Complementary Therapies in Clinical Practice 22 (2016) 44–50

Contents lists available at ScienceDirect

Complementary Therapies in Clinical Practice

journal homepage: www.elsevier.com/locate/ctcp

Effects of pomegranate extract supplementation on inflammation in overweight and obese individuals: A randomized controlled clinical trial☆

Banafshe Hosseini [a], Ahmad Saedisomeolia [a, b, *], Lisa G. Wood [c, **], Mehdi Yaseri [d], Sanaz Tavasoli [e]

[a] Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[b] School of Medicine, Western Sydney University, NSW, Australia
[c] School of Biomedical Science and Pharmacy, University of Newcastle, NSW, Australia
[d] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[e] Urology and Nephrology Research Center, Department of Urology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 29 October 2015
Accepted 3 December 2015

Keywords:
Pomegranate extract
Obesity
Inflammation
Lipid peroxidation
Polyphenols

ABSTRACT

The study was designed to determine the effect of thirty days of pomegranate extract oral supplementation on plasma inflammatory and oxidative stress biomarkers as well as serum metabolic profiles, in overweight and obese individuals. In this randomized, double-blind, placebo-controlled study 48 obese and overweight participants were randomly assigned to receive either 1000 mg of pomegranate extract, or a placebo, daily for 30 days. At baseline, and after 30 days of treatment, anthropometric parameters, dietary intake, plasma concentrations of malondialdehyde, interleukin-6 and hyper sensitive-C reactive protein and levels of serum lipids, glucose and insulin were assessed. Thirty days of PE supplementation resulted in a significant decrease in mean serum levels of glucose, insulin, total cholesterol, LDL-C, and plasma MDA, IL-6 and hs-CRP. HDL-C significantly increased following the PE versus the PL intervention. Our study suggests that pomegranate extract consumption may reduce complications linked with obesity.

1. Introduction

The prevalence of obesity and overweight is increasing exponentially worldwide due to population growth, urbanization, unhealthy eating habits and the increasing prevalence of physical inactivity [1]. Currently, more than one billion adults worldwide are overweight; in addition, at least 300 million are clinically obese [2]. Based on the conducted studies in Iran, the prevalence of overweight is 31.7% and 32.3% among men and women, respectively. Moreover, 11.1% of men and 25.2% of women are suffering from obesity [3]. The major adverse health consequences associated with obesity include cardiovascular disease, type 2 diabetes, hypertension, dyslipidemia, respiratory disorders and cancers [4]. These are diseases that develop as a result of the increased insulin resistance, chronic low-grade inflammation, and increased oxidative stress that occurs with obesity [4,5].

Findings from several studies have revealed that obesity is an inflammatory disorder [6,7]. Tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) are expressed by adipocytes and are correlated with total fat mass [8]. Hence low-grade systemic inflammation occurs in obesity, evident as a two to three-fold increase in plasma concentrations of cytokines such as TNF-α, IL-6 and C-reactive protein (CRP) [6,9]. The cytokines secreted by adipose tissue have a major role in the development of atherosclerosis and, consequently, cardiovascular disease (CVD) [4]. Lipid peroxidation also plays a major role in the development of cardiovascular disease and diabetes [10]. Although, the mechanisms underlying the association between obesity and increased risk of these diseases are not fully

☆ Sponsor: Tehran University of Medical Sciences (grant no: 9306161-27760).
* Corresponding author. School of Medicine, Western Sydney University, Room: 30.3.23, NSW, Australia. Tel.: +61 2 46203212.
** Corresponding author. School of Biomedical Science and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Newcastle, Australia.
E-mail addresses: A.Saedisomeolia@westernsydney.edu.au (A. Saedisomeolia), lisa.wood@newcastle.edu.au (L.G. Wood).

http://dx.doi.org/10.1016/j.ctcp.2015.12.003
1744-3881/

understood, the presence of excessive quantities of reactive oxygen species (ROS) is regarded as a principal factor in the pathophysiology of such diseases [10], as the host antioxidant defenses are inadequate to protect against the quantity of ROS [10].

Studies indicate that treatment with pharmaceuticals has a limited effect on obesity, and complementary nutrition, specifically fruit and vegetable consumption, may assist in achieving weight loss [11]. Pomegranate is a fruit which is a good source of polyphenols such as ellagic acid and gallic acid. Polyphenols have proven anti-oxidant and anti-inflammatory effects in the human body [12]. The antioxidant activity of pomegranate is three times stronger than many other dietary sources of polyphenols, such as green tea [9]. Additionally, recent investigations have indicated that pomegranate extract contains abundant anthocyanins (such as delphinidin, cyanidin and pelargonidin) and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallagic acid and ellagic acid) and possesses strong antioxidant, anti-inflammatory and anti-tumor properties both *in vivo* and *in vitro* [13,14]. There are limited studies that have evaluated the effects of pomegranate extract on inflammatory responses and the results of these studies are inconclusive. Furthermore, most of the studies have been conducted in animal models [15]. A randomized placebo controlled trial of 20 patients with obesity who consumed 120 ml pomegranate juice or placebo for one month demonstrated that weight, body mass index (BMI) and body fat were decreased due to pomegranate juice [16]. Insulin secretion and insulin sensitivity were not modified by administration of pomegranate juice [16]. In another 12-week study, intake of pomegranate juice by type 2 diabetic patients showed a significant reduction in the level of all oxidative stress and inflammatory biomarkers compared to placebo, including plasma IL-6 and CRP. Fasting plasma glucose (FPG) and insulin resistance index were not affected by pomegranate juice [9].

