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Effects of herbal mixture extracts on obesity in rats fed a high-fat diet

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ABSTRACT

The aim of this study was to investigate and compare the effects of three herbal mixture extracts on obesity induced by high-fat diet (HFD) in rats. The prescriptions—*Pericarpium citri reticulatae* and *Fructus crataegi*—were used as matrix components and mixed with *Ampelopsis grossedentata*, *Salvia miltiorrhiza*, and epigallocatechin-3-gallate (EGCG) to form T1, T2, and T3 complexes, respectively. Results revealed that HFD feeding significantly increased body weight gain, fat deposition, plasma lipid profiles, hepatic lipid accumulation, and hepatic vacuoles formation, but decreased plasma levels of adiponectin in rats. Only the T1 complex showed the tendency, although not significantly so, for decreased HFD-induced body weight gain. T1 and T3 complexes significantly reduced HFD-induced fat deposition, and plasma levels of triglyceride, total cholesterol, and low-density lipoprotein cholesterol. Only the T1 complex significantly increased HFD-reduced adiponectin levels in plasma, but decreased HFD-increased triglyceride content in liver tissues. All complexes effectively inhibited HFD-induced vacuoles formation. The content of dihydromyricetin, salvianolic acid B, and EGCG in T1, T2, and T3 complexes was 18.25 ± 0.07%, 22.20 ± 0.10%, and 18.86 ± 0.04%, respectively. In summary, we demonstrated that herbal mixture extracts, especially T1 complex, exhibit antiobesity activity in HFD-fed rats.

1. Introduction

Obesity, one of the global health problems, is characterized by an increase in fat mass accumulation and body weight gain [1]. Obesity is the main risk factor for metabolic syndrome, which is related to an increased risk of several types of chronic diseases, including hyperlipidemia, hypertension, atherosclerosis, fatty liver, insulin resistance, and cancer [2]. The major strategy for prevention and treatment of obesity is lifestyle modification, including dieting control and physical exercise [3]. However, it is difficult to carry out

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these options because of people's busy schedules and sedentary lifestyle. Although several available antiobesity drugs, including orlistat [4], rimonabant [5], and sibutramine [6], have been approved as a therapeutic strategy to combat obesity, the potential toxicity may limit their overall usefulness [7].

Herbal medicines can be defined as raw or extracted products isolated from plants, and they are widely used for prevention and treatment of many chronic diseases, including obesity, because they have fewer side effects compared with pharmacological drugs [8]. *Pericarpium citri reticulatae*, also called Chenpi in Chinese, is the dried ripe fruit peel of *Citrus reticulata* Blanco and its cultivars [9]. The methanol extracts of Chenpi have been shown to inhibit adipogenesis in 3T3-L1 preadipocytes, suggesting that Chenpi may exhibit antiobesity potential [10]. *Fructus crataegi*, also called as Shanzha in Chinese and widely used as traditional medicine, is the ripe fruit of *Crataegus pinnatifida* Bge. var. major N.E. Br. or *C. pinnatifida* Bge. Shanzha has been demonstrated to exhibit hypolipidemic activity and cholesterol lowering effects [11]. *Ampelopsis grossedentata* (Hand.-Mazz.) W. T. Wang, also called Tengcha in Chinese, has been used as a daily drink and as folk medicine for the treatment of hepatitis, hypertension, and hyperglycemia [12]. Danshen (in Chinese), one of the traditional Chinese medicinal herbs, is the dried of root and rhizome of *Salvia miltiorrhiza* Bunge and has been shown to exhibit a variety of biological functions, including improving microcirculation, suppressing the formation of thromboxane, inhibiting platelet adhesion and aggregation, and protecting against myocardial ischemia [13]. Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea with several potential health effects, including antiobesity and weight maintenance properties [14].

Synergistic actions are of vital importance in traditional Chinese medicines for prevention and treatment of chronic diseases [15]. The possible explanations for the synergistic actions of herbal medicines are that different herbal medicines may mediate either the same or different targets in a synergistic manner and may decrease the adverse effects or increase pharmacological activity by herbal–herbal interaction [16]. These specific functions of each component led us to hypothesize that these components when used in combinations could serve as an effective antiobesity prescription to decrease body weight gain, remove excessive body fat accumulation, and improve blood lipid profiles. The prescriptions of herbal mixture extracts are Chenpi and Shanzha used as matrix components and mixed with Tengcha, Danshen, and EGCG to form T1, T2, and T3 complex, respectively. The present study was carried out to investigate and compare the antiobesity effects of three herbal mixture extracts in a rat model fed with high-fat diet (HFD).

