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Hibiscus sabdariffa extract inhibits obesity and fat accumulation, and improves liver steatosis in humans

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Obesity is associated with a great diversity of diseases including non-alcoholic fatty liver disease. Our previous report suggested that *Hibiscus sabdariffa* extracts (HSE) had a metabolic-regulating and liver-protecting potential. In this study, we performed a clinical trial to further confirm the effect of HSE. Subjects with a BMI ≧ 27 and aged 18–65, were randomly divided into control ($n = 17$) and HSE-treated ($n = 19$) groups, respectively, for 12 weeks. Our data showed that consumption of HSE reduced body weight, BMI, body fat and the waist-to-hip ratio. Serum free fatty acid (FFA) was lowered by HSE. Anatomic changes revealed that HSE improved the illness of liver steatosis. Ingestion of HSE was well tolerated and there was no adverse effect during the trial. No alteration was found for serum α-amylase and lipase. The clinical effect should mainly be attributed to the polyphenols of HSE, since composition analysis showed that branched chain-amino acids, which is associated with obesity, is not obviously high. In conclusion, consumption of HSE reduced obesity, abdominal fat, serum FFA and improved liver steatosis. HSE could act as an adjuvant for preventing obesity and non-alcoholic fatty liver.

1. Introduction

Obesity, which is generally characterized by overweight, high body mass index (BMI), and fat deposition, is prevalent in most industrialized countries and even in developing countries.[1,2] The same trend of obesity was noted in Taiwan, which is associated with reduced life expectancy and the increased mortality of cardiovascular disease, cancer, and other related diseases.[3–7]

Accumulation of total body fat and/or abdominal fat, high triacylglycerol (TG), high low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C) and free fatty acid flux (FFA), are common symptoms in metabolic syndrome patients.[8] Since low density lipoprotein (LDL) clearance and high density lipoprotein (HDL) recruitment in the liver regulate the plasma lipid level, liver steatosis must be considered as an important factor in the pathogenesis. The observation of liver disorder is often measured by elevated serum aspartate transaminase (AST), alanine transaminase (ALT), and ultrasonic images.[9]

Hibiscus sabdariffa L. (Malvaceae), whose calyx is used worldwide as a cold or hot beverage, is beneficial for the prevention and treatment of many diseases such as hypertension, inflammation and liver disease.[10–12] In our previous report, *H. sabdariffa* extract (HSE) inhibited low-desity lipoprotein (LDL) oxidation *in vitro* and decreased serum cholesterol levels in cholesterol-fed rats and rabbits.[13] HSE capsule reduced serum cholesterol in human subjects.[14]

The present study performed a clinical trial to further confirm the HSE effect on metabolic regulation. We aimed to observe the effect of HSE on obesity, body fat, waist circumstance, serum lipid profiles, thus prevent the occurrence of fatty liver in obese subjects.

2. Methods and materials

2.1. HSE Capsules

The HSE capsules were prepared from *H. sabdariffa* L. The dried flowers were macerated in hot water (95 °C, 6000 L) for 2 hours, and the aqueous extract was evaporated under vacuum at −85 °C. The extracted solution was filtered and then lyophilized to obtain 75 g of HSE, and then stored at 4 °C before use. The total anthocyanins content in the HSE was determined using the Fuleki and Francis method.[15] The total flavonoids content was determined using the method reported by Jia *et al.*, using rutin as a standard.[16] The final extract was composed of 1.43%

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flavonoids, 2.5% anthocyanins and 1.7% phenolic acid, as measured by quantitative analysis. Amino acids of HSE were determined by automated ion-exchange chromatography.[17] One dose of a HSE treatment capsule contains 450 mg HSE extract and 50 mg starch. The placebo dose treatment contained 500 mg starch.

2.2. Subjects

This study was approved and executed with the permission of the Institute Review Board of Chung Shan Medical University Hospital (CSMUH no.: CS08028). All participants gave informed consent in writing. In this project males or non-pregnant females aged 18–65 with BMI $\geqq$ 27 (the criteria of obesity of the Department of Health in Taiwan), fatty liver and not under a course of treatment were recruited. Those who had one of the following were excluded: a drinking habit ($\geqq$20 g alcohol daily), ALT 3-fold higher or bilirulin above 2 mg dL^{-1}, kidney dysfunction, cardiovascular disease, endocrine or severe systemic disturbance, mental disorder or taking any OTC or prescribed medication and nutraceutics. Forty subjects fulfilled the above criteria and were recruited for the study (Table 1).

