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Protective effect of a litchi (*Litchi chinensis* Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters

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ABSTRACT

Cardiovascular disease (CVD) is the major cause of death in the developed countries. Litchi-flower-water-extract (LFWE) contains plenty of phytochemicals, i.e. phenols, flavonoids, tannins which may own cardio-protection. Ten male hamsters per group were assigned randomly to one of the following dietary groups: chow diet and normal distilled water (LFCD/NDW); high-fat/cholesterol diet and normal distilled water (HFCD/NDW); HFCD and 2.5% litchi-flower-water-extract (HFCD/2.5% LFWE); HFCD and 5% LFWE (HFCD/5% LFWE). Serum lipids, cardiac index, and hepatic lipids were lowered ($p < 0.05$) in high-fat/cholesterol-dietary hamsters by drinking 2.5% and 5% LFWE which may result from higher ($p < 0.05$) LDL receptor and peroxisome proliferator-activated receptor-alpha (PPAR-α) gene expressions and lower ($p < 0.05$) fatty acid synthase (FAS) gene expression, as well as increased ($p < 0.05$) faecal lipid and bile acid excretions. Drinking LFWE also lowered ($p < 0.05$) serum malondialdehyde (MDA) contents in high-fat/cholesterol-dietary hamsters, and even showed the same ($p > 0.05$) serum MDA contents as the LFCD/NDW group's which could be due to increased ($p < 0.05$) serum trolox equivalent antioxidant capacity (TEAC). Therefore, this study indicated that LFWE indeed characterises a protective effect on cardiovascular health *in vivo*.

1. Introduction

Cardiovascular disease (CVD) is the major cause of death in developed countries, and hyperlipidemia is regarded as one of the important risks in the development of CVD. The common epidemic reason for hyperlipidemia is an excessive or improper lipid intake. Otherwise, the oxidative stress due to the lipid peroxidation is also highly related to CVD. Currently, lowering serum levels of total cholesterol (TC), triacylglycerol (TAG), and cardiac index (CI) (TC/HDL-C ratio), and increasing self-antioxidant capacity can be carried out *via* diet therapy or medication.

Reduced cardiovascular and cerebrovascular diseases by high fruit and vegetable consumption have been reported, where rich flavonoids and phenolic acids contribute partially (Prior & Cao, 2000). According to the molecular structures, nature phenols range from simple molecules, i.e. phenolic acids, to highly polymerised compounds, i.e. tannins. The healthy benefits of natural phenols from the plant kingdom are evidenced against lipid peroxidation, hypertension, hyperlipidemia, inflammation, DNA damage, and cancer (Chen et al., 2003; Ho, Hwang, Shen, & Lin, 2007; Osada et al., 2006; Prior & Cao, 2000). For example, tea and apple phenols are well-documented for exhibiting healthy benefits. Yang, Wang, and Chen (2001) investigated the effects of rich-phenol green, oolong, and black tea extracts on serum lipids of a hyperlipidemic animal model induced by a high-sucrose diet. All three different kinds of tea extracts show a hypolipidemic effect, but green tea extract had the greater hypolipidemic effect. Dietary apple phenols also exert hypolipidemic and antiatherogenic effects *via* a promotion of cholesterol catabolism and inhibition of intestinal cholesterol absorption (Osada et al., 2006). Furthermore, an improvement of lipid profiles (HDL-C, non-HDL-C/HDL-C) in a rodent model by supplementing apple phenols into the diet was considered as the inhibition on cholesteryl ester transport protein (Lam et al., 2008).

Due to high phenolic compounds in roselle (*Hibiscus sabdariffa* L.) (Chen et al., 2003) and longan (*Dimocarpus longan* Lour.)-flower-water-extracts (Tsai, Wu, & Huang, 2008), they exhibit improvements against atherosclerosis and metabolic syndrome, respectively. Litchi (*Litchi chinensis* Sonn.) is the tropical and subtropical fruit of *Sapindaceae* family and also an important economic fruit in Taiwan. The litchi fruit is edible and juicy. When litchi flowers are not pollinated, they will drop, and are generally regarded as

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disposable byproducts. Our research team analysed disposable litchi flowers and observed that the acetone, methanol, and water-extracts from those disposable flowers contain a large quantity of phenols, flavonoids, and condensed tannins, as well as good antioxidant capacities *in vitro* (Liu, Lin, Wang, Chen, & Yang, 2009). Hence, the antioxidant capacity of litchi-water-extracts on serum peroxidation, which is highly associated with cardiovascular health, warrants further *in vivo* investigation.

In addition, expressions of serum cholesterol clearance (i.e. LDL receptor), cholesterol biosynthesis (i.e. 3-hydroxy-3-methylglutaryl-CoA reductase, HMG-CoA reductase) and catabolism (i.e. cholesterol 7-α hydroxylase, CYP7A1), triacylglycerol biosynthesis (i.e. fatty acid synthase, FAS), and energy expenditure (peroxisome proliferator-activated receptor-alpha, PPAR-α) are major regulations of lipid homeostasis in mammal animals. Based on our knowledge, no *in vivo* studies of LFWE health benefits were available until right now. Therefore, *via* a high-fat/cholesterol-dietary-hamster model, this study addresses whether LFWE could improve serum lipids *via* improvements of the hepatic lipid and increases in the faecal lipid and bile acid outputs, regulate gene expressions related to lipid homeostasis, such as LDL receptor, HMG-CoA reductase, FAS, CYP7A1, and PPAR-α in hamsters, and meanwhile ameliorate serum lipid peroxidation and antioxidant capacity of hamsters.