The aim of this study was to evaluate the effect of thirty days of oral supplementation of pomegranate extract on plasma malondialdehyde (MDA), hs-CRP, IL-6, BMI, serum lipid profile, glucose levels and homeostasis model assessment insulin resistance (HOMA-IR) in overweight and obese subjects. We hypothesized that oxidative stress would be reduced in the group supplemented with pomegranate extract (1000 mg daily) compared to the control group. Moreover, metabolic parameters such as insulin resistance, blood glucose levels, serum total cholesterol concentration, serum total triglyceride concentration, HDL and LDL cholesterol concentration were hypothesized to be modified by the pomegranate supplement.

2. Methods and material

2.1. Study design

The study was conducted as a randomized, double-blind, placebo-controlled clinical trial for a period of thirty days, to determine the effect of daily 1000 mg pomegranate extract supplementation (equivalent in polyphenol content to approximately 1 L of fresh pomegranate juice [17]) on 48 overweight and obese ($25 < BMI < 40\ kg/m^2$), adults aged 30–60 years. The sample size of the study was calculated according the expected changes in the concentration of IL-6 due to the intervention. According to a previous study [9], we expected that the intervention would achieve a 4.8 ng/L reduction in plasma IL-6, with standard deviation (SD) of 5.6 ng/L. To have 80% power to detect this difference in IL-6, assuming $\alpha = 0.05$, we required 21 subjects per group, a total of 42 individuals to complete the intervention. Assuming 10% dropout rate, a sample size of 48 subjects was required. The duration of intervention was selected according to previous studies [16,18,19]. Each participant provided written and informed consent (approved by TUMS Ethics Committee, reference no: 9306161-27760). This clinical trial has been also registered in the Iranian Registry of Clinical Trials at htpp://www.irct.ir with the following identification number: IRCT2015062722934N1, and was conducted between November 2014 and May 2015 by the School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran.

2.2. Study population

The exclusion criteria were greater than 3 kg of self-reported weight change during the last3 months, a history of coronary artery disease, stroke (including transient ischemic attack), congestive heart failure, or malignancy; a history of use of any dietary supplements within 3 months prior to the study; abnormal liver, kidney or thyroid gland function; clinically significant inflammation within the respiratory, digestive, or genitourinary tract, or in the oral cavity, pharynx, or paranasal sinuses; a history of infection in the 3 months prior to the study.

2.3. Randomization

A stratified randomized permuted block (with block size randomly set at 2 or 4) was generated by an independent biostatistician and stored in a sealed envelope until the completion of the trial. Subjects were stratified according to gender and body mass index (3 levels: $25 \leq BMI < 30$, $30 \leq BMI < 35$ and $35 \leq BMI < 40\ kg/m^2$).

2.4. Supplementation regimen

All participants were randomized to receive pomegranate fruit extract using two capsules of 500 mg pomegranate extract (whole fruit with 40% ellagic acid) per day, or two identical placebo capsules, per day for 30 days. The supplements contained no yeast, dairy, egg, gluten, soy or wheat, sugar, starch, salt, preservatives, artificial color, flavor or fragrance. The other ingredients of the PE capsules namely were microcrystalline cellulose, silicified microcrystalline cellulose, stearic acid, and silica. The study capsules and placebo had identical appearance. The placebo contained pure microcrystalline cellulose. Dietary intake of participations was assessed using a 24-h dietary recall questionnaire (for two weekdays and one weekend day) at baseline and study completion. Participants were advised to maintain their usual diet during the study period, including maintenance of their usual energy intake and previous eating habits. Adherence to the study protocol was enhanced using weekly phone calls. Subjects were asked to return remaining supplements, and adherence was calculated via the pill count back method. Average adherence was 98% and 2 subjects were excluded for non-compliance (<90% of capsules consumed). One subject in the placebo group reported that they experienced stomach cramps during the intervention and was withdrawn from the study. In addition, one subject was withdrawn because medical treatment for an unrelated condition was initiated during the intervention and 2 subjects were lost to follow up (Fig. 1).

2.5. Biochemical assessments

Ten ml blood was collected from all participants after a 12-h overnight fast, at baseline and at study completion, into EDTA-coated and serum tubes. Blood samples were centrifuged at 3000 rpm for10 min. Plasma and serum were separated and stored at −80 °C. Serum glucose, total cholesterol, LDL-C, HDL-C and triglyceride were determined through an enzymatic colorimetric