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Dihydromyricetin-rich herbal mixture extracts as a potential prescription for treatment of metabolic syndrome in rats fed a high-fat diet and subacute toxicity assessment in rats

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ABSTRACT

Dihydromyricetin (DHM)-rich herbal mixture extracts, also called APF complex, comprised of *Ampelopsis grossedentata*, *Pericarpium citri reticulatae*, and *Fructus crataegi*. The content of DHM in APF complex was 362.7 ± 12.5 mg/g. The aims of this study were to investigate the therapeutic effects of APF complex on metabolic syndrome in rats fed a high-fat diet (HFD) and evaluate the subacute toxicity of APF complex in rats. HFD significantly increased body weight gain, fat tissue (epididymal fat, mesenteric fat, and perirenal fat) deposition, body fat index, and hepatic triglyceride (TG) and total cholesterol (TC) accumulation as well as caused abnormal blood biochemical parameters, including TC, TG, low-density lipoprotein-cholesterol (LDL-C), free fatty acid (FFA), and glucose. APF complex has a tendency but not significance to limit HFD-induced body weight gain. APF complex also significantly improved HFD-induced body fat accumulation, as evidenced by decreasing fat tissue deposition and body fat index. In addition, APF complex significantly ameliorated HFD-induced hyperlipidemia and hyperglycemia, as evidenced by reducing levels of blood TG and TC as well as blood glucose and FFA, respectively. Furthermore, APF complex significantly decreased HFD-induced hepatic TG and TC accumulation. In subacute toxicity assessment, APF complex exhibited no toxicological signs, as evidenced by without affecting mortality, food and water consumption, body weight changes, absolute organ weights, hematological system, blood lipids and nutritional status, and electrolyte balance as well as non-toxic to liver and renal function. Overall, APF complex was considered as a non-toxic herbal prescription and could act as adjuvant therapy for metabolic syndrome.

1. Introduction

Metabolic syndrome, the prevalence of approximately 25% of all adults, is a complex and clustering disorder comprising of obesity, hyperlipidemia, hyperglycemia, insulin resistance, and hypertension.[1,2] Several criteria and definitions were used to diagnosis metabolic syndrome when a combination of three or more of the following conditions must be achieved: (1) large waist circumference; (2) elevated blood triglyceride (TG); (3) low high density lipoprotein cholesterol (HDL-C); (4) raised blood pressure; (5) elevated fasting blood glucose.[3–6] Previous researches have indicated that several herbal prescriptions are effective in treatment of metabolic syndrome.[7].

Dihydromyricetin (DHM)-rich herbal mixture extracts, also called APF complex, comprised of *Ampelopsis grossedentata*, *Pericarpium citri reticulatae*, and *Fructus crataegi*. *Ampelopsis grossdentata* has not only been used as medicinal plant in treatment of hyperglycemia, hypertension, and hepatitis, but also as daily drink.[8] *Pericarpium citri reticulatae* is the dried ripe fruit peel of citrus reticulate Balance with traditional application for treatment of cough and detoxification[9] and its extracts were shown to inhibit adipogenesis in 3T3-L1 preadipocytes.[9,10] *Fructus crataegi*, the ripe fruits of Crataegus pinnatifida Bge. var. major N.E. Br. or C. pinnatifida Bge., widely used as traditional herbal medicine with hypolipidemic effects.[11]

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Synergistic actions are of vital importance in traditional Chinese medicines for prevention and treatment of chronic diseases.[12] The possible explanations for the synergistic actions of herbal medicines are that different herbal medicines may mediate either the same or different target in a synergistic way as well as may decrease the adverse effects or increase pharmacological activity by herbal-herbal interaction.[13] These specific functions of each component led us to hypothesize that these components when used in combinations could serve as an effective herbal prescription for the treatment of metabolic syndrome.

Based on the Health Food Control Act established by the Taiwan Ministry of Health and Welfare, commercial health food in Taiwan should be evaluated for their health care effect and toxicity. In health care effect, we employed the model of high-fat diet (HFD)-induced metabolic syndrome in rats to determine the ameliorated effects of APF complex. The test items included body weight, food intake, food efficiency, adipose tissue deposition (epididymal fat, mesenteric fat, and perirenal fat), body fat index, blood lipid profile (TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C), blood free fatty acid (FFA), blood glucose, and hepatic lipid levels (TG and TC). The subacute toxicity of APF complex using 28 days repeated feeding study in male and female rats. The test items included body weight, absolute organ weight, hematological parameters, and blood biochemical parameters.