2.3. Study design

The study was conducted from July 2007 to June 2009. Before and after the experiment, the basal serum parameters (glucose, TG, cholesterol, LDL-C, HDL-C, FFA, AST and ALT), BMI, waist-to-hip ratio, body fat and the fatty liver score (FS, described below) were measured as curative indexes. The subjects were double-blinded, randomized and divided into 2 groups (20 subjects in each): one taking a 2 HSE capsule-dose after meals, 3 times a day, and the other the placebo, respectively. Body weight, body fat, and the waist-to-hip ratio were measured at week 0 and 12. Serum parameters and safety evaluations, including creatine kinase (CK), gamma-glutamyl transpeptidase (γ-GT), blood urea nitrogen (BUN), creatinine (CRE), albumin (ALB), uric acid, blood and urine were also measured. The recruited subjects were asked to take 3-day records of daily meals and physical activity at each of the time points before the trial, first 6 weeks and last 6 weeks, respectively. At the end of the study, 36 subjects had completed all the experiments: 17 in the control (9 males and 8 females) and 19 (12 males and 7 females) in the HSE group. 4 subjects did not adhere to the following appointments thus withdrew from the trial.

Table 1 Baseline demographic data of the subjects[a]

	HSE group ($N = 19$)	Control group ($N = 17$)	p value
Biometrics			
Age (y/o)	37.32 ± 8.61	38.59 ± 10.51	0.692
Height (m)	1.67 ± 0.08	1.66 ± 0.09	0.591
Body weight (kg)	88.52 ± 15.96	84.93 ± 12.79	0.465
BMI (kg m^{-2})	31.51 ± 4.01	30.91 ± 3.71	0.641
Body fat (%)	37.37 ± 6.22	38.44 ± 9.80	0.696
Waistline (cm)	98.00 ± 11.75	95.32 ± 10.43	0.477
Hip (cm)	107.66 ± 7.49	106.32 ± 8.00	0.609
W/H	0.91 ± 0.07	0.90 ± 0.06	0.554
Diabetes indicators			
TCHO (mg dL^{-1})	213.47 ± 28.88	207.53 ± 42.38	0.623
LDL-c (mg dL^{-1})	132.63 ± 24.68	126.29 ± 37.11	0.546
HDL-c (mg dL^{-1})	44.63 ± 8.46	43.35 ± 6.12	0.611
TG (mg dL^{-1})	172.32 ± 71.60	190.29 ± 102.57	0.543
FFA (U min^{-1} $mg_{protein}^{-1}$)	0.81 ± 0.27	0.83 ± 0.35	0.823
Glucose (mg dL^{-1})	106.58 ± 22.53	106.71 ± 13.13	0.984
Hepatic function			
ALT (U L^{-1})	57.21 ± 35.45	35.47 ± 24.04	0.033*
AST (U L^{-1})	33.05 ± 17.82	23.18 ± 9.34	0.049*
F S	5.21 ± 1.72	4.82 ± 2.22	0.560

[a] TCHO: total cholesterol, W/H: waist-to-hip ratio. Data are presented as mean ± SD and analyzed by the Student t-test. $p < 0.05$ was considered statistically significant.

2.4. Serum parameters and safety evaluations

Serum glucose, TG, total cholesterol, LDL-C, HDL-C, AST, ALT, BUN, CK, γ-GT, CRE, ALB, amylase, lipase and uric acid were analyzed on a Beckman Synchron CX9 clinical system. FFA was analyzed using a Free Fatty Acid Quantification Kit (ab65341, abcam).

2.5. Body fat and waist-to-hip ratio

The body fat and waist-to-hip ratio in this work were measured with a Tanita TBF-300GS analyzer. The waist-to-hip ratio was calculated using the waist circumference (just above the upper hip bone) divided by the hip circumference at its widest part.

2.6. Ultrasonic image and fatty liver scores (FS)

Liver ultrasonic imaging was applied using the Aloka system (Prosound SSD-4000, with 5.0 MHz convex transducer). The fatty liver characteristic evaluations included hepatic clearance, far gain attenuation and opaqueness of the bladder wall, portal area and hepatic vein. Each item was classified as 0 = normal, 1 = mild to moderate and 2 = severe. The FS in this research was presented as the sum of these five items. The estimation of sample size: we designed the type I error (α) is 0.05, the type II error (β) is 0.2, therefore the power of this study is 0.8. The main target of the study is fatty liver score (FS), we could expect the FS of the HSE treatment group to go from 6 down 4.5, and the FS of the placebo group from 6 down to 5.5. Due to the standard deviation (SD) being equal to 1, and the expected withdraw rate of 20%, therefore, this study needs 40 participants to examine the hypothesis.

