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Alpinia pricei Hayata rhizome extracts have suppressive and preventive potencies against hypercholesterolemia

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ABSTRACT

The aim of this study was to investigate the effects of 70% ethanol extracts of *Alpinia pricei* (APE) on lipid profiles and lipid peroxidation. Syrian hamsters were fed a chow-based hypercholesterolemic diet (HCD) for 2 weeks to induce hypercholesterolemia (>250 mg/dl). To evaluate the potency of APE in suppressing hypercholesterolemia, hamsters were then fed HCD plus a high dose (500 mg/kg body weight) or a low dose (250 mg/kg body weight) of APE, or only HCD for another 4 weeks. We found that hypercholesterolemic hamsters fed a high dose of APE had lower serum total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) levels, lower thiobarbituric acid reactive substances (TBARS) and alanine aminotransferase (ALT) activities, lower atherogenic indices (LDL-C/HDL-C and TC/HDL-C ratios), and lower hepatic protein expression of peroxisome proliferators activated receptor gamma (PPARγ) than hamsters fed a HCD diet. In addition, we also determined the preventive effects of APE on hamsters fed a HCD for 6 weeks. The hypocholesterolemic effects were also found in hamsters co-fed a high dose of APE and HCD for 6 weeks. These results suggest that APE has both suppressive and preventive potencies against hypercholesterolemia and has the potency to protect against lipid peroxidation.

1. Introduction

Alpinia pricei Hayata (Zingiberaceae) is a perennial rhizomatous herb indigenous to Taiwan. The leaves of *A. pricei* are used to make glutinous rice dumplings and the aromatic rhizome of *A. pricei* is thought to dispel abdominal distension and increase stomach secretion and peristalsis. Many *Alpinia* plants are considered medicinal herbs and have been reported to possess antioxidant (Shi et al., 2006), anti-inflammatory (Israf et al., 2007; Lin et al., 2009), anticancer (Ali et al., 2001; Lee and Houghton, 2005; Yang et al., 2008), immunostimulating (Bendjeddou et al., 2003), hepatoprotective (Kadota et al., 2003) and antinociceptive (Arambewela et al., 2004) activities.

Atherosclerosis is a disease involving both hypercholesterolemia and inflammation. Atherosclerosis is initiated by elevated levels of low-density lipoprotein (LDL) and other proatherogenic lipoproteins. In the presence of oxidative stress, LDL becomes the highly reactive form of oxidized LDL after lipid oxidative modification (Chisolm and Steinberg, 2000). Oxidized LDL and/or oxidized lipids can participate in many of the immunologic and inflammatory processes in the complex progression of the atherosclerotic lesion (Steinberg, 2005). In addition to decreasing LDL cholesterol levels, oxidized LDL should be also taken into consideration for preventing cardiovascular events in hypercholesterolemia with drug therapy. Therefore, oxidized LDL is considered as a marker for hypercholesterolemia. Peroxisome proliferator activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily of ligand activated transcription factors, is a key regulator of adipogenesis and lipid metabolism (Plutzky, 2000). PPARγ has been shown to regulate monocyte/macrophage gene expression (Nagy et al., 1998) and to promote monocyte differentiation and uptake of oxidized LDL through transcriptional induction of the scavenger receptor CD36 (Tontonoz et al., 1998). Current medical treatments of hyperlipidemia involve the use of statins, fibrates, and resins (Knopp et al., 2008). In addition, many medicinal plants and their active metabolites are considered potential hypolipidemic agents, such as curcumin derived from *Curcuma longa* (Zingiberaceae) (Ramírez-Tortosa et al., 1999; Olszanecki et al., 2005). Extensive research on Zingiberaceae plants has shown that *Alpinia zerumbet* (Lin et al., 2008) and *Alpinia officinarum* (Shin et al., 2004) have

Abbreviations: ALT, alanine aminotransferase; APE, *Alpinia pricei* extracts; HCD, chow-based hypercholesterolemic diet; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; PPARγ, peroxisome proliferators activated receptor gamma; TBARS, thiobarbituric acid reactive substances.

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hypolipidemic activities. The effects of *A. pricei* Hayata on lipid metabolism, however, have not been studied.

Animal models of diet-induced hypercholesterolemia are useful for assessing the effects of drugs (Fernandez et al., 1999). Hamsters in particular are extensively used for studying the effects of diet or drug on plasma lipid levels and the mechanisms involved because hamster models of disease present characteristics that resemble those in humans (Fernandez et al., 1999; Sawada et al., 2002; Wilson et al., 1999, 2006). This study was designed to assess the preventive and therapeutic efficacy of 70% ethanol extracts of *A. pricei* Hayata (APE) in hypercholesterolemic hamsters. The serum lipid and lipoprotein profiles, thiobarbituric acid reactive substances (TBARS), and oxidized LDL were measured as markers of hypercholesterolemia. Moreover, the liver lipids, PPARγ protein expression, and serum alanine aminotransferase (ALT) activity were also examined to evaluate hepatic response to APE.

