Effect of Bitter Melon Aqueous Extract and Pomegranate Oil on Glucose Concentration and Lipid Profile in Blood of Rats – Preliminary Study

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Abstract: Conjugated fatty acids is a term given to a group of polyunsaturated fatty acids with conjugated double bonds systems in their carbon chains. Conjugated linolenic acids (CLnA) are present in seeds of certain plants *e.g.* α-eleostearic acid (cis-9, trans-11, trans-13 C18:3) in bitter melon (*Momordica charantia*, *Cucurbitaceae*) or punicic acid (cis-9, trans-11, cis-13 C18:3) in pomegranate (*Punica granatum*, *Punicaceae*), where usually they are most prevalent among fatty acids. Bitter melon and pomegranate have been widely investigated as they are commonly consumed plants which also have been used in traditional medicine, mainly in Asia, for treatment of many diseases, such as diabetes and atherosclerosis.

The aim of this study was to evaluate the influence of a diet supplemented with an aqueous extract of bitter melon fruits and/or with pomegranate oil on health status and lipid profile of blood. Sprague-Dawley female rats were divided into four groups with different diet supplementation: pomegranate oil (G), aqueous extract of bitter melon (M), pomegranate oil and aqueous extract from bitter melon (M+G), and control group (C). During the experiment fasting glucose concentration and total cholesterol (TC), HDL, LDL, and triglyceride (TG) concentration were measured in blood collected intravitally from the tail vein.

The modifications introduced into the diets did not influence negatively overall health condition of the animals. Bitter melon fruits extract slightly decreased the fasting glucose concentration during the experiment but its action was not statistically significant ($p>0.05$). Pomegranate oil caused an increase of fasting glucose level in G group ($p=0.03657$) but in M+G group its influence was diminished by the opposite activity of bitter melon fruits extract ($p>0.05$). TC was the lowest in G group and it did not change during the time of experiment, which can suggest that the diet supplementation with pomegranate oil prevents the age-related increase in cholesterol level. TC in blood of G group was significantly lower than in other groups in 14th ($p=0.01057$) and 21st ($p=0.01433$) weeks respectively. Aqueous extract of bitter melon fruits slightly diminished age-related TG increase, whereas pomegranate oil strongly prevents this tendency, as the TG content in G group was significantly lower than TG content in C and M groups at 14th ($p=0.00060$) and 21st ($p=0.00003$) week respectively. Similar activity, although not so pronounced, was visible as far as the M+G group was concerned.

Keywords: Bitter melon, Pomegranate, Pomegranate oil, Lipid profile.

INTRODUCTION

Conjugated fatty acids is a term used to refer to a group of polyunsaturated fatty acids with conjugated double bonds system in their carbon chains. Amongst those, conjugated linoleic acids (CLA) have been investigated for many years due to their numerous health benefits, such as anti-cancerogenic, anti-atherogenic or anti-obesity activity. They are naturally present, especially in ruminant fat, in rather small amounts (<1%) [1]. Conjugated linolenic acids (CLnA or super CLA) are another group of conjugated fatty acids which, in contrast to CLA, are present in nature in seeds of certain plants, where usually they are most prevalent among all fatty acids (>60%). Several fatty acids, such as α-eleostearic acid (cis-9, trans-11, trans-13 C18:3) from bitter melon (*Momordica charantia*), punicic acid (cis-9, trans-11, cis-13 C18:3) from pomegranate (*Punica granatum*), catalpic acid (trans-9, trans-11, cis-13 C18:3) from catalpa (*Catalpa ovata*), calendic acid (trans-8, trans-10, cis-12 C18:3) from pot marigold (*Callendula officinalis*), and jacaric acid (cis-8, trans-10, cis-12 C18:3) from jacaranda (*Jacaranda mimosifolia*), belong to CLnA group [2]. There is some very promising research with respect to the biological activities of CLnA. Especially bitter melon and pomegranate have been widely investigated as sources of CLnA, having been used in traditional folk medicine (mainly in Asia), for treatment of many diseases, such as diabetes and atherosclerosis.