2. Methods

2.1. Herbal mixture extracts preparation

The prescriptions of three herbal mixture extracts were developed by Ko Da Pharmaceutical Co. Ltd. (Taoyuan, Taiwan). The raw materials, including Chenpi, Shanzha, Danshen, and Tengcha, were obtained from Taiwan and China herbal markets and identified in the Ko Da Pharmaceutical Co. Ltd. Research and Development Center (Taoyuan, Taiwan), where voucher specimens have been kept. The EGCG was purchased from Teavigo (DSM Nutritional Products Ltd., Heerlen, The Netherlands) with purity of 50% as claimed by the supplier. The raw materials were extracted in boiled water individually for two times (1 hour for each time) followed by filtration. All filtrates were collected and subjected to vacuum and reduced-pressure concentration to obtain extracts. The dried powder of each extract was produced by freeze-drying. Chenpi and Shanzha were used as matrix components and mixed with Tengcha, Danshen, and EGCG to form T1, T2, and T3 complex, respectively (Table 1). These three complexes were used as commercial herbal supplements as an antiobesity formula. The recommended daily allowance for the three complexes in humans is 5 g/d. A formula is available for converting the human equivalent dose to the animal dose in mg/kg, i.e., multiply the human dose in mg/kg per day by 6.2 [17]. The dose of three complexes of ~500 mg/kg in rats was obtained using the following equation:

$$83.3 \text{ mg/kg (5 g per 60 kg person)} \times 6.2. \quad (1)$$

2.2. Analysis of marker compounds content in three herbal mixture extracts

Analytical high-performance liquid chromatography was performed on Hitachi D-7000 Interface equipped with L-7100 pump, L-7455 detector, and L-7200 autosampler (Hitachi, Tokyo, Japan) to determine the marker compounds in three herbal mixture extracts. For the analysis of dihydromyricetin, salvianolic acid B, and EGCG content in T1, T2, and T3 complexes, respectively, the test solution was prepared by mixing 10 mg of dried powder with 10 mL of 70% (v/v) methanol under ultrasonic condition at room temperature for 1 hour followed by filtration. These filtrates were filtrated through a 0.45-μm filter and chromatographic separation was carried out on a Mightysil RP-18 column (Kanto Chemical CO., INC., Tokyo, Japan.) with the specification of 250 mm × 4.6 mm, 5 μm using gradient solvent systems as summarized in Table 2. The UV wavelength, flow rate and injection volume were set at 254 nm, 1.0 mL/min, and 10 μL, respectively. The standard solution was prepared by mixing dihydromyricetin, salvianolic acid B, or EGCG, respectively, with methanol to obtain different concentrations through serial dilution.

2.3. Animals and experimental design

Wistar male rats (4 weeks old; 200–250 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). This study protocol was approved by the Animal Research Committee of Chung Yuan Christian University, Taoyuan, Taiwan. Rats were housed two to a cage with controlled temperature (25 ± 2°C) and humidity (65 ± 5%) with 12-hour light/dark cycles and maintained based on the guidelines established by the Taiwan Government Guide for the Care and Use of Laboratory Animals. After 1 week, rats were randomly divided into five groups ($n = 10$ for each group) as follows:

Table 1 – Compositions of three herbal mixture extracts.

	Chinese name	Common name	Scientific name	Part used	Composition (%)
Matrix components	Chenpi	Tangerine peel	*Pericarpium citri reticulatae*	Dried ripe fruit peel	30
	Shanzha	Hawthorn fruit	*Fructus crataegi*	Dried ripe fruit	30
T1	Tengcha	*Ampelopsis grossedentata*		Dried stems and leaves	40
T2	Danshen	Salvia root	*Salvia miltiorrhiza*	Dried roots and rhizome	40
T3	EGCG	Epigallocatechin-3-gallate		Dried leaves of green tea	40

EGCG = epigallocatechin-3-gallate.