2. Materials and methods

2.1. Plant material and extract preparation

The rhizomes of *A. pricei* Hayata were collected from Ping-tung County in southern Taiwan in March, 2007 and were identified by Dr. Yen-Hsueh Tseng (National Chung-Hsin University). A voucher specimen was deposited in the herbarium of the same university. The air-dried rhizomes (2 kg) of *A. pricei* were extracted with 10 L 70% ethanol at room temperature. The total crude extracts were evaporated in a vacuum to yield the ethanol extracts (166 g).

2.2. Identification and quantification of major compounds in APE

The 70% ethanol extracts of *A. pricei* (APE) were separated by semi-preparative high performance liquid chromatography (HPLC). A Luna silica column (250 × 10 mm, Phenomenex Co.) was employed in this study with two solvents systems, H_2O (A) and 100% acetonitrile (B). The gradient elution profile was as follows: 0–3 min, 80% A–B; 3–60 min, 80–0% A–B (linear gradient); 60–80 min 0% A–B; the flow rate was 2.5 mL/min and the detector wavelength was set at 280 nm. The major three compounds (Fig. 1A) in the APE were obtained at retention times of 32.5 min (1), 37.0 min (2), and 46.7 min (3). According to HPLC analysis, the amounts of compounds 1, 2, and 3 in APE were 1.1%, 8.9%, and 5.7%, respectively. The structures of compounds **1–3** were then elucidated using spectroscopic analyses. UV spectra of test compounds were recorded with a Jasco V-550 spectrometer and IR spectra obtained from a Bio-Rad FTS-40 spectrophotometer. Electron-impact mass spectrometry (EIMS) and high resolution electron-impact mass spectrometry (HREIMS) data were collected with a Finnigan MAT-958 mass spectrometer and NMR spectra recorded with Bruker Avance 500 and 300 MHz FT-NMR spectrometers, at 500 MHz (^{1}H) and 75 MHz (^{13}C). According to the mass and NMR analyses, compounds **1–3** were identified as (1) desmethoxyyangonin (Dharmaratne et al., 2002), (2) cardamonin (Ngo and Brown, 1998), and (3) flavokawain B (Dharmaratne et al., 2002) (Fig. 1B).

2.3. Animals and treatments

Seven-week-old male Syrian hamsters (*Mesocricetus auratus*) were purchased from the National Laboratory Animal Center. The hamsters were handled in accordance with the animal care and use guidelines of the China Medical University and the study protocol was approved by the Institutional Animal Care Use Committee. Male hamsters rather than female hamsters were used because previous data (Wilson et al., 1999) indicate that male hamsters are more likely to develop early aortic atherosclerosis compared to age-matched premenopausal female hamsters. The experiments were carried out under controlled conditions with a 12-h light/dark cycle. Hamsters were allowed access to drinking water and diet ad libitum during the experimental periods. All hamsters were weighed every week.

To examine the hypocholesterolemic potency, two doses of APE, 250 or 500 mg/kg body weight, were used in our study according to the previous trials (Chou et al., 2009; Lin et al., 2009). In the study by Chou et al., a chow diet with an APE (1500 mg/kg body weight) was used to assess the effects of APE on metabolic syndrome. In the study by Lin et al., flavokawain B (200 mg/kg body weight) extracted from *A. pricei* was administered to ICR mice by intraperitoneal injection. The dose intake of flavokawain B (200 mg/kg body weight) was equivalent to 3508 mg APE/kg body weight. We considered a mouse body weight is only one fourth of a hamster, thus we selected 250 and 500 mg APE/kg body weight in this study.

A hypercholesterolemic hamster model was established according to the method of Wilson (Wilson et al., 2006). A chow-based hypercholesterolemic diet (HCD) contained 89.9% (w/w) murine powdered chow diet (Fwosow Industry Co., Taiwan), 10% coconut oil (Chung Shing Chemicals Co., Taiwan), and 0.1% cholesterol (Sigma–aldrich, St. Louis, MO, USA). There are four steps for a chow-based hypercholesterolemic diet (HCD) preparation as the followings: (1) chow diet was ground into powder and weighed (89.9%, w/w, S) for HCD preparation, (2) a small amount of S (S1) was mixed with 0.1% (w/w) cholesterol powder (C), (3) coconut oil (10%, w/w, O) was added progressively and mixed with previous mixture (C + S1), and (4) the remaining powdered chow diet (S − S1) was mixed progressively with C + S1 + O mixture. The HAP diet was prepared by mixing 0.86% APE (equivalent to 500 mg APE/kg body weight) with 10% coconut oil, 0.1% cholesterol, and 89.04% powdered chow diet. The LAP diet was prepared by mixing 0.43% APE (equivalent to 250 mg APE/kg body weight) with 10% coconut oil, 0.1% cholesterol, and 89.47% powdered chow diet. To keep the diet stability, all diets were stored at 4 °C before use. Each hamster was housed individually in a polycarbonate cage. Powdered diets were given to the hamsters in stainless steel containers and were refreshed every day.

