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Anti-Obesity Effect and the Associated Amelioration of Glucose Intolerance and Hepatic Steatosis of an Herbal Formula (SSEM) on High-Fat Diet Fed Mice

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Abstract

Traditional Chinese medicine has a long history of application in treating diseases characterized by metabolic dysregulation. The present investigation sought to determine if the herbal formulation SSEM could ameliorate the pathological manifestations of metabolic syndrome in a murine model of high-fat diet (HFD)-induced obesity. Our results demonstrated that concurrent administration of SSEM significantly attenuated body weight gain and reduced adiposity, which was accompanied by improved glucose tolerance. The HFD-induced increases in hepatic lipids were also reduced. The proposed mechanism of action suggests that SSEM exerts its beneficial effects by reducing insulin resistance, likely through decreased adiposity, thereby potentiating insulin-stimulated glucose uptake in skeletal muscle. Therefore, SSEM offers a potential means for ameliorating glucose intolerance and hepatosteatosis, particularly those arising from excessive fat accumulation.

Keywords

Herbal Formulation, Anti-Obesity, Metabolic Syndrome

1. Introduction

Metabolic syndrome (MetS), classified by the World Health Organization (WHO) as a non-communicable disease, is a cluster of conditions that significantly increase the risk of heart disease, stroke, and type 2 diabetes (T2D) [1]. Its defining characteristics include abdominal obesity, insulin resistance, glucose intoerance, fatty liver disease (hepatosteatosis), hyperlipidemia, and hypertension [2]. While

historically prevalent in Western populations, MetS is now experiencing a more rapid rise in urban areas of developing countries, often exceeding the prevalence observed in their Western counterparts [3].

The incidence of MetS is strongly correlated with non-alcoholic fatty liver disease (NAFLD), obesity, and T2D, and its prevalence generally increases with age. Data from the U.S. Centers for Disease Control and Prevention (CDC) in 2024 indicated that approximately 14.7% of U.S. adults had T2D, with this figure rising to 29.2% among seniors [4]. Crucially, this data also revealed that MetS (including prediabetes) was three times more prevalent than diagnosed T2D, suggesting that roughly one-third of U.S. adults may have MetS [4]. The global obesity epidemic further exacerbates this issue, with obesity prevalence having doubled in 73 countries and increased in most others since 1980 [5]. Projections from the International Diabetes Federation (IDF) atlas estimate that the global prevalence of diabetes will reach 10.4% of the world's population by 2040, affecting 642 million people. Extrapolating the observed 3:1 ratio of MetS to T2D suggests that by 2040, nearly 2 billion individuals could be affected by MetS [6].

The primary drivers of MetS are increasingly attributed to the elevated consumption of calorie-dense, low-fiber fast food and a significant reduction in physical activity, largely due to mechanized transportation and sedentary leisure activities. Consequently, recommended preventive strategies for MetS focus on promoting increased exercise and adopting a well-balanced diet [7].

Over time, scientific research has highlighted the beneficial effects of specific food items and dietary patterns in mitigating the adverse impacts of MetS. For instance, studies have demonstrated that soy isoflavones, citrus products, and quercetin can improve lipid metabolism, while green tea has shown a notable ability to reduce body mass index (BMI) and waist circumference [8]-[10]. Furthermore, ketogenic and fasting-mimicking diet cycles have been observed to enhance insulin secretion from pancreatic beta cells and ameliorate both type 1 and type 2 diabetes phenotypes in preclinical mouse models [11] [12].

Given the compelling evidence for the beneficial effects of naturally occurring ingredients on MetS, the present research study aims to investigate the impact of a Chinese herbal formula, designated SSEM, on blood glucose regulation. This formula comprises four component herbs: Cynomorii Herba, Schisandrae Fructus, Eucommiae Folium, and Momordicae Fructus. The study will assess the effects of SSEM in both high-fat diet (HFD)-induced obese mice and cultured C2C12 myotubes.

2. Materials and Methods

2.1. Chemicals and Reagents

Animal diets: PicoLab Rodent Diet 20 model 5053 was used for normal diet (ND) (24% kcal from protein, 13.2% kcal from fat, and 62% kcal from carbohydrate; kcal per gram of diet was 3.02), and Research Diets Formula D12492 was used for high-fat diet (HFD) (20% kcal from protein, 60% kcal from fat, and 20% kcal from

carbohydrate; kcal per gram of diet was 5.24) in the animal model experiments. ND was purchased from LabDiet (St. Louis, MO, USA), and HFD was purchased from Research Diets, Inc. (New Brunswick, NJ, USA).

Cell culture reagents: Gibco Dulbecco's modified eagle medium (DMEM, high glucose), fetal bovine serum (FBS), heat-inactivated hours serum (HIHS) were purchased from Life Technologies Limited (Carlsbad, CA, USA). Sodium pyruvate (P) and sodium palmitate (SP) in were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) and TCI (Shanghai) Development Co., Ltd. (Shanghai, China). Free fatty acid bovine serum albumin (FFABSA) was bought from Gold Biotechnology®, Inc. (St. Louis, MO, USA). Sigma-Aldrich-brand (Burlington, MA, USA) insulin was used.

Kits: Glucose Liqui-UV kit (Hexokinase method), Glucose Liquicolor® kit, Glycohemoglobin kit, Triglyceride Liquicolor®kit and Cholesterol Liquicolor® kit were purchased from Stanbio Laboratory (Boerne, TX, USA). LifeScan OneTouch Ultra blood glucose monitoring system was obtained from Johnson & Johnson company (Milpitas, CA, USA).