Table 2 – Conditions of gradient solvent systems in HPLC analysis for marker compounds in three herbal mixture extracts.

Dihydromyricetin			Salvianolic acid B			EGCG		
Time (min)	ACN (%)	0.03% H_3PO_4 (%)	Time (min)	ACN (%)	1% Acetic acid (%)	Time (min)	ACN (%)	0.03% H_3PO_4 (%)
0	10	90	0	12	88	0	10	90
5	15	85	5	12	88	25	10	90
20	45	55	35	20	80	30	50	50
25	50	50	45	23	77	35	50	50
30	10	90	55	30	70	40	10	90
40	10	90	70	12	88	45	10	90

ACN = acetonitrile; EGCG = epigallocatechin-3-gallate; HPLC = high-performance liquid chromatography.

Group 1, control group fed with normal diet; Group 2, HFD group; Group 3, HFD + T1 group (orally received 500 mg/kg of T1 complex daily for 8 weeks); Group 4, HFD + T2 group (orally received 500 mg/kg of T2 complex daily for 8 weeks); and Group 5, HFD + T3 group (orally received 500 mg/kg of T3 complex daily for 8 weeks). The compositions of normal diet and HFD are summarized in Table 3. Rats were given the T1, T2, and T3 complexes daily via oral tubing. At the end of the experiments, rats were sacrificed with CO_2 asphyxiation. Blood samples were collected in a vacutainer tube containing K_3EDTA followed by centrifugation at 4000*g* for 10 minutes to obtain plasma. The fat tissues—including subcutaneous, mesenteric, perirenal, and epididymal tissues—were isolated and weighed.

2.4. *Analysis of biochemical parameters in plasma*

2.4.1. *Adiponectin levels*

The levels of adiponectin in rat plasma were quantified using quantitative sandwich enzyme immunoassay technique with a specific monoclonal antibody against rat adiponectin that has been precoated onto a microplate. The optical density was set to a wavelength of 450 nm according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA). Plasma was diluted 1000-fold with commercial diluents prior to the assay. Ten separate experiments were each tested in duplicate.

2.4.2. *Levels of total cholesterol and triglyceride*

The levels of total cholesterol (TC) and triglyceride (TG) in rat plasma were determined using enzymatic colorimetric method as described previously [18]. For TC measurement, 10 μL of plasma or cholesterol standard (200 mg/dL) was mixed with a reaction buffer containing 0.3 mM 4-aminoantopyrine, 6 mM phenol, 0.5 U/mL peroxidase, 0.15 U/mL cholesterol esterase, and 0.1 U/mL cholesterol oxidase. For TG measurement, 10 μL of plasma or TG standard (200 mg/dL) was mixed with a reaction buffer containing 0.5 mM 4-aminophenazone, 5.5 mM 4-chlorophenol, 1 mM ATP, 150 U/mL lipase, 0.4 U/mL glycerol kinase, 1.5 U/mL glycerol-3-phosphate oxidase, and 0.5 U/mL peroxidase. The mixtures were allowed to react for 10 minutes at room temperature, and the optical density was set to a wavelength of 500 nm. The plasma TC or TG level (mg/dL) was calculated as follows:

$$(\text{the absorbance of sample at 500 nm}/\text{the absorbance of standard at 500 nm}) \times 200. \quad (2)$$

2.4.3. *Levels of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol*

The levels of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in rat plasma were measured using the enzymatic colorimetric method as described previously [19,20]. For HDL-C analysis, 200 μL of

Table 3 – Compositions of normal diet and high-fat diet.

	Compositions	Company (location)
Normal diet	Casein (26%), corn starch (50%), sucrose (9%), corn oil (5%), cellulose (5%), mineral mixture (4%), vitamin mixture (1%)	Oriental Yeast Co. Ltd. (Tokyo, Japan)
High-fat diet	Casein (26%), corn starch (15%), sucrose (9%), beef tallow (40%), cellulose (5%), mineral mixture (4%), vitamin mixture (1%)	

