با سرب در رت

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چکیده

اثار یک میوه خوراکی با اثرات درمانی زیاد مانند اثرات آنتی اکسیدانی، ضد التهابی و تنظیم کننده چربی خون است که از مدتی قبل شناخته شده است. در این مطالعه به بررسی اثرات درمانی عصاره پوسته سفید اتار در مسمومیت با سرب تجربی در رت پرداخته شده است. برای این منظور استفاده از میوه خوراکی برای روز دریافت کردن، زمان دریافت 1000 ppm درایافت کردن روز دریافت بود. میزان تغییر عصاره پوسته سفید شیره در نیازهای آب و برق تغییر می‌کند، که با استخوان و مغز یا دستگاه چربی اندازه‌گیری می‌شود. عصاره با پوسته سفید و غشاهای داخلی اتار در بازه کاهش شیره در مقدار (30%) و استخوان (55%) ثبات یافته و هیچ تغییر معنی داری در غلغظت مس، رژیم و آهن خون و کبد می‌باشد. این کنونه باعث تغییر عصاره پوسته سفید اتار می‌شود. در این مطالعه سنجش شد که عصاره پوسته سفید و غشاهای داخلی اتار بدون هیچ تغییر معنی داری در اندازه‌های ضروری خون و بافت، دارای اثرات درمانی بر مسمومیت با سرب در رت است.

واژگان کلیدی: اتار، مسمومیت با سرب، میکروالمانها، رت

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