Herbal formulation: SSEM consisted of four component herbs, namely Cynomorii Herba, Schisandrae Fructus, Eucommia Ulmoides, and Momordica Fructus. Standardized herbal extracts, namely, Cynomorium Herba extract (4:1; water: ethanol, v/v), Momordica Fructus extract (4:1; water: ethanol, v/v), Schisandra extract (4:1; water: ethanol, v/v), and Eucommia Floret extract (4:1; water: ethanol, v/v), were purchased from Shaanxi Jiahe Phytochem Co., Ltd. (Xi'an, China). They were mixed in a specific ratio and formed the SSEM.

2.2. Animal Care

Male ICR mice (8 weeks old) were obtained from the Laboratory Animal Facility at the Hong Kong University of Science and Technology (HKUST). Animals were maintained under a 12-hour dark/light cycle at approximately 22℃ and allowed food and water ad libitum. The experimental protocol was approved by the Animal Ethics Committee at HKUST (2018-016).

2.3. Animal Treatment

Eight-week-old male ICR mice were randomly divided into two control groups and two co-treatment groups, with 6 - 13 mice per group. A previous study found that having five animals in each experimental group was adequate for detecting differences in endpoint measurements compared to the control group [13]. Each group was fed either normal diet (ND) control (n = 6), HFD control (n = 11), HFD + 0.21 g/kg SSEM (n = 13), or HFD + 0.63 g/kg (n = 12) SSEM formulation. The control groups were fed ND or HFD ad libitum, while SSEM formulation in the co-treatment groups was delivered via intragastric administration simultaneously with HFD feeding for 8 weeks. Control mice received vehicle only. The low dose of SSEM (0.21 g/kg) was the human equivalent dose. Body weight was measured once a week during the 8 weeks. At the end of the experiment, the mice were

fasted overnight and sacrificed by cervical dislocation. Blood samples were then drawn using syringes containing 0.5% heparin in saline as an anticoagulant by cardiac puncture. Various fat pads, including posterior subcutaneous, epididymal, and mesenteric fat, were dissected and weighed. Samples of livers were excised and then subjected to further biochemical analysis. Biochemical analyses were done in a non-biased manner with established protocols. A blinding approach was not adopted in dosing, tissue processing, and endpoint readouts [13].

Food consumption per cage was measured weekly during the whole period of the experiment. Briefly, each cage was provided with 200 g of ND or HFD according to the assigned grouping. The weight of diet per cage consumed was calculated by (200-gram of diet left in the cage after a week). The food consumption (g of diet/mouse/day) = gram of diet per cage consumed/no. of mice in the cage/7. The endpoint food consumption of each group at week 8 (g of diet/mouse/day) was ND control = 5.48 ± 0.53; HDF control = 3.42 ± 0.19*; HFD + 0.21g/kg SSEM = 3.66 ± 0.79; HFD + 0.63 g/kg SSEM = 2.81 ± 0.17. If in terms of kcal of diet/mouse/day: ND control = 16.5 ± 1.60; HDF control = 17.9 ± 0.98; HFD + 0.21 g/kg SSEM = 19.2 ± 4.13; HFD + 0.63 g/kg SSEM = 14.7 ± 0.90.

2.4. Plasma Glucose and Lipids Measurement

Plasma samples were obtained by centrifuging the collected whole blood samples at 2000 × *g* for 10 min at 4˚C and then stored at 4˚C before use for biochemical analysis. Plasma glucose, cholesterol and triglyceride levels were determined using corresponding assay kits mentioned in Chemicals and Reagents section [13] [14].

2.5. Glucose Tolerance Measurement

After 8 weeks of feeding, oral glucose tolerance test (OGTT) was performed. Briefly, mice were fasted for 6 hours and intragastrically administered glucose (2 g/kg). Blood samples were then collected at 15, 30, 60, and 120-minute intervals from the tip of the tail vein and used to measure blood glucose using blood glucose monitoring system. The extent of glucose tolerance impairment was estimated by computing the area under the curve (AUC) of the graph plotting glucose concentrations against time [13] [14].

2.6. Glycated Hemoglobin (HbA1c) Measurement

Whole blood samples from the mice were hemolyzed, and HbA1c was determined using an assay kit (Stanbio Laboratory Glycohemoglobin) [13] [14].

2.7. Hepatic Lipid Contents Measurement

Liver homogenates were prepared with phosphate buffered saline (without calcium and magnesium, PBS-A) in 10% (w/v). The lipid content was extracted by methanol/chloroform method. The extracted content was dissolved in isopropanol for lipid measurements with Stanbio Laboratory kits mentioned in Chemicals and Reagents section [13] [14].

2.8. Cell Model

2.8.1. Reagents Preparation

SP was dissolved in 0.1M NaOH at 75 mM, then heated to 70˚C to dissolve. The intermediate stock of SP was then prepared with DMEM+ 1 mM P + 10% FFBSA in 5 mM. The working SP medium (0.75 mM) was prepared by dilution of intermediate stock with DMEM (+P) to induce insulin resistance in differentiated cells. Insulin was dissolved in 0.1 M HCl to make 100 μM stock solution. SSEM formulation was dissolved in DMSO to a stock concentration of 150 mg/mL.

2.8.2. C2C12 Myotubes

C2C12 mouse immortalized myoblast cell line was maintained in DMEM + 1 mM P + 10% HIFBS. Cell differentiation was initiated by differentiation medium containing DMEM + 1 mM P + 2% HI Heat-inactivated Horse Serum (HG2%HIHS). Then the working concentration of SSEM was prepared and diluted with medium (finally each group containing 0.2% DMSO (v/v)). The non-toxic concentration for SSEM was determined by crystal violet toxicity test with 24 h incubation, and the assay result indicated that 300 μg/mL of SSEM was non-toxic.