2.4. Experimental protocol

The experimental protocol is shown in Fig. 2. Hamsters (n = 40) were randomly assigned to five groups of eight hamsters in each and fed the respective treatment diets. Hamsters in group 1 served as controls and received a powdered chow diet for 6 weeks. Hamsters in group 2 (HCD) received HCD for 6 weeks. Hamsters in group 3 (4HAP) received HCD for 2 weeks, followed by a HAP diet for 4 weeks. Hamsters in group 4 (4LAP) received HCD for 2 weeks, followed by a LAP diet for 4 weeks. Hamsters in group 5 (6HAP) received a HAP diet for 6 weeks. The experimental design in groups 3 and 4 was used to evaluate the suppressive effects of APE on hypercholesterolemia and that in group 5 was aimed to assess the preventive effects of APE on a high cholesterol diet. This study has been done under Good Laboratory Practice (GLP).

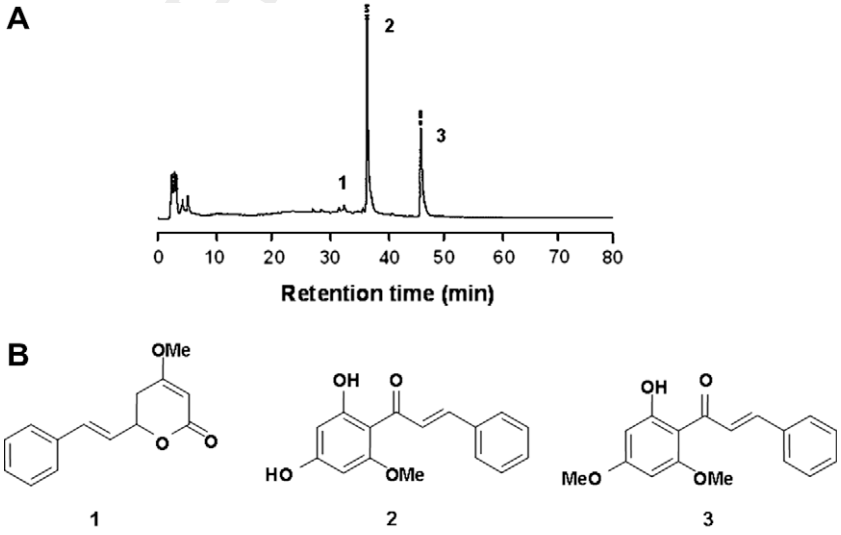

Fig. 1. (A) The HPLC chromatogram of the 70% ethanol extracts of *Alpinia pricei*. Three phenolic compounds (**1–3**) were isolated. (B) The structures of compounds **1–3**. According to the mass and NMR analyses, compounds **1–3** were identified as desmethoxyyangonin (1), cardamonin (2), and flavokawain B (3).

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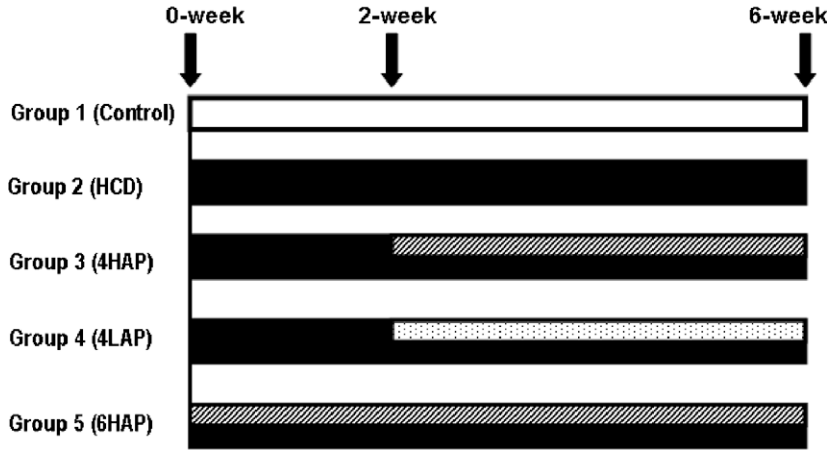

Fig. 2. Experimental protocol. ▭, chow diet; ■, high cholesterol diet (HCD); ▨, high dose *Alpinia pricei* extracts (500 mg/kg body weight); ⬚, low dose *Alpinia pricei* extracts (250 mg/kg body weight).