According to the World Health Organization the number of people suffering from diabetes and atherosclerosis, which often coexist, is increasing all over the world. Amongst the first-stage strategies for reducing the increase in fasting glucose levels and/or

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cholesterol concentration in blood, changes of nutritional habits are recommended. In many cases intensive hypoglycemic therapy is necessary, despite the very effective outcomes of the dietary approach. On the other hand, many patients use alternative medicine therapies or dietary supplements as safe and effective helper of conventional drugs. Especially those with both blood-sugar-lowering capacity and cholesterol lowering capacity are of great concern.

Momordica charantia (*Cucurbitaceae*) is variously known as bitter melon, bitter gourd, balsam pear, bitter apple, bitter cucumber, African cucumber, wild cucumber or karela [3]. It is grown in many parts of Asia, South America, East Africa, and the Caribbean and its fruits are used as a vegetable as well as a medicine [3, 4]. Young fruits, which are oblong and resemble small cucumbers, are emerald green whereas ripe ones are orange-yellow. They are traditionally recommended in Chinese and Indian medicine as anti-inflamatory, anti-leukemic, anti-microbial, anti-tumor, anti-ulcer, and anti-diabetic agents [5]. Among the phytochemicals responsible for blood-glucose-lowering capacity are charantin, vicine, and polypeptide-p [3]. Also α-eleostearic acid (of the CLnA family) is potentially beneficial for health.

The pomegranate *Punica granatum* (*Punicaceae*) comes from the south-east part of Europe but nowadays it is grown in most of the countries of the tropical and subtropical regions of the world [6]. From antiquity it has been known for its multiple beneficial influences on health but its fruit is also consumed fresh or in form of juice, extract, wine or liqueur [7]. Seeds of pomegranate are rich in polyunsaturated fatty acids, among which punicic acid (cis-9, trans-11, cis-13 C18:3) is present in the greatest amount (over 70% of all its fatty acids) [8].

The objective of the present study was to investigate the influence of diet supplementation with pomegranate seed oil and/or an aqueous extract of dried bitter melon fruits (bitter melon tea) on the overall health status, glucose levels, and lipid profiles in the serum of rats.

MATERIALS AND METHODS

Bitter Melon Aqueous Extract

Commercially available dried fruit of bitter melon (*Momordica charantia*) (Tra Kho Qua, Gohyah Tea, CTE JSCO) was purchased from a local grocery store in Warsaw, Poland. Fresh aqueous extracts (tea) were prepared daily according to the manufacturer's description. Briefly, water (80˚C) was added to a weighed amount of dried fruits to obtain 2% (w/v) extract. After 10 min, the extract was filtered and freshly cooled tea was given to animals daily.

Pomegranate Seed Oil

Commercially available cold pressed, unrefined oil from seeds of pomegranate fruits (INCI: Punica Granatum (Pomegranate) Seed Oil) Zielony Klub) was purchased from a local market (Kielce, Poland). It was stored in 8˚C before being given to animals.

Animals

The whole experiment (including the guiding principles for the use and care of laboratory animals) were approved by the Local Ethical Committee on Animal Experiments. Female Sprague–Dawley rats (n = 24, age – 30 days) were purchased from the Division of Experimental Animals, Department of General and Experimental Pathology (Medical University of Warsaw, Warsaw, Poland). They were kept in animal room at 21ºC, with a 12 h light: 12 h dark cycle. During the entire experiment animals were fed *ad libitum* water and the standard laboratory fodder Labofeed H (Feed and Concentrates Production Plant, A. Morawski, Żurawia 19, Kcynia, Poland), which contains 22.0% protein, 4.0% fat, 30.0% starch, 5.0% fibre and 6.5% minerals. After 1-week adaptation, animals were randomly divided into 4 groups of 6 individuals each. The overall characteristics of each experimental group was as follows:

C – control group fed a standard diet and water *ad libitum*,

M – animals fed a standard diet and a 2% (w/v) aqueous extract of dried bitter melon fruits *ad libitum*,

G – animals fed a standard diet and water *ad libitum* and they received pomegranate oil given via a gavage in the amount of 0.15 ml/day,

M+G - animals were fed a standard diet and 2% (w/v) aqueous extract of dried bitter melon fruits *ad libitum* and additionally they received pomegranate oil given via a gavage in the amount of 0.15 ml/day.