2.8.3. Glucose Uptake Assay

The C2C12 myotubes (passage 14 - 24 were used in this study) were seeded and grown in 6-well tissue culture plates for 3 days before being treated with HG2%HIHS over 4 days to differentiate the myoblasts into myotubules. The cells were dispensed into different treatment groups, namely, SSEM (0, 3, 10, 300 μg/mL), SSEM (0, 3, 10, 300 μg/mL) + SP (0.75 mM), SSEM (0, 3, 10, 300 μg/mL) in the presence of insulin, and SSEM (0, 3, 10, 300 μg/mL) + SP (0.75 mM) in the presence of insulin. All groups were first incubated with either SSEM only or SSEM + SP for 18 hours. After 18 hours, cells were washed with PBS-A before changing the glucose uptake assay medium. Insulin and non-insulin groups were then incubated with 100 nM of insulin (in DMEM without glucose, P and phenol red, all no DMEM) or all no DMEM, respectively for 10 minutes. An aliquot (4.8 μL) of 2-NBDG (stock: 25mM in DMSO, then 60 μM working) was then added to the wells of all samples before incubating for another 50 min at 37˚C in the dark. Samples were washed thrice with PBS-A, and trypsinized (with 400 μL 0.25 mM Trypsin with 0.53 mM Na2EDTA in PBS-A) at 37˚C for 10 minutes. The reaction was stopped with 300 μL PBS-A, and samples were collected into centrifuge tubes. Aliquots (200 μL) of the samples were put into wells of a black microplate, and then glucose uptake was analyzed with the Flex Station 3 plate reader (read three times, excitation at 468 nm, emission at 540 nm).

Protein content was measured to standardize the glucose uptake readings. Centrifuged the leftover of collected samples in 20,000 × *g* for 5 min, then removed the supernatant. The cell pellet was lysed with 200 μL 0.1% (v/v) TritonX in PBS-A and then with 10 min sonication. The cell debris was precipitated by centrifugation at 20,000 × *g* for 5 min. The supernatant was used for protein measurement by Braford assay.

The effect of SSEM on glucose uptake in myotubules was then estimated. One-way ANOVA with LSD test was performed to detect significant differences in glucose uptake in different experimental groups (0 μL SSEM, 0 μL SSEM in the presence of insulin, 0 μL SSEM + SP in the presence of insulin), as compared with the respective control group.

2.9. Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). Unless otherwise specified, data from different groups at the same time point were analyzed by one-way analysis of variance (one-way ANOVA), and intergroup differences were detected by post hoc LSD tests, with a value of $P < 0.05$.

3. Results

3.1. Effect of SSEM on Body Weight in Mice Fed ND or HFD

Weekly body weight measurements were taken over the 8-week experimental period. As presented in Figure 1, HFD-fed mice demonstrated a substantial (31.7%) and statistically significant increase in body weight compared to the incremental gain (8.33%) in ND-fed mice. Crucially, SSEM co-treatment, administered at either 0.21 g/kg or 0.63 g/kg, significantly mitigated the body weight escalation in HFD-fed mice throughout the 8-week study.

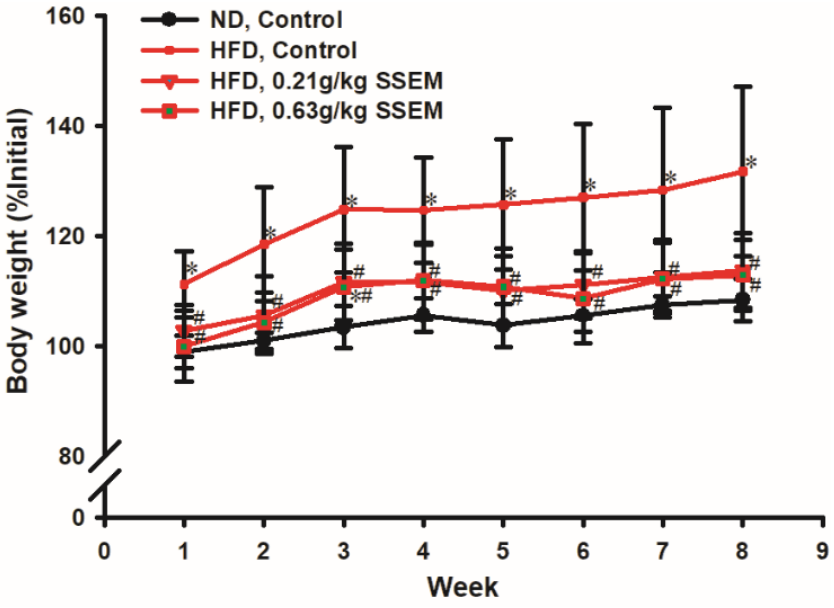


Figure 1. Effect of SSEM on body weight in HFD-fed mice. Data were expressed as a percentage of initial body weight. Each data point represents mean ± standard deviation (SD), with n = 6 - 13 for each group. *Significantly different from the ND control; # Significantly different from the HFD control.

SSEM co-treatment (0.21 or 0.63 g/kg) completely prevented HFD-induced weight gain during the whole course of the experiment, with no significant differ-

ence when compared with that of ND-fed mice. There was no dose-dependent effect of SSEM on the body weight in HFD-treated mice, with significant suppression of HFD-induced weight gain by 76.9 and 80.5% at doses of 0.21 and 0.63 g/kg, respectively, by the end of the 8th week.

The HFD-induced body weight gain was independent of food consumption volume, given that HFD mice consumed less food than their ND counterparts. Additionally, SSEM administration did not alter feeding behavior in HFD mice (data not shown), indicating its efficacy in mitigating weight gain is driven by metabolic modulation rather than appetite suppression.

