Effect of Pomegranate Juice on Bone Calcium Content and Body Weight of Adult Mice

Fatemeh Parvin,*1 Malihezaman Monsefi,1 Tahereh Talaei-Khozani2

1. Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran
2. Department of Anatomy, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Pomegranate juice has several antioxidant components such as flavonoids and mineral materials such as sodium and potassium. In this study the effects of pomegranate juice on bone calcium content and body weight of adult mice were survived.

Materials and Methods: In this study two doses (3.3 and 6.6 ml/kg) of pomegranate juice (PJ) were gavaged to female mice for 30 days. Animals were weighed at days of 0, 5, 10, 15, 20, 25 and 30. Bone calcium contents were measured by flame photometer.

Results: Bone calcium content of PJE treated mice increased but it was not significant statistically. Pomegranate juice did not affect body weight.

Conclusion: Pomegranate juice extracts even its high dose did not show any side effect on body weight and tissues of adult female mice.

Introduction

Pomegranate is grown in different parts of Iran. Its juice has several flavonoids as an antioxidant and mineral materials as sodium and potassium [1]. Pomegranate juice inhibits angiotensin activating enzyme and regulate systolic blood pressure [2]. Pomegranate juice is a rich source of punicic acid and prevents diet-induced obesity and insulin resistance obesity [3]. Pomegranate juice in a fasted state prevents increase in weight, body mass index and fat mass [4]. Administration of pomegranate extract to ovariectomized mice increased bone volume and the trabecular number [5]. Pomegranate extract may be a useful approach for prevention of onset and severity of inflammatory arthritis [6].

Our previous research revealed mice embryos femur length and femur and tibia osteogenesis index increased significantly in pomegranate juice treated group compared to the control group. Bone calcium contents of pregnant mice increased significantly after pomegranate juice administration [7]. These results indicated positive effects of pomegranate in pregnant animals and their fetuses.

Several researches were evaluated pomegranate effects in osteoarthritis, osteoporosis of menopausal syndrome model ovariectomized mice or pregnancy that accompanied with hormonal imbalance. In this study, the effects of pomegranate juice on bone of adult female mice in physiologic condition studied.

Materials and Methods

Extract preparation: In this applied study, pomegranate CV. Rabbab was collected from Eig (Estahban, Fars Province). Pomegranates were peeled then arils were separated. Husks (seeds) and water juice of arils were separated. Husks (seeds) and water juice of arils were desiccator. Eighty grams extract were obtained from 100 g arils. Extract were condensed to 100 ml using of 60 ºC water bath and desiccator. Forty grams extract were obtained from 100 ml condensed pomegranate juice.

Animals grouping: Balb/c female mice weighting between 20-30 g were obtained from the animal house of Razi Institute in Shiraz.

The animals were adapted to the laboratory condition for two weeks at controlled temperature (22±2ºC) and a period of 12 hours light, and 12 hours darkness. Principles of laboratory care established by the National Institute of Health (NIH Publication, No. 85-23, revised 1985) were followed. Mice were divided randomly into one control group and two experimental groups (N=6). Control group were treated with 200 μl distilled water and first experimental group was fed with 0.0033 μl/kg pomegranate juice (PJ) in 200 μl distilled water. For probable side effect evaluation pomegranate juice of 0.0066 μl/kg pomegranate juice in 200 μl distilled water were fed in second experimental group. Animals were gavaged for 30 days in distinct time and weighed in 0, 5, 10, 15, 20, 25 and 30 days of extract administration.

For toxicity examination of high dose administration of extract, the mice liver and kidney were removed and after fixation with formalin, dehydration with ethanol and clearing with xylol were embedded in paraffin. Five μm-thick sections were cut from paraffin-embedded specimens and stained with hematoxylin-eosin. The sections were examined under light microscope and probable changes were evaluated. Bone density assay: Posterior limbs of mice were removed.
The dried bones were ashed at 520°C for 20 h in oven (Model 10500, Thermolyne, USA). The bone was then powdered. 0.03 gram bone powder was dissolved in 250 γ HCl and was diluted with 31 ml distilled water. The Calcium contents were measured with a flame photometer (Model 8515, JENWAY). At first standard solution were done by Tukey and LSD as post hoc tests one-way ANOVA test. Intergroup statistical differences of mice’s body weight in days of 5, 10, 15, 20, 25 and 30 in all treated group with PJE (Table 1).