Supplementation of the diet was conducted for 21 weeks. Rats were also weighed at weekly intervals to determine the body weight. In the 21st week of the

experiment all animals were decapitated and exsanguinated, and the weight of internal organs was determined.

Glucose Analysis

At the beginning of the experiment (week 0) and in the 7th, 14th and 21st weeks fasting glucose concentration in the blood was measured with CardioChek™ P.A. equipped Glucose Test Strips (RedMed Poland Sp. z o.o.). Blood for testing was collected intravitally from the tail vein.

Lipids Analysis

At the beginning of the experiment (week 0) and in the 7th, 14th and 21st weeks fasting total cholesterol (TC), HDL, LDL and triglyceride (TG) concentration in the blood were measured with CardioChek™ P.A. equipped with Lipid Panel Test Strips (RedMed Poland Sp. z o.o.). Blood for testing was collected intravitally from the tail vein.

Statistical Analysis

All data are shown as mean values ± standard deviation. All results were evaluated with Statistica 10.0 (StatSoft, Poland). Results of mass gained and internal organs' mass were evaluated with nonparametric Kruskal-Wallis test with multiple comparison test. Due to the accuracy of measurement method results obtained for glucose and lipids were tested with Q-Dixon test followed by one way ANOVA and RIR Tukey post-hoc test to verify the differences among groups (diet) as well as Friedman ANOVA test to verify the results obtained during the experiment for each group (time). P value ≤ 0.05 was considered significant.

RESULTS

Applied modification of diet did not influence negatively overall health condition of the animals. Although the initial body mass of the supplemented animals was slightly lower than mean body mass of the control animals, they reached high body mass at the end of the experiment. Weight increase in group receiving water extract of bitter melon fruits (M) was the highest, significantly higher than in control group. Although mean initial body mass of animals assigned to the group supplemented with pomegranate oil (G) was the lowest and they reached the lowest end body mass, mass increase in this group was similar to that in the other groups, even slightly higher than in the C and M+G groups.

We compared the influence of applied supplementation on the average weights of internal organs. Mean mass of liver and spleen did not differ among groups whereas the addition of pomegranate oil to the diet caused significant decrease of mean mass of kidneys and heart as the mean mass of those organs from the G and M+G groups was significantly lower than in the M group. However, when we compared those parameters expressed in relation to

Table 1: Body Weight and Internal Organs Weight of Rats Supplemented with an Aqueous Extract of Dried Fruits of Bitter Melon and/or of Pomegranate Oil

	C (n=6)	M (n=6)	G (n=6)	M+G (n=6)	P value
Mass start [g]	107.3 ± 3.9[a]	83.5 ± 11.8	63.0 ± 10.4[a]	79.2 ± 25.4	0.0024
Mass end [g]	229.3 ± 8.9[a,b]	235.7 ± 10.2[c,d]	208.5 ± 11.3[a,c]	209.3 ± 10.9[b,d]	0.0002
Mass increase [g]	122.0 ± 5.8[a]	152.2 ± 15.2[a]	145.5 ± 12.4	130.2 ± 16.7	0.0103
Liver [g]	6.24 ± 0.41	6.32 ± 0.66	5.53 ± 0.81	5.77 ± 0.63	NS
Liver [%]	2.72 ± 0.14	2.69 ± 0.36	2.64 ± 0.24	2.76 ± 0.30	NS
Kidneys [g]	1.84 ± 0.07[a,b]	1.82 ± 0.08[c,d]	1.58 ± 0.12[a,c]	1.62 ± 0.12[b,d]	0.0002
Kidneys [%]	0.80 ± 0.04	0.77 ± 0.03	0.76 ± 0.02	0.76 ± 0.03	NS
Spleen [g]	0.48 ± 0.07	0.48 ± 0.03	0.49 ± 0.06	0.50 ± 0.03	NS
Spleen [%]	0.21 ± 0.03	0.20 ± 0.01[a,b]	0.24 ± 0.02[a]	0.24 ± 0.02[b]	0.0078
Heart [g]	0.82 ± 0.06	0.88 ± 0.04[a,b]	0.77 ± 0.03[a]	0.78 ± 0.04[b]	0.0020
Heart [%]	0.36 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	NS

All data are shown as mean values ± standard deviation. P value ≤ 0.05 - significant differences among groups in Kruskal – Wallis test; NS – not significant - P value > 0.05 in Kruskal – Wallis test; values sharing a letter (a, b, c, d) in one row are significantly different from each other (P ≤ 0.05) in multiple comparison test.

the total body mass as a percentage of total body mass, only spleen weights of groups fed with pomegranate oil (G and M+G) were significantly higher than in other groups (Table **1**).