2. Materials and methods

2.1. Preparation of APF complex

The prescription of APF complex was developed by our company. *Ampelopsis grossedentata*, *Pericarpium citri reticulatae*, and *Fructus crataegi* were purchased from Taiwan herbal markets and identified in our company, where voucher specimens have been kept. *Ampelopsis grossedentata* were extracted in 20-fold (w/v) boiled water for 1 h follow by filtration through 200-sieve mesh. *Pericarpium citri reticulatae* and *Fructus crataegi* were extracted in 12-fold (w/v) boiled water for 1 h follow by filtration through 40-sieve mesh. All filtrates were collected and subjected to vacuum and reduced-pressure concentration to obtain extracts. The *Ampelopsis grossedentata* extracts were mixed with microcrystalline α-cellulose, dried in 60 °C oven for 12 h, and then grinded using pulverizer followed by mixed with the extracts of *Pericarpium citri reticulatae*, and *Fructus crataegi*. The mixtures were collected into the granulator and add sodium carboxymethyl cellulose to conduct granulating followed by sifted granule. The proportion of *Ampelopsis grossedentata*, *Pericarpium citri reticulatae*, *Fructus crataegi*, microcrystalline α-cellulose, and sodium carboxymethyl cellulose in 500 mg of APF complex were 44% (w/w), 20% (w/w), 20% (w/w), 15% (w/w), and 1% (w/w), respectively.

2.2. Analysis of DHM content in APF complex

Analytical HPLC was performed on Hitachi D-7000 interface equipped with L-7100 pump, L-7455 detector and L-7200 auto-sampler (Tokyo, Japan) to determine DHM content in APF complex. The test solution was prepared by mixing 10 mg of APF complex with 50 mL of methanol under ultrasonic condition at room temperature for 40 min followed by filtration through a 0.45 μm filter. The standard solution was prepared by mixing DHM (the purity is higher than 98%, Sigma Chemical Co., St. Louis, MO) with methanol to obtain different concentrations through serial dilution. Chromatographic separation was carried out on a Mightysil RP-18 column (250 × 4.6 mm, 5 μm) using a gradient solvent system comprised of acetonitrile (A) and 0.03% (v/v) H_3PO_4 (B). Gradient profile was set as follows at 0–10 min with the ratio of 15% A and 85% B; at 10–20 min with the ratio of 15–25% A and 85-75% B; at 20–25 min with the ratio of 25–40% A and 75-60% B; at 25–40 min with the ratio of 40–15% A and 60–85% B. The UV wavelength, flow rate, and injection volume were set at 210 nm, 1.0 mL/min, and 10 μL, respectively.

2.3. Rats model of HFD-induced obesity

2.3.1. Experimental design

Wistar male rats (6-wk-old; 180–200 g) were purchased from BioLASCO Co. Ltd, (Yilan, Taiwan). Rats were housed two to a cage with controlled temperature (25 ± 2 °C) and humidity (65 ± 5%) with 12 h-light/dark cycles and maintained based on the guidelines established in Taiwan Government Guide for the Care and Use of Laboratory Animals. After accommodation for 1 wk, rats were randomly divided into five groups (n = 12 for each group) as follows: group 1, control group; group 2. HFD group; group 3, HFD +0.2% APF complex; group 4, HFD +0.4% APF complex; group 5, HFD +0.8% APF complex. The medium- (0.4%) and high (0.8%)-dose of APF complex were selected based on two- and four-fold difference from the low dose. We stipulated that the recommended dietary allowance (RDA) of APF complex in humans is 1 g/day. In rats of HFD-induced obesity model, the low dose (0.2%) of APF complex in rats was obtained by the equation: 1 g/day ÷ 500 g (daily food intake in dry weight for a person) × 100%. The composition and nutritional value of HFD are listed in Table 1. All groups, except the control rats, were fed with HFD for 4 wks, and then received with APF complex for additional 8 wks. The body weight was measured biweekly and the calculation of food efficiency is the ratio of weight gain (g) and total food intake (g).

