

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

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Article information	Abstract
<p>Article history: Received: 11 Apr 2012 Accepted: 21 Aug 2012 Available online: 5 Jan 2013 ZJRMS 2014; 16(3): 64-66</p> <p>Keywords: Bone calcium Body weight Mouse Pomegranate</p>	<p>Background: Pomegranate juice has several antioxidant components such as flavenoids 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.</p> <p>Materials and Methods: In this applied 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.</p> <p>Results: Bone calcium content of PJE treated mice increased but it was not significant statistically. Pomegranate juice did not affect body weight.</p> <p>Conclusion: Pomegranate juice extracts even its high dose did not show any side effect on body weight and tissues of adult female mice.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Pomegranate is grown in different parts of Iran. Its juice has several flavenoids 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 puniceic 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 by mixer. Four hundred milliliters pomegranate water was obtained from 500 g arils. Extract were condensate to 100 ml using of 60°C water bath and desiccator. Eighty 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±2°C) 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.

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

Groups	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	23.90±5.48	23.68±5.23	23.64±5.35	24.11±4.81	24.40±4.83	25.05±4.80	24.93±4.83
Pomegranate juice extract	25.49±4.19	25.51±3.86	25.98±2.93	26.37±3.60	26.83±3.08	26.66±2.38	26.57±2.49
High dose of pomegranate juice extract	25.08±0.99	25.78±0.64	26.67±0.67	26.74±1.14	26.74±1.14	26.11±0.89	27.07±1.30

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

Groups	Bone calcium content (µg/kg dry weight)
Control	0.027800±0.0002683
Pomegranate juice extract	0.027988±0.0005139
High dose of pomegranate juice extract	0.027940±0.0007162

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 µl 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 prepared and their Calcium contents were measured. Linear diagram and its equation were drawn using of Microsoft Excel. $Y=0.0134X+0.0221$ (Y =bone calcium measurement, X =Calcium content of standard solution).

Statistical analysis: The data of body weight and bone Calcium contents were analyzed by SPSS software and one-way ANOVA test. Intergroup statistical differences were done by Tukey and LSD as post hoc tests.

Results

Body weight changes: There are no significant differences of mice's body weight in days of 5, 10, 15, 20, 25 and 30 in all treated group with PJE (Table 1).

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).

Histological examination: There were no pathological changes such as hypertrophy, congestion, malignancy and fibrosis in liver and kidney of mice even in high dose of pomegranate juice administration.

Discussion

Our 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 Vroegrilk 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 coumestrol, 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 female 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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