2. Materials and methods

2.1. Collection and preparation of a litchi-flower-water-extract (LFWE)

Fresh litchi (*L. chinenesis* Sonn.) flowers were obtained from a local fruit farm (Taichung County, Taiwan). The flowers were dried in a hot-air dryer (Chi-Yeh Electric and Machinery Co., Taipei, Taiwan) at 40 °C for 16 h before water-extraction. The 2.5% and 5% (w/v) LFWE were obtained by steeping suitable volumes of dried litchi flowers with boiled distilled water (100 °C) for 30 min, with a stirrer bar and laboratory stirrer/hot plate (Model: PC420, Corning Inc., USA) for efficient extraction. Both extracts were then filtered through No. 1 filter paper and stored at −20 °C until feeding animals as a replacement of normal distilled water.

2.2. Determination of phytochemicals in a litchi-flower-water-extract (LFWE)

The total phenol contents in LFWE were measured by a method with Folin–Ciocalteu's phenol reagent using gallic acid as a standard and expressed as mg gallic acid equivalent (GAE)/100 ml extract (Julkunen-Titto, 1985). Flavonoid contents were determined according to a method with 10% $AlCl_3 \cdot H_2O$ solution using (+)-catechin as a standard and expressed as mg catechin equivalent (CE)/100 ml extract (Zhishen, Mengcheng, & Jianming, 1999). Condensed tannins were determined according to the method of Julkunen-Titto (1985) and expressed as mg CE/100 ml extract. Total monomeric anthocyanins were surveyed through the differential pH method and expressed as mg cyanidin-3-glucoside equivalents (cy-3-gluE)/100 ml extract, using $\varepsilon = 26{,}900$ (Giusti & Wrolstad, 2001). Proanthocyanidin contents were measured at 550 nm, on the basis of a colourmetric reaction with 10% $NH_4Fe(SO_4)_2$ after dissolution in hydrochloric acid (2 M) containing *n*-butanol (Porter & Rossi, 1986). Ascorbic acid was quantified according to the method by Klein and Perry (1982).

2.3. Animal and diets

The animal use and protocol was reviewed and approved by Chung-Shan Medical University Animal Care Committee. 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. Chow diets contain 48.7% (w/w) carbohydrate, 23.9% (w/w) protein, 5.0% (w/w) fat, 5.1% (w/w) fibre, and 7.0% ash (Laboratory Rodent Diet 5001, PMI® Nutrition International/Purina Mills LLC, USA) and water was provided for one week of acclimation. A chow diet was regarded as a low-fat/cholesterol diet (LFCD) while a high-fat/cholesterol diet (HFCD) was formulated as 92.8% (w/w) chow diets supplemented with 7% (w/w) soybean oil and 0.2% (w/w) cholesterol (Tzang et al., 2009). Therefore, HFCD contains 45.2% (w/w) carbohydrate, 22.2% (w/w) protein, 11.7% (w/w) fat, 4.7% (w/w) fibre, and 6.5% ash. After that, 10 hamsters with two hamsters per cage were randomly assigned to one of the following groups:

(1) LFCD and normal distilled water (LFCD/NDW).
(2) HFCD and normal distilled water (HFCD/NDW).
(3) HFCD and 2.5% (w/v) litchi-flower-water-extract (HFCD/2.5% LFWE).
(4) HFCD and 5% (w/v) litchi-flower-water-extract (HFCD/5% LFWE).

All hamsters were fed the assigned diets and drinking water *ad libitum*. The experimental period lasted for 6 weeks. The body weight of hamsters was individually recorded every week. Feed and water were changed everyday. Daily feed and water per cage was determined and further divided by two for obtaining daily feed (g) and water intakes (ml) on a per hamster daily basis.

2.4. Collection of serum, liver, visceral fat, and faeces

Blood samples were collected *via* puncturing the retroorbital sinus with a capillary tube after a 14-h fasting at week 0, 2, and 4. At the end of the experiment (week 6), all feed was removed 14 h before killing. All hamsters were euthanised by CO_2. Liver and visceral adipose tissue in the abdominal cavity from each hamster were removed and weighed. Liver was stored at −80 °C for further analyses. Blood samples were also collected by an intracardiac puncture. Serum was separated from blood samples by centrifugation 3000*g* for 10 min, and then stored at −80 °C for further analyses. Faeces were collected from each cage 72 h before the end of experiment and stored at −80 °C for further analyses.

2.5. Determination of serum lipid parameters

Serum total cholesterol (TC), triacylglycerol (TAG), and high-lipoprotein cholesterol (HDL-C) were measured by using commercial kits (Randox Laboratories Ltd., Antrim, UK). In the HDL-C analysis, low-density-lipoprotein (LDL), very-low-density-lipoprotein (VLDL), and chylomicron in serum were precipitated by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation (3000*g* for 10 min), the cholesterol concentration in HDL fraction was determined by using the TC commercial kit (Randox Laboratories Ltd., Antrim, UK). Those methods are based on detection of coloured end-products at 500 nm. The cardiac index (CI) was calculated by the formulation of TC/HDL-C.

2.6. Determination of hepatic/faecal cholesterol, triacylglycerol, and bile acids

Hepatic and faecal lipid extractions were measured according to the procedures of Tzang et al. (2009). Briefly, hepatic and faecal lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2 and resuspended in isopropanol *via* an

ultrasonic cleaner (Model: DC150H, Taiwan Delta New Instrument Co. Ltd., TW) for an efficient dissolution. Cholesterol and triacylglycerol concentrations were measured using commercial kits (Randox Laboratories Ltd., Antrim, UK). 3α-hydroxy bile acids compromise about 60% of the faecal bile acids in the hamster (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997) and 3α-Hydroxy bile acids can be determined using an enzymatic method (Randox Laboratories Ltd., Antrim, UK).