At the end of experiment, rats were sacrificed with CO_2 asphyxiation and blood samples were collected using cardiac puncture. After collection of the whole blood, allow the blood to clot at room temperature for 1 h followed by centrifuged at 1400 × g for 10 min to obtain serum. Liver tissues were isolated and stored at −80 °C until used. The fat tissues, including mesenteric fat, perirenal fat, and epididymal fat, were isolated and weighted. The percentage of body fat index is the ratio of total fat weight (mesenteric fat + perirenal fat + epididymal fat) and body weight.

2.3.2. Analysis of serum biochemical parameters

The serum levels of TC, TG, FFA, LDL-C, HDL-C, and glucose were determined by enzymatic colorimetric methods using commercial kits (Randox Laboratories, Ltd., Antrim, UK) according to the manufacturer's protocol. The analysis of serum biochemical parameters was carried out by an automatic analyzer (Olympus AU2700, Olympus Co., Tokyo, Japan).

2.3.3. Analysis of TC and TG content in liver tissues

1 g of liver tissues were homogenized with 20 mL of chloroform and methanol mixture (1:2, v/v), and then l mL of filtrate was mixed with 5 mL of chloroform and distilled water (1:1, v/v). After centrifugation (1500 × g) for 10 min, the lower organic phase solution was transferred into a new glass tube followed by lyophilized. The hepatic lipid extracts were obtained by mixing 0.1 g of lyophilized powder with 1 mL of chloroform and methanol mixture (1:2, v/v) and stored at −20 °C until used. The TC and TG content were measured by enzymatic colorimetric methods using commercial kits (Randox Laboratories, Ltd., Antrim, UK).

2.4. Subacute toxicity assay in rats

Male and female Wistar rats of 6 to 8-wk-old were purchased from BioLASCO Co., Ltd (Yilan, Taiwan). Rats were housed in cages with controlled temperature (25 ± 2 °C) and humidity (65 ± 5%) with 12 h-light/dark cycles. After accommodation for 1 wk, rats

Table 1
Composition and nutritional value of experimental normal diet and high fat diet (HFD).

Ingredients (g/kg dietary weight)	Normal diet				HFD			
	Quantity (g)	Nutritional value			Quantity (g)	Nutritional value		
		Items	Quantity (g)	Kilocalories (Kcal)		Items	Quantity (g)	Kilocalories (Kcal)
Beef tallow	–	–	–		400	Fat	400	3608 (59.5%)
Casein	260	Carbohydrate	33.8	126.75 (3.3%)	260	Carbohydrate	33.8	126.75 (2.1%)
		Protein	208	832 (21.9%)		Protein	208	832 (13.7%)
		Fat	18.2	163.8 (4.3%)		Fat	18.2	163.8 (2.7%)
Corn starch	500	Carbohydrate	500	1905 (50.2%)	150	Carbohydrate	150	571.5 (9.4%)
Sucrose	90	Carbohydrate	90	317.7 (8.4%)	90	Carbohydrate	90	317.7 (5.2%)
Corn oil	50	Fat	50	450 (11.9%)	50	Fat	50	450 (7.4%)
Cellulose	50	–	–	–	–	–	–	–
Mineral mixture[a]	40	–	–	–	40	–	–	–
Vitamin mixture[a]	10	–	–	–	10	–	–	–
Total	1000	–	–	3795.25	1000	–	–	6069.75

[a] Mineral and vitamin mixtures were purchased from Oriental Yeast (Tokyo, Japan).

were randomly divided into eight groups (n = 10 for each group) as follows: group 1, male control; group 2, male low dose of APF complex (999 mg/kg); group 3, male medium dose of APF complex (1700 mg/kg); group 4, male high dose of APF complex (1998 mg/kg); group 5, female control; group 6, female low dose of APF complex (oral supplementation with 999 mg/kg); group 7, female medium dose of APF complex (1700 mg/kg); group 8, female high dose of APF complex (1998 mg/kg). The recommended dietary allowance (RDA) of APF complex in human is 1 g per day. The doses of APF complex were 60, 100, and 120-fold relative to RDA of product (1 g per 60 kg person translated into 16.66 mg/kg). Rats were orally treated with APF complex daily for 28 consecutive days. During the accommodation and experimental periods, rats were supplied a standard rodent diet (Lab 5001, Purina Mills) and water ad libitum. The body weights of rats were measured weekly. At the end of experiment, rats were sacrificed with CO_2 asphyxiation and blood samples were collected with cardiac puncture. The organs were isolated and weighed. Biochemical analysis was performed on rats serum for examination of HDL, LDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CRE), TC, TG, globulin, albumin, total protein, glucose, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and phosphorus. All analyses were carried out using Express Plus Automatic Clinical Chemistry Analyzer (Beijing, China). For hematological analysis, the blood samples collected in K_3EDTA to obtain plasma. The parameters, including white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and lymphocytes, were conducted by System-450 Automated Hematology Analyzer (Tokyo, Japan).

