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

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ARTICLE INFO

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.

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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,

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$_2$O (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$_2$O and filtered through Whatman No. 1 paper and then diluted to volume with ddH$_2$O 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$_2$O) 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

(GSH), trolox equivalent antioxidant capacity (TEAC), and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assayed as indices for hepatic antioxidant capacities. They all were performed according to procedures as described by Chou, Wang, Lin, and Chen (2014). The thiobarbituric acid reactive substances (TBARS), GSH values were calculated by taking the extinction coefficients of MDA to be $1.56 \times 10^5\,M^{-1}$ cm^{-1} at 535 nm and 2-nitro-5-thiobenzoic acid to be 1.36×10^4 $M^{-1}\,cm^{-1}$ at 412 nm, respectively. The TEAC value was measured by the decrease in absorption at 734 nm after the addition of reactant via a standard curve for trolox on scavenging $ABTS^+$ capacity. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. A standard curve was plotted for trolox on scavenging $ABTS^+$ capacity was calculated as the TEAC. SOD activity was detected by the inhibitory effect of SOD on purpurogallin of pyrogallol oxidation product, and then was recorded at 420 nm in 3 min. One unit of SOD activity was defined as the amount of enzyme that inhibited the autoxidation of pyrogallol by 50%. SOD activity was expressed by μunit/mg protein. CAT activity was calculated by taking the extinction coefficient of H_2O_2 to be $39.5\,M^{-1}\,cm^{-1}$ at 240 nm and expressed as μunit/mg protein. GPx activity was assayed following the commercial manufacturer's instruction (Randox Laboratories Ltd., Antrim, UK) and expressed as μunit/mg protein.

2.8. Determination of hepatic tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) levels

An aliquot of liver (100–200 ng protein) was used in the foregoing liver homogenate. Hepatic TNF-α and IL-1β levels were assayed following the commercial manufacturer's instructions (TNF-α and IL-1β kits, eBioscience, Inc., San Diego, CA, USA). The optical density value of each well was read at 450 nm in an ELISA reader (Dynex Technologies, UK) and converted to the TNF-α and IL-1β levels expressed by pg/mg protein by using a standard curve.

2.9. Statistical analysis

The experiment was conducted using a completely random design (CRD). A significant difference was used at the 0.05 probability level. Differences between treatments were tested using the Least Significant Difference (LSD) test. All statistical analyses of data were performed using SAS (SAS Institute Inc., Cary, NC, USA, 2002).

3. Results and discussion

3.1. Amino acid, mineral, and polyphenol profiles in black vinegar (BV)

Based on a proximate-analysis report from Success Medical Co., Ltd. (New Taipei City, Taiwan), there was 16.5% (w/w) protein in the of lyophilized BV powders. Hence, to assess the protein quality, we tried to determine the contents of the amino acids in lyophilized BV powders and compared with the recommended values by FAO/WHO/UNU for adults (Table 1). Among the essential amino acids, Leu content in lyophilized BV was the highest, followed by Val, Thr, Ile, Phe, His, Lys, and Met. The top three amounts of non-essential amino acids were Glu, Pro, and Ala. In the mineral profile of lyophilized BV, K and Mg were the major mineral compounds, while Ca, Fe, Mn, and Se were also analysed. Regarding the identified polyphenolic profile in lyophilized BV, the flavonoid category only included catechin, but the phenolic-acid category included gallic acid, chlorogenic acid, p-hydroxybenzoic acid, p-cumeric acid, ferulic acid, and sinapic acid.

To our knowledge, there are no published reports regarding the amino acid analysis of protein in lyophilized BV. Although the biological values of plant-source protein are not higher, the black vinegar owns complete essential amino acid. In a comparison of daily recommended values in essential amino acids from FAO/WHO/UNU Expert Consultation. (1985), the BV should be a good amino-acid supplementation. Besides, according to the adult dietary intake references in Mg, K, Ca, Fe, Mn, and Se from USDA. (2013), the BV is also a suggestive supplementation for the adults. The functionalities of those amino acids, minerals, and polyphenolic compounds were discussed previously. Saiga, Tanabe, and Nishimura (2003) reported that Asp and Glu own antioxidant properties; moreover, Val, Ile, Leu, Met, Phe, Trp, and Cys that are belonging to very hydrophobic amino acids (Betts & Russell, 2003) were indicated to a higher scavenging ability of free radicals than hydrophilic ones in a lipid system (Ren et al., 2008). BV contains high amounts of Leu, Ile, and Val (Table 1). Kobayashi, Hirabayashi, Murakami, and Ueda (2009) reported that

Table 1
Amino acid, mineral, and polyphenolic profiles in black vinegar.