3.2. Effect of SSEM on Fat Pad Indices in Mice Fed ND or HFD

HFD feeding markedly increased the posterior subcutaneous, epididymal, and mesenteric fat indices by 290, 282, and 365%, respectively. In HFD-fed mice, SSEM treatment significantly reduced posterior subcutaneous fat and visceral fat (epididymal plus mesenteric) by 67.1 and 66.7 at 0.21 g/kg, and by 58.7 and 47.8% at 0.63 g/kg, respectively, resulting in overall reductions in total fat mass of 66.9 and 51.1% (Figure 2).

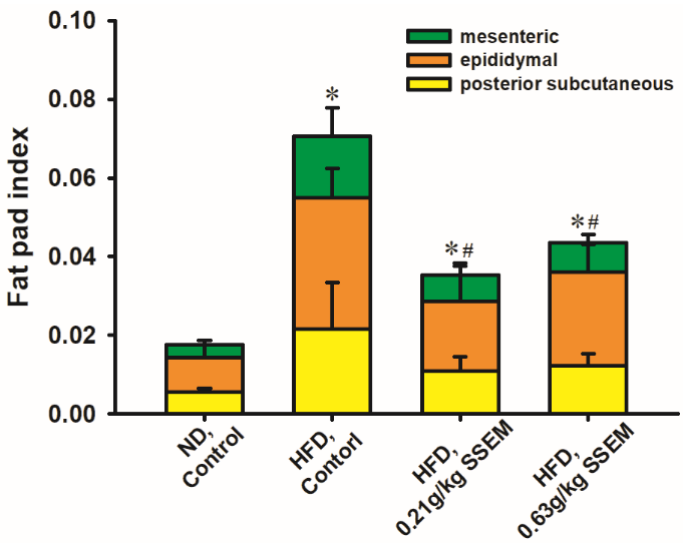


Figure 2. Effect of SSEM on adiposity in HFD-fed mice. Posterior subcutaneous fat, epididymal fat, and mesenteric fat were weighed. Values given are means ± SD, with n = 6 - 13. *Significantly different from the ND control; #Significantly different from the HFD control.

3.3. Effect of SSEM on OGTT in Mice Fed ND or HFD

The effect of SSEM co-treatment on glucose tolerance in HFD-fed mice was assessed via an OGTT performed at the 8-week mark of the experiment. Figure 3 illustrates that HFD feeding led to substantial glucose intolerance compared to ND-fed controls. Crucially, SSEM co-treatment achieved a complete reversal of this diet-induced glucose intolerance. The AUC values in HFD-fed mice receiving SSEM were reduced to 70.8 and 72.7% of the untreated HFD group at SSEM doses

of 0.21 and 0.63 g/kg, respectively, with the values not being different from that of the ND group.

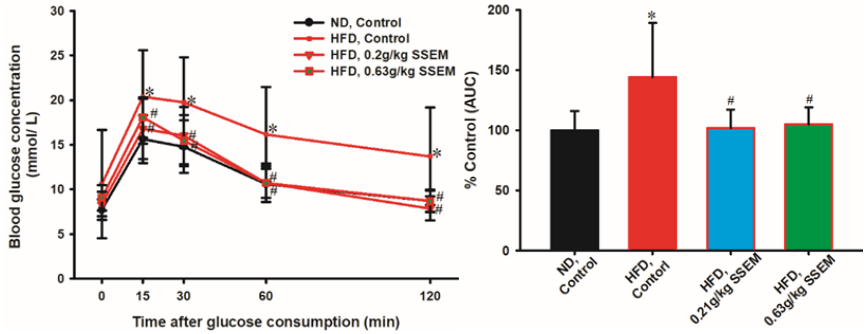


Figure 3. Effects of SSEM on HFD-induced glucose intolerance in mice. Glucose tolerance (estimated by AUC) was expressed as a percentage of ND-fed mice as a control. Each data point represents mean ± standard deviation (SD), with n = 6 - 13. The value for glucose tolerance (AUC) in the ND control was 1364 ± 217. *Significantly different from the ND control; #Significantly different from the HFD control.

3.4. Effect of SSEM on Blood/Plasma Glucose and Lipid Contents in HFD-Fed Mice

We investigated the long-term effect of SSEM on blood glucose control in high-fat diet (HFD)-fed mice by measuring HbA1c levels after an 8-week experimental period. While HFD feeding induced a substantial 35.8% increase in HbA1c compared to normal diet (ND) controls, co-administration of SSEM at daily doses of 0.21 or 0.63 g/kg failed to ameliorate this hyperglycemia, as HbA1c levels remained unchanged in SSEM-treated HFD mice (Table 1).

Table 1. Effects of SSEM on fasting blood/plasma glucose and lipid contents in HFD-fed mice. Data were expressed as a percentage of ND-fed mice. Each data point represents mean ± SD, with n = 6 - 13. The ND control value for HbA1c (%) was 3.10 ± 0.31; plasma glucose (mg/dL) was 76.3 ± 5.19; plasma TG (mg/dL) was 34.7 ± 2.86; plasma TC (mg/dL) was 86.2 ± 5.69; plasma HDL-C (mg/dL) was 57.5 ± 2.92; plasma LDL-C (mg/dL) was 38.8 ± 34.2 and TC/HDL-C ratio was 1.50 ± 0.10. *Significantly different from the ND control; #Significantly different from the HFD control.