2.5. Statistical analysis

Values are expressed as means ± SD and analyzed using one way ANOVA followed by Fisher's protected Duncan's multiple range test for comparisons of group means, when the F value was significant ($P < 0.05$). All statistical analyses were performed using SPSS for Windows, version 10 (SPSS, Inc.); a P value < 0.05 is considered statistically significant.

3. Results and discussion

3.1. Effects of APF complex on body weight changes, food intake, and food efficiency in HFD-fed rats

HFD feeding has been shown to induce obesity and metabolic disorders in rodents due to its high similarity for obesity and metabolic syndrome development in human.[14] All groups, except the control rats, were fed with HFD for 4 weeks, and then received with the different doses of APF complex for additional 8 weeks. HFD-treated alone rats significantly raised body weight gain during entire experimental period with increasing 43% at wk 2 as compared to control rats (Table 2). APF complex has a tendency but not significance to limit HFD-induced body weight gain starting at wk 2 to wk 8 and the optimal effects occurred at wk 2 (Table 2), suggesting that a weight loss plateau effect may involve. The total food intake was not statistical difference among all groups (Table 3). The food efficiency in the HFD (24.8 ± 1.4%) increased to more than 2 folds in comparison with control group (11.6 ± 0.9%), but the supplement of APF complex did not affect food efficiency compared with HFD group (Table 3). In comparison with food intake, the reduction of body weight was not associated with appetite or energy intake, suggesting that the limited weight gain effect of APF complex could be attributed to metabolic regulation.

3.2. Effects of APF complex on body fat deposition in HFD-fed rats

HFD consumption has been shown to create a positive energy balance followed by an increase in visceral fat deposition leading to abdominal obesity.[15] Herein, we found that HFD markedly increased body fat deposition, as evidenced by increased the weight of epididymal fat, mesenteric fat, and perirenal fat, as compared to control group (Table 3). APF complex treatment significantly decreased the weight of epididymal fat and perirenal fat in a dose-dependent manner without affecting the weight of mesenteric fat in HFD-fed rats (Table 3). By calculation, APF complex treatment also significantly and dose-dependently decreased body fat index, with a reduction of 27% ($P < 0.05$), as compared with HFD group (Table 3). Surprisingly, HFD-increased body fat index were suppressed dramatically by treatment of APF complex in a similar extent to levels approximating those in control group (Table 3). These results indicate that APF complex inhibits body fat deposition.

3.3. Effects of APF complex on serum biochemical parameters and hepatic lipid accumulation in HFD-fed rats

Hyperlipidemia and hyperglycemia were used to diagnosis metabolic syndrome.[5] Dysregulation of FFA metabolism is a key factor for development of insulin resistance.[16] Herein, we found that HFD significantly caused dyslipidemic changes in rats as illustrated by increasing serum levels of TC, TG, and LDL-C without

Table 2
Effects of APF complex on body weight change of rats[a].

	Week						
	−4	−2	0	2	4	6	8
Control	219 ± 1	252 ± 7	264 ± 8	285 ± 7	329 ± 10	341 ± 10	373 ± 10
HFD	220 ± 1	288 ± 14	399 ± 17*	499 ± 42*	511 ± 27*	529 ± 21*	551 ± 18*
HFD +0.2% APF	219 ± 1	288 ± 15	399 ± 22*	476 ± 38*	499 ± 39*	504 ± 55*	524 ± 30*
HFD +0.4% APF	219 ± 1	287 ± 12	399 ± 23*	460 ± 26*	484 ± 40*	500 ± 39*	521 ± 27*
HFD +0.8% APF	219 ± 1	288 ± 14	398 ± 26*	457 ± 50*	481 ± 31*	490 ± 30*	520 ± 30*

*$P < 0.05$ compared with the control group.
[a] All groups, except the control rats, were fed with high-fat diet (HFD) for 4 weeks, and then received with the different doses of APF complex for additional 8 weeks. The body weight was measured biweekly. Each value is expressed as the mean ± SD (n = 12 for each group).