2.7. Hepatic mRNA expressions of LDL receptor, HMG-CoA reductase, FAS, CYP7A1, PPAR-α, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Total RNA was isolated from the stored frozen liver tissues by using the protocol described by Rneasy Mini Kits (Qiagen, Valencia, CA, USA). Reverse transcription was carried out with 2-μg total RNA, 8-μl reaction buffer, 2-μl dNTPs, 4.8-μl $MgCl_2$, 4-μl Oligo-dT (10 pmol/l) and 200U RTase (Promega, Madison, WI, USA) with diethyl pyrocarbonate (DEPC) H_2O in a final volume of 40 μl at 42 °C for 1 h. After a heat inactivation, 1-μl cDNA product was used for a PCR amplification. The appropriate primers of target genes were designed for hamster's LDL receptor, HMG-CoA reductase, FAS, CYP7A1, PPAR-α, and GAPDH as follows: LDL receptor sense 5′-ACAGATTCAGTTCCAGGCAG-3′, antisense 5′-TGGGGACAAGAGG TTTTCAG-3′; HMG-CoA reductase sense 5′-AACTGAGAGC ACAAGCAGAG-3′, antisense 5′-ATCACAAGCACGAGGAAGAC-3′; FAS sense 5′-AGCCCCTCAAGTGCACAGTG-3′, antisense 5′-CACGT GTATGCCCTGGCGCC-3′; CYP7A1 sense 5′-TTTGGACACAGAAGCA TT-3′, antisense 5′-TCCATGTCATCAAAGGTA-3′; PPAR-α sense 5′-GGACAAGGCCTCAGGGTACC-3′, antisense 5′-CCACCATCTTGGCCAC AAGC-3′; GAPDH sense 5′-GACCCCTTCATTGACCTCAAC-3′, antisense 5′-GGAGATGATGACCCTTTTGGC-3′. The size of reaction products is as follows: for LDL receptor, 477 bp; HMG-CoA reductase, 583 bp; FAS, 347 bp; CYP7A1, 497 bp; PPAR-α, 421 bp; GAPDH, 264 bp. GAPDH was used as an internal control in all reactions. The PCR amplification was performed under conditions using a DNA thermal cycler (ASTEC PC-818, ASTEC Co. Ltd., Fukuka, Japan) under the following conditions: LDL receptor and CYP7A1: 30 cycles at 94 °C for 1 min, 51 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; HMG-CoA reductase: 30 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; FAS: 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; PPAR-α: 25 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; GAPDH: 25 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. The final products were subjected to electrophoresis on a 2% agarose gel and detected by ethidium bromide staining *via* UV light. The relative expression levels of the mRNAs of the target genes were normalised using the GAPDH internal standard.

2.8. Determination of serum malondialdehyde (MDA) and trolox equivalent antioxidant capacity (TEAC)

The serum MDA and TEAC were analysed at the end of the experiment. The serum MDA content was measured by a 2-thiobarbituric acid reactive substances (TBARS) assay as described by Dhar et al. (2007) with a modification. Serum (0.5-ml) was mixed with 0.75-ml TBA solution in a Teflon tube, and then 4.25-ml trichloroacetic acid–HCl (TCA–HCl) reagent was added. The tube was flushed with nitrogen and closed. A blank was prepared in the same manner, but PBS (pH 7.0) replaced serum. The tubes were boiled for 30 min, and then cooled. The coloured solution was centrifuged at 4000*g* for 15 min. A clear and coloured supernatant was transferred to a cuvette, and the absorbance was measured at 535 nm using an Implen NanoPhotometer (Model 1443, Implen GmbH, Munich, Germany). The serum MDA content was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ at 535 nm.

The serum TEAC was analysed according to a method described by Hung, Fu, Shih, Lee, and Yen (2006). A free radical, $ABTS^+$, can be generated by mixing ABTS (100 μM) with H_2O_2 (50 μM) and peroxidase (4.4 U/ml). The TEAC value was expressed as a scavenging capacity against $ABTS^+$. Briefly, a 0.25-ml mixture of ABTS, H_2O_2, and peroxidase, and 1.5-ml dd H_2O mixed well and placed in a dark room. After 30 min, 0.25-ml diluted serum (1%, v/v) was then added. Absorbance was measured at 734 nm after the interaction of sample solution for 10 min. 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. The higher TEAC value of a sample the stronger its antioxidant activity.

2.9. Statistical analysis

The experiment was conducted using a completely random design (CRD). Data were analysed using analysis of variance (ANOVA). A significant difference was used at the 0.05 probability level and differences between treatments were tested using the Least Significant Difference (LSD) test. All statistical analyses of data were performed using SAS (SAS Institute Inc., 2002).

3. Results and discussion

3.1. Phytochemical contents in litchi-flower-water-extracts (LFWE)

According to previous investigations, water-extracts from *H. sabdariffa* (Chen et al., 2003), longan flower (Ho et al., 2007), and chestnut flowers (Barreira, Ferreira, Oliveira, & Pereira, 2008) contain plenty of polyphenols and flavonoids. Both litchi and longan are tropical and subtropical fruits belonging to the *Sapindaceae* family (Liu et al., 2009; Tsai et al., 2008). Table 1 shows that litchi-flower-water-extracts (LFWE) have considerable amounts of polyphenols, flavonoids, condensed tannins, and proanthocyanindins. The higher amounts of the samples used, the higher the levels of the phytochemicals in LFWE determined. Moreover, our research team has demonstrated that acetone, methanol, and water-extracts of litchi flowers showed a good antioxidant capacity *in vitro* (Liu et al., 2009). Hence, we believe that the protective effects of litchi-water-extracts on cardiovascular health are worthy of an *in vivo* investigation.

