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Mulberry Fruit Extract Consumption is Inversely Associated with Hyperlipidemia in Middle-aged Men

†Ae-Jung Kim, Soo-Jin Park* and Jeong-Ok Rho**

Dept. of Food and Nutrition, Hyejeon College, Choongnam 350-702, Korea
**Dept. of Food and Nutrition, Chonbuk National University, Jeonju 561-756, Korea*
***Dept. of Food and Nutrition, Baewha Women's College, Seoul 110-735, Korea*

오디 추출물이 중년 남성의 항고지혈증에 미친 효과

†김애정 · 박수진* · 노정옥**

혜전대학 식품영양과, *전북대학교 식품영양학과, **배화여자대학 식품영양과

Abstract

In a previous study, a mulberry fruit extract(MFE) supplement exhibited anti-inflammatory activity and improved serum lipid profiles in arthritic rats. The objective of this study was to determine whether dietary MFE could ameliorate inflammatory parameters and serum lipid levels in humans. Twenty-six middle-aged subjects(mean body mass index=27 kg/m²) consumed MFE(100 mℓ/day) after lunch for 4 wks. Anthropometric measurements, serum oxidative stress markers and serum lipid profile analyses were performed at baseline and then at 4 wk following the study. There were no significant differences in anthropometric measurements, including BMI, WHR, and body fat composition. After the 4 wk-intervention, serum levels of C-reactive protein(CRP), ferric-reducing ability of plasma(FRAP), serum triglyceride(TG) and LDL-cholesterol had significantly decreased($p<0.05$), whereas serum levels of HDL-cholesterol significantly($p<0.05$) increased. These findings suggest the consumption of mulberry extract may be protective against inflammation and the atherosclerotic state in elderly obese men at high risk for cardiovascular disease(CVD).

Key words: mulberry fruit extract(MFE), CRP, FRAP, triglyceride, HDL-cholesterol, LDL-cholesterol.

INTRODUCTION

Diets rich in fruits and vegetables have been of interest because of their potential health benefits against chronic diseases such as cardiovascular disease(CVD) and cancer[1]. It has been reported that anthocyanin rich berry varieties including blueberry, cherry, strawberries have anti-inflammatory effect of more 10 times than aspirin without any stomach troubles and also have better antioxidant effect[2].

Mulberry fruit, specifically, has been clinically used for the treatment of inflammatory conditions traditionally in oriental medicine[3]. In our recent study, anti-inflammatory efficiency of mulberry fruit intake was described in experimental arthritic rats[4]. The findings[3,5~8] support a protective role of mulberry fruit supplements in hyperlipidemia and inflammatory conditions, although the results were inconsistent[9]. Only a few previous studies, however, have been performed in which an association between intake of mulberry and bioactivity was observed. Furthermore, the increase in the prevalence of obesity, hyperlipidemia and dyslipidemia has been a major health concern in adult and elderly population in current[10]. Confirmation of the association between foods and disease risk therefore will improve the

† Corresponding author: Ae-Jung Kim, Dept. of Food & Nutrition, Hyejeon College, Choongnam 350-702, Korea.
Tel: +82-41-630-5249, Fax: +82-41-630-5175, E-mail: aj5249@naver.com

recommendations made for healthy diet.

Based on the previous observation, the aim of this study was to examine the effect of mulberry fruit extract intake on serum inflammatory parameters and serum lipid profiles in middle-aged Korean men.

MATERIALS AND METHODS

1. Preparation of Mulberry Fruit Extracts(MFE)

Mulberry fruits [*Morus ihou*(Ser.) Koids] were obtained from a breed registrator, NIAST(National Institute of Agricultural Science and Tech, Suwon, Korea) in June 19, 2006. Fully matured mulberry fruits were harvested and stored at −20℃ until further studies. Mulberry fruits were extracted according to the process by Imsil Herbal Medicine(Jeonbuk, Korea) to minimize the damage of anti-oxidative substances.

