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Beneficial effects of noni (*Morinda citrifolia* L.) juice on livers of high-fat dietary hamsters

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ABSTRACT

Polyphenols in noni juice (NJ) are mainly composed of phenolic acids, mainly gentisic, *p*-hydroxybenoic, and chlorogenic acids. To investigate the beneficial effects of NJ on the liver, hamsters were fed with two diets, normal-fat and high-fat diets. Furthermore, high-fat dietary hamsters were received distilled water, and 3, 6, and 9 mL NJ/kg BW, respectively. After a 6-week feeding period, the increased ($p < 0.05$) sizes of liver and visceral fat in high-fat dietary hamsters compared to the control hamsters were ameliorated ($p < 0.05$) by NJ supplementation. NJ also decreased ($p < 0.05$) serum/liver lipids but enhanced ($p < 0.05$) daily faecal lipid/bile acid outputs in the high-fat dietary hamsters. High-fat dietary hamsters supplemented with NJ had higher ($p < 0.05$) liver antioxidant capacities but lowered ($p < 0.05$) liver iNOS, COX-2, TNF-α, and IL-1β expressions, gelatinolytic levels of MMP9, and serum ALT values compared to those without NJ. Hence, NJ protects liver against a high-fat dietary habit via regulations of antioxidative and anti-inflammatory responses.

1. Introduction

Morinda citrifolia (noni) belongs to the family Rubiaceae (coffee family), subfamily Rubioideae. Noni-related products have been globally commercialized while noni juice (NJ) is claimed to have health-related benefits, such as treatments or prevention of hypertension, arthritis, atherosclerosis, diabetes, inflammation, senility, and poor digestion (Potterat & Hamburger, 2007). Polysaccharides, fatty acid glycosides, iridoids, anthraquinones, flavonoids, phytosterols, and carotenoids exist in NJ (Bui, Bacic, & Pettolino, 2006; Potterat & Hamburger, 2007). Noni has been locally planted in southern Taiwan for decades and its related products are diversified recently in the market as well.

Fatty-liver disease has reached epidemic proportions and is a common cause of chronic liver disease in worldwide (Targher, Day, & Bonora, 2010). Fatty-liver diseases are divided in two categories: alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD). The pathological processes of NAFLD can range from fatty liver alone (steatosis) to non-alcoholic steatohepatitis (NASH), and cirrhosis (irreversible, advanced scarring of the liver). There is a correlation for 70–90% of the population clinically diagnosed as NAFLD with obese, type 2 diabetes, and metabolic syndrome sufferers in western countries (Bellentani, Scaglioni, Marino, & Bedogni, 2010). There are large amounts of triacylglycerol (TAG) and cholesterol (TC) accumulated in hepatocytes that also accelerate the development of steatosis to NASH. Hence, long-term consumption of high-fat foods is considered to increase the lipid accumulation, thus potentially promoting lipid peroxidation and oxidative stress within the hepatocytes and starting a vicious cycle (Chang et al., 2011; Liu et al., 2012). Furthermore, it was observed that the development of NASH by a high-fat diet is always coupled with increased hepatic lipogenesis, oxidative stress, and low-grade inflammatory responses (Chang et al., 2011; Liu et al., 2012; Yang, Tseng, Chang, & Chen, 2009). Hence, food scientists and nutritionists strive to attenuate the development of NAFLD or even NASH by enhancing hepatic antioxidation and anti-inflammation via a dietary modification rather than medicine.

The main antioxidant-defended mechanisms in mammals are through neutralization of ROS with glutathione (GSH) and three major scavenging antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). In addition, matrix metalloproteinases (MMPs) promote the degradation of the extracellular matrix (ECM) molecules which accelerate the progression of liver damage or even fibrosis (Consolo, Amoroso,

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Spandidos, & Mazzarino, 2009). Increased gelatinolytic levels of liver MMP2 and 9 were also observed in high-fat dietary rodents, and they can be considered as a major cause of NASH (Yang et al., 2009). Besides, interleukin- 1 (IL-1) and tumour necrosis factor-alpha (TNF-α) are inflammatory cytokine which stimulate MMPs gene expressions thus inducing a serious inflammatory response in osteoarthritis (Fernandes, Martel-Pelletier, & Pelletier, 2002). As we know, phytochemicals from plants have demonstrated several health properties. For example, polyphenol-rich longan-flower-water extracts can decrease hepatic lipid peroxidation and MMP9 activities, thus reducing liver damage in high-fat dietary rats (Liu et al., 2012). Although the health benefits of NJ are available, the detailed studies on hepatoprotective effects of NJ against a high-fat diet are still lacking. Hence, via an animal model we would like to understand if supplementing NJ can increase hepatic antioxidant capacities, and decrease hepatic inflammatory factors, i.e. TNF-α, IL1-β, iNOS, and COX-2 protein expressions, and gelatinolytic levels of MMP2 and 9 in a high diet.

2. Materials and methods

2.1. Noni juice manufacture

Noni fruit was obtained from a local fruit farm (Xuejia District, Tainan City, Taiwan). Noni fruit was stored in a stainless-steel bottle and fermented at room temperature for one year, and then separated via a wire mesh screen. The experimental NJ materials were further centrifuged from collected NJ at 3000*g* for 15 min, pasteurized at 80 °C for 60 s, and then stored at −20 °C until feeding animals.