2.7. Statistical analysis

Using an unpaired Student's t-test for the control and HSE-treated groups, and a paired Student's t-test for the pre- and post-trial, a p value of less than 0.05 was considered statistically significant. All the analyses were performed with SigmaPlot 11.0.

3. Results

3.1. HSE reduced body weight and BMI

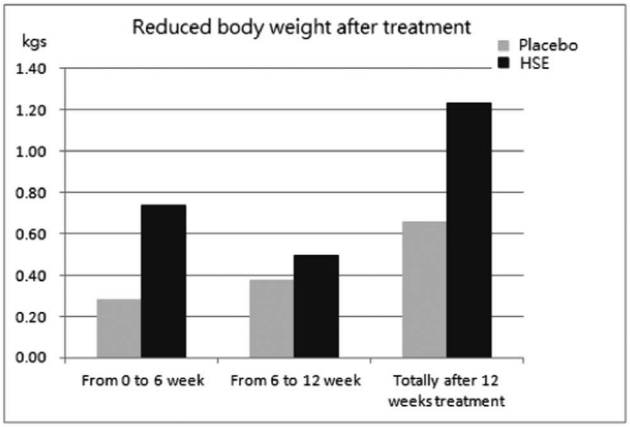
Before the trial, the body weight and BMI showed no significant difference between the HSE and control groups (Table 1). After 12 weeks of treatment, the body weight and BMI significantly decreased in the HSE group (88.53 ± 15.96 kg to 87.28 ± 16.02 kg, $p < 0.008$; 31.51 ± 4.01 kg m^{-2} to 31.09 ± 4.23 kg m^{-2}, $p < 0.009$) (Table 2). The reduction in body weight and the percentage of change during the trial is shown in Fig. 1, indicating that about 60% of the weight change occurred between 0–6 weeks. Almost 70% of the HSE-treated subjects had a reduced body weight and BMI (data not shown).

3.2. HSE reduced body fat and waist-to-hip ratio

During the trial, significant alterations in body fat existed neither in the HSE nor control groups. However, by the end of the treatment, HSE showed a significant effect on the pre–post difference compared with the control group. The waist-to-hip ratio of the HSE group significantly decreased from 0.91 ± 0.07 to 0.90 ± 0.06 ($p < 0.01$), which could be attributed to the lowering of the waist circumference. No alteration was found in the control group (Table 2).

3.3. HSE decreased serum FFA

About 63% of the HSE-treated subjects showed a reduced level of FFA (data not shown). The serum FFA level of the HSE group decreased from 0.81 ± 0.27 to 0.64 ± 0.24 ($p = 0.025$), whereas no alteration was found in the control (Table 2). By the end of



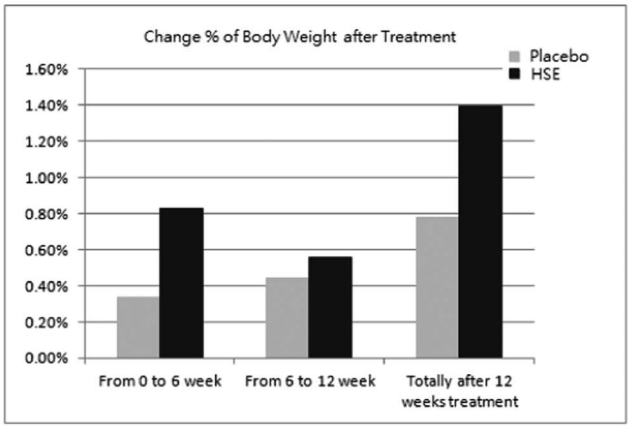


Fig. 1 The reduction in body weight (kg) and percentage of change at 0–6 weeks, 6–12 weeks, and 0–12 weeks.

