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Antihyperlipidemic effects of buckwheat leaf and flower in rats fed a high-fat diet

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ABSTRACT

This study was conducted to investigate the hypolipidemic effects of a powdered whole buckwheat leaf and flower mixture in rats fed a high-fat diet. Male Sprague–Dawley rats were divided into three groups: normal control (NC), high-fat (HF), and high-fat supplemented with a mixture of powdered buckwheat leaf and flower (BLF; 5%, wt/wt) groups. The plasma total cholesterol and triglyceride concentrations were significantly lower in the BLF group than in the other groups. Hepatic cholesterol and triglyceride values of the BLF group were similar to those of the NC group. This plant part mixture elevated the faecal triglyceride and acidic sterol level in the BLF group. The result suggest that the beneficial effect of this buckwheat plant portion on plasma and hepatic lipid profiles in high-fat fed rats is partly mediated by higher excretion of faecal lipids and synergistic effect of phenolic compounds and fibre present in the BLF.

1. Introduction

Buckwheat, a crop utilized throughout the world, is one of our important food sources. Besides various polyphenols, it contains proteins with high biological value and balanced amino acid composition, fibres, vitamins B_1 and B_2, zinc, copper, manganese and selenium.

Recently, attention was paid to the identification of the individual components of the phenolic fraction and to their antioxidant effects. The antioxidant activity in buckwheat exhibited a statistically significant relationship with its total phenolics, as well as rutin content (Holasova et al., 2002). Buckwheat seed contains more rutin than do most plants and the rutin content in buckwheat leaves is 3–8% (wt/wt) (Bruneton, 1999). Also its total phenolics content is higher than that of seeds (Holasova et al., 2002). Kim, Park, Yang, and Shim (1994) and Lee, Kim, and Park (2001) reported that the rutin content of the flower part is higher than that of other parts of the buckwheat (flower > leaves > stem > root). Han and Zhang (2004) reported that total flavones content of buckwheat flower has some protective effects on isoproterenol-induced cardiac hypertrophy in rats. However, metabolic activity regarding the buckwheat leaf and flower has not yet been studied *in vivo*.

Plant polyphenols exert cardiovascular benefits by altering concentrations of blood lipid components and a high intake of polyphenols (flavonoids) can significantly reduce the risk of mortality from cardiovascular diseases (Hertog, Feskens, Hollman, Katan, & Kromhouy, 1993; McNamara, 2000). Since dietary fat is one of the most important environmental factors associated with the cardiovascular diseases incidence (McNamara, 2000), this study investigated lipid-lowering properties of a mixture of powdered buckwheat leaf and flower parts in rats fed a high-fat diet.

2. Materials and methods

2.1. Preparation of powdered buckwheat leaf and flower and analysis of general composition

Buckwheat leaf and flower parts were harvested in Daejon (Korea) and dried in the shade for 1 week. The leaf and flower were powdered and passed through 60 mesh sieves. The crude protein and the crude fat contents were determined by the Kjeldahl method (1983) and Soxhlet extraction method (1879), respectively. The carbohydrate and the total fibre contents were analyzed using the AOAC (1995) method and the Prosky–AOAC (Prosky, Asp, Schweizer, Devries, & Furda, 1988) method, respectively.

2.2. Determination of total phenolic contents in the mixture of powdered buckwheat leaf and flower

Total phenolic content was analyzed spectrophotometrically by a modified Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999; Wolfe, Wu, & Liu, 2003). Briefly, 50 g of a mixture of powdered buckwheat leaf and flower

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were suspended and extracted with 10 volumes of methanol with shaking at room temperature for 15 h. The extracts were filtered through a filter paper and the supernatants were pooled. The residue was re-extracted under the same conditions. Pooled extracts were condensed (and methanol removed) to 9.2 g with a rotary evaporator at 50 °C. Volumes of 0.5 ml of deionized water and 0.125 ml of a known dilution of the extract were placed in a test tube, followed by the addition of 0.125 ml of Folin–Ciocalteu reagent. They were mixed well and then allowed to stand for 6 min before 1.25 ml of a 7% sodium carbonate solution were added. The mixture was diluted to 3 ml with deionized water. The colour was developed for 90 min at room temperature and the absorbance was measured at 760 nm using a spectrophotometer (UVIDEC-50, JAS-CO Co., Tokyo, Japan). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as mean (±S.E.) mg of gallic acid equivalents per gramme for the triplicate extracts and total phenolic content was expressed as mg phenolics per gramme of powdered buckwheat leaf and flower.

