EFFECTS OF CITRUS LIMON BURM. F. ON SOME ATHEROSCLEROSIS RISK FACTORS IN RABBITS WITH ATHEROGENIC DIET

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Abstract

BACKGROUND: Dietary polyphenols have been shown to possess cardio protective effects. Recently, dietary citrus flavonoids have been associated with reduced risk of coronary heart disease in epidemiological studies. So, this study was designed to determine the effects of Citrus limon Burm. f. juice and peel on cardiovascular risk factors in male rabbits with atherogenic diet.

METHODS: Forty rabbits were randomly divided into four groups and treated 2 months as follows: 1: Hypercholesterolemic diet (HCD), 2 and 3: HCD + lemon juice (5cc daily) and lemon dried peel (1g daily), respectively and 4: normal diet. At the baseline and the end of the study in addition to weight, biochemical factors (FBS, TC, TG, ApoA1, ApoB100, LDL, HDL) were measured in the rabbits. Blood pressure measurements were conducted directly after the intervention.

RESULTS: Compared with 4th group total cholesterol was significantly increased during the study in 3 first groups ($p = 0.00$). Results for HDL and LDL cholesterol were the same ($p = 0.00$). But compared with control group (first group), only LDL cholesterol mean value was significantly increased in group 2 (lemon juice consumer) after intervention ($p<0.05$). And for apolipoproteins, only ApoB100 was significantly increased in group 3 (lemon peel consumer) in comparison with control group after the intervention ($p < 0.05$).
Weight and FBS have not been significantly changed between the groups after interventions. Also, blood pressures did not show any significant difference between the groups.

CONCLUSION: We conclude that opposite to high amount of antioxidants in sore lemon, it not only was able to control the hyperlipidemic effects of an atherogenic diet but also increased serum LDL level and Apo B. But decision about antiatherogenic effect of this fruit, will be made after defining pathologic results.

Keywords: Lipid profile, risk factor, flavonoid, rabbit, atherogenic diet, blood pressure.

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Introduction

Cardiovascular diseases (CVD) play a certainly role in the morbidity and mortality rates in the world, both in developed and in developing countries.[1,2]

For a long time, the idea that CVD were genetically determined and little could be done about their prevention has prevailed. However, the recognition of risk factors has changed this view.[3] Some risk factors like age, sex and heredity, cannot be changed or controlled while hypertension, dyslipidemia, obesity, smoking, and etc are controllable ones.[4,5] Dietary interventions remain the initial choice for the restraint controllable risk factors.[6] Also, with the global prevalence of coronary artery diseases(CAD), there is an increasing need for dietary approaches to successfully manage existing, as well as prevent the occurrence of CAD.[7] Meanwhile, the use of natural foods can be expected to offer protection from peroxidative damage- which is one of the early processes of CAD -without side effects.[8]

Over the past decade, there has been increasing interest in a large class of natural plant compounds known as flavonoids, and their potential to improve human health, including cardiovascular health.[9] Epi-

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demiological studies have found an inverse association between the consumption of flavonoid-containing foods and the risk of CVD.[10,11] Three types of flavonoids (flavanones, flavones, flavonols) and more than 60 individual flavonoids ,have been identified in citrus fruits.[12] Lemon fruit as a member of this family has been known as a typical healthy food for a long time. As lemon juice and peel contain various biofunctional nutrients as flavonoids, carotenoids, and ascorbic acid[13,14], it has desmutagenic and antimutagenic effects.[15,16] Citrus limon Burm. f. is a species of sore lemon fruit that is abundant in Iran and use much by our population. Therefore, because of favorite content of the lemon and its abundance in Iran and high prevalence of CAD risk factors of in our society, this study was designed to evaluate the effects of fresh juice and dried peel of this lemon species on some CAD risk factors in male rabbits with an atherogenic diet.

Materials and Methods

The preparation of juice and peel of lemon

Aerial parts (stem, leaves and fruits) of Citrus limon were collected from Shiraz gardens (Fars-Iran) at fruiting stage in 2007 and authenticated by botanist at the Biology department, School of science, Isfahan University. The voucher specimen was deposited in Isfahan University Herbarium under the number 14067.

Peels of the fruits were dried for 3 days at room temperature. The dried peels were ground by an electric blender. Lemon juice was prepared freshly by hand.

Animals and Diets

Forty 10-weeks old male New Zealand white rabbits with the average weight of 2 ± 0.3 kg were purchased from Paustor Institute, Teheran, Iran. The rabbits were housed individually at 24 ± 1°C with a 12-h: 12-h light/dark cycle. Animals were fed a normal rabbit chow (Super Fosskorn, Dam Pars Co., Tehran, Iran) for 3 weeks, then they were randomly divided into four groups of 10 animals. Each group of animals had its specific diet and regular drinking water ad libitum. As the control group, animals in group 1, received a normal diet supplemented with 1% cholesterol dissolving in 2 cc olive oil (HCD) through out the experiment (2 month). Animals in group 2 and 3 received HCD supplemented with 5cc lemon juice and 1g powder of lemon dried peels, respectively. In group 4 animals only received normal diet.

