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Beneficial effects of pomegranate peel extract on plasma lipid profile, fatty acids levels and blood pressure in patients with diabetes mellitus type-2: A randomized, double-blind, placebo-controlled study

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ABSTRACT

Pomegranate peel contains high levels of various phytochemicals. We evaluated the effects of pomegranate peel extract (PoPEx) consumption on plasma lipid profile, fatty acids (FA) level and blood pressure (BP) in patients with diabetes mellitus type 2 (DMT2). Thirty-seven subjects were recruited in this double blind, placebo controlled randomized trial. The study group (n = 19) received over 8 week's capsules containing PoPEx twice a daily, while the placebo group received placebo. Treatment with PoPEx induced a significant lowering of both systolic and diastolic BP. The plasma levels of triglycerides, low-density lipoprotein cholesterol/high-density lipoprotein cholesterol ratio (LDL-C/HDL-C), and HbA1c were significantly decreased, while the level of HDL-C was significantly increased, compared with placebo intake. Moreover, the PoPEX treatment significantly improved the plasma lipids fatty acids content. It is concluded that consumption of PoPEx in DMT2 subject had favourable effects on some metabolic parameters, BP, lipid profile and plasma lipid FA composition.

1. Introduction

Diabetes mellitus[1] (DMT2) is a serious public health problem. According to the 2017 International Diabetes Federation report, approximately 425 million adults (range 20–79 years) were living with diabetes, and by year 2045 it will rise to 629 million globally. The proportion of people suffering from DMT2 is increasing in most countries, but 79% of adults with diabetes were living in low- and middle-income countries (International Diabetes Federation, 2017). The epidemics of obesity and sedentary lifestyle have contributed to the prevalence of diabetes and high blood pressure[2] (BP) (Sowers, 2013). Obesity is linked to insulin resistance and is one of the main characteristics of the DMT2 (Boden, 1997); Lewis, Carpentier, Adeli, & Giacca, 2002). Diabetes is associated with hypertension and dyslipidemia, which lead to increased risk of cardiovascular complications such as angina, coronary artery diseases, myocardial infarction, stroke, peripheral artery disease, and congestive heart failure (American Diabetes Association, 2019). Adults suffering from diabetes have two to three times greater frequency of cardiovascular disease[3] (CVD) in comparison to those without diabetes (Sarwar et al., 2010).

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[1] DMT2, diabetes mellitus type 2.
[2] BP, blood pressure.
[3] CVD, cardiovascular disease.

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Most of the DMT2 patients have dyslipidemia with increased levels of low-density lipoprotein cholesterol[4] (LDL-C) and triglycerides[5] (TG), and low levels of high-density lipoprotein cholesterol[6] (HDL-C) (Parhofer, 2015; Wu & Parhofer, 2014). Changes in lipid metabolism are associated with changes in fatty acids[7] (FA) composition of blood lipids. Patients with diabetes usually have higher levels of saturated fatty acids[8] (SFA), in particular palmitic acid (16:0), as well as FA that may derive from palmitic acid during *de novo* lipogenesis, including palmitoleic acid (16:1n–7) and stearic acid (18:0) (Lemaitre et al., 2015). These FA have been associated with insulin resistance and diabetes risk in several epidemiologic studies (Kröger et al., 2011). Status of n-3 polyunsaturated fatty acids[9] (PUFA) is inversely associated with diabetes, mostly docosahexaenoic acid[10] (DHA, 22:6n-3) although a novel study reported that this relation is sex-dependent (Abbott et al., 2019). Hyperglycemia activates production of reactive oxygen species[11] (ROS) and presents a condition of oxidative stress and inflammation, which is linked to a high production of adhesion molecules and proinflammatory cytokines (Preedy, 2016). Therefore, diabetes is a chronic pro-inflammatory condition which decreases antioxidative potential of cells making them easily damaged (Preedy, 2016).

