Nutrition 30 (2014) 198–203

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Effects of bayberry juice on inflammatory and apoptotic markers in young adults with features of non-alcoholic fatty liver disease

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ARTICLE INFO

Article history:
Received 15 February 2013
Accepted 24 July 2013

Keywords:
Apoptosis
Bayberry
Inflammation
Non-alcoholic fatty liver disease
Oxidative stress

ABSTRACT

Objective: Oxidative stress and inflammation are involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Bayberries contain high levels of polyphenols that possess anti-oxidative and anti-inflammatory properties in vitro. The purpose of this study was to investigate whether the consumption of bayberry juice beneficially alters the levels of oxidative, inflammatory, and apoptotic biomarkers in young individuals with features of NAFLD.
Methods: In this randomized, placebo-controlled, double-blind, crossover study, 44 participants (ages 18–25 y) were given 250 mL of either bayberry juice or placebo twice daily for 4 wk. Several anthropometric characteristics were measured, and fasting blood samples were drawn before and after each intervention period. The levels of plasma glucose, insulin, lipids, and some NAFLD-related biomarkers were determined.
Results: No significant effects on the anthropometric parameters and the homeostasis model assessment for insulin resistance were observed. Compared with placebo, the consumption of bayberry juice significantly decreased the plasma levels of protein carbonyl groups ($P = 0.038$), tumor necrosis factor-α ($P < 0.001$), and interleukin-8 ($P = 0.022$). The apoptosis markers analysis revealed significant differences between the treatment and the placebo in the levels of tissue polypeptide-specific antigen ($P < 0.001$) and cytokeratin-18 fragment M30 ($P < 0.001$).
Conclusion: The consumption of bayberry juice for a period of 4 wk can protect against NAFLD in young adults by improving the plasma antioxidant status and inhibiting the inflammatory and apoptotic responses that are involved in this disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide and encompasses a morphologic spectrum of simple steatosis, nonalcoholic steatohepatitis (NASH), and hepatic cirrhosis [1]. Emerging evidence suggests that hepatic inflammation and necrosis, in addition to oxidative stress and insulin resistance, play a role in the pathogenesis of NAFLD and more precisely in the transition from simple steatosis to NASH, which predicts both disease progression and liver-related complications over a subsequent 10-y period [2]. Excessive fat accumulation in hepatocytes, regardless of its cause, tends to induce the activation of nuclear factor-κB, which is the key regulator of inflammation. Subsequently, the expression of the inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-8 increases, which leads to neutrophil infiltration and inflammatory liver injury [3,4]. Additionally, lipid accumulation in hepatocytes can trigger specific signaling pathways that result in apoptotic and necrotic cell death [5]. The key role of apoptosis and necrosis in the progression from steatosis to NASH has been supported by various studies that demonstrated that the serum levels of total cell death and apoptosis markers are elevated in patients with NASH and that the levels of these markers are correlated with histologic severity [6].

HG and WL conceived the idea and designed the study. XJ, XT, and ZL performed the assay experiments and assisted with the statistical analysis and the interpretation of the data. YL and RZ participated in the participant enrollment and the distribution of beverages. HG drafted the manuscript. MX provided critical corrections to the manuscript. All of the authors read and approved the final manuscript. The authors declare that there are no conflicts of interest.

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http://dx.doi.org/10.1016/j.nut.2013.07.023

An ideal NAFLD treatment remains elusive. Lifestyle modifications, including physical exercise and a well-balanced diet, are the cornerstone of NAFLD treatment [7]. The inclusion of ω-3 polyunsaturated fatty acids, fruits, and vegetables and the reduction of saturated fat may be universally recommended to NAFLD patients [8,9]. Although fruits provide essential vitamins, minerals, and dietary fiber, the antioxidant, anti-inflammatory, and lipid-lowering properties of their polyphenols provide the rationale for the protective effects of fruits against NAFLD [10–12].

Chinese bayberry (*Myrica rubra* Sieb. and Zucc.), which is also called waxberry or Yang-mei, is a subtropical evergreen fruit tree native to China and Southeast Asia, and its cultivation has been recorded in Chinese history for more than 2000 y. Bayberries have a unique sweet, sour taste, and their red, purple, or dark-red color is due to the presence of anthocyanins [13]. Bayberries also contain a variety of phenolic acids, including caffeic, ferulic, sinapic, and salicylic acids [14]. These phytochemicals exhibit a high antioxidant capacity in vitro [15]. Additionally, berries rich in anthocyanins and phenolic acids have been shown to improve features of experimental NASH, such as oxidative stress, dyslipidemia, liver steatosis, and inflammation, in rodents [16–18]. However, there is a paucity of clinical data on the hepatic health benefits of berries.

Thus, this study was designed to test the hypothesis that bayberry supplementation in the form of pasteurized juice can improve features of NAFLD and decrease the levels of plasma biomarkers of oxidative stress, apoptosis, and inflammation in study participants through a randomized, placebo-controlled, double-blind, crossover trial.