	Content	FAO/WHO/UNU (1985) adults (mg/kg BW/day) [**]		Content	Adult DIRs (male, female) [*****]
Amino acid (mg/100 g dried black vinegar)			*Mineral*		
Methionine (Met)	161.33 ± 5.21	13 [***]	Magnesium (Mg) (mg/100 g)	95.37 ± 0.55	(420, 320) mg/day
Leucine (Leu)	734.33 ± 13.86	14	Potassium (K) (mg/100 g)	306.73 ± 9.29	(4.7, 4.7) g/day
Isoleucine (Ile)	443.33 ± 23.33	10	Calcium (Ca) (mg/100 g)	0.31 ± 0.04	(1.0, 1.0) g/day
Lysine (Lys)	222.67 ± 11.57	12	Iron (Fe) (mg/100 g)	2.47 ± 0.05	(8, 18) mg/day
Threonine (Thr)	450.67 ± 22.84	7	Manganese (Mn) (mg/100 g)	3.29 ± 0.05	(2.3, 1.8) mg/day
Histidine (His)	305.67 ± 10.71	8–12	Selenium (Se) (μg/100 g)	1.80 ± 0.31	(55, 55) μg/day
Phenylalanine (Phe)	345.67 ± 19.24	14 [****]	*Polyphenol (g/100 g dried black vinegar)*		
Valine (Val)	653.67 ± 22.15	10	Gallic acid	1.29 ± 0.10	
Tryptophan (Trp)	17.00 ± 1.73		Catechin	2.31 ± 0.14	
Cysteine (Cys)	226.33 ± 13.97		Chlorogenic acid	2.54 ± 0.15	
Arginine (Arg)	600.33 ± 11.29		p-Hydroxybezoic acid	1.62 ± 0.11	
Tyrosine (Tyr)	184.33 ± 7.88		p-Cumeric acid	1.81 ± 0.08	
Aspartic acid (Asp)	827.67 ± 29.41		Ferulic acid	1.47 ± 0.10	
Glutamic acid (Glu)	3306.00 ± 51.86		Sinapic acid	1.62 ± 0.12	
Alanine (Ala)	979.00 ± 46.20				
Glycine (Gly)	754.00 ± 23.12				
Proline (Pro)	1184.67 ± 28.20				
Serine (Ser)	529.67 ± 30.07				

[*] The data are given as mean ± SEM (*n* = 3).
[**] Values are based on people older 12 years old from FAO/WHO/UNU Expert Consultation (1985).
[***] Methionine + cysteine.
[****] Phenylalanine + tyrosine.
[*****] Values are based on people at 19–70 years old from USDA (2013).

Table 2
Growth performance, as well as relative size of heart, liver, and visceral fat of the experimental hamsters.

Group	LFCD	HFCD	1XBV	2XBV
Growth performance				
Initial body weight (g)	82.17 ± 2.34 a	82.60 ± 2.82 a	81.88 ± 1.55 a	81.33 ± 1.45 a
Final body weight (g)	115.79 ± 4.37 a	124.44 ± 3.22 a	115.38 ± 1.98 a	117.76 ± 1.85 a
Weight increase (g)	33.63 ± 2.51 b	41.84 ± 0.54 a	33.51 ± 1.43 b	36.43 ± 0.89 b
Weight increase (%)	40.81 ± 2.38 b	50.85 ± 1.49 a	41.07 ± 2.03 b	44.84 ± 1.18 b
Food intake (g/hamster/day)	5.12 ± 0.20 a	5.10 ± 0.32 a	5.04 ± 0.12 a	5.25 ± 0.17 a
Water intake (mL/hamster/day)	6.44 ± 0.40 a	6.80 ± 0.41 a	6.57 ± 0.89 a	6.50 ± 0.51 a
Relative size (g/100 g body weight)				
Liver	2.66 ± 0.10 b	3.77 ± 0.09 a	3.64 ± 0.04 a	3.58 ± 0.09 a
Heart	0.33 ± 0.012 a	0.33 ± 0.01 a	0.37 ± 0.02 a	0.34 ± 0.01 a
Visceral fat	2.65 ± 0.23 b	3.35 ± 0.17 a	2.82 ± 0.12 b	2.84 ± 0.09 b

*The data are given as mean ± SEM ($n = 8$; except feed and water intakes, $n = 4$). Mean values with different letters were significantly different ($p < 0.05$).
**LFCD: normal diet (chow diet) + 1 mL ddH$_2$O (oral gavage); HFCD: high-fat/cholesterol diet + 1 mL ddH$_2$O (oral gavage); 1XBV: high-fat/cholesterol diet + 0.13 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage); 2XBV: high-fat/cholesterol diet + 0.26 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage).