2.3. Animals and diets

Thirty male Sprague–Dawley rats, aged 3 weeks (40–50 g), were purchased from Bio Orients Inc. (Seoul, Korea). The animals were housed individually in stainless steel cages in a room with a 12:12-h light-dark cycle and an ambient temperature of 24 °C. All rats were fed a pellitized commercial chow diet for 1 week after arrival. They were then randomly divided into three groups and fed with a normal control diet (NC, $n = 10$) and two high-fat diets (HF, $n = 10$, BLF, $n = 10$) for 6 weeks, respectively. High-fat groups were without (HF) or with 5% (wt/wt) powdered buckwheat leaf and flower mixture (BLF). The composition of the experimental diet (Table 1) was based on the AIN-76 semisynthetic diet (American Institute of Nutrition, 1977, 1980). The energies of the high-fat diets in the HF and the BLF groups were 498.7 and 501.1 kcal/100 g whereas that of the NC group was 386.2 kcal/100 g due to differences in dietary fat content. The animals were given food and distilled water *ad libitum* during the experimental period. Food consumption and weight gain were measured daily and weekly, respectively.

Faeces were collected during the final 3 days using metabolic cages, and dried faeces were used for the determination of faecal lipid and sterol levels. At the end of the experimental period, the rats were sacrificed, following a 14 h fast, by removing the feed from the cages at 7 p.m. the day before and collecting the blood samples at 9 a.m. of the following day. Animals were anesthetized with ketamine and blood samples were taken from the inferior vena cava for determination of the plasma lipid profiles and leptin. The livers were removed under an anesthetized condition and rinsed with physiological saline. The adipose tissues (epididymal white adipose tissue, perirenal white adipose tissue, interscapular white adipose tissue, and interscapular brown adipose tissue) were immediately weighed. The livers were removed and rinsed with physiological saline. All samples were stored at −70 °C until analyzed. The current study protocol was approved by the Ethics Committee at Kyungpook National University for animal studies.

Table 1
Compositions of the experimental diets (%).

Component	NC	HF	BLF
Casein	20	20	20
D,L-methionine	0.3	0.3	0.3
Corn starch	15	15	15
Sucrose	50	27.5	22.5
Cellulose powder	5	5	5
Mineral mix[a]	3.5	3.5	3.5
Vitamin mix[b]	1	1	1
Choline bitartrate	0.2	0.2	0.2
Corn oil	5	9.9	9.9
Lard	–	17.6	17.6
BLF[c]	–	–	5
Total (%)	100	100	100
kcal/100 g diet	386.2	498.7	501.1
Calories from fat (%)	11.7	49.6	49.6
Calories from carbohydrate (%)	67.3	34.1	31.3
Calories from protein (%)	21.0	16.3	19.1

[a] AIN-76 mineral mixture contained (in g/kg of mixture): calcium phosphate, dibasic 500.0; sodium chloride, 74.0; potassium citrate, monohydrate, 220.0; potassium sulphate, 52.0; magnesium oxide, 24.0; manganous carbonate, 3.5; ferric citrate, 6.0; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulphate, 0.55; sucrose, finely powdered, 118.03.

[b] AIN-76A vitamin mixture contained (in g/kg of mixture): thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7: niacin, 3.0; D-calcium pantothenate, 1.6; folic acid, 0.2: D-biotin, 0.02; cyanocobalamin (vitamin B_{12}), 1.0; dry vitamin A palmitate (500,000 U/g), 0.8; dry vitamin E acetate (500 U/g), 10.0; vitamin D_3 trituration (400,000 U/g), 0.25; menadione sodium bisulphite complex, 0.15; sucrose, fine powder, 981.08.