There is no absolute cure for diabetes. It can be managed by combination of various approaches, such as antidiabetic medications, physical activity, healthy diet, and herbal remedies (Banihani, Swedan, & Alguraan, 2013; Shishehbor et al., 2016). Growing evidence from animal studies supports the anti-diabetic properties of some dietary polyphenols, suggesting that dietary polyphenols could be one dietary therapy for the prevention and management of DMT2. Studies have shown that polyphenols can improve conditions of glucose and lipid metabolic disorder, and their anti-inflammatory effects have been determined (Shishehbor et al., 2016). Pomegranates contain high levels of various phytochemicals such as polyphenols, fatty acids, amino acids, tocopherols, sterols, terpenoids, alkaloids that have therapeutic effects and are used either for their anti-inflammatory properties, as antioxidants, or for their antineoplastic, hypoglycemic, lipid-lowering or antimicrobial effects (Viuda-Martos, Fernandez-Lopez, & Perez-Alvarez, 2010). Fractions of various pomegranate parts show potential for treating numerous illnesses and disorders including cardiovascular diseases and diabetes mellitus. It is shown that pomegranate extract antagonizes harmful effects of prooxidants and reduces oxidative stress and lipid peroxidation (Esmaillzadeh, Tahbaz, Gaieni, Alavi-Majd, & Azadbakht, 2004; Fenercioglu, Saler, Genc, Sabuncu, & Altuntas, 2010; Viuda-Martos et al., 2010). Besides its antioxidative effects, pomegranate juice reduces TG, LDL-C, increases HDL-C and lowers blood pressure (Vučić, Grabež, Trchounian, & Arsić, 2019; Esmaillzadeh et al., 2004; Fenercioglu et al., 2010). Nonedible part of the pomegranate fruit is its peel, which makes 40% of the total mass of the fruit, and is usually considered as a waste (Çam, Içyer, & Erdoğan, 2014). However, it contains phytochemicals that are medically and nutritionally important (Singh, Chidambara Murthy, & Jayaprakasha, 2002). Almost 48 phenol components (flavonoids, condensed tannins and hydrolysable tannins) have been detected in pomegrante peel and other parts of pomegranate fruit (Akhtar, Ismail, Fraternale, & Sestili, 2015; Singh et al., 2002). Although the beneficial effects of pomegranate juice on various parameters of DMT2 in experimental and clinical studies have been well documented (Atrahimovich, Samson, Khattib, Vaya, & Khatib, 2018; Aviram & Dornfeld, 2001; Banihani et al., 2013; Rosenblat, Hayek, & Aviram, 2006) the effect of pomegranate peel extract[12] (PoPEx) has not been properly examined in clinical trials so far.

The aim of this study was to evaluate the effects of pomegranate peel extract consumption on blood pressure, plasma glycemic and lipid profile, and fatty acids in patients with diabetes mellitus type 2.

2. Material and methods

2.1. Study population

This study was a randomized, double blind, placebo-controlled clinical trial in patients with type 2 diabetes mellitus. The patients were recruited at the Endocrinology Department of the University Clinical Centre of Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia & Herzegovina. All participants were in the age range of 40–65 years, had a body mass index[13] (BMI) $\geq$ 25 kg/m^2, $HbA_1C \geq 6.5\%$ and were treated with oral hypoglycaemic agent, metformin at least one year before beginning of the study, in recommended daily doses. Participants not considered for the study were those with chronic kidney, liver, or inflammatory diseases; those who were taking hormone replacement therapy, or antioxidant supplements; or were on insulin treatment or anti-inflammatory medications.

2.2. Ethical considerations

All participants who expressed an interest to participate in the study signed an informed consent. During the recruitment phase, participants were informed of the study purpose and protocol, risks/benefits related to their participation, proposed testing, and the study time course. The study protocol was approved by the Ethics Committee of the Faculty of Medicine No.:01-9-604-2/17, University of Banja Luka and was conducted in accordance with the Declaration of Helsinki.

2.3. Study design

Thirty-seven patients were randomly allocated into two groups, blinded to both participants and researchers. The study group (PoPEx group; n = 19) received capsules containing pomegranate peel extract (250 mg) twice a day for period of 8 weeks, while the placebo group (n = 18) received visually identical capsules containing placebo (250 mg). Participants were asked not to change their dietary habits, physical activities, and medication regimens during the study period. Each participant was provided with a fixed number of capsules needed for the course of treatment and they were contacted by the principal investigator on a weekly basis to verify if they have taken capsules regularly.

2.4. Pomegranate peel extract

Method of triple percolation, using 70% ethanol as a solvent, was used for the preparation of pomegranate peel extract. The solvent was evaporated to dryness using vacuum oven in order to obtain a dry extract. Phenolic compounds, as the most responsible for the biological activity of the pomegranate peel, were quantified in the extract. Base on high pressure liquid chromatography[14] (HPLC) methodology, the obtained dry extract contained punicalagin 69.67 ± 0.72 mg/g, punicalin 30.41 ± 0.11 mg/g, ellagic acid 23.83 ± 0.07 mg/g and gallic acid 10.46 ± 0.04 mg/g as the main phenolic compounds. HPLC chromatogram is presented on Fig. 1. Toatal tannins were detected spectrophotometrically according to European Pharmacopoeia 8.0 (Council of Europe, 2013) and the amount was 11.8% which is in

[4] LDL-C, low-density lipoprotein cholesterol.
[5] TG, triglycerides.
[6] HDL-C, high-density lipoprotein cholesterol.
[7] FA, fatty acids.
[8] SFA, saturated fatty acids.
[9] PUFA, polyunsaturated fatty acids.
[10] DHA, docosahexaenoic acid.
[11] ROS, reactive oxygen species.
[12] PoPEx, pomegranate peel extract.
[13] BMI, body mass index.
[14] HPLC, high pressure liquid chromatography.

