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RESEARCH ARTICLE

Anti-hyperlipidemic activity of spider brake (*Pteris multifida*) with rats fed a high cholesterol diet

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Abstract

This study evaluates the possible potency of the anti-hyperlipidemic effect of spider brake [(*Pteris multifida* Poiret (Pteridaceae)]. We investigated this by feeding the hyperlipidemic Sprague-Dawley rats, caused by a high cholesterol diet, with lyophilized powder of spider brake (LSB) and compared the result with the rats fed with β-sitosterol. The results indicated that the administration of lyophilized powder of spider brake (LSB) lowered the hyperlipidemic level on rats. The relative weights of the liver, adipose tissue, and relative adipose tissue of 10% substitutions of LSB group (LSB-10) showed a significant decrease ($P < 0.05$) by 6%, 15.9%, and 14.3% in contrast to the untreated counterparts (control), respectively. A significantly lower ($P < 0.05$) plasma TG, low density lipoprotein cholesterol, low density lipoprotein cholesterol/high density lipoprotein cholesterol ratio, liver CH, and TG contents were also observed in LSB-10 compared to the untreated counterparts (by 36.8%, 21%, 18.7%, 10.2% and 14.3% reduction, respectively). Simultaneously, the wet fecal weight, dry fecal weight, nitrogen compounds, excretion of neutral steroids, and bile acids significantly ($P < 0.05$) increased by 9.6%, 10.6%, 23.7%, 9.7%, and 3.4% respectively. The results showed that LSB could cause not only a reduction in CH and TG, but also could increase the excretion of lipids and metabolic by-products via the intestinal tract.

Keywords: *Spider brake* (Pteris multifida *Poiret); anti-hyperlipidemic effect; triglyceride; cholesterol*

Introduction

The herb spider brake [(*Pteris multifida* Poiret (Pteridaceae)], also known as "fong-wei-cao" in China and Taiwan, is one of the most widespread folk medicines that is commonly used as material for herb beverages in Taiwan. In previous studies, it was found that many Taiwanese folk herbs have pharmacological functions such as anti-inflammation, anti-virus, anti-cancer, immune enhancement, antioxidant, anti-aggregation and hepatoprotection (Chang et al., 2005; Hsu et al., 2005; Kuo et al., 2004; Lin et al., 2000, 2002, 2007; Lu et al., 2000; Wu et al., 2004; Yang et al., 2005, 2007; Yen et al., 2007). One report indicated that spider brake could potentially absorb and therefore accumulate arsenic (Wang et al., 2007a). However, no toxic effect of spider brake on rats was found from our previous research (Wang et al., 2007b). Spider brake has various flavonoids and possesses many properties such as anti-pyretic, detoxifying, antibiotic, anti-inflammatory, anti-mutagenic (Lee & Lin, 1988), and free radical-scavenging activities (Wang et al., 2007c). The present study not only investigated the anti-hyperlipidemic effects of spider brake in high CH diet-induced hyperlipidemic Sprague-Dawley (SD) rats, but also compared its therapeutic effects to β-sitosterol.

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Materials and methods

Chemicals

Absolute alcohol and ether were obtained from Riedel-deHaen (Seelze, Germany). Acetone, β-carotene, chloroform, copper (II) sulfate, ethanol, hydrochloric acid, methanol, potassium hydroxide, potassium sulfate, pyridine, pyrogallol, β-sitosterol (purity >95%) and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Acetonitrile and petroleum ether were obtained from TEDIA (Fairfield, OH). Apigenin-7-*O*-β-D-glucoside and luteolin-7-*O*-β-D-glucoside were obtained from Extrasynthese (Genay, France). Bovine serum albumin (BSA) was obtained from Bio-Rad (Anaheim, CA, Los Angeles District). Calcium chloride and K_2-EDTA were obtained from Nacalai Tesque (Kyoto, Japan). Cholesterol, cholic acid, gallic acid, and TDF-kit (α-amylase, protease, and amyloglucosidase) were obtained from Sigma (St. Louis, MO). Cholesterol (CH) CH201 kit, high density lipoprotein cholesterol (HDL-C) CH204 kit, low density lipoprotein cholesterol (LDL-C) CH1350 kit and triglyceride (TG) TR213 kit were obtained from Randox Lab (Antrim, Northern Ireland). Lard was obtained from Chang-Guann (Kaohsiung, Taiwan). All other regents were of analytical grade.