[c] A mixture of powdered buckwheat leaf and flower; Five grammes of buckwheat leaf and flower in a 100 g diet provides 10.7 kcal energy, 1.7 mg carbohydrate, 726 mg protein, 113 mg fat and 1.9 g fibre based on Table 2.

2.4. Plasma, hepatic, and faecal lipids

Triglyceride (TG), total cholesterol, and high-density lipoprotein cholesterol were enzymatically analyzed using a commercial kit (Asan Pharmaceutical, Seoul, South Korea). Non-HDL-C was calculated as (total-C − HDL-C). The hepatic and faecal cholesterol and triglycerides were extracted using the procedure developed by Folch, Lees, and Sloan-Stanley (1957). Triton X-100 and a sodium cholate solution were added to 200 μl of the dissolved lipid solution in 1 ml of ethanol to produce final concentrations of 5 g/l and 3 mmol/l, respectively. The cholesterol and triglyceride concentrations of the liver and faeces were analyzed with the same enzymatic kits as used in the plasma analysis. The faecal bile acid was extracted with methanol and quantified enzymatically with 3-α-hydroxysteroid dehydrogenase (Crowell & Macdonald, 1980).

2.5. Hepatic and fatty morphology

Livers and epididymal white adipose tissue were removed from the rats and fixed in a buffer solution of 10% formalin. Fixed tissues were processed routinely for paraffin embedding, and 4 μm sections were prepared and dyed with haematoxylin–eosin; stained areas were viewed using an optical microscope with a magnifying power of ×200.

2.6. 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase and acyl-CoA:cholesterol acyltransferase (ACAT) activities

The microsomes were prepared according to the method developed by Hulcher and Oleson (1973) with a slight modification. One gramme of liver tissue was homogenized in 4 ml of an ice-cold buffer (pH 7.0) containing 0.1 M triethanolamine, 0.02 M EDTA, and 2 mM dithiothreitol. The homogenates were centrifuged twice at 10,000*g* for 15 min at 4 °C. Next, the supernatants were ultracentrifuged twice at 100,000*g* for 60 min at 4 °C. The resulting microsomal pellets were then redissolved in 1 ml of a homogenation buffer for protein determination (Bradford, 1976) and finally analyzed for their HMG-CoA reductase and ACAT activities.

The HMG-CoA reductase activities were determined as described by Shapiro, Nordstrom, Mitschelen, Rodwell, and Schimke (1974) with a slight modification, using freshly prepared hepatic microsomes. The incubation mixture (60 μl) containing the microsomes (100∼150 μg of protein) and 500 nmol of NADPH (dissolved in a reaction buffer containing 0.1 M triethanolamine and 10 mM EDTA) was preincubated at 37 °C for 5 min. Next, 10 μl of 50 nM

[^{14}C]HMG-CoA (specific activity, 2.1083 GBq/mmol; NEN™ Life Science Products, Boston, MA) were added, and the incubation continued for 15 min at 37 °C. The reaction was terminated by the addition of 15 μl of 10 M HCl, and the resultant reaction mixture was incubated at 37 °C for an additional 15 min to convert the mevalonate into mevalonolactone. The incubation mixture was centrifuged at 10,000*g* for 5 min, and the supernatant was spotted on a Silica Gel 60 F_{254} thin-layer chromatography plate, using mevalonolactone as the standard. The plate was developed in benzene–acetone (1:1, vol/vol) and air-dried. Finally, the ratio of fronts (R_f) 0.3–0.6 region was removed by scraping with a clean razor blade, and its [^{14}C] radioactivity was determined using a liquid scintillation counter (Tricarb 1600TR, Packard Instrument, Meriden, CT). The results were expressed as picomoles of mevalonate synthesized per min per mg of protein.