	%Control	ND, Control	HFD, Control	HFD, 0.21 g/kg SSEM	HFD, 0.63 g/kg SSEM
Blood	HbA1c	100 ± 9.90	136 ± 11.5*	135 ± 16.3*	139 ± 17.9*
Plasma	Glucose	100 ± 6.80	142 ± 24.2*	164 ± 20.4*#	163 ± 17.3*#
	TG	100 ± 8.22	147 ± 28.3*	148 ± 22.9*	120 ± 8.00*#
	TC	100 ± 6.60	152 ± 14.7*	159 ± 17.3*	154 ± 13.8*
	HDL-C	100 ± 5.08	128 ± 6.08*	126 ± 13.7*	150 ± 15.5*#
	LDL-C	100 ± 8.83	224 ± 38.8*	256 ± 41.8*#	222 ± 24.0*#
	TC/HDL-C ratio	100 ± 6.59	119 ± 12.4*	127 ± 16.5*	103 ± 12.8*#

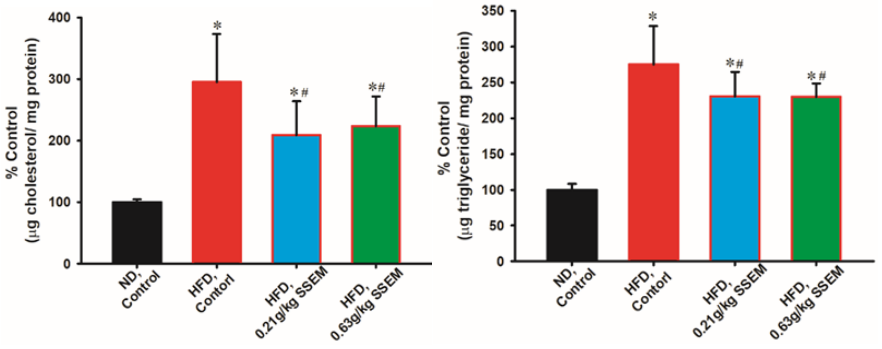
Fasting plasma glucose levels were also measured at the end of the 8-week ex-

perimental period. Table 1 reveals that HFD feeding significantly elevated fasting plasma glucose by 42% in mice compared to the ND group. Notably, SSEM co-treatment (0.21 or 0.63 g/kg) exacerbated this effect, leading to further increases of 64% and 63%, respectively, above the levels observed in ND-fed mice.

HFD feeding significantly elevated plasma lipid profiles, increasing TG, TC, HDL-C, and LDL-C levels by 47% - 124%. Treatment with a high dose of SSEM (0.63 g/kg) effectively attenuated the HFD-induced elevation in TG while further increasing HDL-C levels. Consequently, SSEM administration improved the TC/HDL-C ratio, mitigating the elevated ratio observed in the HFD-control group (Table 1).

3.5. Effects of SSEM on Hepatic Lipid Contents in ND-Fed and HFD-Fed Mice

HFD feeding significantly increased hepatic TC by approximately 195% compared to the ND control. SSEM treatments 0.21 g/kg and 0.63 g/kg effectively counteracted this HFD-induced TC elevation, reducing it by 44.2 and 36.5%, respectively (Figure 4(a)), to a similar extent when compared to untreated HFD mice. HFD also markedly raised hepatic TG levels by 175%. In HFD-fed mice, 0.21 and 0.63 g/kg SSEM treatments significantly reduced these elevated hepatic TG levels by 25.3 and 25.9%, respectively (Figure 4(b)).



(a) (b)

Figure 4. Effects of SSEM on hepatic lipid contents in ND-fed and HFD-fed mice. Hepatic TG and TC level were measured. Data were expressed in percent control with respect to the ND control [control hepatic TC level (μg/mg protein): 0.68 ± 0.03; control hepatic TG level (μg/mg protein): 2.81 ± 0.24]. Values given are means ± SD, with n = 6 - 13. *Significantly different from the ND control; #Significantly different from the HFD control.

3.6. Effect of SSEM on Glucose Uptake in SP-Induced Insulin Resistance in Myotubules

To investigate the cellular mechanism by which SSEM ameliorates HFD-induced glucose intolerance, we examined glucose uptake in differentiated C2C12 myotubules. Insulin resistance was experimentally induced using SP, a free fatty acid, to replicate HFD conditions within skeletal muscle cells. Figure 5 reveals that SSEM treatment alone had no significant impact on glucose uptake in control myotu-

bules. In contrast, co-incubation with SP and SSEM at concentrations of 100 and 300 μg/mL significantly enhanced glucose uptake by 18.6 and 26.7%, respectively, relative to SP-treated cells. When insulin was added, overall glucose uptake increased by 73.3% compared to the non-insulin control. Importantly, SSEM co-incubation in the presence of insulin consistently boosted glucose uptake by 12% - 14% at all tested concentrations, relative to the insulin control. The incubation of SP alone, however, significantly attenuated insulin-stimulated glucose uptake by 32.3%. Notably, SSEM demonstrated a dose-dependent reversal of SP-induced suppression. At 30 and 100 μg/mL SSEM, a partial reversal of SP's inhibitory effect was observed, while at 300 μg/mL SSEM, the effect was fully reversed.

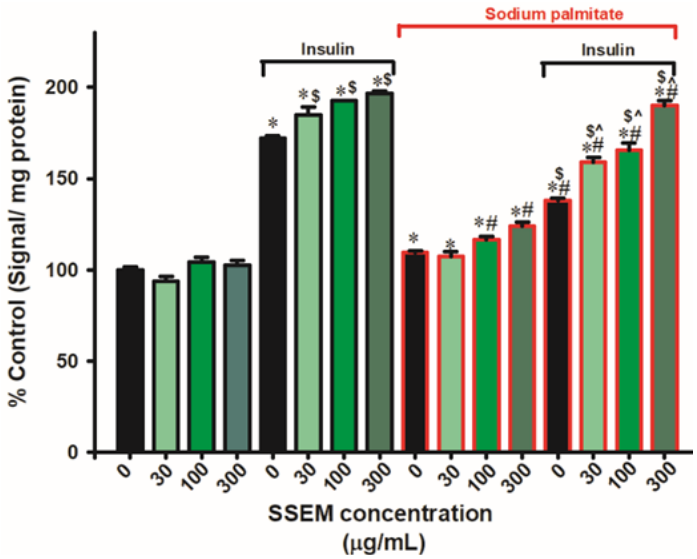


Figure 5. Effect of SSEM on glucose uptake in SP-induced insulin resistance in myotubules. Data were expressed as percentages of initial control values (0 μg/mL SSEM). Each bar represents mean ± standard error (SEM), with n = 6. The value of glucose uptake in the initial control was 150 ± 4.70 signal/mg protein. *Significantly different from the initial control; #Significantly different from the SP control. $Significantly different from insulin control. ^Significantly different from the SP insulin control.