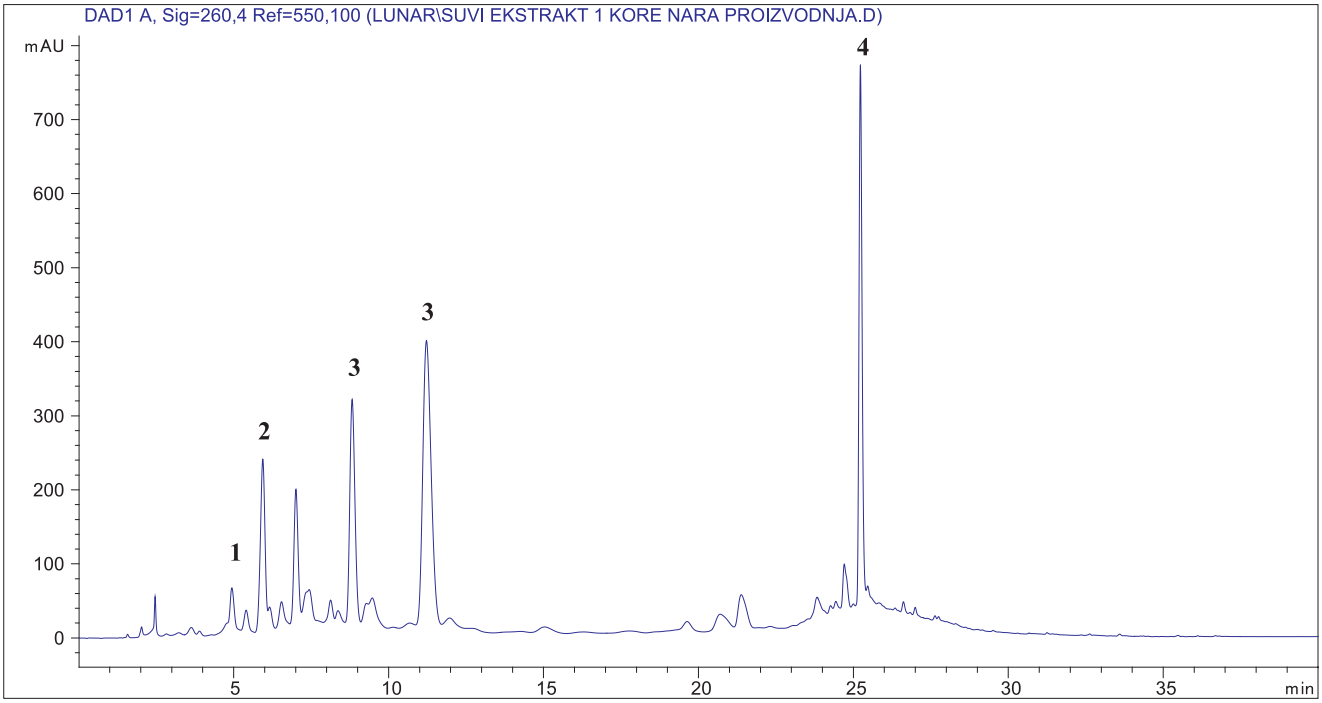

Fig. 1. HPLC chromatogram (λ = 260 nm) of dry pomegranate peel extract. Peak numbers as follows: 1, gallic acid; 2, punicalin; 3, punicalagin α + β; 4, ellagic acid.

accordance with WHO monograph (WHO, 2009). Each capsule contained 250 mg of the dry extract. Placebo capsule contained only excipients, lactose, starch and magnesium stearate. The energy value of pomegranate capsules and placebo capsules was less than 50 kJ/12 kcal per 100 g of product.

2.5. Anthropometric and clinical variables

Anthropometric measurements were taken at the beginning and at the end of intervention period using standard techniques. All measurements were made by trained personnel in the morning with the subject wearing only underwear. Height was determined using a height meter with an accuracy of 1 mm. Waist circumference (WC)[15] was measured with a non-stretchable tape. Weight was measured by standard scale with an accuracy of 100 g. Body composition (fat mass and fat-free mass) was determined using a Tanita bioelectrical impedance analyser (Tanita MC-780MA; Tanita Corporation, Tokyo, Japan). Body mass index was calculated according to the following formula: [weight (kg)/height $(m)^2$]. Blood pressure was measured on the right arm of patient in seating position using a digital sphygmomanometer (Omron Intell Sense, HEM-907XL, Omron Company, Kyoto, Japan) with accuracy of ± 5 mmHg. Three consecutive measurements were taken at 5-min intervals, and the mean values were calculated.

2.6. Energy intake

To estimate energy intake and account for any changes during the intervention period, which could influence the results, a 3 days' food diary was collected at baseline and at the last 3 days of the week 8. Energy intake was generated using the Serbian Food Composition Database, harmonized with EuroFIR standards and embedded in EuroFIR Food Platform and Balkan food platform (Gurinović et al., 2016). Food recording support and training including guidance on portion sizes and household measures, were provided by the researcher.