plasma was mixed with a separation buffer containing 0.55 mM phosphotungstic acid and 25 mM magnesium chloride to precipitate the LDL and VLDL fractions. For LDL-C analysis, 100 μL of plasma was mixed with separation buffer containing 5000 IU/L heparin and 64 mM sodium citrate to precipitate the HDL and VLDL fractions. The mixtures were allowed to react for 10 minutes at room temperature and then centrifuged at 10,000*g* for 15 minutes. Next, 100 μL of the supernatant containing HDL-C and LDL-C or 10 μL of HDL-C and LDL-C standard (200 mg/dL) was mixed with a reaction buffer containing 0.3 mM 4-aminoantipyrine, 6 mM phenol, 0.5 U/mL peroxidase, 0.15 U/mL cholesterol esterase, and 0.1 U/mL cholesterol oxidase. The mixtures were allowed to react for 10 minutes at room temperature, and the optical density was set to a wavelength of 500 nm. The plasma HDL-C or LDL-C level (mg/dL) was calculated as follows:

(absorbance of sample at 500 nm/absorbance of standard at 500 nm) × 200. (3)

2.5. *Preparation of liver homogenates*

The liver samples (0.5 g) were homogenized in 10 mL of chloroform and methanol (2:1, v/v) mixtures with a homogenizer at 4°C to obtain liver homogenate. The homogenates were centrifuged at 10,000*g* for 1 hour at 4°C. The supernatant were collected and used for TC and TG analysis as described above. The values of TC and TG content are expressed as mg/g liver.

2.6. *Histopathological analysis*

Liver tissues were fixed in 10% formalin and embedded in paraffin, then sectioned into 2-μm thickness using a microtome. The paraffin-embedded tissues were dried at 60°C for 1 hour and deparaffinized using a dip of xylene and gradient concentration of ethanol. The microslide was stained with hematoxylin and eosin stain and observed under a microscope (BX51; Olympus, Tokyo, Japan).

2.7. *Statistical analysis*

Values are expressed as means ± standard deviation and analyzed using one-way analysis of variance 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., Chicago, IL, USA); a *p* value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. *Effects of three herbal mixture extracts on body weight in HFD-fed rats*

Although the etiology of obesity is complex, the dietary factor, especially the consumption of HFD, is regarded as one of the major risk factors for obesity development [21]. In the present study, we investigated and compared the effects of three herbal mixture extracts on HFD-induced obesity in rats. Herein, we found that HFD feeding effectively induced obesity in rats, as evidenced by a significance increment of body weight at the beginning of 4 weeks in comparison with the control group (Figure 1). At the end of the experiment, the average body weight of rats in the HFD group (496 ± 29 g) was significantly higher than that in the control group (434 ± 34 g; Table 4). Among these three herbal mixture extracts, only T1 complex supplementation showed a tendency, although not at a significant level, toward reduction of body weight gain caused by HFD, whereas T2 and T3 complexes did not exhibit such effect (Table 4).

3.2. *Effects of three herbal mixture extracts on fat deposition in HFD-fed rats*

HFD feeding is critical for obesity development because excess calorie intake can promote the development of a positive energy balance followed by increased visceral fat deposition leading to abdominal obesity [22]. In this study, the weight of viscera fat tissues was calculated as the sum of the weight of mesenteric, perirenal, and epididymal fat tissues. The body fat index is a ratio of the total fat tissues (viscera and subcutaneous) weight and body weight. Results revealed that HFD feeding significantly increased fat deposition in comparison with the control group, as evidenced by the increased viscera (mesenteric + perirenal + epididymal) and subcutaneous fat tissues weight (Table 4). T1 and T3 complex treatment significantly decreased HFD-induced fat deposition, as compared to the HFD group (Table 4). By calculation, there was an increase in the body fat index in HFD-fed rats in contrast to decreased values from the groups of rats fed with T1 and T3

