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# Effect of pigeon pea (*Cajanus cajan* L.) on high-fat diet-induced hypercholesterolemia in hamsters

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ABSTRACT

Obesity is associated with increased systemic and airway oxidative stress, which may result from a combination of adipokine imbalance and antioxidant defenses reduction. Obesity-mediated oxidative stress plays an important role in the pathogenesis of dyslipidemia, vascular disease, and nonalcoholic hepatic steatosis. The antidyslipidemic activity of pigeon pea were evaluated by high-fat diet (HFD) hamsters model, in which the level of high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and total triglyceride (TG) were examined. We found that pigeon pea administration promoted cholesterol converting to bile acid in HFD-induced hamsters, thereby exerting hypolipidemic activity. In the statistical results, pigeon pea significantly increased hepatic carnitine palmitoyltransferase-1 (CPT-1), LDL receptor, and cholesterol 7α-hydroxylase (also known as cytochrome P450 7A1, CYP7A1) expression to attenuate dyslipidemia in HFD-fed hamsters; and markedly elevated antioxidant enzymes in the liver of HFD-induced hamsters, further alleviating lipid peroxidation. These effects may attribute to pigeon pea contained large of unsaturated fatty acids (UFA; C18:2) and phytosterol (β-sitosterol, campesterol, and stigmasterol). Moreover, the effects of pigeon pea on dyslipidemia were greater than β-sitosterol administration (4%), suggesting that phytosterone in pigeon pea could prevent metabolic syndrome.



## 1. Introduction

Obesity, defined as an excess of white adipose tissue, is associated with a higher risk of developing diabetes and cardiovascular disease. Atherosclerosis and its related complications such as cardiovascular disease and cerebrovascular disease are the major causes of morbidity and mortality in many developed countries. The total triglyceride (TG) content in adipocytes reflects the balance between lipogenesis and lipolysis, which is largely related to cell volume (Marques et al., 1998). Abundant evidence from animal and human research demonstrates that dyslipidemia, including that resulting from elevations in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and TG concentration and decreases in high-density lipoprotein cholesterol (HDL-C) concentration in the blood, is the leading risk factor of atherosclerosis (Plat and Mensink, 2005). Nonalcoholic hepatic steatosis or fatty liver is the abnormal accumulation of triglycerides in the cytoplasm of hepatocytes. As it was found to increase the vulnerability of the liver to progression to steatohepatitis and advanced stages of liver disease (Brunt and Tiniakos, 2010), hepatic steatosis is no longer regarded as a relatively benign condition. Reflecting these findings, the more embracing term nonalcoholic fatty liver disease (NAFLD) has been adopted to cover the full spectrum of metabolic fatty liver disorders (Angulo, 2002).

Diet is closely related to the development of obesity. A diet high in cholesterol content is a major environmental contributor to unbalanced lipoprotein metabolism and is associated with an increased prevalence of atherosclerosis. Although most food components are discussed in relation to their contribution to obesity, certain food components have been reported to exhibit anti-obese activity, including bitter melon and grape seed extract (Huang et al., 2007; Vogels et al., 2004). Furthermore, conjugated linoleic

*Abbreviations:* apo-A1, apolipoprotein-A1; bw, body weight; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; CPT-1, carnitine palmitoyltransferase-1; CYP7A1, cytochrome P450 7A1; DTNB, 5,5-dithiobis[2-nitrobenzoic acid]; EDTA, ethylenediaminetetraacetic acid; FXR, farnesoid X receptor; GC, gas chromatography; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; H&E, hematoxylin and eosin; $H_2O_2$, hydrogen peroxide; HDL-C, high-density lipoprotein-cholesterol; HDP, high-dose (80%) pigeon pea; HFD, high-fat diet; HPLC, high-performance liquid chromatography; LDL-C, low-density lipoprotein-cholesterol; LDP, low-dose (20%) pigeon pea; MDA, malondialdehyde; MDP, medium-dose (40%) pigeon pea; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, nonalcoholic fatty liver disease; ND, normal diet; PC, positive control; SD, standard deviation; SOD, superoxide dismutase; TBA, Thiobarbituric acid; TBARS, thiobarbituric acid reaction substances; TBHQ, tert-butylhydroquinone; TC, total cholesterol; TCA, trichloroacetic acid; TG, total triglyceride; UFA, unsaturated fatty acids.

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acid has been reported to lower body weight and visceral fat mass in mice fed a high-fat diet (HFD) (Bhattacharya et al., 2006). Studies have also indicated that phytosterol can improve dyslipidemia by suppressing increases in LDL-C, TC, and TG levels and inhibiting fatty acid absorption (Homma et al., 2003; Doggrell, 2011), as well as elevate apolipoprotein-A1 (apo-A1) expression (Bañuls et al., 2011).