Table 3
Effects of APF complex on food intake, food efficiency, adipose tissue weight, serum biochemical parameters, and liver lipids content in high-fat diet-fed rats[a].

	Control	HFD			
			APF		
		–	0.2%	0.4%	0.8%
Total food intake (g)	1323 ± 45	1335 ± 35	1322 ± 42	1322 ± 38	1321 ± 45
Food efficiency (%)[b]	11.6 ± 0.9	24.8 ± 1.4*	23.2 ± 2.3*	22.8 ± 1.8*	22.9 ± 2.6*
Adipose tissue					
Epididymal fat (g)	7.5 ± 1.1	13.6 ± 3.4*	11.0 ± 2.3*	10.6 ± 1.3*,#	10.2 ± 3.2*,#
Mesenteric fat (g)	2.2 ± 0.4	5.7 ± 1.6*	5.5 ± 1.9*	5.4 ± 2.0*	5.3 ± 1.8*
Perirenal fat (g)	6.0 ± 0.8	17.7 ± 3.8*	11.5 ± 1.8*	11.0 ± 1.9*,#	10.2 ± 3.1*,#
Body fat index (%)[c]	4.3 ± 0.6	6.7 ± 1.1*	5.3 ± 0.8*,#	5.2 ± 0.5*,#	4.9 ± 1.0#
Serum parameters					
TC (mg/dL)	55 ± 9	77 ± 8*	64 ± 9*,#	62 ± 9*,#	61 ± 10*,#
TG (mg/dL)	43 ± 6	72 ± 23*	56 ± 15*	49 ± 13*	46 ± 9#
LDL-C (mg/dL)	9.0 ± 2.4	13.9 ± 1.7*	10.9 ± 3.2*,#	10.1 ± 2.5#	9.2 ± 2.8#
HDL-C (mg/dL)	30 ± 5	38 ± 5	34 ± 3	33 ± 6	32 ± 5
Glucose (mg/dL)	105 ± 15	187 ± 50*	146 ± 31*,#	140 ± 31*,#	136 ± 36*,#
FFA (U/min/mg protein)	2.9 ± 0.7	5.1 ± 1.4*	3.9 ± 0.7*,#	3.8 ± 0.9*,#	3.5 ± 0.9*,#
Liver parameters					
TC (mg/g)	116 ± 20	219 ± 56*	123 ± 18#	119 ± 17#	114 ± 20#
TG (mg/g)	197 ± 39	587 ± 86*	393 ± 82*,#	391 ± 99*,#	390 ± 107*,#

*Represented as $P < 0.05$ in comparison with the control group.
#Represented as $P < 0.05$ in comparison with the HFD group.
[a] All groups, except the control rats, were fed with high-fat diet (HFD) for 4 weeks, and then received with the different doses of APF complex for additional 8 weeks. Each value is expressed as the mean ± SD (n = 12 for each group).
[b] The formula for calculation of food efficiency as follows: weight gain/total food intake × 100%.
[c] The percentage of body fat index is the ratio of total fat weight (epididymal fat + mesenteric fat + perirenal fat) and body weight.

affecting HDL-C (Table 3). HFD also significantly increased blood glucose and FFA level in rats. Administration of APF complex significantly reversed these changes caused by HFD in a dose-dependent manner (Table 3). Surprisingly, HFD-increased serum TG and LDL-C levels were suppressed dramatically by treatment of APF complex in a similar extent to levels approximating those in control group (Table 3).