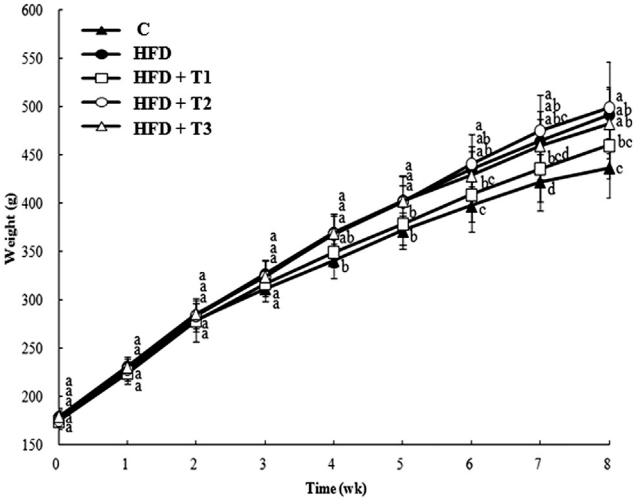

Figure 1 – Effects of three herbal mixture extracts on body weigh changes in high-fat diet (HFD)-fed rat during the 8-week experimental period. Rats were fed a normal diet (C), HFD, or HFD with 500 mg/kg of T1, T2, and T3 complex for 8 weeks and the body weight was measured weekly. Values from 10 rats are expressed as mean ± standard deviation; means without a common letter differ significantly ($p < 0.05$).

Table 4 – Effects of three herbal mixture extracts on body weight and fat tissue weight in high-fat diet fed rats.

	C	HFD	HFD + T1	HFD + T2	HFD + T3
Body weight (g)	434 ± 34^{a}	496 ± 29^{bc}	463 ± 35^{ac}	501 ± 49^{b}	486 ± 37^{bc}
Mesenteric fat tissues (g)	6.0 ± 2.1^{a}	11.2 ± 2.5^{b}	9.4 ± 2.6^{c}	12.0 ± 3.4^{b}	9.5 ± 2.4^{c}
Perirenal fat tissues (g)	7.0 ± 1.4^{a}	20.2 ± 5.0^{b}	15.5 ± 3.8^{c}	19.9 ± 5.1^{bd}	16.3 ± 4.0^{cd}
Epididymal fat tissues (g)	6.3 ± 0.9^{a}	15.8 ± 2.8^{b}	12.7 ± 3.0^{c}	15.8 ± 5.0^{b}	12.1 ± 3.3^{c}
Viscera fat tissues (g)	19.4 ± 4.0^{a}	47.2 ± 8.9^{b}	37.5 ± 8.9^{c}	47.7 ± 12.6^{b}	37.9 ± 9.1^{c}
Subcutaneous fat tissues (g)	8.4 ± 3.2^{a}	23.3 ± 10.0^{b}	15.2 ± 4.3^{c}	20.1 ± 7.0^{bc}	16.5 ± 4.7^{c}
Body fat index (%)	6.4 ± 1.4^{a}	14.1 ± 2.8^{b}	11.3 ± 2.3^{c}	12.4 ± 3.5^{bc}	11.2 ± 2.4^{c}

Rats were fed a normal diet (C), a high-fat diet (HFD), or HFD with 500 mg/kg of T1, T2, and T3 complex, respectively, for 8 weeks ($n = 10$ for each group). Values from 10 rats are expressed as mean ± standard deviation; means without a common letter in a row differ significantly ($p < 0.05$).
The weight of viscera fat tissues were calculated as the sum of the weight of mesenteric, perirenal, and epididymal fat tissues.
Body fat index is the ratio of the total fat tissues (viscera and subcutaneous) weight and body weight.

complexes (Table 4). However, T2 complex did not decrease body fat deposition in HFD-fed rats (Table 4).

3.3. *Effects of herbal mixture extracts on plasma adiponectin levels in HFD-fed rats*

Adiponectin is an adipocyte-specific protein that mediates many metabolic processes such as glucose regulation and fatty acid oxidation [23]. The plasma levels of adiponectin are decreased in patients with obesity, type 2 diabetes mellitus, and coronary artery disease and in rats with HFD-induced obesity [24]. In addition, adiponectin knockout could accentuate HFD-induced obesity in mice [25]. A similar finding was found in this study, wherein the plasma levels of adiponectin in HFD-fed rats were significantly lower than those of the control group (Table 5). The T1 and T3 complex treatments effectively increased HFD-reduced adiponectin levels in plasma to levels approximating those in control rats (Table 5), whereas T2 complex did not exhibit such increased actions (Table 5).