2.2. Polyphenol compounds of NJ

The amount of phenolic acid and flavonoid in NJ were detected by using high performance liquid chromatography (HPLC) method. A *PrimeLine*™ Gradient Model 500 G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA) and an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany) were employed. The analytical conditions were based on those reported by Liu et al. (2012). The stationary phase was a Hypersil GOLD C_{18} column (250 × 4.6 mm, 5 μm; Thermo Fisher Scientific Inc., Waltham, MA, USA), and the gradient solvent system was consisted of methanol (solvent A) and water containing 9% glacial acetic acid (solvent B) (conditions: 5–17% A from 0 to 5 min and kept at 17% A from 5 to 25 min; 17–31% A from 25 to 40 min and kept at 31% A from 40 to 76 min; 31–40% A from 76 to 80 min and kept at 40% A from 80 to 120 min; flow rate = 0.8 ml/min). UV spectra were recorded from 220 to 450 nm. Individual phenolic acid and flavonoid compound were purchased from Sigma Co. (St. Louis, MO, USA) and then run on the HPLC as standards to verify chemical compounds.

2.3. Animals and diets

The animal use and protocol was reviewed and approved by National Taiwan University Care Committee (IACUC No.: 99-050). Forty male Golden Syrian hamsters of 5-week age were purchased from the National Laboratory Animal Center (National Science Council, Taipei, Taiwan). Two hamsters were housed in each cage in an animal room at 22 ± 2 °C with a 12/12 h light–dark cycle. For an induction of chronic nonalcoholic-fatty-liver development, the high-fat diet (12% fat/0.2% cholesterol) was formulated based on an AIN-93G growing-rodent-diet formulation supplemented with coconut oil and cholesterol while the basal AIN-93G growing rodent diet (7% fat/0% cholesterol) was regarded as a control diet. According to our measurement, NJ contained 2.14 g crude polysaccharide/100 mL. Hence, after one week of acclimation 40 hamsters were randomly divided into five groups: (1) Control: basal AIN-93G growing rodent diet and distilled water (oral gavage); (2) HFD: high fat diet (12% fat/0.2% cholesterol) and distilled water (oral gavage); (3) NJ_1X: high fat diet (12% fat/0.2% cholesterol) and 3 mL NJ (including 64.23 mg crude polysaccharides)/kg BW (oral gavage); (4) NJ_2X: HFD and 6 mL NJ (including 128.46 mg crude polysaccharides)/kg BW; (5) NJ_3X: HFD and 9 mL NJ (including 192.69 mg crude polysaccharides)/kg BW for 6 weeks. Hamsters were sacrificed by CO_2 asphyxiation. Blood samples, liver, and visceral tissue in the abdominal cavity of each hamster were obtained after sacrificed and stored at −80 °C for further analyses. Faecal materials were collected from each cage 72 h before the end of the experiment and stored at -80 °C for further analyses.

2.4. Determination of serum biochemical values, hepatic lipids, and fecal lipids/bile acids

The serum biochemical values, i.e. triacylglycerol (TAG), total cholesterol (TC), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined by using commercial enzymatic kits with the SPOTCHEM™ EZ SP-4430 automated analyzer (ARKRAY, Inc., Kyoto, Japan). Hepatic and faecal lipids were extracted according to the procedures of Chang et al. (2011). Hepatic and faecal TAG and TC, and faecal bile acids were measured by using commercial kits (Randox Laboratories Ltd., Antrim, UK). Daily faecal lipids and bile acid outputs per hamsters were calculated by their concentrations multiplied with the daily volume of faecal outputs, respectively.

2.5. Preparation of liver homogenate

The liver (0.5 g) was homogenized in phosphate buffer saline (PBS, pH 7.0, containing 0.25 M sucrose), and the supernatant was collected by a centrifugation at 12,000×*g* for 30 min. The protein content in the supernatant was measured according to the procedures of a Bio-Rad protein assay kit (Cat#: 500-0006, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.6. Determination of liver lipid peroxidation level and antioxidant capacity

The liver triobarbituric acid reactive substances (TBARS) was used to represent the level of liver lipid peroxidation, while glutathione (GSH), trolox equivalent antioxidant capacity (TEAC), and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were assayed as indices for liver antioxidant capacities (Chang, Yang, Chiu, Lin, Chen, & Chen, 2012). They all were performed according to procedures described by Chang et al. (2011). The measurement of hepatic TBARS values was based on the production of TBARS. The liver TBARS level was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmole/mg protein. Due to the unique thiol compound in glutathione (GSH), 2, 2-dithiobisnitrobenzoic acid (DTNB) is commonly used for thiol assay. The hepatic GSH content was calculated by taking the extinction coefficient of 2-nitro-5-thiobenzoic acid (NTB) to be $1.36 \times 10^4\ M^{-1}\ cm^{-1}$ at 412 nm and expressed as μmole/mg protein. Hepatic trolox equivalent antioxidant capacity (TEAC) was measured by the decrease in absorption at 734 nm after 10 min of the addition of liver homogenate. The TEAC of sample was converted with a standard curve for trolox on scavenging 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^+$) capacity and expressed as mmol/mg protein. Hepatic SOD was detected by the inhibitory