3.2. Body weight, weight gain, food and water intake, and sizes of visceral fat and liver

Regardless of the types of drinking water, body weights among a low-fat/cholesterol-dietary group (LFCD/NDW group) and high-fat/cholesterol-dietary groups (HFCD/NDW, HFCD/2.5% LFWE, and HFCD/5% LFWE groups) during the experimental period were not ($p > 0.05$) different, but a higher ($p < 0.05$) weight gain of HFCD/NDW was recorded when compared to the other three groups (Table 2). This result implies that drinking LFWE can reduce body weight gain in a high-fat/cholesterol-dietary habit. Besides, food intakes of hamsters during the experimental period were not ($p > 0.05$) different among treatments, whereas water intakes in the three high-fat/cholesterol-dietary groups were higher ($p < 0.05$) than in the LFCD/NDW groups. After sacrificing hamsters at the end of experiment, visceral fat and liver were collected, and their sizes relative to body weight were also calculated (Table 2). Larger ($p < 0.05$) sizes of visceral fat in high-fat/cholesterol-dietary groups, except the HFCD/5% LFWE group, were obtained than that

Table 1
Phytochemical contents in 2.5% and 5% (w/v) litchi-flower-water-extract (LFWE).

Extract (w/v) (%)	Content (mg/100 ml)[a]					
	Total phenols (GAE)[b]	Total flavonoids (CE)[c]	Condensed tannins (CE)[c]	Anthocyanins (cy-3-gluE)[d]	Proanthocyanidins	Ascorbic acid
2.5	78.29 ± 2.75	31.20 ± 0.46	22.21 ± 0.53	1.30 ± 0.05	23.45 ± 0.68	1.21 ± 0.06
5	160.72 ± 5.51	58.32 ± 1.22	37.24 ± 1.07	2.72 ± 0.09	55.03 ± 1.74	3.42 ± 0.13

[a] Data are given as mean ± SEM ($n = 3$).
[b] GAE, gallic acid equivalent.
[c] CE, catechin equivalent.
[d] cy-3-gluE, cyaniding-3-glucoside equivalent.

Table 2
Body weight, food intake, water intake, and sizes of visceral fat and liver of the experimental hamsters.

Groups	LFCD/NDW[B]	HFCD/NDW[B]	HFCD/2.5% LFWE[B]	HFCD/5% LFWE[B]
Initial body weight (g)[A]	79.52 ± 2.25a	80.43 ± 1.66a	81.98 ± 2.56a	82.67 ± 1.38a
Final body weight (g)[A]	93.33 ± 1.61a	100.24 ± 2.25a	98.46 ± 2.23a	99.20 ± 1.54a
Weight gain (g)[A]	13.81 ± 1.11b	19.80 ± 1.19a	16.48 ± 0.91b	16.54 ± 1.05b
Food intake (g/rat/day)[A]	7.34 ± 0.13a	7.44 ± 0.08a	7.46 ± 0.04a	7.45 ± 0.07a
Water intake (ml/rat/day)[A]	10.10 ± 0.59b	12.18 ± 0.38a	13.41 ± 0.33a	13.43 ± 0.34a
Visceral fat (g/100 g body weight)[A]	1.51 ± 0.06b	1.80 ± 0.06a	1.81 ± 0.09a	1.69 ± 0.07ab
Liver (g/100 g body weight)[A]	3.33 ± 0.07c	4.37 ± 0.06a	4.17 ± 0.05b	4.13 ± 0.05b

[A] Data are given as mean ± SEM ($n = 10$, except food intake and water intake, $n = 5$). Mean values with different letters in each testing parameter were significantly different ($p < 0.05$).
[B] LFCD/NDW: low-fat/cholesterol-dietary + pure water group; HFCD/NDW: high-fat/cholesterol-dietary + pure water group; HFCD/2.5% LFWE: high-fat/cholesterol-dietary + 2.5% (w/v) litchi-flower-water-extract group; HFCD/5% LFWE: high-fat/cholesterol-dietary + 5% (w/v) litchi-flower-water-extract group.

of the LFCD/NDW group. Similar results were also observed in the sizes of livers in high-fat/cholesterol-dietary groups compared to that of the LFCD group; however, drinking LFWE reduced ($p < 0.05$) the sizes of livers in high-fat/cholesterol-dietary hamsters.

Obesity is a serious problem in developed countries. Increasing consumption of more energy-dense, i.e. high-fat or sugar foods is considered as the major cause for the increase in obesity levels. Generally, high-fat diets significantly increase body and liver weights, which leads to obesity, hyperlipidemia, and fatty liver. An *in vitro* study demonstrated that naturally-occurring polyphenols can inhibit lipase activity (He, Ly, & Yao, 2006; Sugiyama et al., 2007). Yoshikawa, Shimoda, Nishida, Takada, and Matsuda (2002) also illustrated that the antiobesity effects of polyphenols-rich *Salacia reticulate*-water-extracts in rats are through suppressions of pancreatic lipase, adipose tissue-derived lipoprotein lipase, and glycerophosphate dehydrogenase, as well as an enhancement of lipolysis. In addition, reductions of the hepatic triacylglycerol and visceral fat deposition in rats fed tea catechins or hot-treated tea catechins are attributed to decreased activities of hepatic fatty acid synthase and malic enzyme (Ikeda et al., 2005). Based on our data, the body weight gain, and sizes of visceral fat and livers were decreased ($p < 0.05$) upon high-fat/cholesterol-dietary hamsters drinking LFWE (Table 2). This is most likely due to inhibitions of pancreatic enzymes or hepatic enzymes related to fatty acid biosynthesis.