3.4. *Effects of three herbal mixture extracts on plasma lipid profiles in HFD-fed rats*

Obesity is deeply related to many chronic diseases, including hyperlipidemia, hypertension, arteriosclerosis, and cancer [26]. We showed that the plasma levels of TG, TC, and LDL-C in HFD-fed rats were significantly higher than those of control group (Table 5), indicating that HFD feeding caused hyperlipidemia in rats. T1 and T3 complex supplementation significantly decreased these changes induced by HFD, whereas T2 complex did not exhibit such ameliorative actions (Table 5). However, plasma HDL-C levels were not significantly different among these five groups (Table 5).

3.5. *Effects of three herbal mixture extracts on hepatosteatosis in HFD-fed rats*

HFD feeding has been shown to induce hepatosteatosis in mice and in rats [27]. In this study, we found that HFD feeding significantly increased liver weight (Table 5), hepatic TG and TC accumulation (Table 5), and hepatic vacuoles (Figure 2). Only T1 complex significantly decreased HFD-increased liver weight and hepatic TG content (T2 and T3 complexes did not exhibit such actions; Table 5). However, all treatments using these complexes did not affect HFD-increased TC levels in liver tissues (Table 5). HFD-induced hepatic vacuoles were suppressed by treatment using all complexes in a similar extent to levels approximating those in control rats (Figure 2).

Table 5 – Effects of three herbal mixture extracts on adiponectin levels and lipid profiles in plasma as well as lipid accumulation in liver tissue in high-fat diet (HFD)-fed rats.

	C	HFD	HFD + T1	HFD + T2	HFD + T3
			Plasma		
Adiponectin (μg/dL)	4.6 ± 0.8^{a}	3.9 ± 0.3^{b}	4.7 ± 0.6^{a}	4.1 ± 0.5^{ab}	4.5 ± 0.8^{ab}
TG (mg/dL)	61 ± 12^{a}	76 ± 13^{b}	57 ± 12^{a}	64 ± 15^{ab}	55 ± 11^{a}
TC (mg/dL)	62 ± 7^{a}	76 ± 13^{b}	64 ± 9^{a}	71 ± 10^{ab}	60 ± 8^{a}
HDL-C (mg/dL)	14.4 ± 1.6^{a}	15.7 ± 2.7^{a}	14.8 ± 2.1^{a}	15.0 ± 1.8^{a}	14.5 ± 2.8^{a}
LDL-C (mg/dL)	41 ± 7^{a}	57 ± 11^{b}	43 ± 7^{a}	50 ± 9^{ab}	40 ± 7^{a}
			Liver		
Weight (g)	10.8 ± 0.9^{a}	12.7 ± 1.4^{b}	11.2 ± 1.2^{c}	13.1 ± 1.7^{b}	11.6 ± 1.7^{bc}
TG (mg/g liver)	103 ± 11^{a}	230 ± 57^{b}	164 ± 33^{c}	209 ± 71^{b}	176 ± 44^{bc}
TC (mg/g liver)	49 ± 4^{a}	57 ± 7^{b}	57 ± 4^{b}	58 ± 8^{b}	56 ± 9^{b}

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglyceride.
Rats were fed a normal diet (C), HFD, or HFD with 500 mg/kg T1, T2, and T3 complex for 8 weeks ($n = 10$ for each group). Values from 10 rats are expressed as mean ± standard deviation; means without a common letter in a row differ significantly ($p < 0.05$).

