Hypocholesterolemic effect of pomegranate's peel (water extract) supplemented yoghurt in hypercholesterolemic rats

Sally S. Sakr and Sherein A. Abou Dawood

Received: December 2014 / Accepted: January 2015

Abstract Uses of agricultural wastes such as juice-making wastes as alternative low-cost sources of phenolic compounds are on increase. In this case, Pomegranate is a good example. About 50% of its weight is peel, which is usually discarded as waste. However, it has a significant portion of polyphenols. For these reasons we used experimental animals to study the effect of pomegranate juice or pomegranate's peel extract incorporated in yoghurt manufacture as a heart healthy dairy product. Our results indicated that yoghurt with either pomegranate's juice or peel's water extract has good effect on lowering Total lipid, Total tri glycerides and Total cholesterol. In contrast the lipid peroxidation determined by Malondialdehyde decreased. In the same trend, the histopathological changes in liver and heart of rat groups fed orally with Pomegranate's juice yoghurt (JG) and Peel's extract yoghurt (EG) showed marked improvement and no histopathological changes to be nearest to negative control group (NC). It could be concluded that JG and EG treatments under our study are useful for the treatment of hypercholesterolemia.

Keywords: Yogurt, pomegranate, peel extract, cholesterol

Introduction

Many epidemiologists have argued that economic development pushes populations through a nutrition transition shifting food preferences from traditional diets characterized by low saturated fat to less healthy diets. Unhealthy diets cause obesity with increased the risk of high cholesterol and fatty streak development, and consequently greater risk of cardiovascular disease (Tuso et al. 2013). A large majority of epidemiological studies have demonstrated that elevated plasma triglycerides and/or reduced plasma concentrations are associated with increased cardiovascular risk (Poli et al., 2008; Da Luz et al., 2008). As a result, a high level of serum cholesterol has been identified clearly as a risk factor for atherosclerosis and coronary heart disease (Chen et al., 2004). High cholesterol diet is however regarded as an important factor in the development of cardiac diseases since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease. Moreover, hypercholesterolemia is shown to be one of the major risk factors of atherosclerosis by increasing plasma low-density lipoprotein (LDL) levels (Levine et al., 1995).

Nowadays, consumers have become more aware of the relationship between good health and food intake, particularly from naturally derived foods such as fruits, vegetables and probiotic dairy products. The concept of "functional food" involves and requires the use of bioactive ingredients or the presence of natural healthy bioactive molecules in foods (Coisson et al., 2005).

Yoghurt is typical fermented milk consumed all around the world. As a major dairy product it has long been recognized as having desirable health effects, and it is not surprising that most consumers consider yoghurt to be 'healthy' (Lourens-Hattingh and Viljoen, 2001). Codex Standard classified yoghurt as fermented milk and could contain a maximum of 50% (m/m) of nondairy ingredients such as fruits and vegetables as well as juices, purees or pulps. Fermented milk products have been recommended as dietary supplements because of their hypocholesterolemic effect in humans (Mann, 1977) and rats (Suzuki, Kaizu, & Yamauchi, 1991).
Pomegranate (Punica granatum L.) is an important fruit of tropical and subtropical regions, which originated in the Middle East and India and it is one of the oldest known fruit. It is mentioned in the Ebers papyrus of Egypt written in about 1550 BC (Faria et al., 2006). And 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. It is widely reported that pomegranate exhibits antiviral, antioxidant, anticancer, and antiproliferative properties. Pomegranate is consumed fresh and in processed form as juice, wines, flavors, and extracts. Commercial pomegranate juice has the highest antioxidant activities compared to other fruit juices, red wine, and green tea and currently is a high value product in the agricultural market. The pomegranate antioxidant activity is typically higher in commercial juices extracted from whole pomegranates than in experimental juices obtained from the arils only. About 50\% of the total fruit weight corresponds to the peel, which they are usually discarded as waste even a significant portion of polyphenols are often present in high concentrations in the outer parts of fruits. Also, it is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ETs), and proanthocyanidin compounds (Li et al., 2006), minerals (Mirdelghani & Rahemi, 2007), and complex polysaccharides (Jahfar et al., 2003). Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of pomegranates have been reported by various researchers (Davidson et al., 2009; Tezcan et al., 2009). Moreover, it attracted attention due to its apparent antibacterial activity, wound-healing properties, anti-cancer activity, anti-atherosclerotic, anti-inflammatory and anti-oxidative capacities. This antioxidant capacity has been mainly attributed to the water-soluble polyphenols, proanthocyanidins, and hydrolysable tannins. Wang et al. (2011) reported that these components can be extracted from peel by using water which has the economic and safety merits as an environmental friendly method for food and pharmaceutical industry because it is nontoxic and gives an acceptable yield of those components.

