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Amino acid, mineral, and polyphenolic profiles of black vinegar, and its lipid lowering and antioxidant effects *in vivo*

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ABSTRACT

Black vinegar (BV) contains abundant essential and hydrophobic amino acids, and polyphenolic contents, especially catechin and chlorogenic acid via chemical analyses. K and Mg are the major minerals in BV, and Ca, Fe, Mn, and Se are also measured. After a 9-week experiment, high-fat/cholesterol-diet (HFCD) fed hamsters had higher ($p < 0.05$) weight gains, relative visceral-fat sizes, serum/liver lipids, and serum cardiac indices than low-fat/cholesterol diet (LFCD) fed ones, but BV supplementation decreased ($p < 0.05$) them which may resulted from the higher ($p < 0.05$) faecal TAG and TC contents. Serum ALT value, and hepatic thiobarbituric acid reactive substances (TBARS), and hepatic TNF-α and IL-1β contents in HFCD-fed hamsters were reduced ($p < 0.05$) by supplementing BV due to increased ($p < 0.05$) hepatic glutathione (GSH) and trolox equivalent antioxidant capacity (TEAC) levels, and catalase (CAT) and glutathione peroxidase (GPx) activities. Taken together, the component profiles of BV contributed the lipid lowering and antioxidant effects on HFCD fed hamsters.

1. Introduction

World Health Organization (WHO) reported that more than 1.4 billion adults were overweight (WHO, 2013). As we know, imbalanced fat or excess energy intake is one of the most important environmental factors resulted in not only increased serum/liver lipids but also oxidative stress, further leading cardiovascular disorders and inflammatory responses. Beside of medical therapies, food scientists strive to improve serum lipid profile and increase serum antioxidant capacity via dietary medication or functional supplementation.

Vinegar is not only used as an acidic seasoning but also proclaimed some beneficial effects, such as digestive, appetite stimulation, antioxidant, exhaustion recovering effects, lipid lowering effects, and regulations of blood pressure (Fushimi et al., 2001; Qui, Ren, Fan, & Li, 2010). Polyphenols exist in several food categories, such as vegetable, fruits, tea, wine, juice, and vinegar while they were evidenced against lipid peroxidation, hypertension, hyperlipidemia, inflammation, DNA damage, and cancer (Lin, Chang, Yang, Tzang, & Chen, 2013; Osada et al., 2006; Prior & Cao, 2000; Yang et al., 2010a). Black vinegar (BV) also called as Kurosu is produced from unpolished rice with rice germ and brain through a stationary surface fermentation and contains higher amounts of amino acids and organic acids than other vinegars (Nishidai et al., 2000). Black vinegar is also characterised as a health food rather than only an acidic seasoning because it was reported to own a DPPH radical scavenging ability (Shimoji et al., 2002) and decrease the adipocyte size (Tong et al., 2010) in rat models. Moreover, Nishidai et al. (2000) indicated that the extract of BV shows the highest radical scavenging activity in a DPPH radical system than rice, grain, apple, and wine vinegars. They also demonstrated that this extract suppresses increased lipid peroxidation in mouse skin treated with 12-*o*-tetradecanoylphorbol-13-acetate.

Based on our literature searchings, reports regarding *in vivo* lipid lowering effects of BV are absent. Hence, this study focused on the nutritional compositions in BV, and its *in vivo* lipid lowering and antioxidant effects. First, the amino acid, mineral, and polyphenolic profile of BV were identified. Hypolipidemic hamsters induced by a high-fat/cholesterol diet (HFCD) were orally administered with different doses of BV. Serum lipid profile and liver damage indices,

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liver and faecal lipid contents, as well as hepatic antioxidant capacities [thiobarbituric acid reactive substances (TBARS), glutathione (GSH), trolox equivalent antioxidant capacity (TEAC), and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)] and hepatic cytokine levels were assayed to demonstrated physiological functions of BV.

2. Materials and methods

2.1. Materials

Lyophilized black vinegar (BV) samples were generously provided by Success Medical Co., Ltd. (New Taipei City, Taiwan). Lyophilized BV contains 16.5% (w/w) protein, 0.3% (w/w) lipid, 73.1% (w/w) carbohydrate, and 361 kcal/100 g. All other chemicals used in this study were of the highest pure grade available.