[15] WC, waist circumference.

2.7. Biochemical analysis

Blood samples were collected after 12–14 h overnight fasting at baseline, and at the end of study protocol. The 5 mL of plasma samples were stored at −80 °C until analysis. Total cholesterol[16] (TC), LDL-C, HDL-C, TG, HbA_{1C} and glucose were determined using standard biochemical methods at Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany). Serum insulin concentration was measured using the ADVIA Centaur XPT immunoassay analyzer (Siemens Healthineers, Tarrytown, NY, USA.).

Insulin resistance was calculated with homeostatic model of insulin resistance[17] (HOMA-IR index), and insulin sensitivity was calculated with quantitative insulin sensitivity check index[18] (QUICKI) as follow:

$$HOMA - IR = [fasting\ insulin\ (\mu IU/mL) * (fasting\ glycemia\ (mmol/L)]/22.5.$$

$$QUICKI = 1/[log(fasting\ insulin\ \mu IU/mL) + log\ fasting\ glycemia\ (mg/dL = mmol/L * 18.182)],$$

The method of Glaser, Demmelmair, and Koletzko (2010) was used for determining FA profile in plasma total lipids. The method was slightly modified, as follows: 100 mL of plasma is mixed with 1.5 mL of 3 M HCL in MetOH. This mixture was heated on 85 °C for 45 min. After the mixture was cooled down to room temperature, 1 mL of hexane was added, vortexed for 30 s and centrifuged on 1800*g* for 10 min. Hexane layer was separated and evaporated to dry in the nitrogen stream. The sample was dissolved in 10 μL of hexane and 1 μL of the solution was injected in gas chromatograph, equipped with capillary column RESTEK Rtc 2330, 60 m, 0.25 mm, 0.2 mm. Temperature was set on 140–210 °C for 3 min. Individual FA were identified in comparison to the retention times of FA methyl esters commercial standards PUFA-2 (Supelco Inc., Bellefonte, Pennsylvania, USA). The results are presented as percentage of total FA composition.

[16] TC, total cholesterol.
[17] HOMA-IR, homeostatic model of insulin resistance.
[18] QUICKi, quantative insulin sensitivity check index.

Index of lipid peroxidation was estimated in the plasma samples by measuring Thiobarbituric acid reactive species[19] (TBARS) according to the method of Ohkawa, Ohishi, and Yagi (1979). Thiobarbituric acid (1% in 0.05 M NaOH) was incubated with the plasma sample at 100 °C for 15 min and measured at 530 nm. Distilled water was used as a blank probe.

2.8. Statistical analyzes

Data were analyzed using IBM SPSS 20 (Chicago, IL, USA), considering $p < 0.05$ as significant. Data obtained from intervention study are expressed as mean ± standard deviation. Normality of variables distribution was assessed by Shapiro-Wilk's test. After checking data distribution, the appropriate parametric or non-parametric test was used. For comparisons between groups at baseline, the following statistical tests were used: Student's *t* test and Mann-Whitney *U* test. Furthermore, for analysis of differences in the outcome variables were performed paired sample *t*-test or Wilcoxon Signed Rang test.

3. Results

3.1. Study population and baseline characteristics

The study was performed between June and November 2018. All recruited patients (20 females and 17 males) completed the study. There were no gender differences between the groups; study group (n = 19) with 10 females' participants, and placebo group (n = 18) with 10 females' participants. No differences were noticed concerning the age and duration of DMT2 between the study and placebo group (57.58 years ± 6.10 vs 57.06 years ± 6.73; and 72.95 months ± 47.57 vs 74.67 months ± 55.75, respectively). Concerning the baseline anthropometric characteristics at the beginning of the study, no significant differences existed between the groups (Table 1).

3.2. Anthropometric and clinical parameters

Eight weeks' treatment with pomegranate peel extract (PoPEx group) did not have any significant effect on anthropometric parameters such as BMI, weight, fat mass and fat free mass. However, it had a significant effect on reduction of waist circumference (−2.46 cm). The intake of pomegranate peel extract induced the significant lowering of both systolic and diastolic blood pressure by 6.06 mmHg and 2.10 mmHg (4.45% and 2.53%), respectively. No such differences were observed in the placebo group (Table 1). Analysis of energy intake showed no significant differences in baseline energy intake between the PoPEx group and the placebo group and at the end of study (Table1).