### Table 1. The mean and standard deviation of body weight (g) of mice treated to different doses of pomegranate juice extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 25</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.90±5.48</td>
<td>23.68±5.23</td>
<td>23.64±5.35</td>
<td>24.11±4.81</td>
<td>24.40±4.83</td>
<td>25.05±4.80</td>
<td>24.93±4.83</td>
</tr>
<tr>
<td>High dose of pomegranate juice extract</td>
<td>25.08±0.99</td>
<td>25.78±0.64</td>
<td>26.67±0.67</td>
<td>26.74±1.14</td>
<td>26.74±1.14</td>
<td>26.11±0.89</td>
<td>27.07±1.30</td>
</tr>
</tbody>
</table>

The effects of pomegranate juice on bone Calcium contents: Bone Calcium contents of pomegranate juice and its high dose treated groups increased at compared to the control group but it was not significant (Table 2).

### Table 2. The mean and standard deviation of bone Calcium contents of mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bone calcium content (µg/kg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02780±0.0002683</td>
</tr>
<tr>
<td>Pomegranate juice extract</td>
<td>0.02798±0.0005139</td>
</tr>
<tr>
<td>High dose of pomegranate juice extract</td>
<td>0.02794±0.0007162</td>
</tr>
</tbody>
</table>

The results indicated that high dose of pomegranate juice elevated bone Calcium contents but it was not significant. Also there were no changes in body weight and bone histological structure. The body weight of mice at the beginning of experiments and after days 5, 10, 15, 20, 25 and 30 in all treated pomegranate juice did not show any different as compared to the control group. Therefore, it concluded that this extract is safe and did not change somatic growth. The results of Vroegrij et al., showed body weight of mice decreased when they treated to high fat diet and pomegranate seed oil for 12 weeks [3].

Our data revealed that bone Calcium contents of pomegranate juice and its high dose treated groups increased but it was not significant. Our previous research showed femur and tibia osteogenesis index and bone calcium contents of pregnant mice and their fetuses increased in PJE treated group [7]. Pomegranate extract was clinically effective on bone loss of menopausal syndrome model ovariectomized mice [5]. In two latest research hormonal changes during pregnancy and menopause could change the effects of pomegranate extract on bone tissue. Pomegranate contains not only the steroidal estrogen, estrone but also non-steroidal phytoestrogens including the comesten, coumestrol, and the isoflavones, genistein and daidzein. Phytoestrogens showed agonists and antagonist effects to estrogen, therefore according to the hormonal balance, pomegranate extract have different effects. Stimulatory effects of Genistein on bone mineralization have reported in vitro [8, 9]. Anabolic effect of isoflavones on bone metabolism reported in animal calcified tissues and cell cultures [10]. Mardon et al., also demonstrated BMD (bone mineral density) increased significantly in fetuses that their mother exposed to diet rich isoflavones [11]. Therefore, dietary phytoestrogens could play a role in bone remodeling [12]. It may be conclude that isoflavones of pomegranate showed estrogenic effects in females mice but these effects is more prominent in pregnant mice and their fetuses than to non-pregnant mice.

With attention to our data, it conclude pomegranate juice did not show any effects on body weight and bone calcium content of female adults mice with normal diet at physiologic condition (not in pregnancy or menstruation).

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**Authors’ Contributions**

All authors had equal role in design, work, statistical analysis and manuscript writing.

**Conflict of Interest**

The authors declare no conflict of interest.
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Shiraz University.

*Corresponding author at:
Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran.
E-mail: parvin-ff@yahoo.com

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