Isfahan Cardiovascular Research Center Ethics Committee which is a member of office for human research protections, US department of health and human services, approved the present study, and the animals were handled according to guidelines of Isfahan University of Medical Sciences for Laboratory Animal Sciences for the care and use of laboratory animals.

Blood samples and biochemical factors measurement

Fasting blood specimens were taken from heart of each rabbit at baseline and at the end of the study. Serum samples were separated by low speed centrifugation (3000 x g for 15 minutes) and used for measurement of biochemical factors.

Total cholesterol (T.Cho), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and fasting blood sugar(FBS) levels were determined using an automated enzymatic assay by Hitachi 902 auto-analyzer and using special kits (Diasys Diagnosis Inc., Holzheim, Germany) performed by Pars–Azmun (Tehran, Iran).

ApoA1 and apoB100 were assayed by immunoturbidometric methods (Diasys Diagnosis Inc.,Holzheim, Germany) performed by Pars - Azmun.

All rabbits were weighted before and after the intervention by an animal Seca scale.

Blood pressure was measured at the end of the study directly from femoral vein by osilograph.

Statistical analysis

Results are expressed as the mean ± SD. All analyses were performed using SPSS (version 12.5) statistical software. One way and Repeated Measure ANOVA with post Hoc test were used to analysis the data.

Results

Effect of lemon juice and peel on FBS, weight and blood pressures, serum lipids and lipoproteins of rabbits have been presented in tables 1 and 2.

Based on data of table 1 weight ($P = 0.795$) and FBS ($P = 0.371$) do not have significantly changed between the groups after interventions. Also, blood pressures do not show any significant difference between the groups ($P > 0.740$).

Compared with 4th group, total cholesterol was significantly increased during the study in 3 first groups ($P = 0.00$). Results for HDL and LDL cholesterol were the same ($P = 0.00$). But compared with control group (first group), only LDL cholesterol mean value was significantly increased in group 2 (lemon juice consumer) after intervention ($P < 0.05$). And for apolipoproteins, only ApoB100 was significantly increased in group 3 (lemon peel consumer) in comparison with control group after the intervention ($P < 0.05$).

Table 1. Changes in serum fasting blood pressure, weight and blood pressures of 4groups after 2 months of intervention

Factor	Group 1		Group 2		Group 3		Group 4		P
	Before	After	Before	After	Before	After	Before	After	
Weight (Kg)	3.0±05.2	3.0±3.2	3.0±1.9	4.0±2.2	2.0±2.2	2.0±4.2	2.0±1.9	0.3±2.3	0.795
FBS (mg/dl)	1.8±1.100	0.18±6.84	0.6±6.98	3.9±74	5.6±0.106	3.13±9.90	3.8±0.105	17.4±79.5	0.371
(mmHg) Systolic Blood Pressure		7.14±3.83		5.19±4.80		6.11±7.84		11.8±86.0	0.896
(mmHg) Diastolic Blood Pressure		1.9±9.55		8.13±8.56		3.10±3.60		12.0±55.0	0.741

(1) Cholesterol + Olive oil, (2) Cholesterol + Olive oil + lemon juice, (3) Cholesterol + Olive oil + lemon peel, (4) Olive oil

Table 2. Change in serum lipids and lipoproteins of 4 groups after 2 months of intervention

Factor	Group 1		Group 2		Group 3		Group 4		P
	Before	After	Before	After	Before	After	Before	After	
T.Cho (mg/dl)	9.9±5.34	8.5±7.978	8.5±6.49	7.197±6.931	.9.9±5.48	1.106±5.999	3.12±7.48	8.18±1.59	000.0
LDL (mg/dl)	2.4±6.13	7.133±9.242	9.3±6.17	3.112±1.454	7.4±0.16	7.121±4.229	8.6±4.13	3.11±1.1.9	000.0
HDL (mg/dl)	4.5±9.13	8.19±7.107	3.5±1.15	8.28±4.114	5.1±15.6	26..3±117.6	0.6±3.18	8.8±6.27	000.0
TG (mg/dl)	1.26±6.61	1.284±2.227	8.13±3.54	8.23±7.96	6.8±6.39	7.162±138.6	8.10±8.38	5.8±9.75	301.0
apo AI (mg/dl)	5.5±7.25	1.6±5.31	4.4±1.26	4.5±8.31	1.4±2.55	0.6±4.36	6.4±1.27	8.7±33.8	0.444
Apo B-100 (mg/dl)	2.1±6.6	3.8±0.23	6.1±7.6	0.16±1.33	5.1±2.7	8.9±2.37	0.2±8.6	6.9±0.21	031.0

(1) Cholesterol + Olive oil, (2) Cholesterol + Olive oil + lemon juice, (3) Cholesterol + Olive oil + lemon peel, (4) Olive oil