Preparation of lyophilized powder of spider brake

Spider brake was obtained from a local herb store and the voucher specimens identified by Chunching Lin were deposited in the herbarium of the Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan. The lyophilized powder of spider brake (LSB) was prepared by first washing and freeze-drying the fresh whole plant, and then grinding the plant into powder through a sieve (40-mesh).

Experimental animals and design

Forty male pathogen-free Sprague-Dawley rats, 4-5 weeks old, were purchased from the National Laboratory Animal Production and Research Center in Taipei. The rats were housed in macrolon cages with stainless steel grid covers and sterilized wood shavings were used as bedding material in a controlled environment (temperature 25° ± 1°C, relative humidity 60% ± 10%, and artificial lighting was sequenced at 12 h light/dark cycles). All animals received humane care in accordance with the guideline by Yu et al. (2001) and the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996). The formulated diet was manufactured from commercial rat chow (Purina 5001, Purina, St. Louis, MO) and added with CH 1%, cholic acid 0.05%, and lard 5%. The LSB was added as a substitution based on 1%, 5%, and 10% raw material/kg diet. The rats had free access to food and water. The rats were randomly divided into five groups, each consisting of eight rats. Group 1: high cholesterol (HC), normal control, fed on formulated diet (containing basal diet and supplement of 1% CH, 0.05% cholic acid, and 5% lard). Groups 2-4: LSB-1, LSB-5, and LSB-10, respectively, treated with LSB, fed on formulated diet and 1%, 5%, and 10% LSB, respectively. Group 5: sitosterol, treated with β-sitosterol, fed on formulated diet and 1% β-sitosterol. The initial body weights and fasting blood lipid of all the animals in each group were measured. Their daily feed intake and weekly body weight changes were recorded, and the feed conversation efficiency was measured. On day 54, the feces of the rats were collected for estimation of fecal neutral steroids, bile acid, and nitrogen compounds. After 56 days, the rats were fasted for 12 h and their final body weights and blood lipids were determined, following which eight rats in each group were sacrificed under anesthesia by ether. Their blood and tissues of liver and adipose were collected for various biochemical estimations.

Plasma, hepatic and fecal lipid levels

The blood was collected in heparinized tubes. Plasma was prepared by centrifugation (2700 *g* for 15 min at 4°C). The liver was immediately taken out and washed twice with ice-cold saline, and then weighed and stored at -80°C. The blood, liver and fecal samples were assayed for their biochemical analysis. The plasma CH, HDL-C, and TG levels were determined using the enzymatic colorimetric method (Randox Laboratories Ltd., Crumlin, County Antrim, UK). The level lipids were extracted using the procedure developed by Folch et al. (1957) and the CH and TG levels were analyzed with the same enzymatic kit as used in the plasma analysis. The feces were collected at week 8 and the wet and dry weights (dehydrated at 150°C for 5 h) were weighed. The fecal neutral steroids and bile acid was estimated according to Grundy et al. (1965). Nitrogen compounds as determined by the Kjeldahl method were multiplied by a factor of 6.25 (AOAC, 1990).

Anti-hyperlipidemic components

The dietary fiber, β-sitosterol, and β-carotene were estimated by the methods of AOAC 985.29 (AOAC, 1995), Farombi et al. (2000), Dutta and Normen (1998), and Kitada et al. (1989), respectively. Flavonoid content was determined using the method of spectrophotometric continuous assay as described by Christel et al. (2000) with slight modification. In brief, an equal volume of 2% $AlCl_3 \cdot 6H_2O$ was added to various concentrations of LSB, followed by vortexing, standing for 10 min, and calculation of the absorbance at 340 nm. The flavonoid content