4. Discussion

According to Traditional Chinese Medicine (TCM) theory, the SSEM formulation is designed to prevent fat accumulation, particularly when influenced by high-fat or high-calorie diets. Each herb is meticulously selected for its specific properties, contributing to the restoration of balance within the Zang-Fu organs and the harmonious flow of Qi. For instance, Cynomorii Herba tonifies Kidney Yang, a crucial factor in fundamental metabolic processes, including energy metabolism and the regulation of water and fat [14]. High-fat or high-calorie diets can induce excessive internal "heat" [15], which Momordica Fructus effectively clears, thereby mitigating inflammation and impaired metabolic function [16]. The "Five Flavors" of Schisandra extract harmonize the Zang-Fu organs, which are central to metabolic regulation [17] [18]. Furthermore, Eucommia leaf extract tonifies both

the Liver and Kidney, reinforcing their roles in metabolism, Qi circulation, and the efficient processing of fats [19]-[21]. In essence, SSEM addresses fat accumulation not through a direct "fat-burning" mechanism, but by restoring the body's internal equilibrium and optimizing the functions of organs involved in digestion, metabolism, and elimination.

In modern medicine, dysregulation of blood glucose is intimately linked to abdominal obesity and insulin resistance, primary contributors to MetS [22]. Consequently, targeting mechanisms that regulate blood glucose holds significant pharmacological interest for MetS prevention [23]. Several key components within SSEM's constituent herbs exhibit relevant bioactivity [14]-[21]. For example, triterpene and steroid-like compounds, such as ursolic acid from Cynomorii Herba and triterpenoids derived from Momordica Fructus, are potent activators of AMP-activated protein kinase (AMPK) [14] [24]. AMPK activation is known to enhance cellular glucose uptake, thereby improving mitochondrial function and increasing energy expenditure, leading to a reduction in body weight [25]. Eucommia leaves are rich in lignans, iridoids, phenolics, steroids, and flavonoids compounds that have demonstrated anti-MetS effects. These effects may be attributed to their ability to regulate adipocytokines and enhance gene expression for fat oxidation, ultimately improving insulin resistance and reducing body weight [26]. The lignan schisandrin B, found in Schisandrae Fructus, has been shown to enhance the mitochondrial antioxidant status [27] [28]. This protective effect combats oxidative stress and ameliorates mitochondrial dysfunction, both of which are implicated in the metabolic alterations characteristic of MetS [29]. In addition to these identified active compounds, other constituents within the four herbs of SSEM likely contribute synergistically to reducing high-fat diet (HFD)-induced obesity and its associated MetS.

The observed improvements in HFD-fed obese mice, namely, reduced body weight and adiposity, and enhanced glucose tolerance and lipid profiles (e.g., TC/HDL-C ratio and hepatic lipid content), are strongly corroborated by the discovery that SSEM can reverse insulin resistance induced by SP in differentiated C2C12 muscle cells. Insulin plays a critical role in skeletal muscle glucose uptake by promoting the translocation of GLUT-4 transporters to the cell surface, thereby increasing glucose entry into the cell [30]. It is plausible that SSEM alleviates insulin resistance, potentially caused by lipid accumulation in tissues, by enhancing insulin-induced GLUT-4 translocation to the cell membrane. Studies have indicated that improvements in glucose tolerance can be mediated by increased GLUT-4 concentrations in skeletal muscle or adipose tissue [31].

Despite these positive findings, fasting blood glucose and HbA1c levels remained elevated or showed only a slight increase in HFD-induced obese mice, even after SSEM treatment. This observation may be linked to the regulation of blood glucose through gluconeogenesis. While insulin normally suppresses gluconeogenesis once normal blood glucose levels are reached [32], hepatic insulin resistance, often present in MetS, can impair this suppression, leading to hyper-

glycemia [33]. Uncontrolled gluconeogenesis can also contribute to the development of non-alcoholic fatty liver disease, a common progression of MetS [32]. The occurrence of gluconeogenesis during periods of hypoglycemia could explain the elevated fasting blood glucose levels observed in the mice, and similarly, uncontrolled gluconeogenesis might account for the high HbA1c levels. SSEM effectively reverses insulin resistance in skeletal muscle but does not appear to correct uncontrolled hepatic gluconeogenesis, even though it did improve hepatic lipid content. This differential effect in the liver may be attributed to alternative insulin signaling pathways that regulate hepatic glucose and lipid metabolism, a notion requiring further study for confirmation [34] [35].