supplements rich in Glu, His, Leu, and Lys can reduce the body weight in high-fat diet fed mice via increasing energy expenditure. Furthermore, Vaskonen (2003) reported that Mg and Ca can lower absorption of dietary fat in the intestine, and Mn and Se are the cofactors of SOD (Iranzo, 2011) and GPx (Maseko, Howell, Dunshea, & Ng, 2014), respectively. Natural phenols from the plant origin work against lipid peroxidation, hypertension, hyperlipidemia, inflammation, and DNA damage; therefore, these beneficial effects have been frequently discussed previously (Lin et al., 2012, 2013; Osada et al., 2006; Prior & Cao, 2000). Ngamukote, Mäkynen, Thilawech, and Adisakwattana (2011) indicated that three major polyphenolic compounds (gallic acid, catechin, and epicatechin) present in grape seeds can lower the cholesterol absorption via an inhibited pancreatic cholesterol esterase, bonded of bile acids, and reduced solubility of cholesterol in micelles. Moreover, Qiu, Ren, Fan, and Li (2009) reported that oat vinegar owns stronger antioxidant activity than rice vinegar in mouse serum and livers due to the higher amounts of polyphenols. In a comparison with the amounts of polyphenols between lyophilized BV (12.66 g/100 g dried base) and oat vinegar (3.99 g/100 g dried base) (Qiu, Ren, Fan, & Li, 2009), the polyphenols in lyophilized BV should also partially contribute lipid-lowering and antioxidant activities. To sum up, a synergetic effect of amino acid, mineral, and polyphenolic compounds in BV may result in lipid-lowering and antioxidant effects in a high-fat diet.

3.2. Effects of BV on growth performance, relative sizes of heart, liver, and visceral fat in hyperlipidemic hamsters

After 9 weeks of experiment, although the initial and final body weights of hamsters among groups were not ($p > 0.05$) different, BV supplementation decreased ($p < 0.05$) weight increases (g and %) in HFCD fed hamsters, which even similar to those of LFCD fed hamsters (Table 2). Food and water intakes were not ($p > 0.05$) different among groups. Moreover, the sizes of heart were not ($p > 0.05$) altered among groups while the HFCD increased ($p < 0.05$) sizes of liver and visceral fat of hamsters. However, BV supplementation decreased ($p < 0.05$) sizes of visceral fat in HFCD fed hamsters to the similar ($p > 0.05$) size of LFCD fed hamsters. There was only a tendency toward lower liver sizes in the 2XBV group.

Dietary fat is regarded as an important environmental factor associated with the incidence of metabolic syndrome, i.e. cardio-vascular disease (CVD), hypertension, diabetes, and so on. Kobayashi et al. (2009) indicated that Glu, Ala, Leu, and Lys can increase energy expenditure and then reduce body weight. Due to a lipase inhibitory effect, rich-polyphenol longan flower-water extract also demonstrated an antiobesity effect (Yang et al.,

2010b). Tong et al. (2010) also indicated that the decreased adipocyte sizes in AIN-76 formula with mildly high fat (10%, v/v) diet fed rat supplemented with Kurozu (black vinegar) were attributed to an inhibition of lipase activity and reductions of PPAR-γ and aP2 mRNA expression levels in adipocytes. Vaskonen (2003) indicated that Mg and Ca as divalent cations can react with fatty acids and form insoluble soaps in the intestine which further results in the lower absorption of dietary fat. Hence, the antiobesity effects of BV can be contributed to its complete amino acid profile, divalent-cation mineral (Mg), and a plenty amount of polyphenols (Table 1).

3.3. Effects of BV on serum, liver, and faecal lipid levels in hyperlipidemic hamsters

Serum TAG, TC, and HDL-C levels, as well as cardiac index (ratio of TC/HDL-C level) of hamsters in a 3-week interval were shown in Fig. 1. HFCD resulted in higher ($p < 0.05$) serum TAG and TC levels than LFCD (Fig. 1A and B). Although there was only tendency toward lower serum TAG in HFCD fed hamsters supplemented with BV (1XBV and 2XBV groups) in the end of experiment, lowered ($p < 0.05$) serum TAG levels were observed in the third and sixth week of experiment (Fig. 1A). The similar results were indicated in serum TC levels as well (Fig. 1B). Meanwhile, serum HDL-C levels were not ($p < 0.05$) affected before the sixth week of experiment, but in the end of experiment the highest ($p < 0.05$) serum HDL-C level in HFCD groups was analysed, followed by 2X BV, 1BV, and LFCD groups (Fig. 1C). Because of the higher serum TC levels observed in HFCD fed hamsters, cardiac index of the LFCD group was still lower ($p < 0.05$) than those of HFCD fed hamsters in the third and sixth weeks (Fig. 1D). The cardiac indices of the 1XBV and 2BXBV groups was however similar ($p > 0.05$) to that of LFCD groups in the end of experiment (Fig. 1D). Liver/faecal TAG and TC levels of different groups for 9 weeks are shown in Fig. 2A and B, respectively. Liver TAG and TC levels in HFCD fed hamsters were higher ($p < 0.05$) than those of LFCD group while HFCD fed hamsters with BV supplementation had lower ($p < 0.05$) liver TC levels than those without BV supplementation. Although no ($p > 0.05$) differences on faecal TAG and TC levels between LFCD and HFCD groups were illustrated, those values in HFCD fed hamsters were increased ($p < 0.05$) by the BV supplementation.