was determined using gallic acid as a standard. The two anti-hyperlipidemic active ingredients, apigenin-7-*O*-β-D-glucoside and luteolin-7-*O*-β-D-glucoside, were determined by the high performance liquid chromatography (HPLC) method. In brief, 1 g LSB was added to a bottle and mixed with 70% ethanol 10 mL ultrasonic for 60 min at 40°C, followed by filtering through 0.45 μm filter. The filtrate (10 μL) was injected onto HPLC system (Agilent 1100, Palo Alto, CA) and compared with the standard chemicals. The HPLC system consisted of a Cosmosil 5C18-MS-II column (4.6 × 250 mm, 5 μm) using a mobile phase of 0.1 N H_3PO_4/acetonitrile (85:15, v/v) with UV detection at 203 nm.

Statistical analysis

Means and standard deviation of data were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A 95% confidence level ($P < 0.05$) is to be considered statistically significant among all the groups.

Results and discussion

No significant ($P > 0.05$) differences of daily feed intake among all groups were found. In addition, the final body weights and feed conversion efficiencies in LSB-10 and sitosterol groups were significantly ($P < 0.05$) lower (about 3.3%–3.9% and 2.4%–2.8% reduction, respectively) than the other groups (Table 1). The body weights were increased for all groups during the experimental period. Administration of LSB neither influenced growth conditions nor reduced feed intake. Furthermore, the lower body weight and feed conversion efficiency might be associated with the inhibitory effect on CH synthesis and promotion of body weight loss mentioned below.

Both the elevated plasma CH and TG levels were due to the high CH diet, which were significantly ($P < 0.05$) reduced in the treated groups in the order of sitosterol > LSB group (Table 2). The content of plasma CH, TG, and LDL-C in both the LSB-10 and sitosterol groups were significantly ($P < 0.05$) reduced by 12.5% and 22.6%, 36.8% and 56.1%, and 21% and 20%, respectively, compared to the HC group. No significant ($P > 0.05$) differences of HDL-C level among all groups were found and only LSB-10 showed the lowest LDL-C/HDL-C ratio (by 18.7% reduction). The atherogenic index of LDL-C/HDL-C ratio was negatively related to the cardiovascular disease (CVD). As a result, lower LDL-C is considered to reduce the occurrence of CVD (Friedman & Brandon, 2001; Grundy, 2002; Little, 1988). Generally, the reduction of blood CH is considered to be associated with the metabolism of CH and bile acid in intestine.

Table 1. Changes of body weight, daily feed intake and feed conversion efficiency in SD rats fed different diets during experiment period.

	Body weight (g)			
Group	Initial	Final	Daily feed intake (g)	Feed conversion efficiency (%)
HC	104.4 ± 1.9[a]	465.9 ± 4.5[a]	26.2 ± 1.3[a]	24.6 ± 2.6[a]
LSB-1	103.5 ± 1.7[a]	460.1 ± 4.6[a]	26.2 ± 0.9[a]	24.3 ± 2.1[ab]
LSB-5	105.7 ± 1.6[a]	454.1 ± 3.1[ab]	25.6 ± 1.2[a]	24.3 ± 2.4[ab]
LSB-10	103.9 ± 2.0[a]	450.3 ± 3.2[b]	25.7 ± 1.2[a]	24.0 ± 2.6[b]
Sitosterol	105.0 ± 1.6[a]	447.3 ± 4.0[b]	25.6 ± 1.1[a]	23.9 ± 2.7[b]

Animals were fed for 8 weeks. HC, high cholesterol diet (1% cholesterol + 0.05% cholic acid +5% lard); LSB-1, HC plus 1% LSB; LSB-5, HC plus 5% LSB; LSB-10, HC plus 10% LSB; Sitosterol, HC plus 1% β-sitosterol. Results were expressed as mean ± SD (n = 8). Means in the same column followed by different letters are significant ($P < 0.05$).

Table 2. Plasma cholesterol, triglyceride, HDL-C, LDL-C concentration, and LDL-C/ HDL-C ratio of rats fed different diets.