3.3. Metabolic parameters

Supplementation with pomegranate peel extract for 8 weeks did not change total cholesterol and LDL-C plasma level, but it significantly decreased plasma levels of TG (2.74 ± 2.31 vs 2.33 ± 2.31 mmol/L), as well as the LDL-C/HDL-C ratio (3.18 ± 1.15 vs 2.41 ± 0.90), and lipid peroxidation index (TBARS; 1.66 ± 0.34 vs 0.40 ± 0.17). At the same time, plasma level of HDL-C was significantly increased in study group (1.13 ± 0.25 vs 1.36 ± 0.23 mmol/L; Table 2). Those differences in metabolic parameters were not observed in the placebo group. At the end of study, there were no differences between the study and placebo groups concerning fasting blood glucose, serum insulin concentration, and insulin sensitivity, but 8 weeks' pomegranate peel extract supplementation significantly decreased HbA_1c in study group (7.65 ± 1.16 vs 7.44 ± 1.09%) (Table 2).

Table 3 displays fatty acids profile in total plasma lipids, in subject with DMT2, before and after the study period. The results show that pomegranate peel extract decreased levels of palmitic (16:0) and stearic acid (18:0) and total saturated fatty acids, after 8 weeks of supplementation ($p < 0.05$, $p < 0.05$, $p < 0.01$ respectively). Also, the level of arachidonic acid (AA)[20] 20:4n-6, was significantly higher in the PoPEx group at the end of study ($p < 0.05$). Furthermore, some change in FA composition in subjects who received placebo was observed. Thus, level of stearic acid was significantly lower ($p < 0.05$) in the control group. No changes in FA profiles were found in the placebo group after the treatment.

4. Discussion

The main purpose of the present study was to investigate whether 8-week long PoPEx consumption could attenuate changes in lipid profile, fatty acids and blood pressure in subjects with diabetes mellitus type 2. To the best of our knowledge, the beneficial effects of pomegranate peel extract have not been studied in DMT2 patients.

In the first step, we made a dry pomegranate peel extract that was obtained in a ratio 8:1 (peel: extract). During the capsule formulation, determination of adequate dosage was guided by the World Health Organization monograph in which the oral daily dose is from 2.5 to 4.5 g (WHO, 2009). Considering that we want to achieve a therapeutic effect, we opted for a dose of 500 mg per day.

Our findings clearly demonstrated that the consumption of PoPEx significantly decreased systolic and diastolic BP in patients with DMT2, compared to the placebo group. The previous studies reported similar blood pressure-lowering effect of pomegranate juice on diabetic patients, hypertensive patients, and also on healthy adults (Asgary et al., 2013; Lynn, Hamadeh, Leung, Russell, & Barker, 2012; Sohrab et al., 2019). Stockton, Farhat, McDougall, and Al-Dujaili (2017) reported that 8-week long administration of concentrated extract of whole pomegranate induced a significant decrease in diastolic BP, but a non-significant decrease in systolic BP in healthy adults. Several mechanisms have been suggested for blood pressure-lowering effect of pomegranate juice[21] (PJ). According to Aviram and Dornfeld (2001), Dos Santos et al. (2016) polyphenols from pomegranate reduce angiotensin converting enzyme. Also, pomegranate juice might exert beneficial effect on vasodilatation of blood vessels promoting Nitric oxide[22] (NO) synthase bioactivity and thus increasing bioavailability of NO (Sahebkar et al., 2017).

The results of the present study showed no significant interaction between the consumption of PoPEx and body weight, body fat mass and fat free mass. Results of previous studies are not consistent (González-Ortiz, Martínez-Abundis, Espinel-Bermúdez, & Pérez-Rubio, 2011; Hosseini, Saedisomeolia, Wood, Yaseri, & Tavasoli, 2016; Sohrab et al., 2019; Stockton et al., 2017). Furthermore, we found a significant decrease in WC in PoPEx group. Waist circumference is independent predictive factor of the chronic disease including diabetes mellitus type 2 (Amato, Guarnotta, & Giordano, 2013). DMT2 risk can decrease when WC was modified no matter whether BMI remains the same (Luo et al., 2013). This finding may provide an interesting avenue for further research.

Glycemic control reminds a constant challenge for diabetic patients. Poor glycemic management is still one of the major causes long-term adverse outcomes such as macro- and microvascular complication in DM subject (International Diabetes Federation, 2017). We observed that administration of PoPEx significantly decreased level of HbA_1c, but exerted no significant change concerning fasting blood glucose, serum insulin concentration, and insulin sensitivity. Similar results according mean glycemia were obtained after 30 days' supplementation of

[19] TBARS, Thiobarbituric acid reactive species.

[20] AA, arachidonic acid.

[21] PJ, pomegranate juice.

[22] NO, nitric oxide.

Table 1
The anthropometric characteristics of Diabetes mellitus Type 2 patients, and responsiveness to pomegranate peel extract and placebo after the 8 weeks' treatment.