The ACAT activities were determined in freshly prepared hepatic microsomes, by the method of Erickson, Schrewsbery, Brooks, and Meyer (1980), as modified by Gillies, Rathgeb, Perri, and Robinson (1986). To prepare the cholesterol substrate, 6 mg of cholesterol and 600 mg of Tyloxapol (Triton WR-1339, Sigma) were each dissolved in 6 ml of acetone, mixed well, and completely dried in N_2 gas. The dried substrate was then redissolved in 20 ml of distilled water to a final concentration of 300 μg of cholesterol/ml. Next, reaction mixtures containing 20 μl of the cholesterol solution (6 μg of cholesterol), 20 μl of a 1 M of potassium phosphate buffer (pH 7.4), 10 μl of 0.6 mM bovine serum albumin, 10 μg of the microsomal fraction, and distilled water (up to 180 μl) were preincubated at 37 °C for 30 min. The reaction was then initiated by adding 20 μl of 5.62 nM [^{14}C]oleoyl-CoA (specific activity, 1.9795 GBq/mmol; NEN Life Science Products) to a final volume of 200 μl; the reaction time was 30 min at 37 °C. The reaction was stopped by the addition of 500 μl of isopropanol–heptane (4:1, vol/vol), 300 μl of heptane, and 200 μl of 0.1 M potassium phosphate (pH 7.4), and the reaction mixture was allowed to stand at room temperature for 2 min. Finally, an aliquot (200 μl) of the supernatant was subjected to scintillation counting. The ACAT activities were expressed as picomoles of cholesteryl oleate synthesized per min per mg of protein.

2.7. Statistical analysis

All data are presented as means ± standard error of the mean. The data were evaluated by a one-way analysis of variance using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA), and the differences between the means assessed using Duncan's multiple-range test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. General composition and total phenolic content of powdered buckwheat leaf and flower mixture

In 100 g of dried powdered plant parts, the following components were included: 34.1 ± 0.09 g carbohydrate, 14.51 ± 0.19 g crude protein, 2.25 ± 0.12 g crude fat and 36.95 ± 1.01 g fibre. Its energy content was 215 kcal per 100 g of diet. Total phenolic content recovered from 50 g of the powdered plant parts was 575 ± 0.7 mg [data not shown; mean ± S.E.M. (n = 3)].

3.2. Feed intakes, weight gains and feed efficiency ratio

The energy density of the two high-fat diets used was higher than that of the normal control diet (498.7 kcal/100 g and 501.1 kcal/100 g vs. 386.2 kcal/100 g), as shown in Table 1. High-fat diet fed groups exhibited significantly lower feed intakes than did the normal diet group (Table 2). Interestingly, feed intake was significantly lower in the BLF group than in the HF when compared within the two high-fat diet groups.

Table 2
Effects of a mixture of powdered buckwheat leaf and flower supplementation on feed intake, body weight gain, feed efficiency ratio and weights of organ and adipose tissues in rats fed a high-fat diet.[a]

	NC[b]	HF[c]	BLF[d]
Feed intake (g/day)	22.34 ± 1.45^{x}	20.38 ± 0.38^{y}	18.24 ± 0.48^{z}
Energy intake (kcal/day)	86.27 ± 2.45^{x}	101.64 ± 3.16^{y}	91.40 ± 2.81^{x}
Body weight gain (g/day)	7.72 ± 0.16^{x}	9.01 ± 0.16^{y}	8.11 ± 0.10^{x}
FER[e]	0.34 ± 0.01^{x}	0.44 ± 0.01^{y}	0.44 ± 0.01^{y}
Organs (mg/g B.W.)			
Liver	34.03 ± 0.78^{x}	33.73 ± 0.43^{x}	31.24 ± 0.67^{y}
Heart	3.15 ± 0.06^{x}	3.18 ± 0.04^{x}	3.08 ± 0.05^{x}
Kidney	6.72 ± 0.10^{x}	6.91 ± 0.15^{x}	6.53 ± 0.12^{y}
Adipose tissue (mg/g B.W.)			
Epididymal WAT[f]	17.78 ± 0.80^{x}	21.75 ± 0.99^{y}	18.74 ± 0.93^{xy}
Perirenal WAT	5.18 ± 0.49^{x}	31.33 ± 1.43^{y}	28.85 ± 1.23^{xy}
Interscapular WAT	26.27 ± 1.43^{x}	7.00 ± 0.53^{y}	6.62 ± 0.66^{xy}
Interscapular BAT[g]	1.25 ± 0.06^{x}	1.58 ± 0.10^{y}	1.37 ± 0.08^{x}
Total	50.48 ± 2.96^{x}	61.66 ± 3.36^{y}	55.58 ± 3.70^{xy}

^{x,y,z}Means in the same row not sharing a common superscript are significantly different ($p < 0.05$) between groups.
[a] Means ± S.E.M. (n = 10).
[b] Normal control group.
[c] High-fat fed group.
[d] High-fat with a mixture of powdered buckwheat leaf and flower part fed group.
[e] Feed efficiency ratio = body weight gain/food intake.
[f] WAT, white adipose tissue.
[g] BAT, brown adipose tissue.