Group	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	LDL-C/HDL-C ratio
HC	2.65 ± 0.19[a]	0.57 ± 0.07[a]	1.01 ± 0.27[a]	1.95 ± 0.19[a]	1.93 ± 0.44[a]
LSB-1	2.59 ± 0.18[a]	0.57 ± 0.06[a]	1.00 ± 0.26[a]	1.73 ± 0.27[a]	1.73 ± 0.65[a]
LSB-5	2.35 ± 0.2[b]	0.44 ± 0.07[b]	1.01 ± 0.30[a]	1.68 ± 0.24[a]	1.66 ± 0.73[a]
LSB-10	2.32 ± 0.21[b]	0.36 ± 0.10[c]	0.98 ± 0.26[a]	1.54 ± 0.23[b]	1.57 ± 0.50[b]
Sitosterol	2.05 ± 0.23[c]	0.25 ± 0.06[d]	0.97 ± 0.23[a]	1.56 ± 0.24[b]	1.70 ± 0.71[a]

Results were expressed as mean ± SD (n = 8). Data with the same superscript in the same column were not different ($P > 0.05$).

Table 3. Liver weight, relative liver weight, liver lipid concentration, adipose weight, and relative adipose weight of rats fed with different diets.

	Liver					
Group	Weight (g)	Relative liver weight (%, w/w)	Cholesterol (μmol/g liver)	Triglyceride (μmol/g liver)	Adipose weight (g)	Relative adipose weight (%, w/w)
HC	22.5 ± 2.6[a]	4.9 ± 0.3[a]	29.78 ± 3.97[a]	42.76 ± 5.32[a]	6.1 ± 1.1[a]	1.4 ± 0.3[a]
LSB-1	21.8 ± 2.3[a]	4.7 ± 0.5[ab]	30.23 ± 4.12[a]	39.99 ± 4.72[a]	6.2 ± 1.2[a]	1.3 ± 0.4[a]
LSB-5	20.7 ± 2.1[a]	4.6 ± 0.4[b]	29.35 ± 4.17[a]	37.56 ± 4.87[a]	5.8 ± 1.4[a]	1.3 ± 0.3[a]
LSB-10	20.5 ± 2.6[a]	4.6 ± 0.3[b]	26.75 ± 3.12[b]	36.65 ± 4.01[b]	5.3 ± 1[b]	1.2 ± 0.2[b]
Sitosterol	20.6 ± 2.6[a]	4.6 ± 0.4[b]	27.62 ± 2.88[b]	37.98 ± 5.60[a]	5.5 ± 1.3[b]	1.2 ± 0.3[b]

Results were expressed as mean ± SD (n = 8). Data with the same superscript in the same column were not different ($P > 0.05$).

Among all groups, including the LSB and sitosterol groups, no significant ($P > 0.05$) differences of liver weight were found (Table 3). In addition, the relative liver weights in LSB-5, LSB-10, and sitosterol groups were significantly ($P < 0.05$) reduced by 6%. The adipose tissue weights and relative adipose tissue weights in LSB-10 and sitosterol groups were significantly ($P < 0.05$) reduced (by 12.7%, 15.9%, 14.3% and 14.3% reduction, respectively). Although the liver CH in sitosterol and LSB-10 groups were significantly ($P < 0.05$) reduced (by 7% and 10.2% reduction, respectively), the LSB-10 was the only group that had a significantly ($P < 0.05$) reduced (by 14.3% reduction) liver TG. The lower CH accumulation in the liver may account for the lower relative liver weight in LSB and sitosterol groups. On the other hand, lower adipose and relative adipose weights could be observed in LSB-10 and sitosterol groups.

The LSB (LSB-5 and LSB-10) and sitosterol addition significantly ($P < 0.05$) increased the wet fecal weight (by 9.1%, 9.6%, and 7.9% increase, respectively), dry fecal weight (by 9.2%, 10.6%, and 9.5% increase, respectively), fecal nitrogen compounds (by 18.4%, 23.7%, and 21.1% increase, respectively), fecal neutral steroids (by 8.2%, 9.7%, and 19.4% increase, respectively), and fecal bile acid (by 3%, 3.4%, and 3.9% increase, respectively) than their untreated counterparts (Table 4). Soybean protein was reported to have anti-hyperlipidemic effect because it can inhibit CH absorption and increase neutral steroids (Potter, 1995), and excrete bile acid (Sugano et al., 1984). LSB can also lower CH and increase the excretion of fecal nitrogen compounds and fecal neutral steroids according to our observations.