It was discussed that natural polyphenols from the plant origins own hypolipdemic effects (Prior & Cao, 2000). In addition, a higher faecal lipid excretion is highly associated to lower serum lipid level, thus alleviating the hepatic lipid accumulation (Yang et al., 2010a, 2010b; Yang, Lin, Liu, & Chen, 2014). Dietary apple phenols exert hypolipidemic and antiatherogenic effects via a
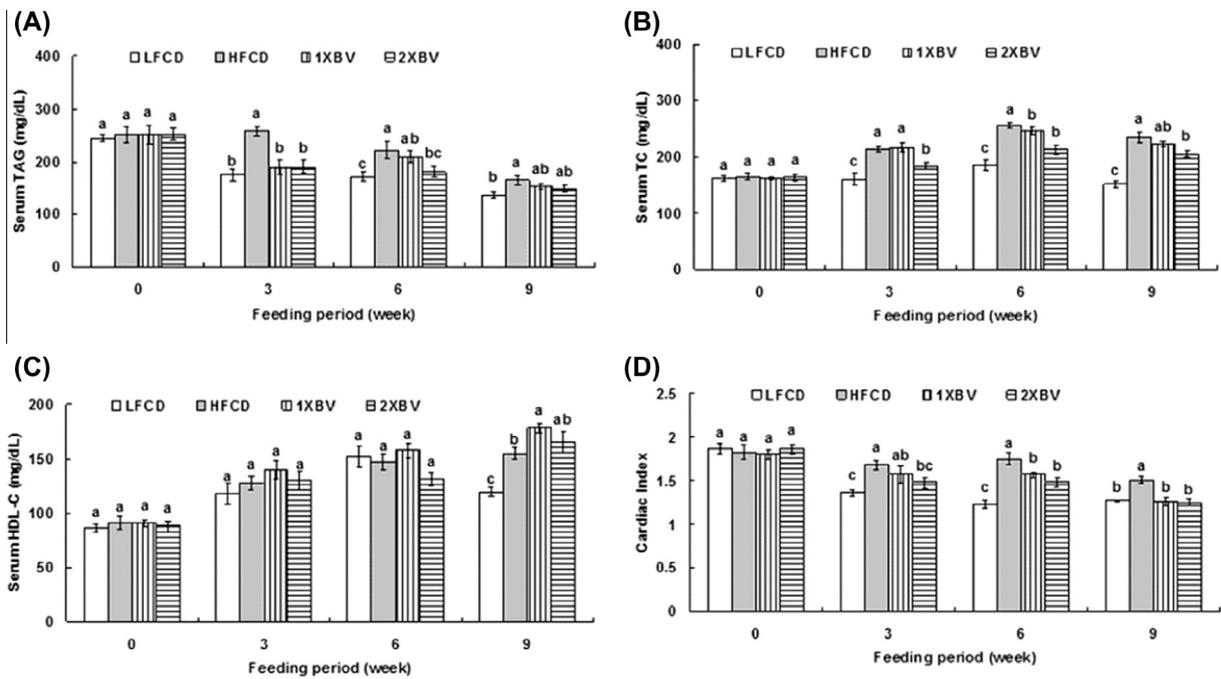
Fig. 1. Effects of black vinegar powders on serum TAG (A), TC (B), HDL-C (C), and cardiac index (D) of experimental hamsters. *The data are given as mean ± SEM ($n = 8$). Different letters on data bars indicate a significant different. **LFCD: normal diet (chow diet) + 1 mL ddH$_2$O (oral gavage); HFCD: high-fat/cholesterol diet + 1 mL ddH$_2$O (oral gavage); 1XBV: high-fat/cholesterol diet + 0.13 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage); 2XBV: high-fat/cholesterol diet + 0.26 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage).
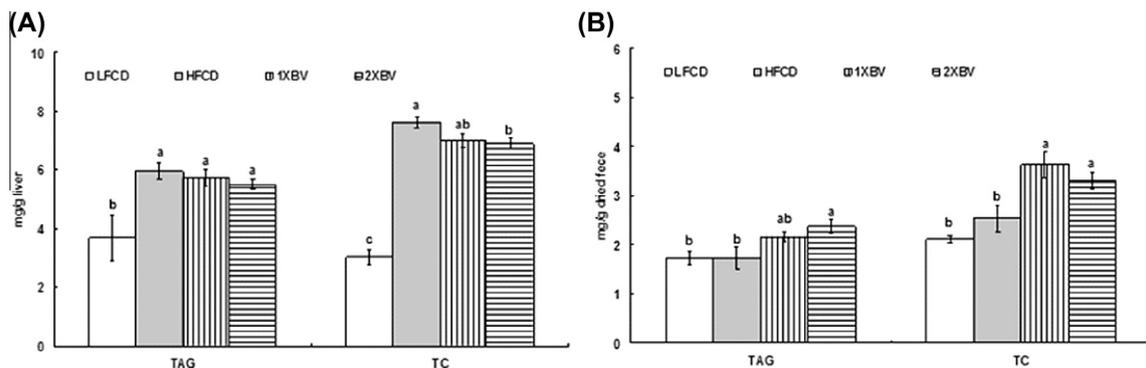
Fig. 2. Effects of black vinegar powders on hepatic (A) and faecal (B) TAG and TC contents of experimental hamsters. *The data are given as mean ± SEM (liver, $n = 8$; fece, $n = 4$). Different letters on data bars indicate a significant different. **LFCD: normal diet (chow diet) + 1 mL ddH$_2$O (oral gavage); HFCD: high-fat/cholesterol diet + 1 mL ddH$_2$O (oral gavage); 1XBV: high-fat/cholesterol diet + 0.13 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage); 2XBV: high-fat/cholesterol diet + 0.26 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage).