2.5. Blood sample collection and analyses

All of the hamsters were sacrificed after an overnight fast of 12 h under CO_2 anesthesia at the end of 6 weeks. Blood was centrifuged (365 × g) for 20 min. The serum was stored at 4 °C before analysis. So far there is no universal anticoagulant that could be used for evaluation of several laboratory parameters in a sample from a single test tube. The reason for using serum but not plasma is based on different requirement of analytic method. For example, anticoagulant EDTA is unsuitable for lipoprotein lipase activity determination because lipoprotein lipase uses metal ion as coenzyme. Therefore, we collect serum for all analysis in this study. Serum total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), and triacylglycerol (TG) were determined by commercial kit enzymatic methods (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). We used precipitation reagent for *in vitro* determination of LDL cholesterol with the CHOP-PAP method by photometric system. LDL was precipitated by addition of heparin. HDL and VLDL remain in the supernatant after centrifugation and were measured enzymatically by the CHOD-PAP method. The concentration of LDL cholesterol was calculated as the difference between total cholesterol and cholesterol in the supernatant (VLDL-cholesterol and HDL-cholesterol). The commercial kits including LDL Precipant (Ecoline®, Dia Sys Diagnotic System GmbH, Germany) and cholesterol FS* (Dia Sys Diagnotic System GmbH, Germany). Cholesterol N Calibrator was the reference standard (Diaiichi Pure Chemicals Co., LTD, Tokyo, Japan). HDL-cholesterol was measured spectrophotometrically following precipitation with phosphotungstic acid and magnesium ions to remove LDL and VLDL. Centrifugation leaved only the HDL in the supernatant. HDL cholesterol content was determined enzymatically using Ecoline S + cholesterol kits. The commercial kits including HDL Precipant (Ecoline®, Dia Sys Diagnotic System GmbH, Germany) and cholesterol FS* (Dia Sys Diagnotic System GmbH, Germany). In addition, the concentration of non-HDL-cholesterol was calculated as the difference between total cholesterol and HDL-cholesterol. Therefore, LDL cholesterol concentrations are not equal to non-HDL cholesterol concentrations. Serum lipoprotein lipase and alanine aminotransferase (ALT) activities were determined enzymatically utilizing Randox kits (Randox Laboratories Ltd. Co. Antrim, UK). Serum thiobarbituric acid reactive substances (TBARS) were determined using a ZeptoMetrix Kit (ZeptoMetrix Corporation, buffalo, NY, USA). Serum oxidized LDL concentrations were measured spectrophotometrically using an ELISA kit (Mercodia AB, Uppsala, Sweden) as previously described (Kopprasch et al., 2002).

2.6. Hepatic lipid measurement

The liver was excised and then divided into two portions. One portion was used to determine the hepatic cholesterol and triacylglycerol concentrations as previously described (Chang et al., 2004). The second portion was used to analyze PPARγ protein by Western blotting.

2.7. Western blot analysis

The liver was homogenized in a sucrose-HEPES buffer containing 0.5% Nonidet P40, protease inhibitors, and dithiothreitol according to the method of Kim et al. (2007). Then, the nuclear proteins were extracted (Kim et al., 2007) and the protein concentration was determined using a protein assay kit (Pierce Biotechnology, Rockford, USA), with bovine serum albumin as the standard. Samples were resolved by SDS–PAGE, transferred to nitrocellulose membrane and probed with antibodies against PPARγ (1:1000, Santa Cruz Biotechnology Inc., California, USA) and β-actin (1:2000, Santa Cruz Biotechnology Inc., California, USA). Signals were developed using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, USA).

2.8. Statistical analysis

All data were analyzed using SPSS 15.0 for windows. All results are shown as mean ± SD Group is the only factor for evaluation and values of total cholesterol, LDL-C, HDL-C, or TG are the outcomes. Therefore, one way analysis of variance (ANOVA) is used in this study. Significant differences between groups were analyzed using ANOVA followed by multiple comparison tests. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of APE on lipid profiles and atherogenic indices

There were no significant differences in dietary intake or body weight of hamsters (data not shown). Our preliminary studies showed that hamsters fed HCD for 2 weeks had significantly higher serum total cholesterol (275.2 ± 5.4 vs. 117.5 ± 12.7 mg/dl), LDL-C

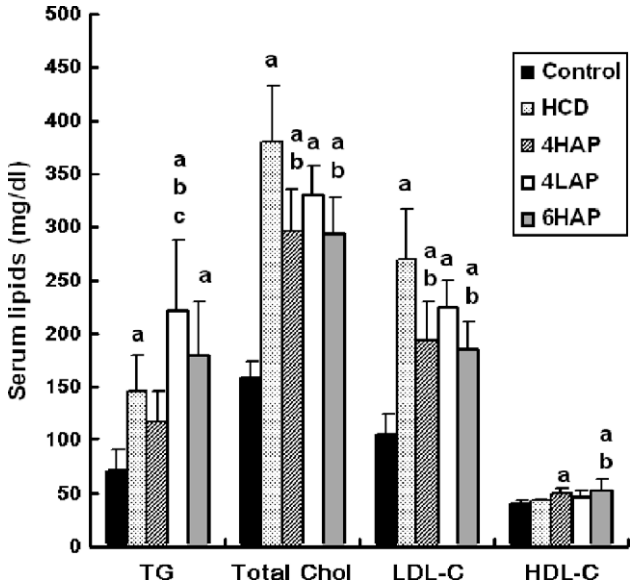

Fig. 3. Serum lipid levels in hamsters fed low dose (250 mg/kg body weight) or high dose (500 mg/kg body weight) *Alpinia pricei* extracts. Values are means ± SD. [a]Significantly different from control ($P < 0.05$). [b]Significantly different from HCD ($p < 0.05$). [c]Significantly different from 4HAP ($p < 0.05$). Groups are described in Fig. 2.