Materials and methods

Study participants

Participants ranging from ages 18 to 25 y with high body mass indices (BMI $\geq$ 23.1 kg/m^2) [19] were recruited from Shaoguan University through flyers and emails. Each recruit was subjected to an initial telephone screening before a screening visit. During the first visit, the health status and medical history of the volunteers were assessed through an interview and laboratory measurements, including routine hematologic measures and measures of thyroid, liver, and kidney functions. The height, weight, waist circumference, blood pressure, and fasting plasma glucose, and lipids were measured. The fat mass (percentage of body weight) was estimated through bioelectrical impedance analysis (UM-41, TANITA, Dongguan, China). An abdominal ultrasonographic examination was performed by two hepatologists who were unaware of the clinical and laboratory data.

Participant inclusion required the presence of two of the three following diagnostic criteria of fatty liver disease: increased hepatic echogenicity compared with the spleen or the kidneys, blurring of liver vasculature, and deep attenuation of the ultrasonographic signal [20].

The exclusion criteria included overuse of alcohol (weekly consumption of $>$ 70 g of alcohol for females and 140 g for males); smoking; viral hepatitis; type 1 or 2 diabetes; gastrointestinal or connective diseases; chronic pancreatitis; liver cirrhosis; kidney stones or renal failure; use of acetylsalicylic acid or other antiplatelet drugs; statins or fibrates; oral hypoglycemic drugs; nitrates; nonsteroidal anti-inflammatory drugs, corticosteroids, or drugs interfering with coagulation; and supplementation with vitamins or antioxidants.

In all, 72 volunteers were screened, and 44 (12 men and 32 women) were enrolled in the study. Written informed consent was obtained from each study participant. The study was conducted according to the guidelines in the Declaration of Helsinki, and all of the procedures involving human participants were approved by the Human Research Ethics Committee of Shaoguan University. All of the participants were compensated for participating in the study. This trial was registered at clinicaltrials.gov as NCT01707914.

Test beverages

Gre-Health Foods, Inc. (Shaoguan, China) supplied both the bayberry juice and the placebo beverage using coded labels to ensure double-blinding. The bayberries (cultivar Wusu) were harvested at maturity in Shantou, Guangdong Province, China, and transported to the factory within 24 h. The fruit was squeezed, and the juice was filtered, pasteurized at 75°C, and stored at −5°C in aliquots of 250 mL. The bayberry-flavored placebo beverage matched the 100% juice profile in color, caloric content, acidity, sugar content, and ascorbic acid, but did not contain polyphenols. The characteristics of these beverages, which were determined through routine laboratory techniques, are shown in Table 1.

Study design

The 44 participants were randomized by gender and BMI into the bayberry juice-first and the placebo-first groups. The study followed a randomized, 2 × 2 crossover, placebo-controlled, double-blind design. Each participant was observed over two periods of 4 wk (periods 1 and 2), and these two periods were separated by a 2-wk interval. Participants were randomly assigned to consume 500 mL of bayberry juice/d (250 mL twice daily) or 500 mL of placebo/d (250 mL twice daily). The placebo was comprised of water, bayberry aroma, colorants (coccinellin), 47 g of sucrose, 1.1 g of citric acid, and 0.5 g of ascorbic acid. The participants who received the bayberry juice during the first period were administered the placebo during the second period and vice versa. To ensure compliance, participants were given their test beverages at the students' dining hall daily between 1100 h and 1230 h, and the beverage cans were collected the next day. The participants were advised to maintain their usual dietary habits and physical exercise level except to replace an equivalent amount of dietary carbohydrates with carbohydrates from the test beverages during the experimental period. The beverages provided approximately 184 kcal or 769 kJ, which represented approximately 8% of the daily energy intake. The participants were asked to stop all of their consumption of wine, grape products, green or black tea, dark juices (e.g., cranberry and pomegranate juice), and dietary supplements for the duration of the study. Moreover, a 3-d 24-h dietary recall was conducted before and after each intervention period to ascertain whether the nutrient and energy intakes of the participants changed during the study.

Experimental determinations

Before and after each intervention period, anthropometric characteristics were measured, and fasting blood samples were drawn. The blood samples were immediately transported to the Center Laboratory of Affiliated Hospital of Shaoguan University for the analysis of the fasting glucose, lipid profile (total cholesterol [TC], triglycerides [TGs], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]), total bilirubin, direct bilirubin, total protein, albumin, and liver enzyme (aspartate transaminase [AST] and alanine transaminase [ALT]) levels using an automated diagnostic instrument (Aeroset C8000, Abbott Laboratories, Abbott Park, IL, USA). The other blood variables (hemoglobin, platelets, and white blood cells [WBCs]) were determined using an automatic hematology analyzer (Sysmex 9000, Toa Medical Electronics, Kobe, Japan). The plasma insulin was measured with a commercial radioimmunoassay kit (Weigao Biotech, Weihai, China). The insulin resistance was evaluated

Table 1
Characteristics of the test beverages

	Bayberry juice	Placebo	Method used to determine composition
Reducing sugars (g/100 mL)	9.2	9.5	Fehling reagent method
Titratable acidity (g of citric acid/100 mL)	0.34	0.28	Titration method
Ascorbic acid (mg/100 mL)	98.6	101.3	Iodine titration method
Total polyphenols (mg/100 mL)	270.2	ND	Folin-Ciocalteu method
Total anthocyanin (mg/100 mL)	83.5	ND	pH differential method
TEAC (mmol Trolox/100 mL)	4.62	1.06	ABTS·$^+$ method

ND, not detected; TEAC, trolox equivalent antioxidant capacity
The data are the means of at least three duplicate experiments