Briefly, After adding 3 times water than mulberry fruits' weight, the substance was filtrated using by hydrothermal extractor(Jin Young Machinery Co. Ltd, Seoul, Korea) at 55℃ for 100 minutes. After the primary filter(pore size: 1 ㎛ filter, Jin Young Machinery Co. Ltd, Seoul, Korea), filtrated material went through enzyme decomposition with adding pectinase (Ducsan Co Ltd, Daejeon, Korea) of 0.3%. These were then concentrated in a vacuum decompression until the solid content became 12%. The second filter(Misung Scientific Co. Ltd, Seoul, Korea) was taking by the same filtering machine(Misung Scientific Co. Ltd, Seoul, Korea). After the second filtration, citric acid(Ducsan Co. Ltd. Daejeon, Korea) of 0.01% to promote the stability and storage of anthocyanin pigment and functional oligosaccharide(Ducsan Co. Ltd, Daejeon, Korea) of 0.01% for sweeteners was added in filtrated material. It was then mixed and sterilized at 90℃ for 30 minutes. This mulberry fruit extract was then frozen after filling in pouch at 85℃. The frozen mulberry fruit extracts were stored in a freezer(Deep Freezer, NuAire, Plymouth, MN, USA) of −70℃ for 4 weeks to minimize the decrease of anti-oxidative activity and to maintain the stability of subjects.

2. Subjects

Thirty five voluntary healthy individuals aged 30~60 years were recruited for the study and 3 weeks before the start they were invited to the screening visit. Among them, 26 subjects completed the study. All participants did not report any of the following exclusion criteria: hypertension, diabetes mellitus, coronary heart disease(CHD), alcoholic liver disease, malnutrition, acute infectious diseases, fasting serum glucose(120 ㎎/dℓ), BMI<30 ㎏/㎡, LDL-C>160 ㎎/dℓ, HDL-C<40 ㎎/dℓ, and no alcohol abuse(less than 60 g ethanol/day). At baseline, all individuals were subjected to a medical evaluation by a physician, including a full medical history and physical examination. In addition, none were receiving any medication or taking any vitamin supplements. The procedures followed were in accordance with Good Clinical Practice. The protocol was approved by the research ethics committee of Heojun Medical center, and the informed consent was obtained from each subject.

3. Clinical Study Design

The study was based on an open, prospective, and single blinded design, and carried out from October 20 to November 17 in 2006. During the intervention period for 28 days, each subject consumed 100 ㎖ MFE(200 g for fresh fruit) per day after lunch. All subjects were allowed to eat their normal diet and encouraged to maintain their lifestyle and dietary habits.

4. Anthropometric Measurements

Anthropometric measurements were taken by Inbody 3.0(Bioelectrical Impedance Fatness Analyzer, Biospace Co, Seoul, Korea) while the subjects were dressed in light clothing, before and after the final intervention period. Height and weight were measured by an automatic height-weight scale. Waist circumference was measured at the mid point between the lower border of the rib cage and the iliac crest, and hip circumference was measured at the widest part of the hip region. All anthropometric measurements were checked by one person throughout the study to minimize interpersonal variations.

5. Biochemical Analysis

Blood samples were taken three times, at the beginning of the experimental period(day 0), in the middle of the study(day 15^{th}) and at the end of the experimental period(day 28^{th}) after 12 h fast. Subjects were not allowed to drink alcohol or to smoke in the morning of the blood sampling day. Serum was obtained by centrifugation at 3,000 rpm for 10 min at 4℃ directly after blood sampling then stored at −70℃ until other tests were done.

Serum levels of triglyceride(TG), total cholesterol(TC), HDL-cholesterol(HDL-c), LDL-cholesterol(LDL-c) were measured using commercial kits(Sigma Co. Ltd, NY, USA) according to

manufacturer's description. Atherogenic index(AI), high density lipoprotein cholesterol and total cholesterol ratio(HTR) and low density lipoprotein cholesterol and high density lipoprotein cholesterol(LHR) values were determined by the formula of Fiedeward *et al*[11].

Inflammation related markers such as serum uric acid was measured with ADVIA 1650(Bayer, Leverkusen, Germany) based on the uricase enzyme method. The quantitative analysis of either serum C-reactive protein(CRP) or serum rheumatoid factor (RF) was conducted using a Hitachi(Hitachi 7150, Tokyo, Japan), respectively, based on the immuno-turbidimetric assay. Serum levels of alanine aminotransferase(ALT) and aspartate aminotransferase(AST) were measured using a commercial kit (YD Diagnostics) based on the Reitman-Frankel method[12]. Serum TBARS level was analyzed using serum for hiobarbituric acid reactive substance(TBARS) as a peroxidation index based on Ohkawa method[13] and standard solution used 1,1,3,3-terraethoxpropane. Serum Ferric reducing ability plasma(FRAP) level was analyzed by Bennzie and Strain method[14] and standard solution used 2,4,6-tripyridyl-s-triazine.

6. Statistical Analysis

To compare the differences in the parameters obtained from the subjects, we used standard statistical methods from the SAS (SAS Institute, Cary, NC). The results of this experiment were verified within $p<0.05$, based on the student *T*-test.