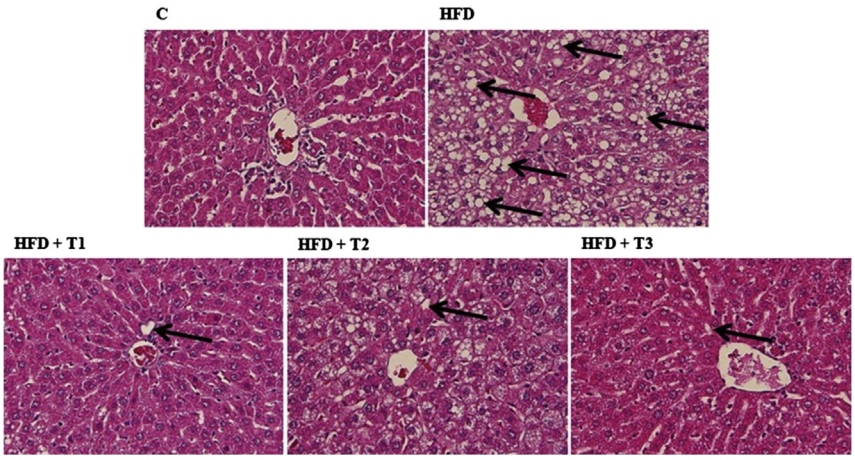

Figure 2 – Effects of three herbal mixture extracts on hepatic vacuoles in high-fat diet (HFD)-fed rats at the end of experiment. Rats were fed a normal diet (C), HFD, or HFD with 500 mg/kg of T1, T2, and T3 complex for 8 weeks. Histopathological analysis of liver tissues by hematoxylin and eosin stain.

Table 6 – Comparison of three herbal mixture extracts on obesity-related parameters in HFD-fed rats.

	T1	T2	T3		T1	T2	T3
Body weight	+	–	–	Plasma TG	++	–	++
Viscera fat tissues	++	–	++	Plasma TC	++	–	++
Subcutaneous fat tissues	++	–	++	Plasma LDL-C	++	–	++
Body fat index	++	–	++	Liver weight	++	–	+
Adiponectin	++	–	+	Liver TG	++	–	+

++ = significant effect; + = a tendency but not a significant effect; – = no effect.
HFD = high-fat diet; LDL-C = low-density lipoprotein cholesterol; TG = triglyceride.

3.6. *Comparison of three herbal mixture extracts on obesity-related parameters in HFD-fed rats*

Among these three herbal mixture extracts, T1 complex exhibited the highest antiobesity activity in HFD-fed rats as summarized in Table 6. Interestingly, T2 complex did not affect obesity-related parameters in HFD-fed rats (Table 6). We ruled out that the matrix components, including Chenpi and Shanzha, are involved in the modulation of obesity, although Chenpi and Shanzha have been shown to exhibit antiobesity potential [10] and hypolipidemic activity [11], respectively. We considered that the active components, such as Tengcha in T1 complex and EGCG in T3 complex, may play an important role in antiobesity activity in HFD-fed rats.

3.7. *Marker compounds content in three herbal mixture extracts*

We speculated that dihydromyricetin, salvianolic acid B, and EGCG can function as marker compounds with contents of 18.25 ± 0.07% (Figures 3A and 3B), 22.20 ± 0.10% (Figures 3C and 3D), and 18.86 ± 0.04% (Figures 3E and 3F) in T1, T2, and T3 complex, respectively. Dihydromyricetin, one of the flavonoids, is the major bioactive component in Tengcha with several biological functions, including antioxidation, anti-inflammation, hepatoprotective activity, and regulation of plasma lipids [28]. Similarly, our results also revealed that the dihydromyricetin-rich T1 complex exhibited hypolipidemic effects in HFD-fed rats (Table 5). This study may provide a clue for dihydromyricetin, which can be regarded as a novel compound for antiobesity treatment.

Salvianolic acid B is a water-soluble compound in Danshen with protective effects in the cardiocerebrovascular system and therapeutic effects against HFD-induced obesity in mice [29]. However, we found that the salvianolic acid B-rich T2 complex did not affect obesity-related parameters in HFD-fed rats. EGCG, the most abundant catechin in green tea, has been shown to have antiobesity activity [14]. Consistently, the EGCG-rich T3 complex has the potential to reduce fat tissue accumulation and improve abnormal plasma lipid profile in HFD-fed rats.

3.8. *Conclusion*

In this study, we demonstrated that herbal mixture extracts, especially T1 complex (the mixtures of Chenpi, Shanzha, and Tengcha), effectively improve HFD-induced obesity and hepatosteatosis in rats. Further studies are necessary to determine the exact synergistic effects of herbal mixture extracts as an antiobesity and hepatoprotective agent.