3.3. Changes of serum lipids and cholesterol profile, as well as liver and faecal cholesterol and triacylglycerol levels

Serum triacylglycerol (TAG) and total cholesterol (TC) levels of hamsters in a 2-week interval are shown in Fig. 1. After 2 weeks of feeding, TAG and TC were increased ($p < 0.05$) in high-fat/cholesterol-dietary groups compared to the LFCD/NDW. However, drinking LFWE apparently showed a lipid-lowering ($p < 0.05$) effect in high-fat/cholesterol-dietary hamsters. Although higher ($p < 0.05$) HDL-C levels in the high-fat/cholesterol-dietary groups were observed compared to that of the LFCD/NDW group (Fig. 1C), TC in the high-fat/cholesterol-dietary groups was still about 1.5–2 times higher ($p < 0.05$) than that of LFCD/NDW groups (Fig. 1B). Thus, the higher ($p < 0.05$) cardiac index (TC/HDL-C) of the high-fat/cholesterol-dietary groups was calculated (Fig. 1D). Apparently, lowered ($p < 0.05$) cardiac indexes of the high-fat/cholesterol-dietary hamsters drinking LFWE was seen compared to those drinking NDW.

Liver/faecal cholesterol and triacylglycerol levels of hamsters that consumed different diets for 6 weeks are shown in Table 3. Liver cholesterol and triacylglycerol levels in the high-fat/cholesterol-dietary groups were dramatically increased ($p < 0.05$) than those in LFCD groups. LFWE (2.5% and 5%) showed the lowering ($p < 0.05$) effects on liver cholesterol in the high-fat/cholesterol-dietary hamsters, but the lowering ($p < 0.05$) effect of liver triacylglycerol levels was only observed in the 5% LFWE groups. Faecal cholesterol, triacylglycerol, and bile acid outputs were also measured in the present study (Table 3). The HFCD/5% LFWE group had the highest ($p < 0.05$) faecal cholesterol level among all groups while drinking either 2.5% or 5% LFWE in the high-fat/cholesterol-dietary groups resulted in higher ($p < 0.05$) faecal triacylglycerol levels than drinking NDW. Interestingly, drinking 2.5% and 5% LFWE resulted in approximately 1.5 and 1.8 times higher ($p < 0.05$) bile acid outputs in high-fat/cholesterol-dietary hamsters than drinking NDW, respectively.

It was reported that rich-phenol green, oolong, and black tea extracts show a hypolipidemic effect, but green tea extracts have a greater hypolipidemic effect than the others, which can be explained by lower fat absorption in rats drinking green tea extracts (Yang et al., 2001). Dietary apple phenols exert hypolipidemic and antiatherogenic effects *via* a promotion of cholesterol catabolism and inhibition of intestinal absorption of cholesterol (Osada et al., 2006), while an improvement of lipid profiles (HDL-C and non-HDL-C/HDL-C) by supplementing apple phenols was reported (Lam et al., 2008). Hence, we tried to use a high-fat/cholesterol-dietary-hamster model to investigate the effects of rich-phenol LFWE on serum lipids. Our data demonstrated that LFWE not only reduced serum lipids but also improved the serum cholesterol profile (Fig. 1). A high-fat/cholesterol-dietary habit always couples with higher cholesterol and triacylglycerol accumulations in the liver. Yang et al. (2001) reported that rich-phenol green, oolong, and black tea extracts normalised the liver triacylglycerol contents. Hence, they suppose that this decrease may be related to less fat