Initial body weights of the three groups were not significantly different (88–91 g); however, after 6 weeks, the body weight gains were significantly lower in the BLF group than in the HF group (Table 2). The average weight gain of the BLF group was not significantly different from that of the NC group. However, the feed efficiency ratio (FER) of the HF and BLF groups exhibited identical values that appeared to be significantly higher than that of the NC group.

3.3. Weights of organs and adipose tissues

Organ weights were expressed as their relative weight per body weight. The relative weights of liver and kidney were significantly lower in the BLF group than in the NC and HF groups. It was supposed that the weights of the liver and the kidney in the high-fat fed groups were increased by the high-fat diet. However, the heart weights were not significantly different between the groups (Table 2). Epididymal WAT, perirenal WAT, interscapular WAT, and total adipose tissue weights were the highest in the HF, and then BLF and NC group in order. In particular, the supplementation of BLF significantly lowered the weight of brown adipose tissue compared to the HF group (Table 2).

3.4. Plasma, hepatic and faecal lipids

Concentrations of plasma and hepatic lipids are shown in Table 3. The supplementation of BLF significantly lowered plasma total cholesterol concentration by 35.9% and 29.1%, and triglyceride concentration, by 26.2% and 34.2% compared to the HF and NC groups, respectively ($p < 0.05$). Non-HDL-cholesterol concentration was also significantly lowered by the BLF supplement. Although HDL-cholesterol concentration was significantly lower in the BLF group than in the NC or HF groups, the ratio of HDL-C/total-C exhibited higher values in the BLF and NC groups than in the HF group. For this reason, the atherogenic index was signifi-

Table 3
Effects of a mixture of powdered buckwheat leaf and flower supplementation on plasma, hepatic and faecal lipid concentration in rats fed a high-fat diet.[a]

	NC[b]	HF[c]	BLF[d]
Plasma			
Triglyceride (mmol/l)	1.58 ± 0.13^{x}	1.41 ± 0.08^{x}	1.04 ± 0.05^{y}
Total cholesterol (mmol/l)	3.88 ± 0.32^{x}	4.29 ± 0.23^{x}	2.75 ± 0.12^{y}
HDL-cholesterol (mmol/l)	0.83 ± 0.05^{x}	0.71 ± 0.03^{y}	0.58 ± 0.02^{z}
Non-HDL-cholesterol[e] (mmol/l)	3.05 ± 0.24^{x}	3.58 ± 0.19^{y}	2.17 ± 1.10^{z}
HDL-cholesterol/total-cholesterol (%)	21.4 ± 1.07^{y}	16.5 ± 1.44^{x}	21.1 ± 0.97^{y}
AI[f]	3.68 ± 0.23^{x}	5.06 ± 0.44^{y}	3.74 ± 0.21^{x}
Liver			
Triglyceride (mmol/g)	2.10 ± 0.28^{x}	3.27 ± 0.17^{y}	2.04 ± 0.15^{x}
Cholesterol (mmol/g)	2.70 ± 0.23^{x}	3.92 ± 0.14^{y}	2.35 ± 0.09^{x}
Faeces			
Daily faecal weight (g)	2.24 ± 0.11^{x}	2.08 ± 0.13^{x}	2.35 ± 0.10^{x}
Triglyceride (mmol/g)	0.54 ± 0.06^{x}	0.58 ± 0.08^{x}	1.12 ± 0.12^{y}
Cholesterol (mmol/g)	8.10 ± 0.71^{x}	8.45 ± 0.69^{x}	9.14 ± 0.60^{x}
Acidic sterol (mg/g)	5.98 ± 0.18^{y}	2.85 ± 0.10^{x}	8.92 ± 0.70^{z}