Nonalcoholic fatty liver disease, the hepatic manifestation of metabolic syndrome, is associated with excessive fat and sugar consumption.[2] HFD significantly increased hepatic TG and TC levels by 1.8 and 2.9 folds, respectively, as compared to control group (Table 3). Administration of APF complex significantly reversed these changes caused by HFD in a dose-dependent manner (Table 3). Surprisingly, HFD-increased hepatic TC levels were suppressed dramatically by treatment of APF complex in a similar extent to levels approximating those in control group (Table 3). These results indicated that APF complex has a potential on improvement of metabolic syndrome-related hyperlipidemia, hyperglycemia, insulin resistance, and fatty liver. Herein, we found that the content of DHM in APF complex was 362.7 ± 12.5 mg/g (Fig. 1). DHM is a main bioactive component in *Ampelopsis grossdentata* with several biological functions, including hypoglycemic, antioxidant, anti-inflammatory, antitumor, hepatoprotective, and neuroprotective effects.[17] A drink containing DHM-rich *Ampelopsis grossdentata* has been shown to decrease plasma levels of TC and TG in patients with primary hyperlipidemia at a dose of 9 g/day after 45 days supplementation, indicating a hypolipidemic effect of DHM.[17] In accordance with these finding, DHM-rich APF complex effectively improved HFD-induced abnormalities of serum lipids profile and hepatic lipid accumulation in rats, suggesting that DHM may be involve in the treatment of metabolic syndrome of APF complex. However, the in-depth studies for exploring the mechanism underlying such actions are needed in the future.

3.4. Subacute 28 days repeated toxicity assessment

No toxicity signs, including mortality, food and water consumption (data not shown), body weight changes (Table 4), and absolute organ weights (Table 4), were observed during entire experimental period following orally supplementation of APF complex for 28 consecutive days, indicating that APF complex did not adversely affect the basic metabolic processes of the experimental rats. Hematological system has been regarded as an important marker for monitoring the physiological changes in humans and animals because it is sensitive to toxic substances.[18] Our finding revealed that APF complex administration did not produce any significant alteration in hematological parameters, including WBC, RBC, Hb, Hct, lymphocytes etc. (Table 4), indicating

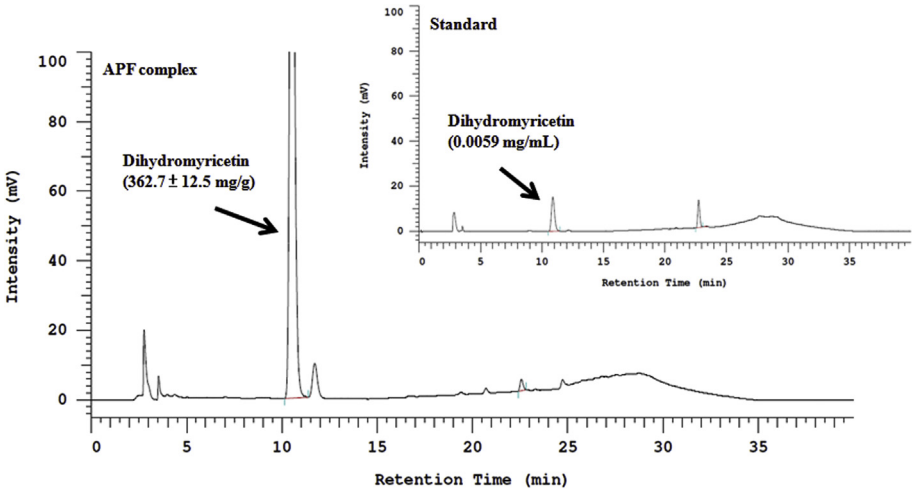

Fig. 1. HPLC chromatogram of dihydromycricetin in APF complex. The inset denotes the dihydromycricetin standard.

Table 4
Effects of APF complex on body weight, relative organ weight, hematological parameters, blood biochemical parameters in rats at the end of experiment.