promotion of cholesterol catabolism and inhibition of intestinal absorption of cholesterol to improve lipid profiles (Osada et al., 2006). Besides, the amount of Mg is abundant in BV (Table 1). Chen et al. (2013) demonstrated that rich-magnesium deep-sea-water drinking water can lower serum and liver lipids via an enhancement of faecal lipid output. Due to quite amounts of lysine (Table 1), as well as the literatures (Iwami, Sakakibara, & Ibuki, 1986), Yang et al. (2014) speculated that decreased lipase activities of pepsin-digested chicken-liver hydrolysates are due to its lysine. Hence, the lipid lowering effects of BV may be associated to the higher faecal lipid outputs due to the Mg and polyphenolic contents in BV.

3.4. Effects of BV on hepatic antioxidant and damage in hyperlipidemic hamsters

According to Fig. 3, the HFCD apparently increased ($p < 0.05$) hepatic TBARS values and decreased ($p < 0.05$) hepatic GSH and

TEAC levels while hepatic SOD, CAT, and GPx activities were also declined ($p < 0.05$). However, among HFCD fed groups, supplementing BV decreased ($p < 0.05$) hepatic TBARS values, restored ($p < 0.05$) GSH levels, and increased ($p < 0.05$) TEAC levels in livers; meanwhile, decreased hepatic CAT and GPx activities were enhanced ($p < 0.05$) as well. Serum AST and ALT values, and hepatic TNF-α and IL-1β contents were applied to evaluate the levels of liver damage. There was not ($p > 0.05$) a difference on serum AST values among groups, but LFCD group had lower ($p < 0.05$) serum ALT values than HFCD fed groups (Fig. 4A). However, BV supplementation decreased ($p < 0.05$) serum ALT values in HFCD fed hamsters. Similar results were observed in hepatic TNF-α and IL-1β contents. The HFCD group had the highest ($p < 0.05$) levels of hepatic TNF-α and IL-1β among groups while supplementing BV reduced ($p < 0.05$) them which even also similar ($p > 0.05$) to those of the LFCD group.