[x,y,z]Means in the same row not sharing a common superscript are significantly different ($p < 0.05$) between groups.
[a] Means ± S.E.M. ($n = 10$).
[b] Normal control group.
[c] High-fat fed group.
[d] High-fat with a mixture of powdered buckwheat leaf and flower part fed group.
[e] Non-HDL-C = total-C − HDL-C.
[f] Atherogenic index: (total-cholesterol − HDL-cholesterol)/HDL-cholesterol.

cantly higher in the HF group than in the NC and BLF groups. Hepatic cholesterol and triglyceride concentrations were also significantly lower in the BLF group than in the HF group by 37.6% and 40.1%, respectively ($p < 0.05$). Hepatic lipid concentration showed no difference between the NC and BLF groups.

Dried faecal weight and faecal cholesterol level were not significantly different between the groups. However, BLF supplementation elevated faecal triglyceride by approximately twofold as compared to the HF or NC group (Table 3). The faecal acidic sterol levels for the three groups were all significantly different; the value in the BLF group was the highest, that is 3.1-fold higher than the HF group, followed by the NC group and the HF group in that order.

3.5. Morphological comparisons in epididymal adipocytes and hepatocytes

Fig. 1a shows the histological appearance and the size of epididymal adipose tissue. The size of the adipocytes in the NC and BLF groups was smaller than those of the HF group when compared under the light microscopy (Fig. 1b). The accumulation of the hepatic lipid droplets (indicated by the arrow) appeared to be highest in the HF group, while it was relatively lower in the NC and BLF groups (Fig. 2).

3.6. Hepatic HMG-CoA reductase and ACAT activities

Both hepatic HMG-CoA reductase and ACAT activities were significantly higher in the BLF group than in the HF or NC groups (Table 4). There was no significant difference between the HF and NC groups in these enzyme activities.

4. Discussion

It was reported that a high-fat diet (60% energy as fat) enhances feed intake and weight gain relative to a high-carbohydrate diet (76% energy as carbohydrate) when both energy density and palatability are equated (Warwick & Weingarten, 1995). But in this study, the food intake was lower in the two high-fat fed groups. Since, the NC diet was a high-carbohydrate diet (67.3% energy as carbohydrate), whereas the BLF and HF diets were high-fat diets (both 49.6% energy as fat), the NC diet was not isocaloric to high-fat diets (HF or BLF diet). The food intake of the HF group was reduced when compared to the NC group, although body weight gain of the HF group was significantly higher than the NC group and that resulted in higher FER values in the HF group than in the NC group. Interestingly, although the FER value of the BLF group is equal to that of the HF group, supplementation of the mixture of

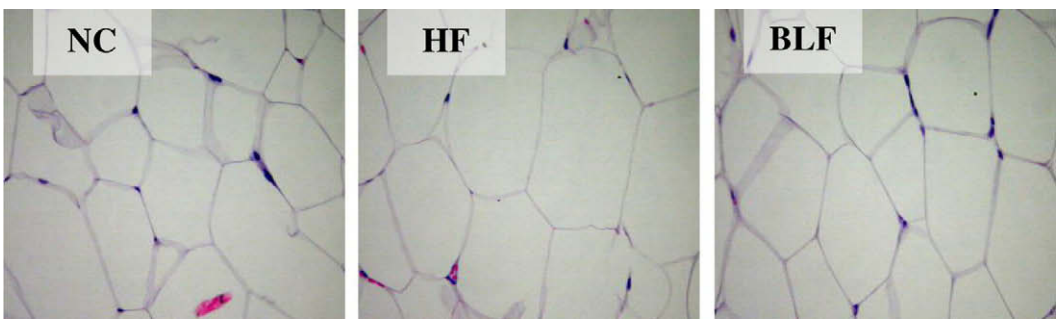

Fig. 1a. Light micrography of epididymal adipocytes in rats fed a normal or a high-fat diet (×200). Representative pictures of haematoxylin and eosin-stained sections of epididymal adipocytes from rats fed a normal control diet (NC) or a high-fat diet supplemented with buckwheat leaf and flower part mixture (BLF) show smaller size of adipocytes than in rats fed a high-fat diet (HF).