Parameter	PoPEx group (n = 19)		Placebo group (n = 18)	
	Before treatment	After treatment	Before treatment	After treatment
BMI (kg/m^2)	31.82 ± 4.94	31.94 ± 4.90	32.51 ± 4.72	32.58 ± 4.76
Waist circumference	109.48 ± 10.13	107.02 ± 10.03***	109.60 ± 10.70	108.98 ± 11.06
Weight (kg)	93.81 ± 16.36	94.20 ± 15.83	95.08 ± 17.05	95.30 ± 17.18
Fat mass (%)	31.73 ± 6.85	31.49 ± 7.07	32.33 ± 8.63	32.38 ± 9.23
Fat free mass (%)	65.25 ± 6.23	65.61 ± 6.51	64.48 ± 8.36	64.31 ± 8.41
Energy intake (kcal)	2274.58 ± 303.57	2301.89 ± 310.13	2273.56 ± 380.96	2282.28 ± 380.74
Systolic BP (mmHg)	136.32 ± 19.57	130.26 ± 14.58**	139.44 ± 14.54	141.94 ± 15.35
Diastolic BP (mmHg)	82.89 ± 8.22	80.79 ± 7.32*	84.88 ± 9.52	85.44 ± 7.69

Data are expressed as the mean ± SD; PoPEx, Pomegranate peel extract; BMI, Body mass index; BP, Blood Pressure.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 2
Effects of pomegranate peel extract (PoPEx) and placebo treatment on metabolic parameters in patients with Diabetes mellitus Type 2.

Parameter	PoPEx group (n = 19)		Placebo group (n = 18)	
	Before treatment	After treatment	Before treatment	After treatment
Total Cholesterol mmol/L	5.22 ± 1.20	5.02 ± 1.32	5.43 ± 0.71	5.41 ± 0.74
HDL-C mmol/L	1.13 ± 0.25	1.36 ± 0.23***	1.08 ± 0.28	1.13 ± 0.30
LDL-C mmol/L	3.44 ± 0.98	3.19 ± 1.13	3.73 ± 0.64	3.74 ± 0.71
TG mmol/L	2.74 ± 2.31	2.33 ± 2.31**	2.13 ± 1.20	2.11 ± 0.87
LDL-C/HDL-C	3.18 ± 1.15	2.41 ± 0.90***	3.66 ± 1.00	3.53 ± 1.02
FBG mmol/L	9.1 ± 2.10	8.99 ± 2.48	8.57 ± 2.61	8.61 ± 2.40
HbA_1c %	7.65 ± 1.16	7.44 ± 1.09*	7.44 ± 1.10	7.41 ± 1.09
Insulin μIU/mL	12.06 ± 6.47	11.74 ± 6.44	14.43 ± 6.75	13.72 ± 5.84
QUICKI index	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.03	0.31 ± 0.02
HOMA-IR	4.84 ± 2.90	6.70 ± 1.96	5.32 ± 3.65	5.14 ± 2.93
TBARS mmol/L	1.66 ± 0.34	0.40 ± 0.17***	1.54 ± 0.12	1.41 ± 0.14

Data are expressed as the mean ± SD. PoPEx, Pomegranate peel extract; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TG, triglycerides; FBG, fasting blood glucose; QUICKI index, Quantitative insulin sensitivity check index; HOMA-IR, Homeostatic model of insulin resistance; TBARS, Thiobarbituric acid reactive substances.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Effects of pomegranate peel extract (PoPEx) and placebo treatment on plasma fatty acids composition (% of total fatty acids) in patients with diabetes mellitus type 2.

Fatty acids	PoPEx group (n = 19)		Placebo group (n = 18)	
	Before treatment	After treatment	Before treatment	After treatment
SFA	43.22 ± 1.14	41.84 ± 1.49**	42.66 ± 1.36	42.11 ± 1.34
16:0	30.37 ± 1.71	29.79 ± 1.80*	29.18 ± 1.25	28.55 ± 1.42
18:0	12.85 ± 1.31	12.05 ± 1.31**	13.48 ± 0.83	13.56 ± 0.76*
MUFA	14.78 ± 1.59	15.33 ± 2.41	15.50 ± 1.42	16.04 ± 2.15
16:1	1.07 ± 0.42	1.08 ± 0.36	1.17 ± 0.43	1.23 ± 0.53
18:1n-7	1.17 ± 0.36	1.24 ± 0.25	1.65 ± 0.40	1.72 ± 0.65
18:1n-9	12.54 ± 1.44	13.00 ± 2.11	12.67 ± 1.48	13.08 ± 1.88
PUFA	42.03 ± 2.01	42.83 ± 2.24	41.84 ± 1.77	41.85 ± 2.38
18:2	23.87 ± 3.42	23.67 ± 3.26	23.59 ± 2.54	23.38 ± 3.06
18:3n-3	0.15 ± 0.05	0.27 ± 0.37	0.18 ± 0.09	0.19 ± 0.14
18:3n-6	0.25 ± 0.12	0.25 ± 0.14	0.20 ± 0.10	0.21 ± 0.09
20:3	2.44 ± 0.58	2.46 ± 0.68	2.91 ± 0.80	3.02 ± 0.64
20:4	11.15 ± 2.34	11.99 ± 2.48*	10.79 ± 2.18	10.80 ± 2.03
20:5	0.26 ± 0.17	0.28 ± 0.18	0.40 ± 0.35	0.44 ± 0.28
22:4	1.40 ± 0.43	1.45 ± 0.50	1.01 ± 0.61	1.09 ± 0.58
22:5	0.49 ± 0.19	0.48 ± 0.11	0.49 ± 0.13	0.53 ± 0.14
22:6	2.03 ± 0.69	1.98 ± 0.54	2.26 ± 0.52	2.19 ± 0.54
n-3	2.93 ± 0.93	3.01 ± 0.97	3.34 ± 0.88	3.35 ± 0.83
n-6	39.10 ± 2.21	39.82 ± 2.37	38.50 ± 1.98	38.50 ± 2.59