Levels of fasting glucose in the blood of all experimental groups during the experiment are presented in Table **2**. Fasting glucose concentration in blood of the C group was the highest at the beginning of the experiment and it was decreasing during the experiment (p=0.0220). Unlimited availability of bitter melon fruits extract (group M) slightly decreased the fasting glucose concentration during the experiment but its action was not statistically significant (p>0.05). Diet supplementation with pomegranate oil caused an increase of fasting glucose level in the G group (p=0.0366) but in the M+G group its influence was diminished by the opposite activity of bitter melon fruits extract (p>0.05). However, when we compared the mean fasting glucose levels at the end of the experiment, there were no significant differences among all experimental groups (p>0.05).

Changes of the total cholesterol concentration are given in Table **2**. Total cholesterol concentration in blood of the C group was increasing during the experiment (p=0.0307). Similarly, cholesterol levels in

Table 2: Effect of an Aqueous Extract of Dried Fruits of Bitter Melon And/or of Pomegranate Oil on Glucose Level and Lipid Profile in Serum

	Group				P value
	C	M	G	M+G	diet
Glucose [mg/dl]					
Week 0	91.8 ± 6.1[a,b]	82.2 ± 14.8[c,d]	60.0 ± 8.1[a,c]	61.3 ± 5.6[b,d]	<0.0000
Week 7	74.2 ± 12.6	81.8 ± 7.8	77.2 ± 3.6	72.6 ± 2.2	NS
Week 14	67.3 ± 5.1[a]	68.2 ± 12.2[b]	88.2 ± 7.1[a,b]	72.2 ± 16.8	0.0149
Week 21	66.7 ± 9.4	74.5 ± 6.0	73.8 ± 10.0	72.7 ± 10.2	NS
TC [mg/dl]					
Week 0	146.3 ± 12.8[a]	133.2 ± 6.2	119.8 ± 18.4[a]	133.0 ± 15.6	0.0318
Week 7	134.3 ± 8.8	136.8 ± 26.5	125.8 ± 15.4	145.7 ± 15.3	NS
Week 14	154.3 ± 19.4[a]	156.5 ± 19.6[b]	122.0 ± 14.0[a,b]	152.2 ± 18.1	0.0106
Week 21	159.2 ± 13.9[a]	164.8 ± 5.5[b]	128.3 ± 16.6[a,b]	148.0 ± 27.1	0.0143
HDL [mg/dl]					
Week 0	86.7 ± 9.8[a]	71.5 ± 6.9[a]	77.0 ± 9.8	77.2 ± 8.2	0.0488
Week 7	89.2 ± 10.7	93.0 ± 6.5	90.2 ± 11.0	92.0 ± 7.5	NS
Week 14	84.5 ± 5.6	88.8 ± 8.1	82.7 ± 11.5	93.3 ± 10.4	NS
Week 21	97.0 ±3.8[a]	95.5 ± 6.4	83.8 ± 12.8[a]	97.2 ±3.9	0.0237
LDL [mg/dl]					
Week 0	46.5 ± 15.2	47.5 ± 9.0	33.7 ± 27.7	37.8 ± 10.2	NS
Week 7	29.5 ± 15.9	19.0 ± 2.0	29.0 ± 5.5	33.8 ± 8.7	NS
Week 14	44.5 ± 19.9	48.4 ± 14.6	83.3 ± 10.6	47.0 ± 21.2	NS
Week 21	25.0 ± 13.1	43.5 ± 4.8	83.3 ± 11.7	12.5 ± 9.2	0.0297
TG [mg/dl]					
Week 0	65.5 ± 9.8	70.8 ± 17.1	68.0 ± 25.7	89.7 ± 17.5	NS
Week 7	79.0 ± 11.3	95.8 ± 19.1[a]	73.0 ± 10.6	77.0 ± 9.7	0.0352
Week 14	128.8 ± 24.0[a]	115.2 ± 41.2[b]	49.2 ± 21.7[a,b]	97.8 ± 22.8	0.0006
Week 21	180.7 ± 20.3[a,b]	138.5 ± 36.9[c]	87.7 ± 25.8[a,c]	99.0 ± 21.0[b]	<0.0000