RESULTS

Twenty six male subjects completed the 4-wk MFE intervention study. Baseline subject characteristics and anthropometric measurements including height, weight, BMI and body fat composition were summarized in Table 1. The age of subjects ranged from 34 to 56 years with mean±standard deviation of 45.25±11.62 years. Since the mean of BMI(27.23±3.27) in our subjects was higher than 23 kg/m², which proposed by World Health Organization(WHO) Expert Consultation[15], the subjects were defined obese at the beginning of the study. After the 4-wk intervention, there was no significant difference in the characteristics of subjects(Table 1). No adverse events on weight or body fat composition occurred at the end of the intervention in the present study.

Either AST or ALT is discharged into the blood due to the liver cell damage and their activity levels are increased[16]. Table 2 show the initial levels of AST or ALT were able to be accepted as normal range(less than 38 IU/dℓ or 43 IU/dℓ) clinically[17]. These initial enzyme concentrations tended to be reduced after mulberry extract, however, there were no significant difference through 4-wk intervention. Inflammatory processes play an integral part in the development and exacerbation

Table 1. Anthropometric measurements and changes after MFE intervention

Variables	Beginning	End	Change
Age(y)	45.25± 5.62[1)]	-	-
Height(㎝)	173.06± 5.79	-	-
Weight(㎏)	79.85±11.00	80.18±11.29[NS2)]	0.33±0.09
BMI(㎏/㎡)	27.23± 3.27	27.36± 3.49[NS]	0.13±0.22
WHR	0.91± 0.03	0.90± 0.12[NS]	0.01±0.09
Body Fat(%)	23.57± 4.33	23.56± 4.50[NS]	−0.01±0.17

BMI: body mass index, WHR: waist hip ratio,
1) Data are presented as mean±standard deviation(n=26),
2) The differences in values within a row before and after the intervention were analyzed using student *t*-test,
NS: not significant.

Table 2. Biochemical data and changes after MFE intervention

Variables	Beginning	End	Change
AST(IU/dℓ)	35.00±18.51[1)]	32.60± 19.81[NS]	2.40± 1.30
ALT(IU/dℓ)	38.31±21.91	37.46± 27.35[NS]	0.85± 5.44
RF(IU/dℓ)	4.60± 2.25	4.06± 2.54[NS]	0.54± 0.29
Uric acid (㎎/dℓ)	5.79± 1.63	5.38± 1.49[NS]	0.41± 0.14
CRP(㎎/dℓ)	0.48± 0.05	0.35± 0.02*	0.13± 0.03
TBARS (μmol/ℓ)	63.04±12.20	57.44± 11.16[NS]	5.60± 1.04
FRAP (μmol/ℓ)	1239.02±63.22	1456.21±134.24*	217.19±71.02

AST: aspartate aminotransferase, ALT: alanine aminotransferase, CRP: C-reactive protein, FRAP: ferric reducing ability plasma, TBARS: thiobarbituric acid reactive substance, RF: rheumatoid factor, TG: triglyceride, TC: total cholesterol, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol,
1) Data are shown as mean±standard deviation(n=26),
2) Values within a row with * are significantly different before and after the intervention by using student *t*-test($P<0.05$),
NS: not significant.

of atherosclerotic lesions. Having an elevated level of the acute-phase reactant, C-reactive protein(CRP) is considered a strong, independent predictor of coronary heart disease[18]. Therefore, we checked those inflammatory parameters including rheumatoid factor(RF) and uric acid whether they were altered by mulberry extract supplement. The initial RF was included in normal clinical range(less than 10 IU/dℓ)[18] but uric acid level was higher than normal clinical range(2.4～5.7 ㎎/dℓ)[18] in the present obese male subject. These inflammatory parameters tended to reduce after mulberry consumption but there was no statistical significance. In case of CRP level, the initial CRP level was higher than normal clinical concentration(less than 0.4 ㎎/dℓ) and it was significantly decreased as the duration of mulberry intake($p<0.05$)(Table 2). After 4wk-mulberry intervention, while the level of serum TBARS had no significant difference, serum FRAP level showed a significant increase($p<0.05$)(Table 2).