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(190.6 ± 11.3 vs. 80.5 ± 4.8 mg/dl), HDL-C (45.5 ± 6.3 vs. 26.0 ± 5.7 mg/dl), and TG (157.7 ± 37.4 vs. 63.3 ± 9.7 mg/dl) levels than hamsters fed a control chow diet. Therefore, the effects of APE on lipid levels in hypercholesterolemic hamsters were examined after a 2-week HCD induction.

Fig. 3 shows the serum lipids levels in hypercholesterolemic hamsters fed low or high doses APE. The levels of serum TG in the HCD (106%, $p = 0.026$) , 4LAP (213%, $p < 0.001$) and 6HAP (156%, $p = 0.001$)groups were significantly higher than those in the control group, while the TG level in the 4HAP group did not differ significantly from that in the control group or that in the HCD group ($p = 0.718$). Serum TG level in the 4LAP group, however, was significantly higher than that in the HCD group (52%, $p = 0.025$) and in the 4HAP group (88%, $p = 0.001$).

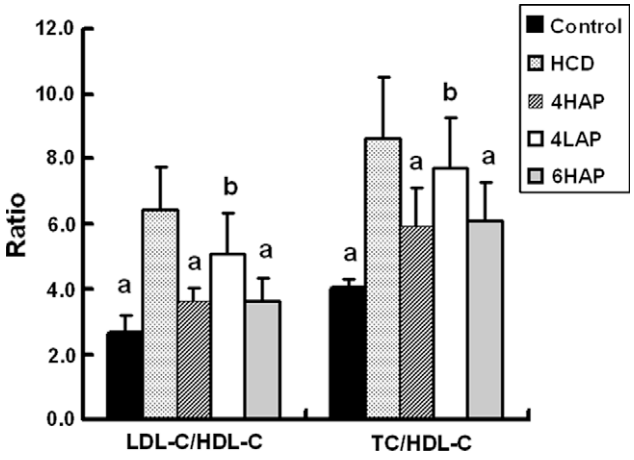

Fig. 4. Serum LDL-C/HDL-C ratios and TC/HDL-C ratios in hamsters. TC: total cholesterol. Values are means ± SD. [a]Significantly different from HCD ($p < 0.01$). [b]Significantly different from group 1 ($p < 0.01$). Groups are described in Fig. 2.

Feeding hamsters with HCD for 6 weeks resulted in a 2.4-fold increase in serum total cholesterol (Fig. 3). Serum total cholesterol and LDL-C levels significantly decreased in animals after receiving high doses of 4HAP or 6HAP. However, LDL-C levels remained unchanged after feeding with low-dose APE (4LAP). HDL-C levels were significantly higher in the 4HAP and 6HAP groups than in the control group ($p < 0.05$). There was no significant difference in HDL-C levels between the 4LAP group and control group. Also, no significant differences were observed between HCD and 4HAP groups. Serum HDL-C levels after treatment in the 6HAP group were 26% higher than those in the HCD group ($p < 0.05$).

In addition, we found that animals fed high-dose APE (4HAP or 6HAP) had lower LDL-C/HDL-C and TC/HDL-C ratios than animals in the HCD group (Fig. 4, $p < 0.01$). There was no significant difference in ratios between 4LAP and HCD groups.

3.2. Effects of APE on serum TBARS, lipoprotein lipase, and ALT activities and oxidized-LDL levels

According to the study of Verhagen et al. (2003), 10 basic requirements for a scientific paper reporting antioxidant, antimutagenic or anticarcinogenic potential of test substances in *in vitro* experiments and animal studies *in vivo* were suggested. In the valid and invalid test systems section, they mentioned that the measurement of lipid peroxidation *in vivo* should use TBARS (Griffiths et al., 2002). Serum TBARS activity and oxidized LDL level were used to evaluate lipid peroxidation which is associated with atherosclerosis. No significant differences in TBARS activity were observed between HCD and control groups ($p = 0.413$, Fig. 5A). When compared with HCD, 4HAP and 6HAP significantly decreased TBARS activity ($p < 0.01$), but this effect was not observed after treatment with low-dose APE (4LAP). There were significant decreases in oxidized LDL between the 4HAP, 6HAP and control groups ($p < 0.05$, Fig. 5C). No significant differences in serum oxidized LDL levels between hamsters fed the HCD (group 2) and those fed HCD supplemented with high dose of APE (group 3).