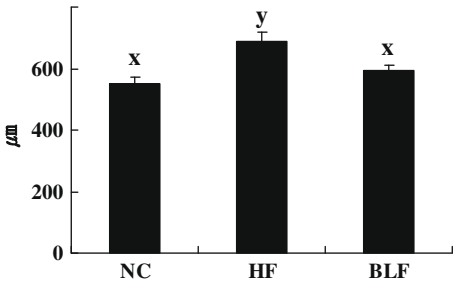

Fig. 1b. Effects of a mixture of powdered buckwheat leaf and flower supplementation on size of epididymal adipocytes in rats fed a high-fat diet[a]. [a]Means ± S.E.M. (n = 10). [b]Normal control group. [c]High-fat fed group. [d]High-fat with a mixture of powdered buckwheat leaf and flower part fed group. [x,y]Means in the figure not sharing a common superscript are significantly different (p < 0.05) between groups.

powdered BLF significantly lowered the weight gain in rats fed a high-fat diet by seemingly suppressing feed intake and/or lowering fat-pad weights. High energy density of the HF diet can partially be responsible for the reduced feed intake, as observed in the NC group vs. HF and BLF groups. However, the supplementation of BLF itself contributed to the decreased feed intake and body weight gain when compared to the HF group, which suggests that other functional factor(s) could be present in the BLF, for instance, phenolic compounds and fibre.

There was no abnormality in the growth performance. However, the relative weights of liver and kidney were significantly lower in the BLF group than in the HF group. Supplementation with these powdered plant parts significantly lowered the weight of interscapular brown adipose tissue compared with the HF group (1.37 ± 0.08 mg/g B.W. vs. 1.58 ± 0.10 mg/g B.W.). This seemed to indicate that the lower body weight observed in the BLF group was not due to the elevated heat production of brown adipose tissue. Interestingly, the powdered BLF mixture reduced the epididymal adipocyte size (similar to those of the NC group). It is normally increased in the obese condition.

Epidemiological studies indicate an inverse relationship between dietary flavonoid intakes and a risk of cardiovascular disease (Hertog et al., 1993). Also, a high fibre diet is associated with the prevention of the coronary atherosclerosis and other diseases (Rimm et al., 1996). In this study, the supplementation of powdered buckwheat leaf and flower parts improved lipid profiles by lowering plasma total cholesterol and triglyceride concentrations compared with the HF and NC groups. The plasma HDL-cholesterol concentration was lower in the BLF group than in the HF group; however, the ratio of HDL-C/total-C significantly increased by 128% compared with the HF group due to differences in the total cholesterol level. Also the non-HDL-C concentration was significantly lowered in the BLF group. Park et al. (2002) reported that rutin glycoside lowered the serum cholesterol and triglyceride level in rats. The mixture of buckwheat leaf and flower parts used in the current study improved the high-fat diet-induced atherogenic index. Generally, a high-fat diet significantly increases the total cholesterol levels in serum and liver in rats. Supplementation of powdered BLF significantly lowered hepatic cholesterol and triglyceride levels, by 40.1% and 37.6%, compared to the HF group and diminished the accumulation of hepatic droplets. This could be related to the high contents of fibre and flavonoids in buckwheat leaf and flower parts.

Table 4
Effects of a mixture of powdered buckwheat leaf and flower supplementation on activities of hepatic ACAT and HMG-CoA reductase in rats fed a high-fat diet.[a]

	NC[b]	HF[c]	BLF[d]
ACAT (pmol/min/mg protein)	1.76 ± 0.04[x]	1.94 ± 0.10[x]	2.19 ± 0.07[y]
HMG-CoA reductase (pmol/min/mg protein)	129 ± 7.67[x]	130 ± 4.16[x]	187 ± 12.30[y]

[x,y]Means in the same row not sharing a common superscript are significantly different (p < 0.05) between groups.
[a] Means ± S.E.M. (n = 10).
[b] Normal control group.
[c] High-fat fed group.
[d] High-fat with a mixture of powdered buckwheat leaf and flower part fed group.