Table 2 Treatment effects[a]

	HSE group ($N = 19$)			Control group ($N = 17$)			
	0 week	12 week	p value*	0 week	12 week	p value*	p value †
Biometrics							
Body weight (kg)	88.52 ± 15.96	87.28 ± 16.02	0.008*	84.93 ± 12.79	84.27 ± 13.14	0.086	0.307
BMI (kg m^{-2})	31.51 ± 4.01	31.09 ± 4.23	0.009*	30.91 ± 3.71	30.65 ± 3.76	0.062	0.396
Waist (cm)	98.00 ± 11.75	97.16 ± 10.94	0.089	95.32 ± 10.43	95.94 ± 10.25	0.222	0.038 †
Hip (cm)	107.66 ± 7.49	108.13 ± 7.05	0.281	106.32 ± 8.00	106.76 ± 8.25	0.311	0.957
W/H ratio	0.91 ± 0.07	0.90 ± 0.06	0.010*	0.90 ± 0.06	0.90 ± 0.06	0.571	0.026 †
Body fat (%)	37.37 ± 6.22	36.67 ± 6.61	0.16	38.44 ± 9.80	39.08 ± 9.82	0.144	0.044 †
Diabetes indicators							
TCHO (mg dL^{-1})	213.47 ± 28.88	209.68 ± 31.66	0.507	207.53 ± 42.38	209.88 ± 36.66	0.707	0.464
LDL-c (mg dL^{-1})	132.63 ± 24.68	133.16 ± 26.31	0.922	126.29 ± 37.11	128.88 ± 21.79	0.682	0.802
HDL-c (mg dL^{-1})	44.63 ± 8.46	44.58 ± 8.41	0.967	43.35 ± 6.12	44.76 ± 5.29	0.264	0.411
TG (mg dL^{-1})	172.32 ± 71.60	154.47 ± 52.87	0.114	190.29 ± 102.57	167.82 ± 103.68	0.025*	0.747
FFA (U min^{-1} mg$_{protein}^{-1}$)	0.81 ± 0.27	0.64 ± 0.24	0.025*	0.83 ± 0.35	0.89 ± 0.49	0.421	0.026 †
Glucose (mg dL^{-1})	106.58 ± 22.53	111.84 ± 26.42	0.365	106.71 ± 13.13	108.12 ± 9.62	0.425	0.540
Hepatic function							
ALT (U L^{-1})	57.21 ± 35.45	55.63 ± 35.62	0.741	35.47 ± 20.04	28.94 ± 11.69	0.075	0.410
AST (U L^{-1})	33.05 ± 17.82	31.11 ± 17.25	0.427	23.18 ± 9.34	19.53 ± 3.97	0.062	0.583
FS	5.21 ± 1.72	4.42 ± 2.01	0.018*	4.82 ± 2.22	4.06 ± 2.42	0.043*	0.957

[a] Data are presented as mean ± SD and analyzed by paired t test. * $p < 0.05$ indicates the significance of each difference at 12 week compared with the baseline. † $p < 0.05$ indicates the significance of each 12–0 week difference between the control and HSE-treated groups.

the treatment, no other significant change of lipid profile existed between the HSE and control groups.

3.4. HSE improved the FS

In the HSE-treated subjects, the FS was significantly decreased by about 15%, from 5.21 ± 1.72 to 4.42 ± 2.01 ($p = 0.018$) (Table 2). No significant alteration was found in the AST or ALT levels in both the HSE and control groups. Also, the pre–post differences between the two groups were not significant.

3.5. HSE did not change the safety evaluation markers

The safety evaluation markers almost remained the same during the trial, except for ALB and BUN. Serum ALB slightly decreased in the HSE group. BUN decreased in both the HSE and control groups, while the pre-post difference was significant only in the control group (Table 3). Noticeably, α-amylase and lipase was not altered, implying the safety and lack of metabolic side effects on the pancreas.

4. Discussion

In the present study, we demonstrated the anti-obesity and liver-protection potential of HSE. HSE decreased body weight, BMI and body fat, and reduced abdominal fat distribution. HSE decreased serum FFA, exerting a beneficial effect on metabolic regulation, while improving the liver steatosis. Noticeably, the safety evaluation revealed that HSE did not harm the human body. This is the first study to investigate HSE for the attenuation of human obesity and fatty liver.