In Table 3, when subject's initial serum lipid levels were compared with the normal range of serum lipids levels for Korean[18] including TC(<200 ㎎/dℓ), HDL-C(42～74 ㎎/dℓ) and LDL-C(<130 ㎎/dℓ), they were nearly cut off level. Initial serum TG level of obese subjects especially was higher than normal range(<150 ㎎/dℓ). After the 4 wk intervention, serum TG level was more decreased within normal range($p<0.05$). Serum HDL-C concentration was significantly increased($p<0.05$), whereas serum LDL-C concentration was significantly decreased($p<0.05$) after the 4-wk intervention of mulberry extract(Table 3). Atherogenic Index($p<0.05$) and the LDL-C/HDL-C ratio($p<0.05$) were therefore significantly lower, whereas the HDL-C/total cholesterol ratio was significantly higher($p<0.05$) after 4-wk mulberry extract supplement(Table 3).

DISCUSSION

In this study, we observed that effectiveness of MFE supplement on reduction in serum inflammatory parameters, serum lipids in middle aged men until 4 wks.

Epidemiological observation have documented that dietary anthocyanin-rich fruit and vegetables such as grapes, elderberry, and red cabbage is related to a reduced risk of several degenerative diseases like chronic arthritis, atherosclerosis, cardiovascular disease, cancer, and diabetes[19].

The intake of anthocyanins in humans has been estimated to be 180～215 ㎎/day in United States[20], which is much higher than the intake(23 ㎎/day) of other flavonoids, including quercetin, kaempferol, apigenin and luteolin[21]. The ripe mulberry contain about 0.2% of major anthocyanin, cyanidin 3-glucoside (C3G). Therefore, the amount of mulberry fruit in this study, 100 mℓ/day(200 g/day for fresh fruit), can be assumed to have 40 ㎎ /day anthocyanin. Also, it can be estimated as a reasonable amount in life.

Our volunteer subject number was relatively small and limited living in South Western area of Korea but typical middle-aged Korean men due to their average BMI was 27 ㎏/㎡, considering

Table 3. Serum lipid profiles and changes after MFE intervention

Variables	Beginning	End	Change
Total cholesterol(㎎/dℓ)	196.33±29.72[1)]	175.43±35.18[NS]	20.90± 5.46
Triglyceride(㎎/dℓ)	170.93±83.16[2)]	137.31±10.71*	33.62±72.45
HDL-cholesterol(㎎/dℓ)	43.68± 8.85	50.13± 9.35*	6.45± 0.50
LDL-cholesterol(㎎/dℓ)	118.46±25.36	97.84±29.61*	20.62± 4.25
AI[3)]	2.91± 1.58	1.74± 0.27*	1.17± 1.31
LHR[4)]	2.71± 0.64	1.95± 0.73*	0.76± 0.09
HTR[5)]	0.26± 0.05	0.37± 0.06*	0.11± 0.01
CRF[6)]	4.50± 0.33	3.47± 0.04	1.03± 0.29

1) Data are shown as mean±standard deviation(n=26),

2) Values within a row with * are significantly different before and after the intervention by using student *t*-test($P<0.05$),

3) AI: Atherogenic index=(Total cholesterol－HDL-cholesterol)/HDL-cholesterol,

4) LHR: Low density lipoprotein cholesterol ratio=LDL-cholesterol/HDL-cholesterol,

5) HTR: High density lipoprotein cholesterol and total cholesterol ratio=HDL-cholesterol/total cholesterol,

6) CRF: Cardiac risk factor=Total cholesterol/HDL-cholesterol.

the recent prevalence of obesity 33% in Korean men[10]. Although a limited number of subjects, the results can be expanded to apply large intervention study.

Obesity has been explained as low-grade inflammation state[22]. It plays a role in both the initiation and progression of atherosclerosis and is associated with cardiovascular and metabolic diseases. In this study, since mulberry intake markedly reduced levels of an inflammatory acute phase reactant including CRP, FRAP as well as ameliorated serum lipid profile significantly, it confirms to improve the recommendations made for healthy diet. Moreover, the TG level showed early decrease at 2 wk-intervention, while most significant responses has been shown at 4 wk-intervention, long term intake of mulberry thus can be recommended to improve the effectiveness.

As previously reported[4], free radical scavenging activities of mulberry extract was very high 78.2% of BHA control, we speculated part of hypolipidemic efficacy of mulberry extract led from antioxidant action.

We found that the consumption of mulberry extracts effectively suppress the production of CRP but increase FRAP, also normalized the circulation level of TG and HDL-C and LDL-C in Korean obese men. The present study primarily focused on the efficacy of the whole extracts of mulberry fruit more than specific individual constituents derived from the extracts, with an objective of predicting the potential effects of mulberry fruit consumption. Conclusively, intake of mulberry extract might improve lipid metabolism and alternative way to treat patients with hypercholesterolemia because of their complex ingredients.

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