As we know, a high fat dietary habit always brings a lipid accumulation in livers that stimulates an oxidative stress and further
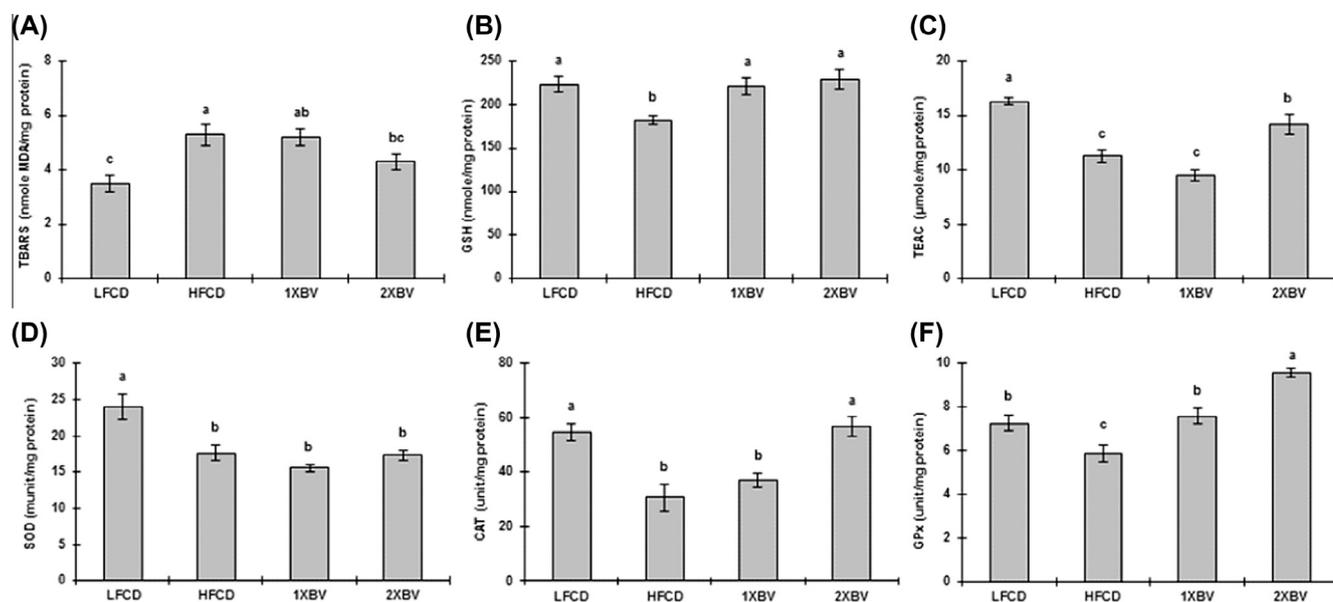
Fig. 3. Effects of black vinegar powders on hepatic TBARS (A), GSH (B), and TEAC (C) levels, as well as SOD (D), CAT (E), and GPx (F) activities of experimental hamsters. *The data are given as mean ± SEM ($n = 8$). Different letters on data bars indicate a significant different. **LFCD: normal diet (chow diet) + 1 mL ddH$_2$O (oral gavage); HFCD: high-fat/cholesterol diet + 1 mL ddH$_2$O (oral gavage); 1XBV: high-fat/cholesterol diet + 0.13 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage); 2XBV: high-fat/cholesterol diet + 0.26 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage).
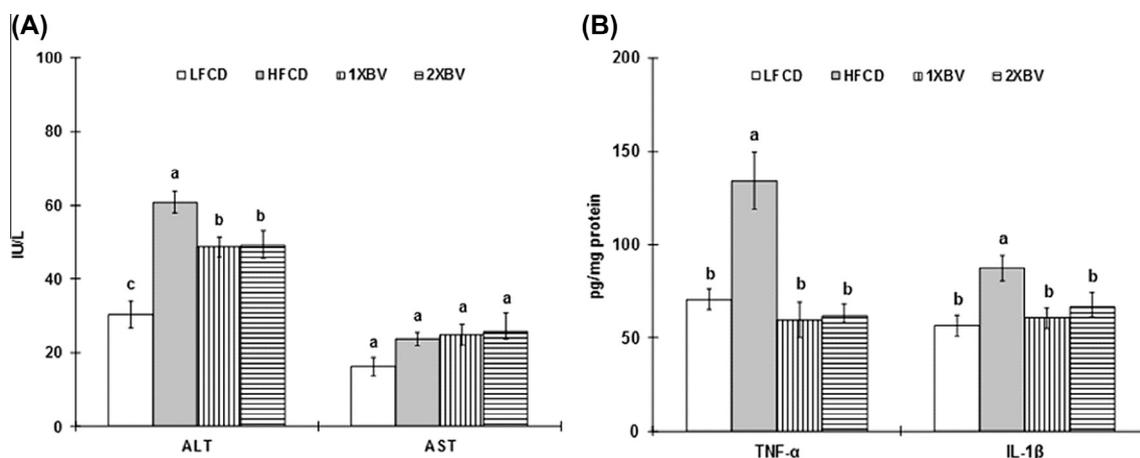
Fig. 4. Effects of black vinegar powders on hepatic damage indices (A) and cytokines (B) of experimental hamsters. *The data are given as mean ± SEM ($n = 8$). Different letters on data bars indicate a significant different. **LFCD: normal diet (chow diet) + 1 mL ddH$_2$O (oral gavage); HFCD: high-fat/cholesterol diet + 1 mL ddH$_2$O (oral gavage); 1XBV: high-fat/cholesterol diet + 0.13 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage); 2XBV: high-fat/cholesterol diet + 0.26 g black vinegar/kg BW in 1 mL ddH$_2$O (oral gavage).