All data are shown as mean values ± standard deviation. P value ≤ 0.05 - significant differences among groups in one-way ANOVA; NS – not significant - P value > 0.05 in one-way ANOVA; values sharing a letter (a, b, c) in one row are significantly different from each other (P ≤ 0.05) in RIR Tukey test.

blood of rats receiving bitter melon extract as a drink (group M) were slightly increased during the experiment, reaching by the end of experiment the highest level of all experimental groups (164.8 ± 5.5 mg/dl). As far as the G group is concerned, the total cholesterol level was the lowest in comparison with the other groups and it did not change during the time of experiment. This suggests that the diet supplementation with pomegranate oil prevents the age-related increase in cholesterol level. Total cholesterol content in blood of the G group was significantly lower than in other groups in 14th ($p=0.0106$) and 21st ($p=0.0143$) week respectively. Simultaneous supplementation of the rats' diet with pomegranate oil and an aqueous extract of the dried fruits of bitter melon prevented the positive action of the pomegranate oil, as the total cholesterol content in blood of the M+G group did not differ from its concentration in the C and M groups.

Profiles of HDL fraction concentrations in blood of all experimental groups is presented in Table **2**. Content of HDL in the blood of control animals did not change during the experiment, and only at the end was slightly higher than before. The level of this fraction in the blood of experimental animals has increased significantly ($p=0.0076$) by the unlimited consumption of an aqueous extract of bitter melon fruits (group M). Moreover, supplementation of the diet with both bitter melon extract and pomegranate oil elevated HDL concentration ($p=0.0094$) even more strongly as the HDL content in blood of the M+G group by the 21st week was the highest of all experimental groups. However, supplementation of the rats' diet with pomegranate oil (group G) only negatively influenced the HDL concentration and the level of this fraction was the lowest in blood of the G group at the end of the experiment (83.8 ± 12.8 mg/dl). This suggests that the apparent decrease of total cholesterol content caused by the pomegranate oil supplementation may be the negative effect of HDL level decreasing.

Changes of LDL fraction profiles are presented in Table **2**. As far as this parameter is concerned, we observed extremely large fluctuations among individuals in each experimental group. This makes it difficult to determine the influence of applied modification of diet. Due to the fact, that LDL concentration in blood of the G group seems to have been stable for the whole period of experiment, it is possible that pomegranate oil supplementation may influence the LDL concentration.

Table **2** shows also the TG profiles of all the experimental groups during the experiment. Concentration of TG in the blood of control animals increased significantly with age ($p=0.0007$). Addition of aqueous extract of bitter melon fruits as a drink slightly diminished this tendency. However, supplementation of rats' diet with pomegranate oil strongly suppresses this tendency, as the TG content in blood of G group was significantly lower than TG content in C and M groups at 14th ($p=0.0006$) and 21st ($p<0.0000$) week respectively. Similar activity but not as pronounced was visible as far as the M+G group was concerned.

DISCUSSION AND CONCLUSION

To our knowledge, this is the first study to assess the influence of a combined administration of pomegranate seed oil and bitter melon tea on health status in female rats. We do not report any harmful impact of applied diet modification on body and organ weight. Our observations are in accordance with those made by other authors. Jayasooriya *et al*. fed male Sprague-Dawley rats with diet supplemented with different levels of freeze dried powder of bitter melon for 14 days and did not observe any differences in food intake, body weight gain or relative liver weight in comparison with a control group [9]. Also in studies of Saha *et al*. who supplemented diet of rats with different levels of bitter gourd seed oil, no changes in body weight were observed [10]. Castellano *et al*., who fed piglets with the mixture of cis-9, trans-11, cis-15 C18:3 and cis-9, trans-13, cis-15 C18:3, did not report any influence of such a dietary treatment for 15 days on body and organ weights [11]. Also Plourde *et al*. observed no differences in food intake and final body weight in hamsters fed with a control diet and diet supplemented with similar CLnA mixture [12]. However, male, wild-type CD-1 mice fed with a high-fat chow and with pomegranate seed oil (20 g oil/kg chow) reached significantly lower body weight ($p=0.002$), absolute weight gain ($p=0.002$) and percentage weight gain ($p=0.01$) compared to mice fed high-fat chow alone [13]. Results of Miranda *et al*. who fed male Wistar rats with a diet supplemented with 0.5% of punicic did not confirm this observation as they did not find any significant differences in final body weight between the experimental and control groups [14].