2.2. Amino acid, mineral, and polyphenolic profiles of black vinegar (BV)

In the amino acid analysis, the lyophilized black vinegar was hydrolysed in 6 N HCl for 24 h. Amino acids were quantified using the Hitachi L8800 amino acid analyser (Hitachi High-Technologies Co., Tokyo, Japan) employing sodium citrate buffers as step gradients with the cation exchange postcolumn ninhydrin derivatization method. The data were described as grams of amino acid per 100 g of lyophilized BV. In the mineral analysis, all glassware was soaked overnight in a solution of 10% HCl in ddH_2O (v/v) prior to use. Ashed BV samples (550 °C, 6 h) were dissolved in 2 mL of 70% nitric acid. The acidified samples were neutralized in 5 mL of ddH_2O and filtered through Whatman No. 1 paper and then diluted to volume with ddH_2O in a 50 mL volumetric flask. Major minerals: magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), manganese (Mn), and selenium (Se) were determined using inductively coupled plasma optical emission spectrometry (ELEMENT 2* ICP-MS, Thermo Fisher Scientific Inc., MA, USA). The polyphenolic compounds in lyophilized BV were identified according to the methods from Liu et al. (2012) with a slight modification. The high performance liquid chromatography (HPLC) system is composed of a Shimadzu LC-10AT HPLC pump system pump system and a Shimadzu SPD-10A UV–vis detector (Shimadzu SCL-10A system controller module, Kyoto, Japan). A Diamonsil C_{18} column (250 × 4.6 mm, 5 μm; Dikma Technologies Inc., Lake Forest, CA, USA) and a gradient solvent system consisting of MeOH (solvent A) and deionized distilled water (dd H_2O) with 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) were used for separation of components whose UV spectra were recorded from 220 to 450 nm. Phenolic acid compounds: gallic, gentisic, chlorogenic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, sinapic, syringic, *p*-anisic and rosmarinic, and flavonoid standards: including catechin, epicatechin, rutin, naringin, myricetin, hesperidin, quercitrin, neohesperidin, eriodictyol, diosmin, morin, daidzein, quercetin, glycitein, naringenin, luteolin, genistein, hesperetin, kaempferol, apigenin and isorhamnetin were purchased from Sigma Co. (St. Louis, MO, USA). Those phenolic acid and flavonoid compounds were also run on the HPLC as standards to verify chemical compounds of lyophilized BV.

2.3. Animal and diets

The animal use and protocol was reviewed and approved by the National Taiwan University Animal Care Committee (IACUC No.: 100-062). Thirty-two male Golden Syrian hamsters of 5-week age were purchased from the National Applied Laboratories (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. Chow diets (Laboratory Rodent Diet 5001, PMI® Nutrition International/Purina Mills LLC, USA) and water were provided for 1 week of acclimation. For an induction of hyperlipidemia of hamsters (Lin et al., 2013), the high-fat/cholesterol diet (HFCD, 12% fat/0.2% cholesterol) based on an AIN-93G formulation supplemented with coconut oil and cholesterol was used while the basal AIN-93G (7% fat/0% cholesterol) was regarded as a low-fat/cholesterol diet (LFCD). After 1 week, hamsters with two hamsters per cage were randomly assigned to one of the following diet: (1) LFCD and 1 mL distilled water (LFCD); (2) HFCD and 1 mL distilled water (HFCD); (3) HFCD and 0.13 g BV/kg BW in 1 mL distilled water (1XBV); (4) HFCD and 0.26 g BV/kg BW in 1 mL distilled water (2XBV). The experimental period lasted for 9 weeks. All hamsters were allowed free access to the assigned diets and water. The feed and water intakes of hamsters were recorded every week. Daily feed (g) and water intake (mL) were calculated on a per hamster daily basis.

2.4. Collection of serum, liver, abdominal fat, and feces of experimental animals

At the third, sixth, and ninth week of the experimental period, blood from each hamster was collected via puncturing the retroorbital sinus with a capillary tube after an over-night fasting. At the end of the experiment (week 9), all hamsters fasted overnight before sacrificing. Hamsters were euthanised by CO_2. Heart, liver, and visceral fat tissues in the abdominal cavity of each hamster were removed and weighed individually. Livers were stored at −80 °C for further analyses. Sera were separated from blood samples by a centrifugation 3000×*g* for 10 min and then stored at −80 °C for further analyses. Feces were collected from each cage 72 h before the end of the experiment and stored at −20 °C for further analyses.

2.5. Determination of serum biochemical values and liver/faecal lipids

The serum biochemical values, i.e. triacylglycerol (TAG), cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined by using commercial enzymatic kits with the SPOTCHEM™ EZ SP-4430 automated analyser (ARKRAY, Inc., Kyoto, Japan). Cardiac index was calculated by the formulation of TC level/HDL-C level (Yang et al., 2010a). Liver and faecal lipid levels were measured according to the previous procedure (Yang et al., 2010b). Briefly, faecal lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2 and then resuspended in isopropanol. Faecal cholesterol and triacylglycerol concentrations were also measured using commercial kits (Randox Laboratories Ltd., Antrim, UK).

2.6. Preparation of liver homogenate

The liver homogenate (10%, w/v) was made with 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, California, USA).

2.7. Determination of hepatic lipid peroxidation level and antioxidant capacity

The hepatic malondialdehyde (MDA) content was an indicator to determine hepatic lipid peroxidation levels, while glutathione