leads to liver damage. Higher serum AST, ALT, and free fatty acids, as well as hepatic cholesterol, triacylglycerol, MDA, hydroperoxide, and cytokine (IL-1β and TNF-α) levels were easily observed in a high-fat-consumption rodent models (Lin et al., 2013; Liu et al., 2012). Several reports indicated that some of amino acids own antioxidant activities *in vitro* and *in vivo*. Acidic amino acids, such as Asp and Glu (Saiga et al., 2003) and hydrophobic amino acids, such as Ile, Leu, and Val (Ren et al., 2008) display high antioxidant properties. Recently, an *in vivo* study indicated that a pepsin hydrolyzation significantly enhanced Asp, Glu, Leu, and Val contents in chicken livers; meanwhile, chicken-liver hydrolysates showed an antioxidant capacity in brain and liver of D-galactose treated mice (Chou et al., 2014). In addition, it was also reported that Mg and Se play important roles in SOD and GPx activities, respectively (Iranzo, 2011; Maseko et al., 2014). Uzun and Kalender (2013) used chlorpyrifos, an organophosphorus insecticide, to induce hepatotoxic and hematologic changes in rats, but they observed that catechin can attenuate the chlorpyrifos-induced hepatotoxicity

by increasing GPx and glutathione-S-transferase activities and decreasing MDA contents. Meanwhile, chlorogenic acid elevated SOD, CAT, and GPx activities with concomitantly decreased lipid peroxidation of liver and kidney in streptozotocin-nicotinamide-induced type-2 diabetic rats (Pari, Karthikesan, & Menon, 2010). Hence, it is reasonable to assume that increased antioxidant capacities and decreased damage in livers of HFCD fed hamsters supplemented with BV should be highly related to the components, i.e. amino acid profile, mineral profile, and polyphenol contents, as well as the lowered liver lipid accumulations.

4. Conclusion

In analyses of amino acids, minerals and polyphenols, BV contained abundant essential amino acids and hydrophobic amino acids. Mg, K, Ca, Fe, Mn, and Se were measured in BV where K and Mg were major. Gallic acid, catechin, chlorogenic acid,

p-hydroxybezoic acid, *p*-cumeric acid, ferulic acid, and sinapic acid were also identified in BV where catechin and chlorogenic acid were the majorities. Meanwhile, the lipid-lowering and antioxidant effects of BV were also investigated via a hamster model. BV supplementation apparently decreased weight gain (g and %), relative size of visceral fat, serum/liver TC levels, serum cardiac index, and hepatic TBARS values and damage indices (serum ALT and hepatic TNF-α and IL-1β) but increased faecal lipid contents and hepatic antioxidant capacities (GSH level, TEAC level, CAT activity, and GPx activity) in HFCD fed hamsters. To sum up, those benefits could be attributed to a synergetic effect of compounds in BV.

References

Betts, M. J., & Russell, R. B. (2003). Amino acid properties and consequences of substitutions. In M. R. Barnes & I. C. Gray (Eds.), *Bioinformatics for geneticists*. Hoboken, NJ, USA: Wiley.

Chen, I. S., Chang, Y. Y., Hsu, C. L., Lin, H. W., Chang, M. H., Chen, J. W., et al. (2013). Alleviative effects of deep-seawater drinking water on hepatic lipid accumulation and oxidation induced by a high-fat diet. *Journal of the Chinese Medical Association, 76*, 95–101.

Chou, C. H., Wang, S. Y., Lin, Y. T., & Chen, Y. C. (2014). Antioxidant activities of chicken-liver hydrolysates by pepsin treatment. *International Journal of Food Science & Technology, 49*, 1654–1662.

FAO/WHO/UNU Expert Consultation. (1985). *Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Technical Report Series No. 724*. Geneva: World Health Organization. Available from <http://www.fao.org/docrep/003/aa040e/aa040e00.HTM>.

Fushimi, T., Tayama, K., Fukaya, M., Kitakoshi, K., Nakai, N., Tsukamoto, Y., et al. (2001). Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *Journal of Nutrition, 131*, 1973–1977.

Iranzo, O. (2011). Manganese complexes displaying superoxide dismutase activity: A balance between different factors. *Bioorganic Chemistry, 39*, 73–87.

Iwami, K., Sakakibara, K., & Ibuki, F. (1986). Involvement of post-digestion hydrophobic peptides in plasma cholesterol-lowing effect of dietary plant proteins. *Agricultural and Biological Chemistry, 50*, 1217–1222.

Kobayashi, H., Hirabayashi, Y., Murakami, H., & Ueda, T. (2009). Anti-obesity effects of amino acid in high-fat diet induced obese mice. *FASEB Journal, 23* (Meeting Abstract Supplement) 227.5.

Lin, Y. L., Chou, C. H., Yang, D. J., Chen, J. W., Tzang, B. S., & Chen, Y. C. (2012). Hypolipidemic and antioxidative effects of noni (*Morinda citrifolia* L.) juice on high fat/cholesterol-dietary hamsters. *Plant Foods for Human Nutrition, 67*, 294–302.