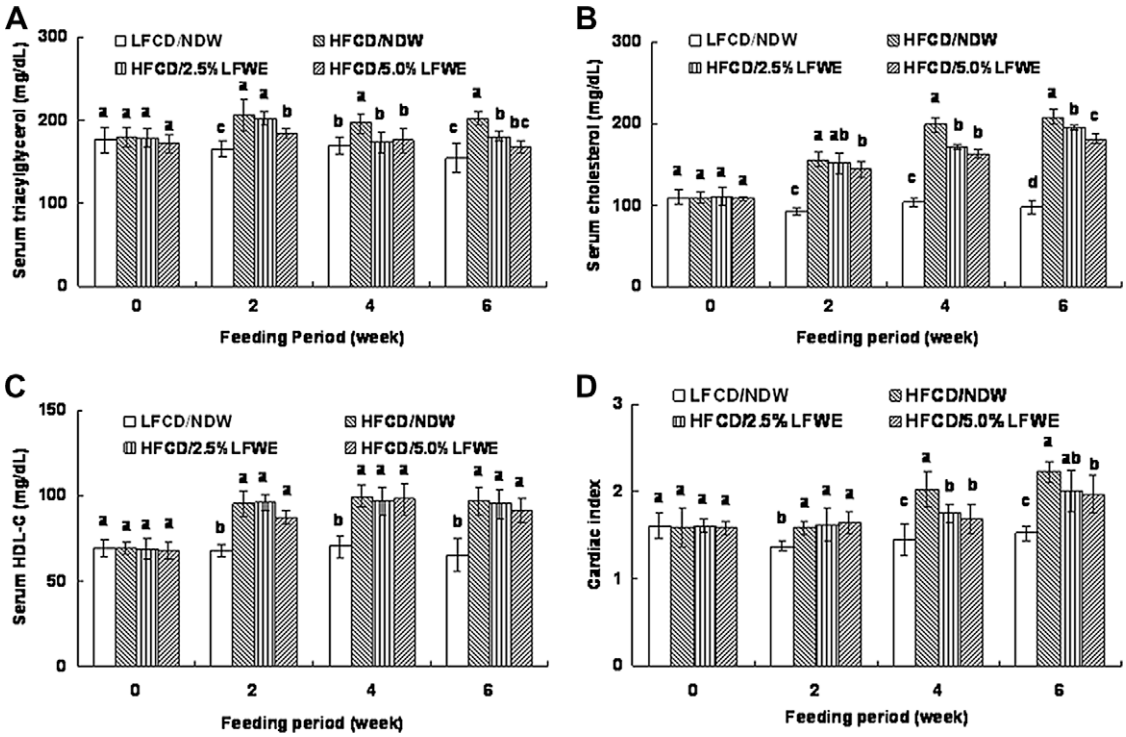

Fig. 1. (A) Serum triacylglycerol, (B) serum cholesterol, (C) serum HDL-C, and (D) cardiac index of the experimental hamsters. *The data are given as mean ± SEM (n = 10). Mean values in each feeding period with different letters indicate a significant difference ($p < 0.05$). **Cardiac index = TC/HDL-C. ***LFCD/NDW: low-fat/cholesterol-dietary + pure water group; HFCD/NDW: high-fat/cholesterol-dietary + pure water group; HFCD/2.5% LFWE: high-fat/cholesterol-dietary + 2.5% (w/v) litchi-flower-water-extract group; HFCD/5% LFWE: high-fat/cholesterol-dietary + 5% (w/v) litchi-flower-water-extract group.

Table 3
Liver and faecal cholesterol and triacylglycerol and faecal bile acid contents of the experimental hamsters.

Groups	LFCD/NDW[B]	HFCD/NDW[B]	HFCD/2.5% LFWE[B]	HFCD/5% LFWE[B]
Liver[A]				
Cholesterol (mg/g tissue)	2.22 ± 0.21c	20.14 ± 0.64a	16.44 ± 1.14b	14.89 ± 0.77b
Triacylglycerol (mg/g tissue)	6.55 ± 0.34c	10.74 ± 0.41a	9.58 ± 0.79ab	8.88 ± 0.50b
Faeces[A]				
Cholesterol (mg/g dry faeces)	1.13 ± 0.19b	0.77 ± 0.11b	0.98 ± 0.07b	1.62 ± 0.26a
Triacylglycerol (mg/g dry faeces)	19.54 ± 1.17a	15.53 ± 0.58b	20.90 ± 1.47a	21.55 ± 1.00a
Bile acids (μmol/g dry faeces)	0.37 ± 0.02a	0.22 ± 0.05b	0.34 ± 0.02a	0.40 ± 0.04a

[A] Data are given as mean ± SEM (liver, n = 10; faeces, n = 5). Mean values with different letters in each testing parameter were significantly different ($p < 0.05$).
[B] LFCD/NDW: low-fat/cholesterol-dietary + pure water group; HFCD/NDW: high-fat/cholesterol-dietary + pure water group; HFCD/2.5% LFWE: high-fat/cholesterol-dietary + 2.5% (w/v) litchi-flower-water-extract group; HFCD/5% LFWE: high-fat/cholesterol-dietary + 5% (w/v) litchi-flower-water-extract group.

absorption. The reduced liver cholesterol content was accounted for by higher cholesterol secretion into bile, thus leading to a depletion of intrahepatic pool of cholesterol (Vijaimohan et al., 2006). In addition, a higher faecal lipid excretion is highly associated with lower serum lipid level, thus alleviating the hepatic lipid accumulation (Tzang et al., 2009). Therefore, it is speculative that the lipid-lowering effect of LFWE on serum and liver lipids is highly associated with higher faecal cholesterol, triacylglycerol, and bile acid excretions (Fig. 1 and Table 3).

3.4. LDL receptor, HMG-CoA reductase, FAS, CYP7A1, and PPAR-α mRNA expressions

After 6 weeks of feeding, gene expression related to lipid homeostasis of hamsters, i.e. LDL receptor, HMG-CoA reductase, FAS, CYP7A1, and PPAR-α were analysed (Fig. 2). The LDL receptor mRNA expressions were higher ($p < 0.05$) in HFCD/2.5% LFWE and HFCD/5% LFCD groups compared to the HFCD/NDW group. In the gene expression of cholesterol biosynthesis and catabolism, HMG-CoA reductase mRNA expressions were not ($p > 0.05$) influenced in different dietary habits; however, CYP7A1 mRNA expressions were lower ($p < 0.05$) in the high-fat/cholesterol-dietary groups than the normal dietary group (LFCD/NDW). Similarly, FAS gene expressions were also lower ($p < 0.05$) in the high-fat/cholesterol-dietary groups than that in the normal dietary (LFCD/NDW) group, but the FAS gene expression was lowered ($p < 0.05$) by drinking 5% LFWE in the high-fat/cholesterol-dietary groups. Drinking LFWE also increased ($p < 0.05$) PPAR-α mRNA expressions in the normal dietary groups, even higher than that in the LFWE/NDW group.