Data are expressed as the mean ± SD. PoPEx, Pomegranate peel extract; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.
* $p < 0.05$.
** $p < 0.01$.

pomegranate extract in overweight and obese participants (Hosseini et al., 2016). Our finding is not in line with other studies that reported no changes in long-term glycemic control after administrated pomegranate juice (González-Ortiz et al., 2011; Huang, Liao, Chen, Chen, & Zhu, 2017; Sumner et al., 2005). The lack of PoPEx activity on fasting blood glucose level and serum insulin concentration in our study could be explained by the fact that all studied individuals were DMT2 patients on regular metformin therapy; therefore, the glycemic control had been already established. Glycated protein HbA_1c is considered as the standard measure of long-term glycemic control, and does not reflect glycemic variability. However, HbA_1c is strongly associated with complication of diabetes (Huang et al., 2017). Increase in 1% of the level of HbA_1c is associated with 7.5% increase in cardiovascular disease risk in DMT2 subjects (Juutilainen, Lehto, Rönnemaa, Pyörälä, & Laakso, 2008). Encouraging findings from animal studies suggested that pomegranate peel polyphenols could improve glycemic metabolism and ameliorate insulin sensitivity. It has been suggested that administration of PoPEx might have hypoglycemic effect by inhibiting activities of α-glucosidase and α-amylase (Arun, Jayamurthy, Anusha, Mahesh, & Nisha, 2017; Middha, Usha, & Pande, 2014).

Dyslipidemia is typical feature of DMT2 and is characterized by elevated TG and LDL-C level, and low level of HDL-C (Matheus et al., 2013). The characteristic lipid changes are present also in patients with metabolic syndrome, implicating that disturbed lipid transport in diabetic patients is a result of insulin resistance, rather than hyperglycemia (Parhofer, 2015). The results of the present study clearly demonstrated that 8-week consumption of pomegranate peel extract induced statistically significant lipid profile improvement. The PoPEx group had significantly reduced the plasma level of TG and the ratio of LDL-C/HDL-C, and significantly increased of HDL-C level. These outcomes are relatively in agreement with previous studies (Esmaillzadeh et al., 2004; Fenercioglu et al., 2010) who used pomegranate juice supplementation. Furthermore, some studies have reported that pomegranate polyphenols did not have any significant effect on lipid profile (Manthou et al., 2017; Sohrab et al., 2019). Discrepancies between results of different trials could be explained by several reasons: small sample size, different duration of supplementation period, dose and form of pomegranate used (Sahebkar, Simental-Mendía, Giorgini, Ferri, & Grassi, 2016).

Previous *in vivo* and *in vitro* studies suggested that extracts of different pomegranate fraction regulate lipid metabolism in metabolic disorder-associated diseases (Manthou et al., 2017). Some authors have indicated that pomegranate peel polyphenols, and their main components punicalagin and ellagic acid had lipid lowering effect in dose-dependent manner. The molecular mechanism may consist of the activation peroxisome proliferator-activated receptor[23] γ (PPARγ) and the enhanced cholesterol metabolism in LO2 cells (Lv, Wang, Li, Ma, & Zhao, 2016).

Plasma TBARS levels are frequently used marker of lipid peroxidation. A positive effect of PoPEx treatment on lipid peroxidation resulted in the significant reduction in TBARS levels by 75%. This result is in line with previous reported in healthy, DM and metabolic syndrome subject (Aviram et al., 2000; Kojadinovic et al., 2017; Rosenblat et al., 2006). Studies have reported that PoPEx had significantly higher antioxidative efficacy than the pulp, seed or juice extract (Orak, Yagar, & Isbilir, 2012; Orgil et al., 2014) in scavenging capacity against hydroxyl, superoxide anion and peroxyl radicals, thus exhibiting protective effects against methotrexate induced oxidative stress and lipid changes in rats (Doostan et al., 2017).