BMI is one of the most popular anthropometric indices. In 2000, WHO defined the BMI cut-off points as 23 kg m^{-2} (overweight), 25 kg m^{-2} (obesity class I), and 30 kg m^{-2} (obesity class II) for people living in the Asia Pacific region. All the subjects in the trial had a BMI > 25 kg m^{-2} (obesity class I) and had been diagnosed with fatty liver for more than one year. However, although the BMI is widely used and adopted in this study, it may still have limitations. For some populations who have shorter lower limbs, using standing height alone may overestimate the number of individuals that are overweight and obese, and at risk for type 2 diabetes mellitus and cardiovascular disease.[18]

On the contrary, central obesity predicts a high prevalence of hepatic steatosis and related disorders. A previous analysis revealed that the waist circumference and waist/height ratio had a significant association with the development of fatty liver, whereas the BMI did not. In this study, we measured the waist circumference and used the waist-to-hip ratio as an index, which should more adequately reflect the regulatory effect of HSE on abdominal fat distribution and central obesity.[19]

Non-alcoholic fatty liver is generally considered to be the liver component of metabolic syndrome, including an excessive waist circumference, dyslipidaemia, hyperglycaemia, and hypertension.[20] In a clinical situation, the ultrasonic examination of fatty liver is usually qualitative but not quantitative. To overcome this limitation, we cited and mimicked the semi-quantitative FS scores, and demonstrated the effect of HSE on improving fatty liver.[21]

H. sabdariffa improved the lipid profiles of patients with metabolic syndrome.[22] Recently, HSE was reported to prevent hepatic steatosis through down-regulation of PPAR-γ and SREBP-1c, which plays an important role in obesity-induced inflammation, especially in the liver, adipose tissue, and vascular system.[23,24] According to the previous report, the calyx of *H. sabdariffa* L. is rich in polyphenols, including anthocyanins, flavonoids and phenolic acids.[25] *H. sabdariffa* polyphenols prevented hyperglycemia and hyperlipidemia, inhibited hepatic lipogenesis, while they promoted hepatic lipid clearance.[26,27] Many of them, such as gallic acid derivative, chlorogenic acid, caffeic acid, quercetin, and tiliroside, were demonstrated to be effective on reducing obesity and related disorders (Table 4). Galloyl ester decreased the body weight, liver weight, and

Table 3 Safe evaluation markers[a]

	HSE group ($N = 19$)			Control group ($N = 17$)		
	0 wk	12 week	p value	0 week	12 week	p value
WBC	7.56 ± 1.47	7.28 ± 0.97	0.328	7.70 ± 1.34	7.77 ± 1.66	0.811
RBC	5.11 ± 0.44	5.09 ± 0.44	0.68	5.13 ± 0.55	5.04 ± 0.62	0.211
HB	15.22 ± 1.38	15.14 ± 1.42	0.462	14.74 ± 1.52	14.51 ± 1.74	0.255
CK (U L^{-1})	46.42 ± 14.47	46.21 ± 19.73	0.97	46.18 ± 12.81	46.70 ± 17.10	0.92
r-GT (U L^{-1})	49.26 ± 45.39	50.05 ± 40.00	0.812	40.88 ± 32.57	35.65 ± 26.08	0.051
ALB (g dL^{-1})	4.55 ± 0.33	4.42 ± 0.25	0.004*	4.31 ± 0.94	4.43 ± 0.23	0.554
BUN (mg dL^{-1})	13.21 ± 2.44	12.26 ± 2.90	0.098	12.41 ± 1.77	10.88 ± 2.17	0.035*
CRE (mg dL^{-1})	1.00 ± 0.20	1.01 ± 0.19	0.63	0.97 ± 0.19	0.94 ± 0.20	0.311
UA (mg dL^{-1})	6.99 ± 1.56	7.04 ± 1.56	0.837	6.57 ± 0.37	6.27 ± 0.34	0.202
TSH (μIU mL^{-1})	1.69 ± 1.00	1.67 ± 1.05	0.916	1.75 ± 1.07	1.80 ± 0.79	0.837
Free T4 (ng dL^{-1})	1.10 ± 0.13	1.08 ± 0.11	0.598	1.09 ± 0.13	1.04 ± 0.13	0.138
Urine PH	6.32 ± 0.56	6.16 ± 0.44	0.301	6.38 ± 0.45	6.44 ± 0.68	0.773
Amylase (U L^{-1})	69.32 ± 21.01	66.95 ± 16.94	0.704	67.35 ± 15.20	72.47 ± 20.83	0.413
Lipase (U L^{-1})	33.02 ± 9.88	35.55 ± 13.68	0.517	34.73 ± 9.53	33.07 ± 7.99	0.589

[a] WBC: white blood cell, RBC: red blood cell, Hb: hemoglobulin, TSH: thyroid stimulating hormone, T4: thyroxine, UA: uric acid. Data are presented as mean ± SD and analyzed by paired t-test. *$p < 0.05$ indicates the significance.