In addition, a high-fat diet supplemented with the BLF significantly elevated both the HMG-CoA reductase and ACAT activities compared to the other groups. Plasma cholesterol-lowering action, mediated by the BLF supplement, possibly resulted in upregulating HMG-CoA reductase and ACAT activities to maintain cholesterol homeostasis. Similar results were observed in a previous study in which rats supplemented with a persimmon leaf, with high contents of fibre and flavonoids, were lower in plasma and hepatic cholesterol level (Lee et al., 2006).

Daily faecal weight tended to be higher in the BLF group than in the HF group which seemed to be due to the additional fibre content included in the BLF diet since 5 g (wt/wt) of powdered BLF provided 1.85 g of fibre. In general, the increase in faecal weight might vary widely with the type and quantity of dietary fibre being consumed. Chau, Huang, and Lin (2004) reported that faecal dry weight was significantly higher when hamsters were supplemented with the water-insoluble fibre-rich fraction isolated from the peel of *Citru sinensis* L. cv. Liucheng and cellulose diets (55–56% increase relative to the fibre-free diet) and suggested that the consumption of insoluble fibre could significantly increase the faecal weight, as well as faecal bulk. Faecal cholesterol levels, as well as daily faecal weights, were not different between the groups. However, faecal acidic sterol was significantly higher in the BLF group by 3.1-fold and the NC group by 1.5-fold than in

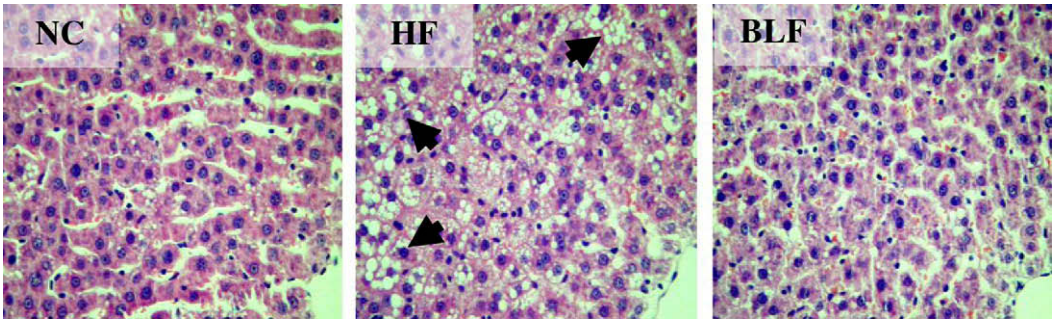

Fig. 2. Effects of a mixture of powdered buckwheat leaf and flower part supplementation on hepatic tissue morphology in rats fed a high-fat diet (×200). Fat accumulation, indicated by the arrowheads, in the form of large fat droplets is present in liver of rats fed a high-fat diet (HF). Representative pictures of haematoxylin and eosin-stained sections of liver tissue from rats fed a normal control diet (NC) or high-fat diet supplemented with a mixture of buckwheat leaf and flower (BLF) show few fat droplets.

the HF group. Interestingly, faecal cholesterol level was not altered, in spite of the additional fibre included in the BLF. Chisaka et al. (1988) and Matsumoto, Okushio, and Hara (1998) suggested that the hypocholesterolemic effect of polyphenols was mainly due to increased faecal excretion of cholesterol and bile acids. It is plausible that, in this study, the high phenolic content in BLF itself could enhance the faecal excretion of acidic sterol, not faecal cholesterol, although the exact mechanism cannot be elucidated at the present time. Faecal triglyceride concentration was significantly higher in the BLF group (1.12 ± 0.12 mmol/g) than in the HF group (0.58 ± 0.08 mmol/g). Functional components in BLF that are responsible for regulating cholesterol and fat metabolism may include various phenolic compounds, or other bioactive compounds, such as a dietary fibre.

In conclusion, the supplementation of the powdered BLF mixture, that is rich in phenolic compounds and fibre, seemingly suppressed the body weight gain and lowered plasma and hepatic lipid concentrations with a simultaneous increase in faecal lipids in rats fed a high-fat diet. An efficacy test of lipid-lowering action of the BLF mixture, suggests that these plant parts would be beneficial for regulation of lipid metabolism or prevention of hyperlipidemia in experimental animal models.

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