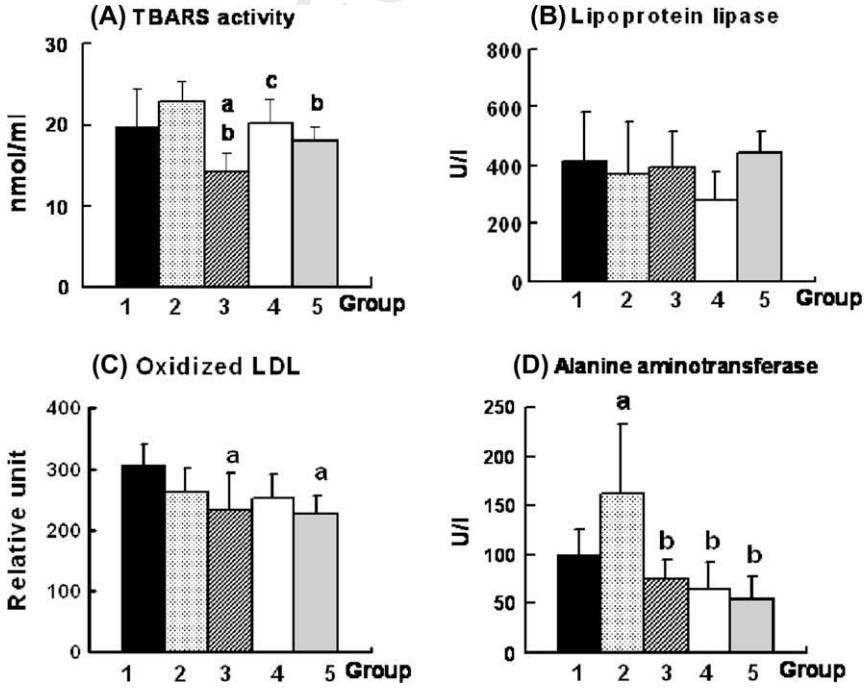

Fig. 5. Serum TBARS (A), lipoprotein lipase (B), and alanine aminotransferase (C) activities and oxidized LDL levels (D) in hamsters. Values are means ± SD. [a]Significantly different from group 1 ($p < 0.05$). [b]Significantly different from group 2 ($p < 0.05$). [c]Significantly different from group 3 ($p < 0.01$). Group 1: control; Group 2: HCD; Group 3: 4HAP; Group 4: 4LAP; Group 5: 6HAP. Groups are described in Fig. 2.

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Factors such as malnutrition, physical inactivity, uremic toxins, and inflammation are known to influence the activity of lipoprotein lipase, an important enzyme in metabolism of blood lipids. Through the action of lipoprotein lipase, triglycerides in plasma lipoproteins are hydrolyzed to fatty acids and monoglycerides, a prerequisite for their utilization in tissues. Lipoprotein lipase is synthesized mainly in adipocytes and myocytes, but is secreted from these cells and transported to the luminal side of the vascular endothelium. Disturbances in the activity of lipoprotein lipase can be coupled to proatherogenic lipid profiles including elevated levels of plasma triglycerides and low levels of HDL-cholesterol (Twickler et al., 2005; Mead and Ramji, 2002). Therefore, the lipoprotein lipase activity, which involved the changes of plasma TG levels, was determined in our study. No significant differences in lipoprotein lipase activity were observed between groups ($p > 0.05$, Fig. 5B). Serum ALT activity was used to detect liver function. ALT activity was significantly lower in animals after high- or low-dose APE treatment than in animals fed only a high cholesterol diet ($p < 0.05$, Fig. 5D).

3.3. Effects of APE on hepatic TG and cholesterol levels and on hepatic PPARγ expression

Liver weight and liver weight/body weight ratio were measured after 6-week treatment. The liver weights are 3.20 ± 0.48 g in control group (a chow-based diet), 4.54 ± 0.46 g in HCD group, 4.23 ± 0.29 g in 4HAP group, 4.54 ± 0.47 g in 4LAP group, 4.74 ± 0.56 g in 6HAP group. The ratios of liver weight/body weight are 0.0308 ± 0.0021 in control group, 0.0402 ± 0.0015 in HCD group, 0.0393 ± 0.0015 in 4HAP group, 0.0414 ± 0.0019 in 4LAP group, 0.0421 ± 0.0024 in 6HAP group. The liver weight and the liver weight/body weight ratio were significantly higher in animals treated with HCD-based diets (HCD, 4HAP, 4LAP, and 6HAP) than in animals fed only a chow-based diet. No differences in liver weight/body weight ratio were observed between hamsters fed the HCD and those fed HCD supplemented with HAP or LAP. Hepatic TG level in 4LAP group was significantly 80% higher than that in HCD group ($p < 0.05$, Fig. 6). Dietary administration of cholesterol significantly increased liver cholesterol levels in groups HCD (3.05-fold), 4HAP (3.31-fold), 4LAP (3.14-fold), and 6HAP (3.15-fold) when compared with control group ($p < 0.001$, Fig. 6). However, liver cholesterol levels in all APE feeding groups were not significantly different from the HCD group. In addition, the protein expression of PPARγ, which related the formation of atherosclerosis, was examined. We found the expression of PPARγ in the 4HAP group was the lowest among the five groups and was 54% and 51% lower than that in the HCD group ($p < 0.01$) and the 4LAP group ($p < 0.05$, Fig. 7).