According to the therapeutic roles of fermented milk and pomegranate's peel extract, the objective of this research was to produce a heart - healthy diet by incorporating pomegranate's juice or pomegranate's peel extract in yoghurt manufacture.

**Materials and Methods**

Full ripened high quality pomegranates were purchased from Egyptian local markets. Pomegranates were cleaned with water and dried with a clean cloth. Fruit pulps with its seeds (edible part) were separated manually. The juice was extracted from pulp using an electric juicer, and then freeze dried (The final product was seemed to be sticky). The moisture content of freeze dried sample was determined by using hot air oven drying at 105°C until constant weight was achieved according to the AOAC methods (AOAC,1990) and the total solids was 80%.

Fruit peels were then cut into small pieces, washed and dried in an air oven at 50°C for 24h. The dried clean peels were then ground and peel's water extract was prepared according to Wissam et al., 2012 and Wang et al., 2011. Briefly, dried and ground peel was extracted in a thermostatic water bath with a 15:1 (w/w) ratio water/sample at 90°C for 2 min. The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 min. and the total solids of the water extract was determined by using hot air oven (AOAC, 1990) and it was 6%.

Fresh cow skim milk with 9.3 % TS was obtained from the Dairy science Department, Faculty of Agriculture, Cairo University. Skim milk powder with 1.25% fat, 36% protein, 51% lactose and 4% moisture was obtained from Arla Foods, Sweden and it was used to standardize the yoghurt milk total solids content to 16%. Freeze-dried lactic culture for Direct Vat Set (YC-X11, thermophilic yoghurt culture Yo-Flex®) was a gift from Misr Food Additives Company, Giza, Egypt and it was used as a starter.

**Preparation of yoghurt**

Fresh cow skim milk was divided into three portions. First portion was converted to normal plain yoghurt after standardization with skim milk powder to 16% TS and it served as control. The second portion (initial TS = 9.3%) was standardized to 16% TS with skim milk powder (3.35 grams) and freeze dried pomegranate juice (4.19 grams) both to cover 6.7% TS. The third portion (initial TS = 9.3%) was also standardized to 16% TS by using skim milk (3.35 grams) and peels' water extract (55.8 grams) both to cover 6.7% TS.

**Animal experiments and diet**

Thirty male albino rats of Wister strain weighing 180-200g were used in this study. The animals were obtained from the Agriculture Research Center (ARC), Giza, Egypt. All animal experiments were done under the experimental animal ethics applied in the ARC. The rats were fed ad libitum on basal diet (BD) and water for seven days as an adaptation period. Rats were then randomly divided into five groups; each consists of 6 rats. Only the first group (Negative control group) was fed on basal diet, but all other groups were fed on hypercholesterolemic diet all over the experimental period (3 months long). Composition of the experimental diets is shown in Table 1.

After increasing total blood serum cholesterol level, different
animal groups were treated according to information in Table 2.

Blood analysis

At the end of the experimental period Rats were sacrificed and blood samples were collected and serum were separated and frozen at -20º C. The total lipids (TL), total triglycerides (TG), total cholesterol (TC), Malondialdehyde was measured using assay kits (Bio diagnostic, 29 Tahreer st., Dokki, Giza, Egypt) following the manufacturer recommendations.