The 8-week treatment with PoPEx induced marked changes in FA composition of plasma lipids. The intervention group had significantly lower levels of individual SFA – palmitic and stearic acid, as well as total SFA. Saturated fats, in particular palmitic acid, have traditionally been considered proatherogenic (Micha & Mozaffarian, 2010; Vučić et al., 2015). Epidemiological studies have demonstrated a positive correlation between SFA intake and level of LDL-cholesterol (Lefevre, Champagne, Tulley, Rood, & Most, 2005; van Dijk et al., 2009). The atherogenic and thrombogenic potential of SFA is exerted through elevated production of very low density lipoprotein[24] (VLDL) particles and ApoA1, increased platelet aggregation, with decreased specific activity of LDL-receptors (Iggman & Risérus, 2011). Moreover, it has been documented that palmitic acid increases insulin resistance, thus worsening DMT2. Namely, SFA activate serine kinases, thereby inhibiting insulin-phosphorylation cascade, diminishing glucose uptake and increasing level of blood glucose (Hotamisligil, 2006). Palmitic acid also activates protein kinase C that leads to a higher insulin resistance and reduced glucose uptake by skeletal muscle cells (Snook, Park, Williams, Tsai, & Lee, 1999). However, all these effects are mostly attributed to palmitic acid rather than to stearic acid. Consumption of PoPEx significantly reduced proportions of all SFA, indicating antiatherogenic and cardioprotective effects of pomegranate peel extract in patients with DMT2. It has been shown that punicic acid found in pomegranate, can bind and activate PPAR α, thus upregulating PPAR α and its responsive genes, among them Stearoyl-CoA desaturase-1[25] (SCD-1) (Viladomiu, Hontecillas, Lu, & Bassaganya-Riera, 2013). The decrease in the level of SFA may result from increased activity of SCD-1 that leads to an increased synthesis of monounsaturated FA. Our study showed an increase in both oleic and vaccenic acids after consumption of pomegranate peel pills, but these changes are not significant. Nevertheless, we found no significant increase in oleic and vaccenic acids after consumption of pomegranate peel pills, that could be due to a limited length of this study.

Besides SFA, PoPEx treatment induced an increase in AA as well. Arachidonic acid is a precursor for a wide spectrum of potent proinflammatory eicosanoids, including prostaglandins series 2 and leukotriens series 4 (Innes & Calder, 2018; Ristic-Medic, Vucic, Takic, Karadžic, & Glibetic, 2013), and high levels of AA have often been found in inflammatory states. However, an increase of AA in plasma lipids of patients with DMT2 who consumed PoPEx, has not been followed by a decrease in its precursor linoleic acid[26] (LA, 18:2n-6) suggesting that PoPEx had no effect on conversion of LA to AA, but rather that conversion of AA to eicosanoids was inhibited. Therefore, we can speculate that intake of PoPEx diminished inflammation in DMT2 patients, which is in line with decreased levels of interleukin 6[27] (IL-6) in these patients (Shishehbor et al., 2016). The mechanism of action of PoPEx could also be attributed to punicic acid, which binds PPARs. PPARs are the receptors for endogenous lipid molecules (i.e., prostaglandins or hydroxy-containing PUFA) and molecular targets for drugs against type 2 diabetes (Mokdad et al., 2001). They also represent promising new targets for the treatment and prevention of inflammatory disorders (Dubuquoy et al., 2003; Katayama et al., 2003). Namely, punicic acid activate PPAR thereby inhibiting generation of prostaglandins and diminishing inflammation, with concomitant accumulation of AA.

Alterations in FA profiles in the intervention group led to decreased n-6/n-3 PUFA ratio, which is an important inflammatory marker. The World Health Organization[28] (WHO) recommended the ratio in the diet of 4:1. However, in most Western diets this ratio is 15–20:1 (Burdge & Calder, 2006). Wall, Ross, Fitzgerald, and Stanton (2010) have reviewed that lowering this ratio in the diet may reduce the incidence of several chronic inflammatory diseases, such as CVD, autoimmune diseases, as well as psychiatric and neurodegenerative illnesses.

[23] PPAR, peroxisome proliferator-activated receptor.

[24] VLDL, very low density lipoprotein.

[25] SCD-1, stearoyl-CoA desaturase-1.

[26] LA, linoleic acid.

[27] IL-6, interleukin 6.

[28] WHO.

5. Conclusion

The present study clearly demonstrated that 8-weeks supplementation with pomegranate peel extract attenuated the systolic and diastolic blood pressure in patients with type 2 diabetes. This treatment has also shown its beneficial effects on blood levels of TG, HDL-C, TBARS, HbA_1c and fatty acids profile in total plasma lipids of these patients, suggesting on hypolipaemic, hypoglycemic, and antioxidative potential of pomegranate peel extract. Further studies are needed in order to get deeper insights of molecular mechanisms underlying these very complex effects of pomegranate peel poyphenols on glucose and lipid metabolism pathways in metabolic disorders.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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