Pigeon pea (*Cajanus cajan* L.) is an important grain legume crop of rain-field agriculture in the tropics and subtropics. Although consumed as a whole/split pulse and as a green vegetable to supplement a cereal-based diet, utilization of red gram for human nutrition may be constrained by the presence of protease inhibitors (Grover et al., 2002; Ambekar et al., 1996). At the same time, extracts and derivatives of pigeon pea have assumed a medicinal role and are commonly used to treat diabetes, febrifuge, dysentery, hepatitis, measles, jaundice, bronchitis, cough, genital irritation, pneumonia, and skin problems throughout the world (Grover et al., 2002; Ambekar et al., 1996). In China, pigeon pea leaves are widely applied as a treatment for blood diseases as well as both analgesic and anthelminthic agents (Tang et al., 1999). In recent years, extracts of pigeon pea have been found to exert a number of therapeutic effects in treating diseases such as sickle cell anemia, plasmodiosis, and hepatic disorders (Akinsulie et al., 2005; Duker-Eshun et al., 2004; Ghosh et al., 2006).

Stilbenes have been identified in the pigeon pea plant, and their impact on lipid metabolism in mice fed a high-cholesterol diet has been investigated (Luo et al., 2008). However, it remains unclear whether the phytosterol in pigeon pea acts directly on lipidemia regulation to improve obesity. This study addressed this knowledge gap by investigating whether treatment with pigeon pea oil exerted hypolipidemic activity in hamsters in which obesity and high cholesterol levels had been induced by administration of a HFD.

## 2. Materials and methods

### 2.1. Materials and chemicals

5,5-Dithiobis[2-nitrobenzoic acid] (DTNB), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), glutathione reductase (GR), glutathione disulfide (GSSG), nicotinamide adenine dinucleotide phosphate (NADPH), buffered formalin, eosin, hematoxylin, xylene, β-sitosterol, tert-butylhydroquinone (TBHQ), and cholesterol were purchased from Sigma (St. Louis, MO, USA). n-Butanol was purchased from Fisher Co. (NJ, USA). Enzymatic kits were purchased from Randox Laboratories Ltd. (Antrim, United Kingdom). Ethyl ether was obtained from Tedia Co. (Fairfield, Ohio, USA). Thiobarbituric acid (TBA) was purchased from Acros Organics Co. (NJ, USA). Soybean oil was purchased from TTET Union Co., Tainan, Taiwan). Pigeon pea was provided from Taitung District Agricultural Research and Extension Station of Council of Agriculture (Taitung, Taiwan). After wishing, pigeon pea was steam cooked at 121 °C for 30 min by autoclave, and was dried at 40 °C (Azevedo et al., 2003), subsequently, the pigeon pea powder was obtained by grind.

### 2.2. Assay for fatty acid of pigeon pea

Sample was pre-treated with the method of Christie (1982), and fatty acid was quantified by gas chromatography (GC). Briefly, the analysis of fatty acid in pigeon pea was carried out on an Hitachi gas chromatograph system (TRACE 2000, Japan) equipped with a column: fused silica column Rt-2330 (30 m × 0.32 mm, ID. 0.2 μm thickness), a detector (flame ionization detector), and carrier gas ($N_2$, 1.5 ml/min). The injector temperature and detector temperature were 230 °C and 240 °C, respectively. The air flow rate and $H_2$ flow rate were 300 ml/min and 30 ml/min, respectively, and the split ration was 30:1. And the ovan program: 50 °C → 10 °C/min → 180 °C (5 min) → 2 °C/min → 200 °C.

### 2.3. Assay for phytosterol of pigeon pea

Sample was pre-treated with the method of Park and Addis (1986), and phytosterol was quantified by GC. Briefly, the analysis of phytosterol in pigeon pea was carried out on an Hitachi gas chromatograph system (TRACE 2000, Japan) equipped with a column: fused sillica capillary column DB-I (30 m × 0.25 mm ID., 0.25 μm thickness), a detector (flame ionization detector), and carrier gas ($N_2$, 0.7 ml/min). The injector temperature and detector temperature were 200 °C and 240 °C, respectively. The air flow rate and $H_2$ flow rate were 350 ml/min and 35 ml/min, respectively, and the split ration was 30:1. And the ovan program: 180 °C (5 min) → 7 °C/min → 250 °C → 3 °C/min → 280 °C (20 min).