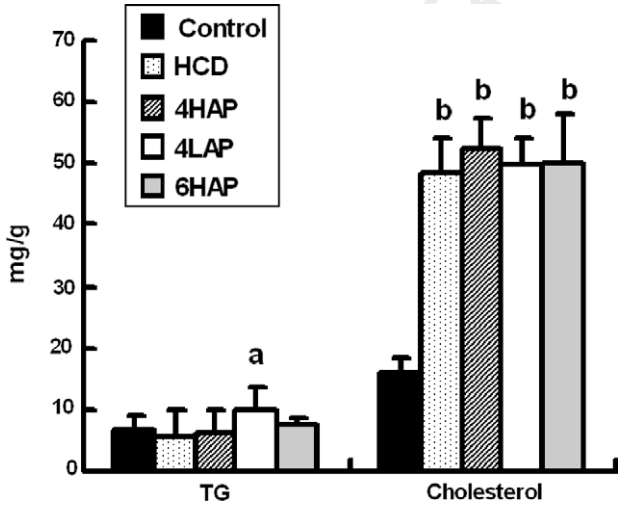

Fig. 6. Liver lipid levels in hamsters fed a low dose (250 mg/kg body weight) or a high dose (500 mg/kg body weight) *Alpinia pricei* extracts. Values are means ± SD. [a]Significantly different from HCD ($p < 0.05$). [b]Significantly different from control ($p < 0.001$). Groups are described in Fig. 2.

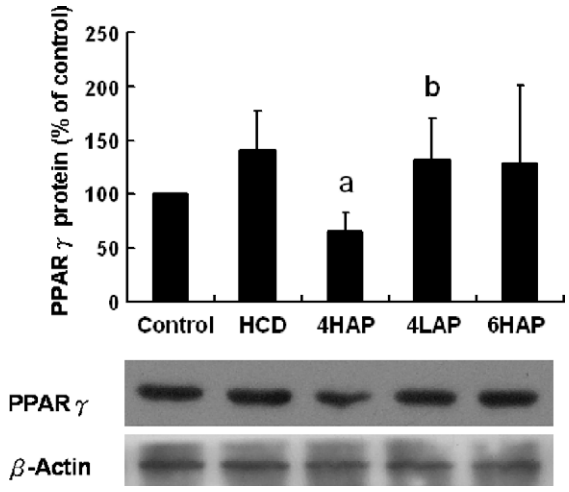

Fig. 7. The expression of PPARγ in hamsters. Livers were homogenized after 6 weeks and nuclear proteins isolated for Western analysis. Results represent four independent experiments. Densitometric analysis and normalization to β-actin are expressed as mean ± SD. [a]Significantly different from HCD ($p < 0.01$). [b]Significantly different from 4HAP ($p < 0.05$). Groups are described in Fig. 2.

4. Discussion

Risk of coronary heart disease is positively associated with total cholesterol and LDL-C and inversely associated with HDL-C (Knopp et al., 2008). According to the study by Wilson et al. (2006), 100 hamsters were fed a chow-based hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol for 2 week. Dietary treatments were fed for 8 or 12 weeks. Wilson et al. indicated that plasma HDL-C concentrations were 68.3 ± 3.38 mg/dl after an 8-week and 94.4 ± 17.1 mg/dl after a 12-week HCD feeding. We used the similar HCD formula (89.9% (w/w) murine powdered chow diet, 10% coconut oil, and 0.1% cholesterol) to induce hypercholesterolemia in hamsters and found HDL-C concentration was 45.5 ± 6.3 mg/dl after a 6-week HCD feeding. In this study, we found that high-dose APE (500 mg/kg body weight) showed a stronger effect in lowering serum TC and LDL-C levels than low-dose APE (250 mg/kg body weight) (Fig. 3) in hypercholesterolemic hamsters. In the 4HAP group, serum TC level was 22% lower and serum LDL-C level was 28% lower than those levels in the HCD group, but the TC and LDL-C levels remained unchanged in the 4LAP group. The LDL-C/HDL-C and TC/HDL-C ratios were 44% and 33% lower in the 4HAP group than those ratios in the HCD group (Fig. 4). Increased LDL-C/HDL-C and TC/HDL-C ratios are associated with high risk for the development of cardiovascular disease and atherosclerosis (Castelli, 1992). The hypocholesterolemic effects were also found in hamsters co-fed a high dose of APE and HCD for 6 weeks; the atherogenic indices (LDL-C/HDL-C and TC/HDL-C ratios) in the 6HAP group were 44% and 30% lower than those in the HCD group (Fig. 4). The results indicate that high-dose APE has both preventive and suppressive effects on hypercholesterolemia.