Histopathological examination

At the end of the experiment, changes in liver and heart were evaluated. Livers and hearts were prepared for histopathological examination at the Department of Pathology, Faculty of Veterinary Medicine; Cairo University; Giza, Egypt. Samples of liver and heart were embedded in paraffin and cut into 3-5 μm thick sections. The sections were stained with hematoxylin-eosin (H&E, 400X) for examination by light microscope.

Statistical analysis

A randomize complete design with one factor was used for statistical analysis. All data were expressed as mean value ±SD for 6 rats in each group. The treatment means were compared by least significant difference (L.S.D.) by using Assistat-Statistical Assistant software according to Snedecor and Cochran (1976).

Results and Discussion

Changes in lipid profile parameters and antioxidant capacity

Data presented in table 3 shows the changes in lipid profile parameters of blood and lipid peroxidation (MDA) between the five different groups. The levels of total lipids significantly differed between groups. It was 350 mg/dl in PC group with a high significant increase comparing with 140 mg/dl for NC group. While, YG, JG and EG groups showed significantly reduction of TL levels especially for JG (130 mg/dl) and EG (170 mg/dl) groups. PC group (positive control group) showed a significant increase in TG level (136 mg/dl) by about 97% increase with an opposite trend for YG, JG and EG groups (74, 83 and 113 mg/dl). Our results also showed a significant decrease in the concentrations of total cholesterol (TC) for EG group indicating the good effect of incorporating peel's water extract in yogurt for producing a heart healthy dairy product. This result seemed to be parallel to those found by Abdel-Rahim et al., 2013 who demonstrated the effect of beans, seeds and peel as well as their mixture on lipid profile and reducing TC in albino rats. Regarding the three treated groups YG, JG and EG it seemed to be no significant effect between each other as well as in comparison to the negative control group (NC). As a lipid peroxidation parameter, MDA in serum was determined on all rat's groups under the experiment, to understand the effect of using yogurt, pomegranate’s juice and peel’s water extract in prevention of peroxidative tissues damage. In the present study, lipid peroxidation concentration was the highest in PC group among all other groups. It increased by 170 % from NC group. While, it significantly decreased by 56% in EG group. MDA for EG group was the closest to those of NC group which indicate the good antioxidant effect of incorporating pomegranate's products in

<table>
<thead>
<tr>
<th>Component %</th>
<th>Basal diet</th>
<th>hypercholesterolemic diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Starch</td>
<td>60</td>
<td>37.75</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mixture**</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Bile salts</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Sheep tail fat</td>
<td>-</td>
<td>20</td>
</tr>
</tbody>
</table>

*Mineral mixture consists of: Cal di phosphate (150g), Sodium chloride (100g), Magnesium oxide (4g), Zinc oxide (2g), ferrous sulphate (3g), Copper sulphate (1g), Calcium iodide (100mg), Cobalt sulphate (20mg), Sodium selenite (20mg).

**Vitamin mixture consists of: Vit. A (80000000 IU), Vit. D3 (15000000 IU), Vit. E (10000000 IU), Vit. K3 (20000 mg), Vit. B1 (5000 mg), Vit. B2 (5000 mg), Vit. B6 (2000 mg), Vit. B12 (80 mg), Folic acid (500 mg), Nicotinic acid (60000 mg), Pantothenic acid (400000 mg), Copper (100 mg), Manganese (1200 mg), Zinc (375 mg), Iron (1000 mg), Cobalt (20 mg).
Table 2  Different animal groups treatments

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control group (NC)</td>
<td>Fed on basal diet only.</td>
</tr>
<tr>
<td>Positive control group (PC)</td>
<td>Fed on hypercholesterolemic diet only.</td>
</tr>
<tr>
<td>Yogurt group (YG)</td>
<td>Fed on orally dose (2g per day for 6 weeks) of plain yogurt by gavage feeding.</td>
</tr>
<tr>
<td>Pomegranate juice group (JG)</td>
<td>Fed on orally dose (2g per day for 6 weeks) of pomegranate juice yogurt by gavage feeding.</td>
</tr>
<tr>
<td>Peel extract group (EG)</td>
<td>Fed on orally dose (2g per day for 6 weeks) of peel extract yogurt by gavage feeding.</td>
</tr>
</tbody>
</table>