	Male				Female			
	APF complex (mg/kg)				APF complex (mg/kg)			
	Control	999	1700	1998	Control	999	1700	1998
Body weight (g)	408 ± 36	403 ± 22	398 ± 34	401 ± 24	267 ± 8	271 ± 18	275 ± 12	268 ± 19
Absolute organ weight (g)								
Heart (g)	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Liver (g)	11.5 ± 1.0	10.7 ± 0.8	10.5 ± 1.4	10.6 ± 1.1	7.2 ± 0.4	7.0 ± 0.9	7.2 ± 0.3	7.0 ± 0.8
Spleen (g)	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.6	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Lung (g)	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Kidney (g)	3.0 ± 0.4	2.9 ± 0.3	2.8 ± 0.4	2.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
Testes (g)	3.3 ± 0.4	3.6 ± 0.5	3.8 ± 0.3	3.5 ± 0.3	–	–	–	–
Ovary (g)	–	–	–	–	0.9 ± 0.4	0.6 ± 0.1	0.7 ± 0.3	0.7 ± 0.2
Hematological parameters								
WBC (10^3/μL)	8.2 ± 1.6	9.0 ± 1.0	9.6 ± 2.7	9.8 ± 1.7	4.8 ± 1.1	4.8 ± 1.1	5.6 ± 1.2	6.3 ± 2.0
RBC (10^6/μl)	8.5 ± 0.7	8.5 ± 0.3	8.7 ± 0.3	8.6 ± 0.3	8.2 ± 0.3	8.3 ± 0.3	8.4 ± 0.5	8.3 ± 0.3
Hb (g/dL)	15.7 ± 1.1	15.6 ± 0.5	16.0 ± 0.4	15.8 ± 0.6	14.7 ± 0.4	14.8 ± 0.4	15.3 ± 0.7	15.0 ± 0.6
Hct (%)	51.4 ± 3.7	50.3 ± 1.7	51.5 ± 1.6	50.8 ± 2.2	46.7 ± 1.7	46.7 ± 1.69	47.9 ± 2.6	46.6 ± 2.4
MCV (fl)	60.5 ± 1.7	59.3 ± 1.4	59.2 ± 1.1	58.9 ± 1.3	56.9 ± 1.3	56.0 ± 1.0	56.9 ± 2.2	56.0 ± 2.1
MCH (pg)	18.4 ± 0.5	18.3 ± 0.4	18.4 ± 0.4	18.3 ± 0.4	18.0 ± 0.5	17.8 ± 0.4	18.1 ± 0.6	17.9 ± 0.5
MCHC (g/dl)	30.5 ± 0.3	30.7 ± 0.3	30.8 ± 0.4	31.1 ± 0.3*	31.6 ± 0.4	31.7 ± 0.5	31.9 ± 0.4	32.1 ± 0.5
PLT (10^3/μl)	1006 ± 154	1014 ± 111	975 ± 93	1007 ± 136	873 ± 93	901 ± 90	885 ± 103	904 ± 127
Lymphocytes (%)	75.2 ± 4.4	74.1 ± 1.9	70.7 ± 9.7	74.4 ± 3.0	78.7 ± 6.4	78.8 ± 7.3	76.5 ± 5.9	83.6 ± 4.4
Blood biochemical parameters								
ALT (U/L)	35.6 ± 3.2	31.5 ± 4.6*	29.6 ± 2.3*	32.6 ± 6.3	34.6 ± 5.8	33.9 ± 4.7	30.3 ± 4.7	31.8 ± 5.6
AST (U/L)	114 ± 9	107 ± 13	104 ± 11	89 ± 4*	132 ± 11	130 ± 20	127 ± 24	113 ± 13*
BUN (mg/dL)	17.9 ± 3.5	16.4 ± 3.9	13.1 ± 2.4*	15.7 ± 1.4	16.5 ± 2.9	17.1 ± 2.5	17.9 ± 2.8	16.2 ± 2.9
Creatinine (mg/dL)	0.38 ± 0.02	0.32 ± 0.03*	0.31 ± 0.04*	0.35 ± 0.04	0.40 ± 0.04	0.38 ± 0.05	0.39 ± 0.05	0.37 ± 0.04
Glucose (mg/dL)	87.4 ± 17.3	92.0 ± 19.3	86.0 ± 21.0	107.6 ± 33.7	72.4 ± 10.1	83.9 ± 21.0	81.8 ± 18.0	80.2 ± 9.1
TC (mg/dL)	59.6 ± 12.2	64.7 ± 10.2	58.8 ± 16.8	53.5 ± 11.1	67.6 ± 10.2	63.9 ± 11.4	62.6 ± 13.3	60.5 ± 4.8
TG (mg/dL)	51.5 ± 10.6	55.9 ± 17.2	53.7 ± 15.0	60.7 ± 16.3	48.5 ± 5.6	48.2 ± 5.9	56.9 ± 13.6	60.7 ± 16.4
HDL (mg/dL)	11.4 ± 2.3	10.5 ± 1.4	10.6 ± 3.1	10.0 ± 2.8	19.6 ± 3.2	17.8 ± 3.3	17.7 ± 3.4	16.4 ± 1.8
LDL (mg/dL)	8.4 ± 1.7	9.4 ± 2.1	8.7 ± 2.8	7.3 ± 2.2	6.6 ± 0.8	6.7 ± 1.3	6.3 ± 1.8	6.6 ± 1.6
Globulin (g/dL)	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.2	2.2 ± 0.1
Albumin (g/dL)	3.9 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	3.8 ± 0.1	4.1 ± 0.1	4.1 ± 0.2	4.1 ± 0.2	4.0 ± 0.2
Na^+ (meq/L)	143 ± 2	145 ± 2	143 ± 2	144 ± 2	144 ± 1	144 ± 1	145 ± 1	144 ± 1
Cl^- (meq/L)	99 ± 3	100 ± 2	100 ± 1	101 ± 3	101 ± 2	101 ± 1	102 ± 2	102 ± 2
Mg^{2+} (mg/dL)	3.5 ± 0.2	3.4 ± 0.2	3.2 ± 0.2	3.2 ± 0.3	3.6 ± 0.3	3.4 ± 0.3	3.4 ± 0.4	3.2 ± 0.2
Ca^{2+} (mg/dL)	10.3 ± 0.3	10.4 ± 0.3	10.3 ± 0.3	10.6 ± 0.4	9.9 ± 0.9	10.3 ± 0.4	10.2 ± 0.3	10.2 ± 0.2
P (meq/L)	9.8 ± 1.1	9.4 ± 0.7	9.0 ± 0.5	8.9 ± 0.7	7.6 ± 1.1	7.2 ± 0.9	7.6 ± 0.7	7.6 ± 0.4