Table 4 Functional ingredients of HSE on reducing obesity and hepatic disorder

Ingredient	Effect	Ref.
Galloyl ester	Decrease body weight	28
	Decrease hepatic lipid	28
Chlorogenic acid	Decrease body weight	30
	Regulate obesity-related hormone	30
	Regulate lipid metabolism	30
	Attenuate fatty liver	29
Caffeic acid	Decrease body weight	30
	Regulate obesity-related hormone	30
	Regulate lipid metabolism	30
	Inhibit hepatic lipogenesis	31
	Promote hepatic lipolysis	31
Quercetin	Attenuate fatty liver	32
	Inhibit adipocyte differentiation	33
	Induce adipocyte apoptosis	33
Tiliroside	Regulate obesity-related hormone	34
	Promote hepatic lipolysis	34
Anthocyanines	Decrease body weight	35–37
	Decrease body fat	35 and 36
	Improve serum and liver lipid profiles	37
	Ameliorate impaired hepatic function	37

Table 5 Amino acid composition of HSE

Amino acid	Content (mg/100 g)
Aspartic acid	1811.90
Threonine	nd[a]
Serine	106.41
Glutamic acid	227.31
Glycine	123.61
Alanine	109.74
Cysteine	48.30
Valine	49.21
Methionine	13.35
Isoleucine	42.19
Leucine	75.34
Tyrosine	27.94
Phenylalanine	73.68
Lysine	137.05
Histidine	58.41
Arginine	68.81
Proline	53.14
Total	3026.11

[a] nd: not detected.

hepatic lipid.[28] Chlorogenic acid lowered serum cholesterol and attenuated fatty liver by up-regulating the expression of PPAR-α.[29] Chlorogenic acid and caffeic acid improved body weight, lipid metabolism and obesity-related hormone levels in high-fat fed mice.[30] Recently, it was reported that caffeic acid inhibits hepatic lipogenesis but promotes lipolysis *via* regulating AMPK in HepG2 cells.[31] Non-alcoholic fatty liver disease rats (NAFLD) have higher serum levels of IL-18 but lower levels of IL-10 than their healthy counterparts. Quercetin treatment reversed the cytokine expressions and helped to delay the progression of NAFLD.[32] An *in vitro* experiment showed that quercetin exerts anti-adipogenesis activity by activating the AMPK signal pathway in 3T3-L1 preadipocytes, while it induces the apoptosis of mature adipocytes by modulation of the ERK and JNK pathways.[33] Tiliroside, a glycosidic flavonoid, ameliorates hyperinsulinemia and hyperlipidemia in obese-diabetic mice by activating adiponectin signaling and the hepatic lipid oxidation.[34] In addition, anthocyanins contained in *H. sabdariffa* L. could exert anti-obesity and liver-protective effects. It was reported that purified anthocyanins reduced the body weight and body fat of rats fed with a high-fat diet.[35] Recently, Wu *et al.* reported that anthocyanins inhibit body weight gain, reduce insulin resistance, increase serum adiponectin while decrease leptin, lower the adipocytes and lipid accumulation, improve serum and liver lipid profiles, and ameliorate the impaired hepatic function in diet-induced obese mice.[36,37] Under normal circumstances, anthocyanins even have the capability to reduce body weight and food intake through its modulation of NPY and GABAB1R in the hypothalamus.[38] Some literature has reported that branched chain-amino acids are associated with obesity and insulin resistance.[39] We have analyzed the amino acid composition of HSE (Table 5), whereas only aspartic is obviously high. Hence the clinical effect of HSE should mainly be attributed to the polyphenols.

In this trial, after HSE treatment, no significant difference was observed in the lipid profile except for FFA. These results are in accordance with that of Kuriyan, Kumar and Kurpad (2010),[40] which might attribute to the dose of HSE (1 g day^{-1}) being too low. The optimum dose of HSE intake should be determined in future clinical work. Further research on the bioavailability and pharmacokinetics of HSE is needed. In conclusion, HSE has the potential to act as an adjuvant for preventing obesity and related fatty liver.

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