Table 3  Lipid profile (mg/dl) and changes in malondialdehyde (n mol/ml) of rats in different examined groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>PC</th>
<th>YG</th>
<th>JG</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>140±40c</td>
<td>350±50a</td>
<td>226±15b</td>
<td>130±44c</td>
<td>170±10bc</td>
</tr>
<tr>
<td>TG</td>
<td>69±31c</td>
<td>136±19a</td>
<td>74±8c</td>
<td>83±16c</td>
<td>113±6ab</td>
</tr>
<tr>
<td>TC</td>
<td>62±24b</td>
<td>96.67±6a</td>
<td>72±12ab</td>
<td>70±12ab</td>
<td>66±5b</td>
</tr>
<tr>
<td>MDA</td>
<td>1.063±.0.16cd</td>
<td>2.867±.45a</td>
<td>2.333±.67ab</td>
<td>1.900±.79bc</td>
<td>0.467±.25d</td>
</tr>
</tbody>
</table>

Each value represents the mean of 6 rats (mean ± SD) Means having different letters in the same row are significantly different (p<0.05)

NC: Negative control group, PC: positive control group, YG: Yogurt group, JG: Pomegranate juice group, EG: Peel extract group

Figure 1. Histopathological examination of liver from some rats in different examined groups (H&E, 400 X)

NC: Negative control group, PC: positive control group, YG: Yogurt group, JG: Pomegranate juice group, EG: Peel extract group
yogurt manufacture. In this regards, our results agreed with the finding of Elbandy and Ashour, 2012, who reported that the levels of MDA were reduced according to the supplementation of rat's diet with different pomegranate's preparations. In general, our results agreed with Basu and Penugonda, 2008, who concluded that Pomegranate has shown significant antiatherosclerotic, anti-hypertensive, antioxidant, and anti-inflammatory effects in human subjects and mouse models and the principal mechanisms of action of pomegranate may include increased serum antioxidant capacity and decreased lipid peroxidation.

Histopathological changes

The histopathological changes in liver and heart of rats from different groups are shown in Figs. 1 and 2. Negative control group (NC) liver showed the normal histopathological structure of hepatic lobule with slight hydropic degeneration of some hepatocytes. Positive control group (PC) liver represented the bad effect of hypercholesterolemic diet which showed congestion of central vein and hepatic sinusoids as well as vacuolar degeneration with ballooning of hepatocytes. In comparison with negative control group (Fig. 1), yogurt group (YG) shows slight congestion of central vein and slight hydropic degeneration of hepatocytes while both Pomegranate juice group (JG) and Peel extract group (EG) appeared to be closer in structure to NC group with decreased hepatocyte degeneration. EG group rat liver showing marked improvement in hepatocytes than other treated groups.

As shown in Fig. 2, NC group's photo showed normal cardiac myocytes with no histopathological changes. As an opposite effect, the PC group's photo shows focal inflammatory cells infiltration and vacuolation of cardiac myocytes. Heart of rat...
from group YG showed congestion of myocardial blood vessel and a slight intramuscular oedema. Rat's heart from group JG showed no histopathological changes in all photos except for one individual (small photo inside the main one), which presented focal myocarditis and intramuscular oedema. In the same direction rat heart from group EG showing no histopathological changes.

**Conclusions**

Hypercholesterolemia is defined as excessive high blood cholesterol levels, and is a strong risk factor for many negative cardiovascular events (Stapleton, 2010). According to our results we can conclude the promising effect of pomegranate's peel water extract as a food supplement especially when combined with fermented dairy products as a therapeutic food with hypocholesterolemic effect. More work need to be carried out for application of pomegranate's peel water extract in different food especially dairy products.

**References**

Abdel-Rahim EA, El-Beltagi HS, Romela RM (2013) White bean seeds and pomegranate peel and fruit seeds as hypercholesterolemic and hypolipidemic agents in albino rats. Grasas Y Aceites, 64: 50-58