*Represented as $P < 0.05$ in comparison with the control group.

that APF complex was not toxic to blood cells nor their production.

Transaminases, including AST and ALT, are good biomarkers for liver function evaluation.[19] BUN and creatinine are the most commonly used indicators of renal function. The plasma level of BUN more than 60 mg/dL indicates a moderate-to-severe degree of kidney failure in rats,[20] and an elevated blood creatinine level is associated with production of nephron damage.[21] Although serum levels of ALT (in male rats of low and medium dose APF), AST (in male and female rat of high dose APF), BUN (in male rats of medium dose APF), and creatinine (in male rats of low and medium dose

APF) exhibited statistical difference in comparison with control rats (Table 4), the detected values are still within normal physiological range.[22] APF complex can be considered non-toxic to liver and renal function.

APF complex treatment did not cause abnormalities in serum levels of glucose TC, TG, HDL, LDL, globulin, and albumin (Table 4), indicating that sub-chronic administration of APF complex neither affected blood lipids and nutritional status nor the normal metabolism of experimental rats. In addition, the serum electrolyte levels, including Na^{+}, Cl^{-}, Mg^{2+}, Ca^{2+}, and P, were found to be no statistical difference in comparison with control rats (Table 4) which reflects that APF complex has no adverse effect on electrolyte balance in rats. These results indicated that the no observed adverse effect level (NOAEL) of APF complex is greater than 1998 mg/kg/day in rats. A formula is available for converting animal dose to human equivalent dose (HED) in mg/kg, i.e., multiply the rat dose in mg/kg/day by 0.16.[23] By calculation, the NOAEL of APF complex for the 60-kg healthy person consumption is 19.2 g/day which tower over the RDA of 1 g/day for 60-kg healthy person, indicating the increase leukocyte counts, blood glucose, and blood TG in rats exhibited no toxicity qualms about APF complex for human use.

4. Conclusion

In this study, we demonstrated that DHM-rich APF complex improved body fat deposition, serum lipid profile, blood glucose and hepatic lipid accumulation in HFD-fed rats. We also demonstrated that APF complex had no subacute toxicity in assay of 28 days repeated feeding study. Taken together, DHM-rich APF complex could act as an adjuvant therapy for metabolic syndrome and as non-toxic herbal prescription for dietary supplementation.

Conflicts of interest

We declare no conflict of interest involved in this study.

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