5. Conclusion and Future Directions

In summary, this study demonstrates that the SSEM formulation effectively reduces HFD-induced obesity, improves glucose tolerance, and alleviates hepatosteatosis. The anti-MetS effects of SSEM appear to be primarily mediated by ameliorating obesity-induced insulin resistance in skeletal muscle, as evidenced by the normalization of glucose tolerance. However, SSEM's inability to attenuate hepatic insulin resistance, indicated by persistently high fasting blood glucose and HbA1c levels, represents a key limitation. Future research should aim to develop more balanced herbal formulations for comprehensive management of the multifaceted aspects of MetS.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Loson-Kawalec, M., Sawina, P., Kowalczyk, A., Pazek, E., Szydłowska, D., Pawlowska, J., *et al.* (2026) Metabolic Syndrome in Focus: Emerging Causes, New Diagnostic Approaches and Criteria, and Long-Term Health Consequences. *Cureus*, **18**, e101012. https://doi.org/10.7759/cureus.101012

[2] Noubiap, J.J., Nansseu, J.R., Lontchi-Yimagou, E., Nkeck, J.R., Nyaga, U.F., Ngouo, A.T., *et al.* (2022) Geographic Distribution of Metabolic Syndrome and Its Components in the General Adult Population: A Meta-Analysis of Global Data from 28 Million Individuals. *Diabetes Research and Clinical Practice*, **188**, Article ID: 109924. https://doi.org/10.1016/j.diabres.2022.109924

[3] Noubiap, J.J., Nansseu, J.R., Nyaga, U.F., Ndoadoumgue, A.L., Ngouo, A.T., Tounouga, D.N., *et al.* (2025) Worldwide Trends in Metabolic Syndrome from 2000 to 2023: A Systematic Review and Modelling Analysis. *Nature Communications*, **17**, Article No. 573. https://doi.org/10.1038/s41467-025-67268-5

[4] Centers for Disease Control and Prevention (CDC) (2024) National Diabetes Statistics Report, 2024. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services.

[5] Friedrich, M.J. (2017) Global Obesity Epidemic Worsening. *The Journal of the American Medical Association*, **318**, 603. https://doi.org/10.1001/jama.2017.10693

[6] Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L.,

Cho, N.H., *et al*. (2017) IDF Diabetes Atlas: Global Estimates for the Prevalence of Diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*, **128**, 40-50. https://doi.org/10.1016/j.diabres.2017.03.024

[7] Mason, K.E., Pearce, N. and Cummins, S. (2018) Associations between Fast Food and Physical Activity Environments and Adiposity in Mid-Life: Cross-Sectional, Observational Evidence from UK Biobank. *The Lancet Public Health*, **3**, e24-e33. https://doi.org/10.1016/s2468-2667(17)30212-8

[8] Abdulghani, M.F. and Al-Fayyadh, S. (2024) Natural Products for Managing Metabolic Syndrome: A Scoping Review. *Frontiers in Pharmacology*, **15**, Article ID: 1366946. https://doi.org/10.3389/fphar.2024.1366946

[9] Noce, A., Di Lauro, M., Di Daniele, F., Pietroboni Zaitseva, A., Marrone, G., Borboni, P., *et al*. (2021) Natural Bioactive Compounds Useful in Clinical Management of Metabolic Syndrome. *Nutrients*, **13**, Article No. 630. https://doi.org/10.3390/nu13020630

[10] Zhang, S., Xu, M., Zhang, W., Liu, C. and Chen, S. (2021) Natural Polyphenols in Metabolic Syndrome: Protective Mechanisms and Clinical Applications. *International Journal of Molecular Sciences*, **22**, Article No. 6110. https://doi.org/10.3390/ijms22116110

[11] Ghasemi Gojani, E., Rai, S., Norouzkhani, F., Shujat, S., Wang, B., Li, D., *et al*. (2024) Targeting β-Cell Plasticity: A Promising Approach for Diabetes Treatment. *Current Issues in Molecular Biology*, **46**, 7621-7667. https://doi.org/10.3390/cimb46070453

[12] Cheng, C.W., Villani, V., Buono, R., Wei, M., Kumar, S., Yilmaz, O.H., *et al*. (2017) Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. *Cell*, **168**, 775-788.e12. https://doi.org/10.1016/j.cell.2017.01.040

[13] Chen, J., Wong, H.S., Leung, H.Y., Leong, P.K., Chan, W.M. and Ko, K.M. (2014) An Ursolic Acid-Enriched *Cynomorium songarium* Extract Attenuates High Fat Diet-Induced Obesity in Mice Possibly through Mitochondrial Uncoupling. *Journal of Functional Foods*, **9**, 211-224. https://doi.org/10.1016/j.jff.2014.04.021

[14] Wong, H.S., Chen, J., Leong, P.K., Leung, H.Y., Chan, W.M. and Ko, K.M. (2014) Cistanches Herba Reduces the Weight Gain in High Fat Diet-Induced Obese Mice Possibly through Mitochondrial Uncoupling. *Journal of Functional Foods*, **10**, 292-304. https://doi.org/10.1016/j.jff.2014.06.019

[15] Chen, J., Leong, P.K., Leung, H.Y., Chan, W.M., Wong, H.S. and Ko, K.M. (2020) Biochemical Mechanisms of the Anti-Obesity Effect of a Triterpenoid-Enriched Extract of *Cynomorium songaricum* in Mice with High-Fat-Diet-Induced Obesity. *Phytomedicine*, **73**, Article ID: 153038. https://doi.org/10.1016/j.phymed.2019.153038

[16] Zhang, C.H., Sheng, J.Q., Xie, W.H., Luo, X.Q., Xue, Y.N., Xu, G.L. and Chen, C. (2021) Mechanism and Basis of Traditional Chinese Medicine against Obesity: Prevention and Treatment Strategies. *Frontiers in Pharmacology*, **12**, Article ID: 615895. https://doi.org/10.3389/fphar.2021.615895

[17] Bortolotti, M., Mercatelli, D. and Polito, L. (2019) *Momordica charantia*, a Nutraceutical Approach for Inflammatory Related Diseases. *Frontiers in Pharmacology*, **10**, Article No. 486. https://doi.org/10.3389/fphar.2019.00486

[18] Ko, K.M. and Leung, H.Y. (2024) How Schisandrae Fructus Benefits the Body: Mechanisms in Traditional Chinese Medicine and Modern Medicine. *Chinese Medicine*, **15**, 75-81. https://doi.org/10.4236/cm.2024.153006

[19] Xv, M. and Peng, H.Y. (2022) The Characteristics of Prescription and Clinical Application of Wu WeiZi Decoction. *International Journal of Chinese Medicine*, **6**, 59-63.