The TG level in plasma was associated with the CH from daily diet and will increase the TG level in liver (Liu et al., 1995). One way of reducing tissue TG level is reducing its absorption in intestine. Our observation in the present study indicated that LSB can effectively inhibit the elevation of TG from high CH diet. On the other hand, it is suggested that another possible mechanism to reduce blood TG is by excreting bile acid. Also, the LSB-5, LSB-10, and sitosterol groups had increased excreting effect on fecal nitrogen compounds and possess similar dietary fiber activity, which are similar to the findings of the previous surveys (De Deckere et al., 1993; Ranhotra et al., 1996).

The wet and dry weights of feces increased from week 4 in LSB-5, LSB-10, and sitosterol groups (data not shown); and the phenomenon abidingly existed until the experiment ended. This might be due to the existence of plant steroids and dietary fiber, which resulted in lipid excretion from feces. The dietary fiber can retain water and enlarge the feces volume to shorten its retention time in intestine. Despite the fact that spider brake could enrich arsenic (Wang et al., 2007a), intake of spider brake did not cause liver swelling (Wang et al., 2007b). Administration of a high fat diet for a long period of time can not only weaken the LDL-C receptor scavenging effect, but also reduce the chance of acquiring liver CH and increase CH self-synthesis (Spady & Dietschy, 1985). There are two ways to maintain regular CH levels: first, reduce the CH absorption and synthesis, and second, increase the CH metabolism and excretion. Lower TG level, relative liver weight, adipose tissue weight, and relative adipose tissue weight in the LSB-10 group were obtained. According to these results we were convinced that the reduction of TG in plasma and liver were the result of the effective excretion rather than the transfer of body fat. According to our experiment, we suggest that the effect of LSB toward liver TG reduction is similar to that of garlic, which inhibits the conversion from fatty acid to TG (Yeh & Yeh, 1994). Moreover, the acceleration of liver lipid metabolism, fecal steroids, and bile acid excretion were shown to be the most potent in terms of reducing lipid levels in blood and liver.

The selected anti-hyperlipidemic components of LSB are shown in Table 5. Dietary fiber has physiological functions such as promoting excretion and lowering blood CH and glucose level (AACC Report, 2001). Plasma lipids and liver CH level could be reduced by the intake of dietary fiber (Chen et al., 1984; Demigné & Rémésy, 1982; Morand et al., 1992; Muramatsu et al., 1986). LSB

Table 4. Fecal wet weight, dry wet, neutral steroids, bile acid, and nitrogen compounds of rats fed different diets.

Group	Wet weight (or dry weight) (g/day)	Nitrogen compounds (mg/day)	Neutral steroids (mmol/L)	Bile acid (mmol/L)
HC	5.73 ± 0.29^{b} (4.44 ± 0.53^{b})	114 ± 2^{b}	39.53 ± 6.2^{c}	2.32 ± 0.43^{b}
LSB-1	5.86 ± 0.34^{b} (4.59 ± 0.58^{b})	117 ± 4^{b}	38.83 ± 6.38^{c}	2.29 ± 0.51^{b}
LSB-5	6.25 ± 0.39^{a} (4.85 ± 0.49^{a})	135 ± 3^{a}	42.76 ± 5.42^{b}	2.39 ± 0.49^{a}
LSB-10	6.28 ± 0.36^{a} (4.91 ± 0.38^{a})	141 ± 4^{a}	43.37 ± 5.88^{b}	2.40 ± 0.58^{a}
Sitosterol	6.18 ± 0.42^{a} (4.86 ± 0.41^{a})	138 ± 5^{a}	47.18 ± 7.64^{a}	2.41 ± 0.47^{a}

Results were expressed as mean ± SD (n = 8). Data with the same superscript in the same column were not different ($P > 0.05$). Values in parenthesis mean dry weights.