Alipinia species has been found to have antioxidant activity (Chen et al., 2008). According to HPLC and spectroscopic analyses, we found that the main compositions of ethanol extracts of *A. pricei* Hayata were desmethoxyyangonin (1.1%), cardamonin (8.9%), and

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flavokawain B (5.7%). Cardamonin (2′,4′-dihydroxy-6′-methoxychalcone) is a potential anti-inflammatory drug that targets the NF-κB pathway (Israf et al., 2007). The chalcone moiety of cardamonin has anti-oxidation and free-radical scavenging activity (Bors et al., 1990). In our study, the activity of TBARS was 37% lower in the 4HAP group and 21% lower in the 6HAP group than in the HCD group (Fig. 5A). A decrease in TBARS activity is important in preventing lipid peroxidation which is associated with atherosclerosis (Korantzopoulos et al., 2003). Lipid peroxides frequently decompose to reactive aldehydes like malondialdehyde and 4-hydroxynonenal that react with nucleic acids, proteins and lipids, causing tissue and organ damage (Slatter et al., 2000). Oxidative modification of lipids, in particular LDL, is suggested to play a key role in atherosclerosis (Chisolm and Steinberg, 2000). We found that the levels of serum oxidized LDL in the 4HAP and 6HAP groups were lower than in the HCD group, although there was no significant difference in level between the two groups. Our interpretation is that APE delays the start of lipid oxidation in LDL by reducing the formation of TBARS through its anti-oxidation and free-radical scavenging activity (Bedwell et al., 1989; Esterbauer et al., 1987). These results indicate that high-dose APE has the potency to protect against lipid peroxidation. We did not determine how APE delays the start of lipid oxidation in LDL but we directly determined the serum oxidized LDL level. The reason is that the blood volume per hamster is not enough to do various biochemical analysis and to ultracentrifuge for LDL preparation. In addition, Dr. Yen et al., the other team group who is also studying *A. pricei* Hayata, have found that APE possessed inhibitory effect on the oxidative modification of LDL induced by Cu^{2+} (unpublished data).

In addition, the expression of hepatic PPARγ in the 4HAP group was significantly lower than that in the HCD group (Fig. 7). A decrease in PPARγ expression in the 4HAP group may be due to APE-induced reduction in lipid peroxidation. Several endogenous oxidized lipids have been identified as ligands of PPARγ (Tontonoz et al., 1998; Nagy et al., 1998). Stimulation of PPARγ activity by oxidized lipids enhances the transcription of CD36, leading to further uptake of oxidized LDL and differentiation of monocytes into foam cells (Tontonoz et al., 1998; Nagy et al., 1998). Oxidized lipids promote macrophage aggregation and up-regulate the process of atherosclerosis by enhancing the expression of PPARγ (Schild et al., 2002). Therefore, one possible mechanism for the decrease in the expression of PPARγ in the HAP group could be explained by reducing endogenous oxidized lipids. Oxidized LDL represented a variety of modification of both lipid and apolipoprotein B components by lipid peroxidation. Among lipid oxidation products of oxidized LDL, oxidized forms of linoleic and arachidonic acids, 9-hydroxyoctadecadienoic acid (9-HODE), 13-HODE, and 15-hydroxyeicosatetraenoic acid (15-HETE), were efficient stimuli of PPARγ-mediated reporter gene transcription (Nagy et al. 1998). Another class of oxidized lipids is short-chain phosphatidylcholine (PC) formed by cleavage of a double bond in 18–20 carbon polyunsaturated acyl chains of the lipid (Itabe et al., 1994). In fact, more studies on the anti-atherosclerotic mechanism, changes in the early aortic atherosclerosis, and potential effects of APE on regulation of PPARγ expression should be examined in the future.

Increases in liver cholesterol level occurred in all animals that received a high cholesterol diet. No significant differences in liver cholesterol were observed between hamsters fed HCD or HCD supplemented with low- or high-dose APE. An increase in cholesterol accumulation in liver may be due to the HCD diets. In addition, ALT is an enzyme produced mainly in the liver. The release of the enzyme is thought to occur during damage of the liver, which results in an elevation of ALT in serum. Accordingly, it has been well accepted that when serum activity is measured, the enzyme, ALT, provides as a marker of hepatic diseases (Sherman, 1991; Iwaki et al., 2007). ALT activity can be measured both in serum and in liver. However, in an animal study of liver fattening, Bogin et al.(1986) found that in the serum from fatty liver rats, there were significant changes in the level of ALT (+68.7%) when compared with normal rats. In contrast, the level of ALT was reduced (−23%) upon detected in the fatty liver (Bogin et al., 1986). Comparison the sensitivity of detected samples from serum with hepatic cells showed detection of ALT from serum is superior to liver cells (Bogin et al., 1986). Therefore, we used the serum samples for detection of ALT to reflect the dysfunction of liver in our animal model. In this study, serum ALT activity in hamsters fed HCD for 6 weeks was 64% higher than that in control animals. Notably, ALT levels in animals exposed to an APE-admixed HCD were significantly lower than those in hamsters fed HCD only (Fig. 5D) indicating that APE has the potency to protect liver function.

In conclusion, the present study demonstrates that APE causes a marked decrease in levels of TC, LDL-C, TBARS, ALT, and PPARγ protein expression as well as a marked reduction in LDL-C/HDL-C and TC/HDL-C ratios in hypercholesterolemic hamsters. These results suggest that APE has both suppressive and preventive potencies against hypercholesterolemia and has the potency to protect against lipid peroxidation. The APE-induced anti-atherosclerotic mechanism may be, at least in part, responsible for a decrease in the expression of PPARγ by reducing endogenous oxidized lipids.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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