Grover *et al*. who summarized the potential use of bitter melon fruits, claimed that their consumption is safe with no adverse effects in most of the experimental models. However, abortifacient activity is observed as well as traditionally attributed to bitter

melon. Fruits and seeds seem to demonstrate grater toxicity than the leaves and aerial parts of a plant. Some adverse effects were reported in mice (reduced fertility, favism-like syndrome), children (hypoglycemic coma, convulsions) and adults (headaches) but they were rather rare [5]. As far as pomegranate is concerned, no adverse effects are attributed to its consumption. Randomized controlled trial of Yuan *et al*. who administered 3 g of punicic acid for 28 days to healthy young humans, revealed no side effects of such a dietary intervention [15]. Moreover, dietary supplements containing oil from pomegranate seeds and air dried or lyophilized fruits of bitter gourd are commercially available in many countries all over the world.

Bitter melon has been widely studied with regard to its antidiabetic effect and many researchers confirm its antidiabetic and hypoglycaemic properties [5, 16]. We have decided to study the influence of bitter melon tea both on glucose level and on lipid profiles as in diabetes there are disturbances not only in glucose but also in circulating lipids levels. In diabetes transport of glucose into the body cells is diminished and therefore lipids are used much more as fuel to generate ATP. The lipids are made available from fat depots and this results in a high level of circulating lipids. Chaturvedi *et al*. administered a methanol extract of bitter melon fruits to albino rats for 30 days. This caused enhancement of oral glucose tolerance. Such a treatment resulted also in a significant reduction of LDL and TG levels ($p<0.001$) and in significant increase of HDL level ($p<0.001$) [17]. Also addition of bitter melon seed oil to the diet of diabetic rats significantly lowered TC and non-HDL-cholesterol levels as well as the TG level [18]. Jayasooriya *et al*. observed hypoglycaemic effect of bitter melon freeze-dried powder supplemented to rats fed with a cholesterol-free diet. However, these effects were reversed in rats fed a cholesterol-enriched diet. Dietary bitter melon also caused an elevation of HDL level but there was little effect on other serum lipid parameters [9]. On the other hand, Saha *et al*. reported an explicit action of CLnA isomers, as they observed normalization of TC, LDL, HDL and TG levels in diabetic rats [10]. Our results seem not to confirm this above-mentioned observation, as the influence of aqueous extract of bitter melon dried fruits on examined parameters was negligible. However, in our experiment there was no pathological condition as diabetes, which can be the main reason for the small impact observed by us.

Influence of punicic acid or pomegranate seed oil on glucose and lipids levels in serum was also investigated in many experimental models, however, the results are not consistent. McFarlin *et al*. supplemented the high-fat diet of mice with pomegranate seed oil and they did not observed any differences in glucose concentration among the groups investigated. A diet modification administered for 14 weeks also did not influence TC, TG and HDL levels [13]. Also Miranda *et al*. who modified rats diet with 0.5% addition of punicic acid, did not observe any significant differences in TC, TG, HLD and non-HDL levels [14]. Moreover, punicic acid given to healthy young humans for 28 days also did not cause any differences in TC, TG, HDL and LDL levels [15]. Above-mentioned results do not confirm our observations, as pomegranate seed oil given to female Sprague-Dawley rats for 21 weeks caused distinct changes in their lipid profiles, especially in the TC and TG levels.

This is the first report demonstrating the influence of separate and combined administration of pomegranate seed oil and bitter melon tea on health status and glucose and lipids levels in female rats. As our results are only partially in agreement with the results obtained by other researchers, it is necessary to extend this project with other experimental models, especially with respect to pathological processes, such as atherosclerosis and diabetes.

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