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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 diseases^{12–14} 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

lactate on pyruvate method and immunoturbidimetric method were respectively used to determine the activities of Creatinine kinase (CK), Lactate dehydrogenase (LDH) and CRP. The intra-assay coefficient of variation for these parameters kit were respectively 1.3%, 4.6%, 2.3%, 0.2% and 1.16%. Aspartate aminotransferase (ASAT) activities were determined by measuring NADH oxidation with 1.1% intra-assay coefficient of variation for the kit.

2.6. Statistical analyses

All statistical tests were processed using STATISTICA Software 20 (Stat-Soft, France). Following normality confirmation was assessed using the Shapiro–Wilks W-test. To analyze the effect of POMj supplementation in the tested parameters paired simple *t*-test was used. Effect sizes were calculated as Cohen's *d* to assess the practical significance of our findings. Significance was set at $p < 0.05$.

3. Results

Paired simple *t*-test (Table 1) showed a significant difference between PLA and POMj supplementation effects on SAP, hematological parameters (i.e., NEU, RBC, HGB, HCT and PLT), muscle damage markers (i.e., CK, LDH, ASAT) and CRP ($p < 0.01$) levels. Indeed, immediately after stopping the PLA consumption, all these parameters showed similar values ($p > 0.05$) compared to the control levels, whereas, immediately after stopping the PLA consumption, (i) the levels of SAP, HCT ($p < 0.001$), NEU, RBC, HGB and CRP ($p < 0.05$) were significantly reduced and (ii) PLT levels were significantly increase ($p < 0.05$) compared to the control levels. It should be noted that at any time point (using PLA or POMj), the values of all biological parameters were in the normal range of elderly.

4. Discussion

The lower SAP level registered using the POMj supplementation confirms –in healthy elderly population- the previous result of Aviram et al.⁹ who showed – in adults with high SAP- a reduction of high systolic blood pressure by 12% after a daily drink of 500 ml of POMj. Additionally, the lower levels of CRP and muscle damage level using POMj condition confirm –in elderly population- previous findings indicating that the fermented pomegranate polyphenols have a powerful anti-inflammatory effect explained by an inhibition of some inflammatory markers such as NF- κ -B, TNF α and COX-2.^{16–18} Furthermore, the higher values of the platelets levels using the POMj supplementation compared to the PLA one,

suggest that POMj could be also an effective treatment for patient who suffer from the thrombocytopenia disease (i.e., disorder in which there is an abnormally low amount of platelets sometimes associated with abnormal bleeding).²⁵ However, confirming this suggestion using patients with thrombocytopenia disease as participants is necessary.

Likewise, the present effect of POMj – which consists in a potent antioxidant supplementation^{9,10,16,17} – in reducing levels of some hematological parameters, of muscle damage and inflammatory levels, suggests a strong relation between the muscle damage, inflammatory, immune and oxidative process. This suggestion is in line with previous studies of (i) Lui et al.²⁶ who showed a strongly correlation between the malondialdehyde (MDA) and the CK, WBC and NE, (ii) of Cudney et al.²⁷ who suggested that lipid peroxidation could be in the origin of hematological and muscle damage alteration, and (iii) of Tidball et al.²⁸ who suggested a causal relation between inflammatory and muscle damage responses.

This efficacy of POMj could be attributed to its high content of polyphenol and nitrate. Indeed, previous studies on polyphenols supplementation have reported an increase in blood flow, vessel dilation²⁹ and endothelial function³⁰ and indicate a protective role of polyphenols against cardiovascular diseases,^{31–33} oxidative stress^{34–37} and inflammation.^{30,38} This potent protective effect of polyphenol-rich food has been explained by the effect of the reducing polyphenols and their metabolites in plasma (i) on the concentrations of other endogenous antioxidants and reducing agents, and (ii) on the absorption of pro-oxidative food components, such as iron.³⁹ Similarly, supplementation with nitrate has been shown to reduce blood pressure⁴⁰ and oxygen uptake⁴¹ and to enhance oxygenation (e.g., blood flow) to skeletal muscle,⁴² mitochondrial efficiency⁴³ and exercise performance and tolerance.^{41,44} This potential ergogenic effect of the nitrate-rich supplementations could be explained by enhanced endothelial nitric oxide synthase expression and nitric oxide production.⁴⁵

In the other hand, in comparison with POM Wonderful bottle (Los Angeles, CA) previous study by Trombold et al.²³ (i.e. total polyphenol content of 650 mg in each 480-ml bottle, during 9 days), has reported no beneficial effect of the commercial POMj on muscle damage levels; while in the present study using natural POMj that contains 2.56 g/500 ml, we showed a reduction in the levels of muscle damage markers among elderly population. The discrepancy between results could be attributed to the high content of polyphenols used in the present study compared to the commercial one (i.e., 2.56 g vs 650 mg/0.5 L) used by Trombold et al.²³ Indeed, it has also been found that supplement rich on polyphenol possess a high biological activity during stressed or pathological situation (i.e., characterized by higher inflammatory and oxidative levels) and have also protective effects against cardiovascular disease, cancer, atherosclerosis and rheumatoid arthritis.^{9,13,15}

5. Conclusion

In conclusion, natural POMj seems to be a potent anti-inflammatory, anti-muscle damage and anti-thrombocytopenia treatment among elderly population. Therefore, it's advised to add such supplementation in the behavioral nutrition of elderly population.

6. Limits

This is the first study that investigates the effect of natural POMj on some physiological parameters in elderly population. However, some limits should be noted. Indeed, the number of participants is too limited; so it would be better to confirm these results using

Table 1
Mean values (mean \pm SD) of the tested parameters following 15 days POMj and PLA supplementations.

Variables	PLA	POMj
SAP (mmHg)	12.73 \pm 0.96	11.89 \pm 0.37 ^P
<i>Hematological parameters</i>		
NEU (10 ³ / μ l)	05.24 \pm 2.12	04.63 \pm 1.90 ^P
RBC (10 ⁹ / μ l)	06.17 \pm 0.54	05.49 \pm 0.59 ^P
HGB (g/dL)	18.10 \pm 2.8	16.01 \pm 1.46 ^P
HCT (%)	59.14 \pm 4.72	47.69 \pm 4.07 ^P
PLT (10 ³ / μ l)	142.1 \pm 32.8	186.3 \pm 43.6 ^P
<i>Muscle damage markers</i>		
CK (UI/L)	292.4 \pm 42.2	221.1 \pm 19.6 ^P
LDH (U/l)	169.7 \pm 24.9	146.0 \pm 23.3 ^P
ASAT (UI/l)	24.11 \pm 7.61	20.32 \pm 5.29 ^P
<i>Inflammatory marker</i>		
CRP (mg/l)	01.41 \pm 0.91	01.20 \pm 0.88 ^P

P: Significant difference between POMj and PLA effect at $p < 0.05$.

large population. Additionally, the tested parameters cover only some physiological functions; therefore it is suggested to investigate in further research the effect of POMj on liver and renal function and calcium, and vitamin D level important in muscle function.

Declaration of interest

The authors report no conflicts of interest and no funding source. The results of the present study do not constitute endorsement by ACSM. The authors alone are responsible for the content and writing of the paper. None of the authors have any competing interests in the manuscript

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