The LDL receptor plays an important role in clearance of cholesterol levels from blood and its gene expression is downregulated by high-fat/cholesterol diets (White, Bennett, Billett, & Salter, 1997). Our data demonstrated that a lower LDL receptor gene expression in the high-fat/cholesterol-dietary groups was normalised by drinking LFWE (Fig. 2). HMG-CoA reductase is the control point for cholesterol biosynthesis while CYP7A1 is the rate-limiting enzyme for cholesterol catabolism/output. High-fat/cholesterol

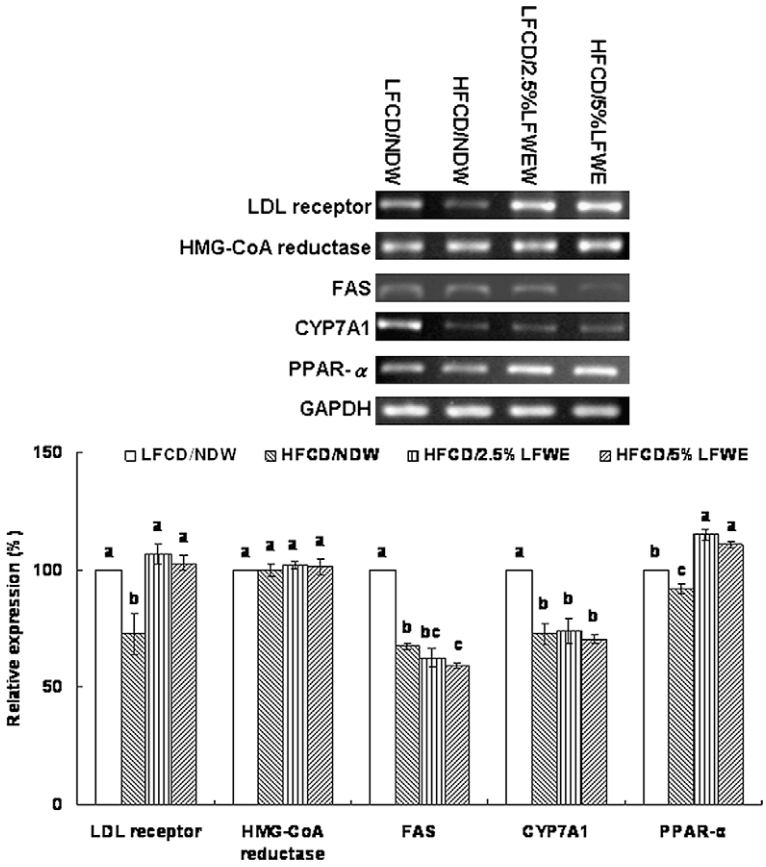

Fig. 2. LDL receptor, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), fatty acid synthase (FAS), cholesterol 7-α hydroxylase (CYP7A1), and peroxisome proliferator-activated receptor-alpha (PPAR-α) mRNA expressions of the experimental hamsters. *The data are given as mean ± SEM (n = 10). Mean values in each target gene with different letters indicate a significant difference ($p < 0.05$). **LFCD/NDW: low-fat/cholesterol-dietary + pure water group; HFCD/NDW: high-fat/cholesterol-dietary + pure water group; HFCD/2.5% LFWE: high-fat/cholesterol-dietary + 2.5% (w/v) litchi-flower-water-extract group; HFCD/5% LFWE: high-fat/cholesterol-dietary + 5% (w/v) litchi-flower-water-extract group. ***The values of LDL receptor, HMG-CoA reductase, FAS, CYP7A1, and PPAR-α mRNA were normalised to the value of GAPDH, and values for the hamsters in HFCD/NDW, HFCD/2.5% LFWE, and HFCD/5% LFWE groups were expressed relatively to the average values for hamsters in the LFCD/NDW group, which was set to 100.

diets cause a down-regulation of HMG-CoA reductase genes; whereas HMG-CoA reductase is upregulated when exogenous cholesterol decreases (White et al., 1997). Our data showed that regardless of cholesterol additions in the diets, no ($p > 0.05$) changes in HMG-CoA reductase expressions of hamsters were observed. Meanwhile, lower CYP7A1 expressions in the high-fat/cholesterol-dietary groups may be explained by a lower cholesterol catabolism than that in the normal dietary group (Fig. 3), which may result in higher liver cholesterol levels (Table 3). Although an activation of CYP7A1 expression can accelerate cholesterol catabolism, faecal bile acid levels represent the true cholesterol excretion. Therefore, it is supposed that drinking LFWE increased faecal bile acids in the high-fat/cholesterol-dietary hamsters, thus lowering liver cholesterol contents (Table 3), although CYP7A1 expressions were not ($p > 0.05$) increased (Fig. 2). FAS is in charge of triacylglycerol biosynthesis. Hence, the suppression of FAS expression can decrease deposition of visceral fat, and serum and liver triacylglycerol levels (Ikeda et al., 2005). In addition, increasing PPAR-α expression results in higher β-oxidation in livers, thus increasing energy expenditure and decreasing a development of non-alcoholic fatty liver (Matsuo et al., 2007; Seo et al., 2008). Therefore, it is supposed that lower liver triacylglycerol results from higher faecal triacylglycerol outputs (Table 3) and hepatic PPAR-α expression (Fig. 2), as well as a suppression of FAS expression (Fig. 2).

Miyake et al. (2006) investigated the hypolipidemic effects of eriocitrin, the main flavonoid in lemon fruit, and their observation indicates that eriocitrin can increase faecal bile acid and cholesterol outputs. Besides, the hypocholesterolemic effect of flaxseed oil was associated with the up-regulation of plasma cholesterol clearance (LDL receptor expression), and an increase of faecal cholesterol excretion (Tzang et al., 2009). Our data was not only in accordance with the previous literature but also indicated that the regulations of FAS and PPAR-α gene expressions are involved in the hypolipidemic effects of LFWE.

