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Original Article

Natural pomegranate juice reduces inflammation, muscle damage and increase platelets blood levels in active healthy Tunisian aged men

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ABSTRACT

Currently, it's well established that aging is associated with various health problems that may interfere with the maintenance of a good nutritional status. Otherwise, pomegranate (POM) was shown to prevent or treat various disease risk factors in adults. However, its efficacy is still not well widespread in elderly population. Therefore, the purpose of the present study is to investigate the effect of natural pomegranate juice (POMj) rich in polyphenols on the blood levels of selected biochemical parameters using older adults.

Twelve active healthy aged men (age: 60 ± 5 years) volunteered to participate in this randomized study. Before and after the supplementation period fasting blood samples were collected, heart rate (HR) and systolic arterial pressure (SAP) were recorded. Supplements of placebo (PLA) or POMj were taken twice daily (250 ml \times 2) for 15 days.

Paired simple *t*-test showed a significant difference between PLA and POMj supplementation effects on systolic blood pressure (SAP), creatinine (CRE), hematological and muscle damage parameters and C-reactive protein (CRP) ($p < 0.01$) with lower values using POMj. Similarly, a significant differences were shown for platelets PLT ($p < 0.01$) with higher values using POMj supplementation. POMj rich in polyphenols seems to have a power anti-inflammatory effect and to be an effective treatment for patients who suffer from the thrombocyto-penia disease. Therefore, aged populations are advised to add natural POMj to their daily nutrition behavior.

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1. Introduction

Currently, it's well established that aging is associated with various changes in body composition¹ and physiological functions² that affect metabolism and make older adults more prone to age-related diseases, functional impairment, and physical inability.³ Indeed, with advancing age, there is a progressive decrease in lean body mass and an increase in body fat.¹ These changes in body composition were shown to reduce (10–15%) muscle mass and contractile force (Sarcopenia syndrome)⁴ and increase muscle damage and pigments and fatty substance inside cells which begin

to function abnormally.⁵ In this context, studies in elderly population showed that cells of the immune system act more slowly and may develop an autoimmune disorder.^{6,7} Additionally, a reduction in total body water, blood volume, active bone marrow and blood cells (i.e., red and white blood cells) production was observed with aging and was found to create a slower response to blood loss and anemia and to reduce the ability to resist infection.⁶ Concerning the hormonal and cardiovascular functions, continual decrease in growth hormone and testosterone which stimulate muscle development and continual rise in blood pressure and artery stiffness were found with aging.^{4,5} In the other hand, it's well documented that pomegranate juice (POMj) has several health benefits.⁸ Pomegranates can help prevent or treat various disease risk factors including high blood pressure, high cholesterol, oxidative stress, hyperglycemia, and inflammatory activities.^{9,10} In fact, supplementation on POMj –contains high levels of polyphenols– was shown

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to: (i) reduce free radicals, oxidative stress and lipid peroxidation (-65%),¹¹ (ii) reduce risk of cardiovascular diseases¹²⁻¹⁴ by reducing high systolic blood pressure (-12%), carotid artery thickness (-30%), low-density lipoprotein cholesterol (LDL) oxidation (-90%), and by enhancing myocardial blood flow (+17%)¹⁵ and antioxidant status (+130%),⁶ and (ii) promote inhibition of some cellular transcription factors such as the nuclear factor NF- κ B (NF- κ B), tumor necrosis factor α (TNF α) and cyclooxygenase-2 (COX-2),¹⁶⁻¹⁸ block their production and combat inflammatory degeneration of cartilage to protect articulations¹⁹. Additionally, in a spectrophotometric comparative study between POMj, red wine, blueberry juice, cranberry juice, orange juice and green tea,²⁰ POMj was found to have the highest capacity to destroy free radicals and reducing LDL oxidation and inhibit cellular oxidative stress in macrophages, with an antioxidant activity (Trolox equivalent antioxidant capacity: TEAC = 18–20) three times higher than red wine and green tea (6–8 TEAC).¹³ The efficacy of POMj is mainly due to its high bioavailability of total phenolic content and antioxidant capacity compared to other polyphenols such as resveratrol²¹ and to its important contain of polyphenols.²² In this context, despite the higher dose of polyphenols in natural POMj, most of studies in this field have investigated the commercial POMj.^{9,12,23} Additionally, the available studies in this field have only investigated the effect of Pomegranate on the oxidant/antioxidant balance in adult population. However, to the knowledge of the authors no previous studies have investigated the effect of this natural “super fruits”¹⁷ on the muscle damage and hematological parameters in older adults. Therefore, the purpose of the present study is to investigate the effect of natural POMj on the blood levels of selected biochemical parameters (i.e., blood levels of C-reactive protein, muscle damage and platelets) among elderly male subjects.