### 2.4. Assay for total phenolic compounds and flavonoids of pigeon pea

For the determination of total phenolic compounds, 95% ethanol extracts of pigeon pea was measured spectrophotometrically using Folin–Ciocalteu's reagent (Taga et al., 1984) Total flavonoid content was calculated according to a standard curve established with quercetin (Jia et al., 1999).

### 2.5. Assay for phenolic compound of pigeon pea by high-performance liquid chromatography (HPLC)

HPLC performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan) consisting of a model L-2135 pump, and a model UV–VIS L-7455 detector (GL Sciences Inc., Japan) set at 280–520 nm. The analyses were carried out on a Mightysil RP-18 column (250 mm × 4.6 mm, 5 μm). Pigeon pea extracts were filtered through a 0.45 μm filter before use. The mobile phase A was 2% acetic acid/water, and the mobile phase B was 0.5% acetic acid/water: acetonitrile (50:50; v/v) (1 ml/min). Gallic acid, epigallocatechin, vallic acid, caffic acid, (−) epigallocatechin gallate, p-coumaric acid, ferulic acid, rutin, querccetin-3-glucoside, querccetin were determined by ultraviolet detector. These compounds were identified by comparison of their retention time (Rt) values and UV spectra with those of known standards and determined by peak areas from the chromatograms (Schieber et al., 2001).

### 2.6. Animals and diets

The 4-week old of male Syrian hamsters were obtained from the National Laboratory for Animal Breeding and Research Center (Taipei, Taiwan). All animals were acclimatized for 1 week prior to the treatment. They were randomly divided into 6 treatment groups (6 animals per group), including (1) normal diet (ND), (2) high-fat diet (HFD), (3) low-dose (20%) pigeon pea + HFD (LDP + HFD), (4) medium-dose (40%) pigeon pea + HFD (MDP-HFD), (5) high-dose (80%) pigeon pea + HFD (HDP + HFD), and (6) positive control (PC-HFD; 4% of β-sitosterol) groups to induce dyslipidemia for 8 weeks. The HFD formula was presented in Table 1. Animals were maintained at 12 h/12 h light/dark cycle, 60% relative humidity, and 25 °C temperature conditions. All animal procedures were followed as per the experimental protocols approved by the institutional animal ethics committee (Chiayi University, Chiayi, Taiwan, ROC).

### 2.7. Histopathologic studies

Liver tissues were trimmed (2 mm thickness) and fixed (buffer formaldehyde). The fixed tissues were processed including embedded in paraffin, sectioned and rehydrated. The histological examination by above conventional methods was evaluated the index of ethanol-induced necrosis by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H&E) (Gray, 1964).

### 2.8. Assays for serum and hepatic TC, TG, HDL-C, and LDL-C

Levels of TC, TG, HDL-C, and LDL-C in serum was determined by commercial kits from Randox Laboratories Ltd. (Antrim, United Kingdom). In addition, liver tissues were homogenized in cold Tris–HCl (pH 7.4) (1:10, w/v) of 20 mM. The homogenate was centrifuged for 30 min at 2500*g*. And then, hepatic TC and TG were measured by commercial kits from Randox Laboratories Ltd.

### 2.9. Measurements of lipid peroxidation products and antioxidase activity

Malondialdehyde (MDA), which is one of the lipid peroxidation products, was determined by the method of Buege and Aus (1978). Briefly, 1 ml of the liver homogenate was mixed with 1 ml of cold trichloroacetic acid (TCA) (75 mg/ml) to precipitate proteins and then centrifuged at 1500 rpm. The supernatant was reacted with 1 ml of thiobarbituric acid (TBA) (8 mg/ml) in boiling water for 45 min. Lipid peroxidation products were estimated by measuring the concentration of thiobarbituric acid reaction substances (TBARS) in fluorescence at ex 530 nm/em 552 nm. Glutathione peroxidase (GPx) activity was determined as previously described (Mohandas et al., 1984). Briefly, 0.1 ml of homogenate was mixed with 0.8 ml of 100 mM potassium phosphate buffer (1 mM EDTA, 1 mM NaN3, 0.2 mM NADPH, 1 unit/ml GR, and 1 mM GSH, pH 7.0) and incubated for 5 min at room temperature. Thereafter, the reaction was initiated after adding of 0.1 ml of 2.5 mM hydrogen peroxide ($H_2O_2$). GPx activity was calculated by the change of the absorbance at 340 nm for 5 min. The reduced GSH content of liver homogenate was determined as previously described (Van Dam et al., 1999). Liver homogenate was mixed with TCA (50 mg/ml) mixture and incubated for 5 min, centrifuged at 8000*g* for 10 min under 4 °C. The homogenate was reacted with DTNB for 5 min