[20] Bao, L., Sun, Y., Wang, J., Li, W., Liu, J., Li, T., *et al*. (2024) A Review of "Plant Gold"

Eucommia ulmoides Oliv.: A Medicinal and Food Homologous Plant with Economic Value and Prospect. *Heliyon*, **10**, e24851. https://doi.org/10.1016/j.heliyon.2024.e24851

[21] Xie, W., Xiao, Y., Xu, P., Zheng, H., Zeng, X., Wu, Y., *et al.* (2026) From Traditional Medicinal Plant to Modern Pharmacotherapy: A Comprehensive Review of the Bioactive Compounds and Health Applications of *Eucommia ulmoides*. *Nutrients*, **18**, Article No. 234. https://doi.org/10.3390/nu18020234

[22] Wang, Z., Sun, Y., Han, Y., Chen, X., Gong, P., Zhai, P., *et al.* (2023) Eucommia Bark/Leaf Extract Improves HFD-Induced Lipid Metabolism Disorders via Targeting Gut Microbiota to Activate the Fiaf-LPL Gut-Liver Axis and SCFAs-GPR43 Gut-Fat Axis. *Phytomedicine*, **110**, Article ID: 154652. https://doi.org/10.1016/j.phymed.2023.154652

[23] Islam, M.S., Wei, P., Suzauddula, M., Nime, I., Feroz, F., Acharjee, M., *et al.* (2024) The Interplay of Factors in Metabolic Syndrome: Understanding Its Roots and Complexity. *Molecular Medicine*, **30**, Article No. 279. https://doi.org/10.1186/s10020-024-01019-y

[24] Chalotra, R., Gupta, A., Singh, T.G. and Singh, R. (2025) Metabolic Syndrome: Risk Factors and Molecular Drug Targets. *EXCLI Journal*, **24**, 1193-1223.

[25] Liu, Z., Gong, J., Huang, W., Lu, F. and Dong, H. (2021) The Effect of *Momordica charantia* in the Treatment of Diabetes Mellitus: A Review. *Evidence-Based Complementary and Alternative Medicine*, **2021**, Article ID: 3796265. https://doi.org/10.1155/2021/3796265

[26] Chung, M.Y., Choi, H.K. and Hwang, J.T. (2021) AMPK Activity: A Primary Target for Diabetes Prevention with Therapeutic Phytochemicals. *Nutrients*, **13**, Article No. 4050. https://doi.org/10.3390/nu13114050

[27] Hussain, T., Tan, B., Liu, G., Oladele, O.A., Rahu, N., Tossou, M.C., *et al.* (2016) Health-Promoting Properties of *Eucommia ulmoides*: A Review. *Evidence-Based Complementary and Alternative Medicine*, **2016**, Article ID: 5202908. https://doi.org/10.1155/2016/5202908

[28] Ko, K.M. and Chiu, P.Y. (2006) Biochemical Basis of the "Qi-Invigorating" Action of Schisandra Berry (Wu-Wei-Zi) in Chinese Medicine. *The American Journal of Chinese Medicine*, **34**, 171-176. https://doi.org/10.1142/s0192415x06003734

[29] Guo, S., Yu, F., Wang, C., Zhao, W., Li, X., Liu, J., *et al.* (2026) Schisandrin B Confers Multi-Organ Protection via Regulation of Mitochondrial Homeostasis: Mechanistic Integration, Organ-Specific Differences, and Translational Challenges—A Review. *Frontiers in Pharmacology*, **17**, Article ID: 1781376. https://doi.org/10.3389/fphar.2026.1781376

[30] Vona, R., Gambardella, L., Cittadini, C., Straface, E. and Pietraforte, D. (2019) Biomarkers of Oxidative Stress in Metabolic Syndrome and Associated Diseases. *Oxidative Medicine and Cellular Longevity*, **2019**, Article ID: 8267234. https://doi.org/10.1155/2019/8267234

[31] van Gerwen, J., Shun-Shion, A.S. and Fazakerley, D.J. (2023) Insulin Signalling and GLUT4 Trafficking in Insulin Resistance. *Biochemical Society Transactions*, **51**, 1057-1069. https://doi.org/10.1042/bst20221066

[32] Chadt, A. and Al-Hasani, H. (2020) Glucose Transporters in Adipose Tissue, Liver, and Skeletal Muscle in Metabolic Health and Disease. *Pflügers Archiv—European Journal of Physiology*, **472**, 1273-1298. https://doi.org/10.1007/s00424-020-02417-x

[33] Hatting, M., Tavares, C.D.J., Sharabi, K., Rines, A.K. and Puigserver, P. (2018) Insulin

Regulation of Gluconeogenesis. *Annals of the New York Academy of Sciences*, **1411**, 21-35. https://doi.org/10.1111/nyas.13435

[34] Santoleri, D. and Titchenell, P.M. (2019) Resolving the Paradox of Hepatic Insulin Resistance. *Cellular and Molecular Gastroenterology and Hepatology*, **7**, 447-456. https://doi.org/10.1016/j.jcmgh.2018.10.016

[35] Bo, T., Gao, L., Yao, Z., Shao, S., Wang, X., Proud, C.G., *et al.* (2024) Hepatic Selective Insulin Resistance at the Intersection of Insulin Signaling and Metabolic Dysfunction-Associated Steatotic Liver Disease. *Cell Metabolism*, **36**, 947-968. https://doi.org/10.1016/j.cmet.2024.04.006