Discussion

In the present study which was conducted on rabbits taking an atherogenic diet, it was revealed that lemon juice decreases only serum LDL level significantly and it has no effect on glucose or other serum lipids. Sour lemon peel had no effect on the aforementioned factors. Dissimilar effects of this fruit,e have been reported till now. Results of a research have showed a hypocholesterolemic effect for it.[17] However in another study on diabetic rats, there was no positive effect of citrus juice on blood cholesterol and glucose. This study also revealed that citrus juice has little influence on food absorption or cholesterol metabolism.[18]

It is better to focus on effective ingredients of sour lemon on CAD in order to find the possible role of citrus on CAD risk factors. In addition, it should be noted that the evidences of the possible effect of flavonoids and their derivatives on lipid metabolism, are limited.[19]

Eriocitrin, haspiridin and narinjin are the most important flavonoids in sour lemon juice.[20,21] Some studies have revealed the hypolipidemic effect of haspiridin and narinjin.[22,23] Moreover, Miyake et al. reported a decreasing effect of eriocitrin on serum total cholesterol, LDL+VLDL and phospholipids in rats with a hypercholesterolemic diet.[24]

In addition, we measured serum apo AI and apo B-100 which their metabolism is directly related to

lipid metabolism and it was observed that lemon peel can significantly increase only serum apo B. There were paradoxes in comparing the results of our study with the results of other researches.

There are not enough researches about the hypolipidemic effect of this fruit and the present results are not sufficient to conclude. Also, these variations are related to different dosages, usage duration, and species of lemon used in various studies. We used fresh and whole lemon juice which may lead to the observed differences. Even the way of squeezing, may influence the type of flavonoids and their results.[14] As most of the researches have studied the effects of a single flavonoid of lemon, the whole lemone juice or peel (with combination of flavonoids) could have a different effect rather than a single one. Also, maybe the taken dosage in our study didn't have hypolipidemic effect.

Understanding of the metabolism of the flavonoids and their metabolites action in live systems, are very important for finding their effects.[25] The first point to be considered is that the mechanisms of flavonoids effects on lipid metabolism, is not clear yet.[26] So finding the reason for difference of our results with the mentioned studies, is not possible. This contrast could be explained by the aggregated effect of flavonoids of lemon. Although most of the flavon glycosides and flavonones in sour lemon juice have been identified,[27,28] perhaps the observed effects are related to some unknown compounds in lemon.[29]
Maybe the other reason is that although most herbal poly phenols have antioxidant effects in usual, they can act conversely in special condition. Morine et al. have reported that hesperidin and nobiletin, increase LDL receptor gene expression.[30]

It is obvious that, dose enhancement of many flavenoids, residue the indiction effect; however the others present the inhibitory influence which caused the inverse U shape pattern in the dose-response curve.[30] According to in vitro investigations, useful effects of most of flavonoids are observed in non physiologic concentrations (> 10 µmol/l).[31-33] This implies that most flavonoids effect is observed in high doses.

The other point which needs to be mulled over, is the slight increasing effect of lemon juice on LDL compared with lemon peel ($P < 0.001$). This difference can be referred to the different type and concentration of flavonoids content of lemon peel and juice.[14]

Flavonoids of lemon juice are commonly of flavonone type (which contain B circle including haspiridin, naringin and eriocitrin)[34] while in lemon peel there is a great deal of flavone like PMF and limonoid which contain A circle.[34,35] In vivo studies have shown that PMF content of citrus peel decreases plasma cholesterol level in a lower dose than flavonones like haspiridine.[31] Moreover, Hydroxy cinamic acid glycoside (a type of flavonoid),[36] 6,8-c-b-diglucosylapigenin and 6,8-C-ß-diglucosyldiosmetin[37] and diosmin[14] have been found in lemon peel which were not found in their juice. Also, all citrus fruits contain valuable fibers especially in the white part of peel[38] that their hypolipidemic effects have been proved.[39]

In our study, maybe atherogenic diet (with very high amount of cholesterol) caused not to findany possible effect of lemon juice or peel on serum lipids and lipoproteins. Probably the used dosage of lemon juice and peel was not sufficient for controlling this level of cholesterol.

The other studied risk factor was blood pressure which was not significantly different among the groups after the intervention. While in a research hypotensive effects have been reported for lemon juice.[16] A part of this effect can be referred to poly phenols in fruits and vegetables.[40,41] Probably, the type and amount of flavonoids are effective in this regard.[42] A research showed that nine various flavonoids of lemon have different effects on blood pressure.[43] So, the aggregated effect could be different from the effect of each one of them alone. Also, may be the reported hypotensive effect is in hypertensive population not normotensive ones. Lack of lemon effect on blood pressure was consistent with lack of its effect on weight.

To sum up, taking the results of this study into consideration, it can be concluded that the Iranian type of sour lemon which is Citrus Limon BURM f. has no considerable effect on CAD risk factors in people with atherogenic diet considering the dosage used in this study. These findings cannot be generalized to the presence or absence of its effect on atherosclerosis.

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