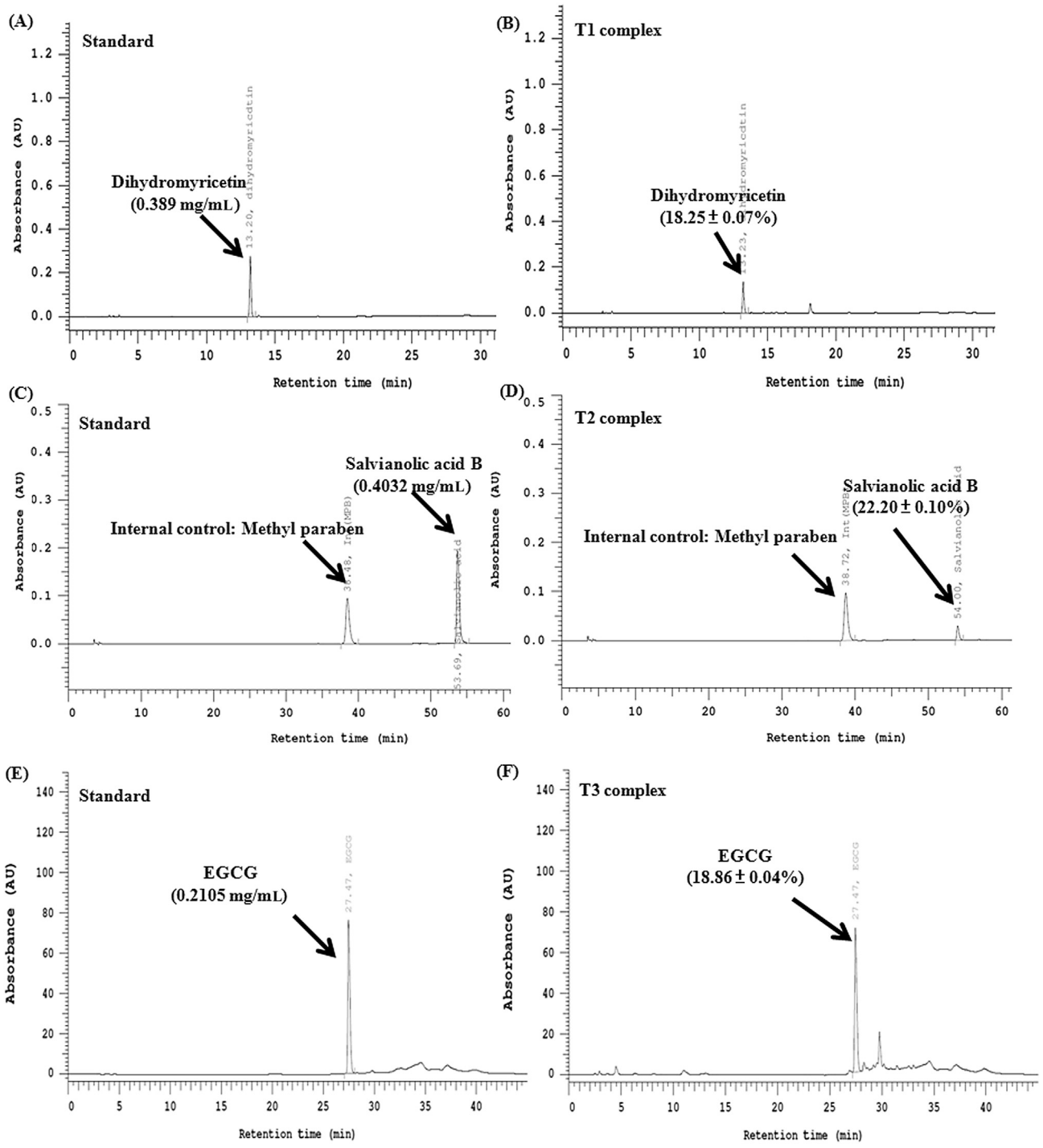

Figure 3 – HPLC chromatogram of marker compounds in three herbal mixture extracts. (A) HPLC chromatogram of standard dihydromyricetin (0.389 mg/mL). (B) HPLC chromatogram of dihydromyricetin in T1 complex. (C) HPLC chromatogram of standard salvianolic acid B (0.4032 mg/mL). (D) HPLC chromatogram of salvianolic acid B in T2 complex. (E) HPLC chromatogram of standard EGCG (0.2105 mg/mL). (F) HPLC chromatogram of EGCG in T3 complex. EGCG = epigallocatechin-3-gallate; HPLC = high-performance liquid chromatography.

Conflicts of interest

We declare no conflict of interest involved in this study.

Acknowledgments

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