Lin, Y. L., Chang, Y. Y., Yang, D. J., Tzang, B. S., & Chen, Y. C. (2013). Beneficial effects of noni (*Morinda citrifolia* L.) juice on livers of high-fat dietary hamsters. *Food Chemistry, 140*, 31–38.

Liu, C. W., Yang, D. J., Chang, Y. Y., Hsu, C. L., Tseng, J. K., Chang, M. H., et al. (2012). Polyphenol-rich longan (*Dimocarpus longan* Lour.)-flower-water-extract attenuates nonalcoholic fatty liver via attenuating lipid peroxidation and downregulating matrix metalloproteinases-2 and -9. *Food Research International, 45*, 444–449.

Maseko, T., Howell, K., Dunshea, F. R., & Ng, K. (2014). Selenium-enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione peroxidase-2 in rat colon. *Food Chemistry, 146*, 327–333.

Ngamukote, S., Mäkynen, K., Thilawech, T., & Adisakwattana, S. (2011). Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules, 16*, 5054–5061.

Nishidai, S., Nakamura, Y., Torikai, K., Yamamoto, M., ishihara, N., Mori, H., et al. (2000). Kurosu, a traditional vinegar produced from unpolished rice, suppresses lipid peroxidation *in vitro* and in mouse skin. *Bioscience, Biotechnology, and Biochemistry, 64*, 1909–1914.

Osada, K., Suzuki, T., Kawakami, Y., Senda, M., Kasai, A., Sami, M., et al. (2006). Dose-dependent hypocholesterolemic actions of dietary apple phenol in rats fed cholesterol. *Lipids, 41*, 133–139.

Pari, L., Karthikesan, K., & Menon, V. P. (2010). Comparative and combined effect of chlorogenic acid and tetrahydrocurcumin on antioxidant disparities in chemical induced experimental diabetes. *Molecular and Cellular Biochemistry, 341*, 109–117.

Prior, R. L., & Cao, G. (2000). Flavonoids: Diet and health relationships. *Nutrition in Clinical Care, 3*, 279–288.

Qui, J., Ren, C., Fan, J., & Li, Z. (2010). Antioxidant activities of aged oat vinegar *in vitro* and in mouse serum and liver. *Journal of the Science and Food Agriculture, 90*, 1951–1958.

Ren, J. Y., Zhao, M. M., Shi, J., Wang, J. S., Jiang, Y. M., Cui, C., et al. (2008). Purification and identification of antioxidant peptides from grass carp muscle hydrolysates by consecutive chromatography and electrospray ionization–mass spectrometry. *Food Chemistry, 108*, 727–736.

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry, 51*, 3661–3667.

Shimoji, Y., Tamura, Y., Nakamura, Y., Nada, K., Nishidai, S., Nishikawa, Y., et al. (2002). Isolation and identification of DPPH radical scavenging compounds in Kurosu (Japan unpolished rice vinegar). *Journal of Agricultural and Food Chemistry, 50*, 6501–6503.

Tong, L. T., Katakura, Y., Kawamura, S., Baba, S., Tanaka, Y., Udono, M., et al. (2010). Effects of Kurozu concentrated liquid on adipocyte size in rats. *Lipids in Health and Disease, 9*, 134–142.

USDA. (2013). Dietary Reference Intakes: Recommended Intakes for Individuals, Dietary Guidance, Food and Nutrition Information Center. Available from <http://iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/RDA%20and%20AIs_Vitamin%20and%20Elements.pdf>.

Uzun, F. G., & Kalender, Y. (2013). Chlorpyrifos induced hepatotoxic and hematologic changes in rats: the role of quercetin and catechin. *Food and Chemical Toxicology, 55*, 549–556.

Vaskonen, T. (2003). Dietary minerals and modification of cardiovascular risk factors. *Journal of Nutritional Biochemistry, 14*, 492–506.

World Health Organization. (2013). Obesity and overweight. Available from <http://www.who.int/mediacentre/factsheets/fs311/en/index.html>.

Yang, K. T., Lin, C., Liu, C. W., & Chen, Y. C. (2014). Effects of chicken-liver hydrolysates on lipid metabolism in a high-fat dietary habit. *Food Chemistry, 160*, 148–156.

Yang, D. J., Chang, Y. Y., Hsu, C. L., Lin, C. W., Wang, Y., & Chen, Y. C. (2010a). Protective effect of litchi (*Litchi chinensis* Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters. *Food Chemistry, 119*, 1456–1561.

Yang, D. J., Chang, Y. Y., Hsu, C. L., Liu, C. W., Lin, Y. L., Lin, Y. H., et al. (2010b). Antiobesity and hypolipidemic effects of polyphenol-rich longan (*Dimocarpus longans* Lour.) flower water extract in hypercaloric-dietary rats. *Journal of Agricultural and Food Chemistry, 58*, 2020–2027.