Table 5. Selected hypolipidemic components of lyophilized powder of spider brake powder.

Components	Content
Total dietary fiber (%)	4.51 ± 0.13
Insoluble dietary fiber (%)	4.20 ± 0.11
Soluble dietary fiber (%)	0.31 ± 0.07
β-sitosterol (mg/g)	1.38 ± 0.04
β-carotene (mg/g)	9.71 ± 0.16
Phenolic content (mg/g)	2.48 ± 0.25
Apigenin-7-*O*-β-D-glucoside (μg/g)	5.46 ± 0.01
Luteolin-7-*O*-β-D-glucoside (μg/g)	8.28 ± 0.01

Results were expressed as mean ± SD (n = 3).

had about 4.51% of dietary fiber, which can decrease the liver CH accumulation and increase its metabolism (Jonnalagadda et al., 1993). Hence, it is reasonable that LSB can help in modulating lipid levels.

Although β-sitosterol ($C_{29}H_{50}O_1$) has similar structure to CH, β-sitosterol has less absorption than CH in intestine (Vahouny & Kritchevsky, 1981; Subbiah, 1973). Regarding the mechanism of how β-sitosterol reduces CH, many hypotheses were suggested: that it competes against CH (Ikeda et al., 1988a), that it modulates lipid metabolism (Coleman et al., 2002), and that it inhibits CH absorption in intestine and CH synthesis (Ling & Jones, 1995). Administration of β-sitosterol everyday can also control blood CH level and lower TG and LDL-C concentrations in blood (Grundy, 1997). Moreover, LSB contained β-sitosterol, which can facilitate fecal neutral steroid excretion (Ikeda et al., 1988b).

Antioxidants possess modulating effects on blood lipid and therefore can oppose LDL-C oxidation (Gallaher et al., 2000). LSB has antioxidant effects. One is β-carotene, which can reduce blood CH and LDL-C levels (Tsai et al., 1992). LSB β-carotene content is higher than that of citrus (Noga & Len, 1983). Supplying diet diary with β-carotene can reduce TG level (Seo et al., 2004), inhibit CH synthesis (Furhman et al., 1999), and mitigate the aggravation of atherosclerosis (Levy et al., 2000). In this present study we find that LSB can effectively reduce LDL-C/HDL-C ratio and the effect might be due to the mechanism of β-carotene.

Administration of abundant phenolic compounds can inhibit atherosclerosis injury and LDL-C oxidation. Hertog et al. (1993) reported that the intake of products rich in flavonoids was helpful in preventing the threat of CVD. The excretion of neutral steroids could decrease when the enzyme 3-hydroxy-methylglutaryl CoA reductase (HMG-CoA reductase) is inhibited (Manorama & Rukmini, 1992; Quintão & Sperotto, 1987). Many antioxidants, such as β-carotene and flavonoids, possess anti-hyperlipidemic effect by inhibiting the HMG-CoA reductase activity (Hertog et al, 1995). In commonly known flavonoids, astilbin possesses the best inhibitory effect on HMG-CoA reductase, followed by luteolin-7-*O*-β-glucoside (Chen et al., 2001). We indicated that LSB contained two flavones, luteolin-7-*O*-β-D-glucoside and apigenin-7-*O*-β-D-glucoside. These flavones might contribute to the inhibition of HMG-CoA reductase activity, thus strengthening the anti-hyperlipidemic effect.

In conclusion, this study indicates that LSB not only has a very definite anti-hyperlipidemic effect in animals fed a high cholesterol diet, but also has components beneficial in modulating experimental animal blood lipid metabolism. The mechanisms of LSB are to decrease the absorption of CH and increase the excretion of nitrogen compounds, neutral steroids, and bile acid from feces. The constituents of LSB might also contribute crucially to these mechanisms because they have both the therapeutic effects and synergistic action. Similar conclusions (Kahkonen et al., 2001; Parejo et al., 2002) were also found in accord with this study.

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