3.5. Serum malondialdehyde (MDA) and trolox equivalent antioxidant capacity (TEAC)

Serum antioxidant status is also related to cardiovascular health. Hence, the serum MDA content was used to analyse the serum peroxidation, and TEAC was used to evaluate serum antioxidant capacity in the present study. Drinking LFWE decreased ($p < 0.05$) serum MDA contents in the high-fat/cholesterol-dietary hamsters compared to only drinking NDW, and even showed a similar level of serum peroxidation as the normal-dietary hamsters (LFCD/NDW group) (Fig. 3A). In the serum antioxidant capacity, lower ($p < 0.05$) serum TEAC was observed in the high-fat/cholesterol-dietary hamsters, but drinking LFWE increased ($p < 0.05$) TEAC compared to only drinking NDW (Fig. 3B), where drinking 5% LFWE normalised the same serum TEAC in the high-fat/cholesterol-dietary hamsters as the normal-dietary hamsters.

A high-fat/cholesterol-dietary habit results in higher oxidative stress in the body and increased serum MDA contents (Sena

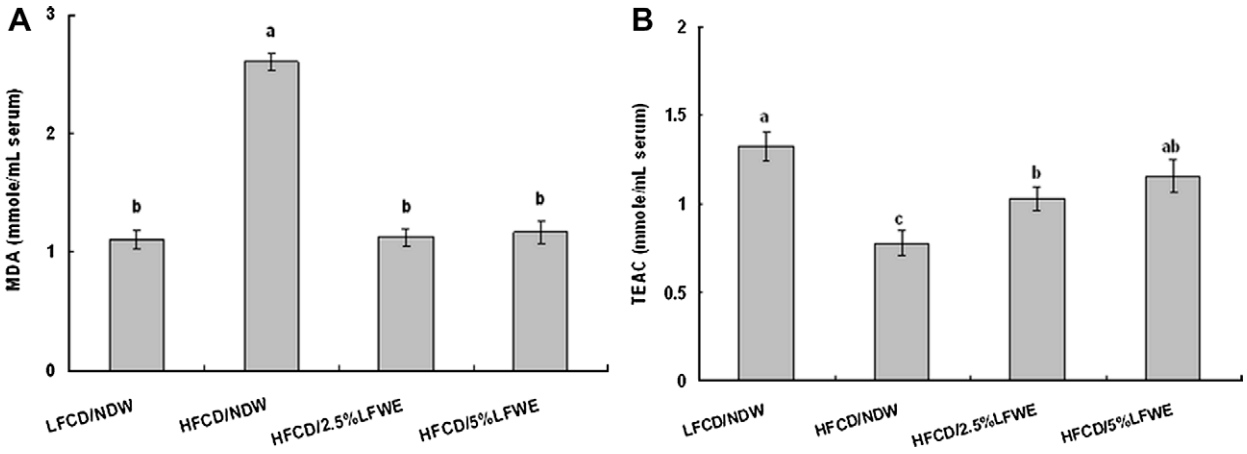

Fig. 3. Serum (A) malondialdehyde (MDA) and (B) trolox equivalent antioxidant capacity (TEAC) of the experimental hamsters. *The data are given as mean ± SEM ($n = 10$). Mean values of serum MDA and TEAC at the end of experiment with different letters indicate a significant difference ($p < 0.05$). **LFCD/NDW: low-fat/cholesterol-dietary + pure water group; HFCD/NDW: high-fat/cholesterol-dietary + pure water group; HFCD/2.5% LFWE: high-fat/cholesterol-dietary + 2.5% (w/v) litchi-flower-water-extract group; HFCD/5% LFWE: high-fat/cholesterol-dietary + 5% (w/v) litchi-flower-water-extract group.

et al., 2008). Frequent intakes of antioxidants, i.e. alpha-lipoic acid, are suggested to decrease serum MDA contents (Sena et al., 2008). Prior and Cao (2000) indicated that the natural phenols from the plant kingdom are evidence against lipid peroxidation. Moreover, a TEAC assay is often used to evaluate overall antioxidant levels in blood or tissue homogenates (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993). It was reported that anthocyanin-rich purple potato flake extract (Han et al., 2006) and rich phenolic green tea (Chan, Hosoda, Tsai, Yamamoto, & Wang, 2006) can augment serum antioxidant capacity (TEAC level) in rats and mice fed a chow diet, respectively. Besides, rich phenolic Du-Zhong (*Eucommia ulmoides* Oliv.)-water-extract also elevate serum TEAC levels in chronic-hepatic-injured rats induced by a gavage of CCl_4 (Hung et al., 2006). Our data demonstrated 2.5% or 5% LFWE used in the present study contains plenty of antioxidant phytochemicals (Table 1); therefore, lower serum TEAC in the high-fat/cholesterol-dietary hamsters could be reversed by drinking LFWE (Fig. 3B). Hence, elevated serum TEAC could be explained by the fact that serum lipid peroxidation (lower serum MDA contents) in the high-fat/cholesterol-dietary hamsters was retarded by drinking 2.5% and 5% LFWE (Fig. 3A and B).

4. Conclusions

Based on the results from this study, drinking LFWE indeed lowers serum lipids and peroxidation and amends the serum cholesterol profile and total antioxidant capacity in the high-fat/cholesterol-dietary hamsters. In the gene expressions of lipid homeostasis in the high-fat/cholesterol-dietary hamsters, LFWE normalised the LDL receptor gene expression, downregulated FAS gene expression, and upregulated PPAR-α gene expression. Furthermore, increased faecal lipid and bile acid excretions of hamsters drinking LFWE may be associated with the lipid-lowering effect of LFWE. Besides, LFWE enhanced serum overall antioxidant capacity (TEAC), thus lowering serum lipid peroxidation (MDA content) in the high-fat/cholesterol-dietary hamsters. Overall, LFWE showed a good protective effect on cardiovascular health and it is worth developing as a health food in some niche markets.

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