2. Method

2.1. Participants

Twelve active healthy aged men (age: 60 \pm 5 years (mean \pm SD)) volunteered to participate in this study. The participants were classified as active since they were engaged in regularly physical activities during the past three years. Additionally, participant were recruited on the basis that they didn't have any acute/chronic diseases or injuries, they avoid highest polyphenol rich foods and they didn't use any antioxidant (e.g., vitamin E, A, C etc.) or anti-inflammatory during the experimentation period and one month before. After receiving a thorough explanation of the protocol, each participant provided written informed consent to take part in the experiment. The study was conducted according to the Declaration of Helsinki. The protocol and the consent form were fully approved by the institutional review board “Habib Bourguiba University hospital ethics committee before the commencement of the assessments.

2.2. Experimental design

Upon recruitment, the subjects visited the laboratory (08:00–09:00 in the morning) the first day and each subject received 30 packs (\times 250 ml) of POMj and PLA. Then subjects were randomized to daily consume 0.5 L of POMj and PLA during 15 days²⁴ for each one with 4 weeks of wash-out period in between. This long wash-out period was chosen based on the results of Matthaïou et al.²⁴ who showed that the delayed beneficial effects of POMj could persist up to 3 weeks after the interruption of consumption. Before and after the supplementation period fasting blood samples were collected, heart rate (HR) and systolic arterial pressure (SAP) were

recorded. Each 500-mL of the tested POMj contained 2.56 g of total polyphenol while, Placebo juice didn't contain polyphenols.⁸ The antioxidant polyphenolic content of juices was determined using the HPLC method and their quantization was performed by comparing with calibration curves constructed using various concentrations of authentic samples.^{8,24}

2.3. Dietary records

To assess the adequacy of nutrient intake, a consecutive dietary record over 7 days was completed. All participants received a detailed verbal explanation and written instructions on data collection procedures. Participants were asked to continue with their usual dietary habits during the period of dietary recording with avoiding foods high in polyphenols and to be as accurate as possible in recording the amounts and types of food and fluid consumed. A list of common household measures, such as cups and tablespoons, and specific information about the quantity in each measurement (grams, etc.) were given to each participant. Each individual's diet was calculated using the Bilnut 4 software package (SCDA Nutrisoft, Cerelles, France) and the food composition tables published by the Tunisian National Institute of Statistics in 1978. Estimated nutrient intakes were referred to reference dietary intakes for healthy olds people and the daily nutriment data showed that total calorie, macronutrient, and micronutrient intakes are situated in the interval of the reference dietary intakes for healthy Tunisian elderly.

2.4. Pomegranate and placebo supplementations

Supplements (7500 ml) of placebo (PLA) or pomegranate juice (POMj) were taken twice daily (i.e., 250 ml \times 2) during 15 days.^{13,14} The tested quantity of the natural POMj were prepared from a fresh pomegranate fruit 48 h before the beginning of the experimentation and were shipped frozen and stored at -4°C . No additional chemical products were added to the natural POMj. Each 500-mL of the tested POMj contained 2.56 g of total polyphenol, 1.08 g of orthodiphenols, 292.59 mg of flavonoids and 46.75 mg of flavonols.⁸ To ensure the accuracy of POMj consumption, subjects were reminded verbally through phone communication to consume the required quantity of supplements at the required times. Placebo juice consisted of an Pomegranate-flavored commercial drink contained water, citric acid, natural flavor and natural identical flavor (Pomegranate), sweeteners (aspartame \times (0.3 g/l), acesulfame K (0.16 g/l)), stabilizers (Arabic gum) and lacked antioxidants, fruit and vegetable extracts or vitamins.⁸ Placebo juice contains no polyphenols.

2.5. Blood sampling and analysis

Blood samples (6 ml) were collected for each participant from a forearm vein (i.e., 2.5 ml in tube contains EDTA for hematological parameters and 3.5 ml in tube contains Heparine for CRE, CRP and muscle damage parameters. Samples were placed in an ice bath and centrifuged immediately at 2500 rpm (\times g) for 10 min. Aliquots of the resulting plasma were stored at -80°C until analyses. To eliminate inter-assay variance, all samples were analyzed in the same assay run. All assays were performed in duplicate in the same laboratory with simultaneous use of a control serum from Randox. Haematological parameters (i.e., neutrophils (NEU), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) and PLT were generally performed within 3 h in a multichannel automated blood cell analyser Beckman Coulter Gen system-2 (Coulter T540, Germany). Muscle damage markers, CRE and CRP were determined spectrophotometrically using Architect Ci 4100 d'ABOTT (Germany). N-acetyl-L-cysteine method, the oxidation of

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