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Anti-obese related pharmacological effects of standard potato protein extracts on the 45%Kcal high fat diet supplied mice

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Abstract

Objectives : In present study, therefore, possible beneficial pharmacological activities of standard potato protein extracts (SPE) were observed on the mild diabetic obese mice.

Methods : After end of 12 weeks of continuous oral administrations of three different dosages of SPE 400, 200 and 100 mg/kg, or metformin 250 mg/kg, analyzed the hepatoprotective, hypolipidemic, hypoglycemic, nephroprotective and anti-obesity effects, separately. In addition, liver antioxidant defense systems were additionally measured with lipid metabolism-related genes expressions and hepatic glucose-regulating enzyme activities for action mechanism.

Results : All of diabetes and related complications including obesity were significantly inhibited by treatment of SPE 400, 200 and 100 mg/kg, dose-dependently, and they also dramatically normalized the hepatic lipid peroxidation and depletion of liver endogenous antioxidant defense system, the changes of the hepatic glucose-regulating enzyme activities, also changes of the lipid metabolism-related genes expressions including hepatic AMPKα1 and AMPKα2 mRNA expressions, dose-dependently. Especially, SPE 200 mg/kg constantly showed favorable inhibitory activities against type II diabetes and related complications as comparable to those of metformin 250 mg/kg in HFD mice, respectively.

Conclusions : The present work demonstrated that SPE 400, 200 and 100 mg/kg showed favorable anti-diabetic and related complications including obesity refinement activities in HFD mice, through AMPK upregulation mediated hepatic glucose enzyme activity and lipid metabolism-related genes expression, antioxidant defense system and pancreatic lipid digestion enzyme modulatory activities.

Key words : Standard Potato Protein Extracts, 45%Kcal high fat diet, mouse, obese, diabetes

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I. Introduction

Obesity contributes to the etiologies of a variety of comorbid conditions, such as cardiovascular disease, hypertension, and type II diabetes[1]. In addition to storing lipid for energy, adipose secretes a variety of adipokines, many of which affect metabolism and inflammation in adipose and nonadipose tissues. Modulation of the endocrine functions of adipose tissue can contribute to a chronic state of inflammation, which leads to the pathogenesis of associated disorders, specifically insulin resistance[2]. Recently, there has been a worldwide increase in the incidence of obesity associated with a metabolic syndrome known as type II diabetes, the development of which seems to be as a result of high-caloric diet intake and physical inactivity[3] and predicted estimates suggest that the population with this syndrome may double to over 300 million by the year 2025[4]. One of the critical determinants for the development of this obesity may be an increase in the regional distribution of body fat, especially abdominal obesity. The latter often shows clustering of atherogenic risk factors[5], like hypertension, dyslipidemia, alterations in coagulation and inflammatory cytokine profiles, and hyperinsulinemic insulin resistance. As a consequence, there is an expected increase in morbidity and mortality of cardiovascular disease[6].

Excessive intake of fatty acids leads to an accumulation of triglyceride (TG) in many tissues, particularly in the fat tissue, in which lipolysis is increased. The increased circulation of fatty acids, associated with rising lipolysis in adipocytes with insulin resistance, results in a plethora of fatty acids to non-adipose tissues, such as muscle, pancreas and liver. In individuals with insulin resistance, increased levels of tissue fatty acid-binding and transport proteins in adipose and non-adipose tissues facilitate the uptake processes. The exaggerated availability of free fatty acid and deposition in muscle induces a negative loop in insulin-mediated muscle insulin signaling and glucose utilization. In the pancreas, prolonged exposure to FFA might cause impairment of insulin release through the mechanism of lipotoxicity[7]. In the liver, high free fatty acid concentration contribute to resistance to the action of insulin by enhancing glucose output from liver[8]. The accumulation of TG in liver by high fatty acids also brings about non-alcoholic fatty liver disease (NAFLD). NAFLD does damage to liver, which is the main organ of glucose metabolism, such as steatosis, steatohepatitis, and hepatocellular necrosis to fibrosis[9]. The balance between hepatic lipogenesis and lipolysis is important to improving insulin resistance and NAFLD. These are generally characteristic features of metabolic syndrome[10].

Currently available pharmacological agents for metabolic syndrome, however, have a number of limitations, such as various adverse effects and high rates of secondary failure[11]. Due to these factors, metabolic syndrome patients and healthcare professions are increasingly considering complementary and alternative approaches[12-15]. Since control of postprandial hyperglycemia and inhibition of oxidative stress are suggested to be important in the treatment of diabetes[16], many efforts had been made to search for effective and safe α-glucosidase inhibitors and antioxidants from natural materials to develop a physiological functional food or lead compounds for curing diabetes[17]. Potato tuber is the source of various proteins active in anti-obese response[18,19]. While several methods to isolate and purify potato protein have been developed over the years on a laboratory scale, all of them are laborious and expensive[20]. Potato protein recovery is also often complicated by interactions with non-protein components of potato tubers that lead to poor solubility and reduced biological activity of the

protein fraction, thus hampering the potential therapeutic applications[21]. Given the low yield and complexity of potato protein isolation, a crude potato protein concentrate that contains several thermostable proteins were developed and it also showed potential anti-obese activity *in vivo*[21]. We already observed beneficial effects of standard potato protein extracts (SPE) on genetically obese diabetic ob/ob mice[22] and anti-adipogenic and antioxidant effects on the 3T3-L1 preadipocytes[23].

Ob/ob mice have a defect in the gene for leptin, a protein involved in appetite regulation and energy metabolism[24] and are hyperphagic, obese and insulin resistant[25], hence serving as a good animal model for the evaluation of anti hyperglycemic and insulinsensitizing drugs[26]. Ob/ob mice also have an unique lipoprotein referred to as low-density lipoprotein, LDL[27]. They also have fatty liver leading to hepatic steatosis[28]. The obesity in rodent mice is developed by feeding HFD, and the obese mice have the characters of hyperglycemia, insulin resistance, hepatic steatosis and hypolipemia[13,14]. HFD-induced hyperglycemia in ICR mice could rely upon the development of obesity that follows protracted access to the HFD. Although many kinds of animal model are used to develop the new drugs for metabolic syndromes, most of all show serious obesity and hyperglycemia, and are suitable to investigation for treating established diabetes[29]. On the other hand, HFD-induced animal models show mild obesity and hyperglycemia, and are appropriate to develop the preventive agent for metabolic syndromes[30]. Therefore, we selected 45%/Kcal rodent HFD supplied mice model for detecting various pharmacological effects of SPE as compared with metformin.

In the present study, therefore, we intended to observed the possible dose-dependent beneficial potentials of SPE on mild diabetic obese mice "the 45%Kcal HFD Supplied Mice"[15] as compared with metformin, a representative anti-obese and anti-diabetic drugs for type II diabetes[31].

II. Materials & methods

1. Animals and husbandry

The female SPF/VAF CrljOri:CD1[ICR] mice (6-wk old upon receipt; OrientBio, Seungnam, Korea) were used after acclimatization for 7 days. Animals were allocated four to five per polycarbonate cage in a temperature (20-25℃) and humidity (40-45%) controlled room. Light : dark cycle was 12hr : 12hr, and standard rodent chow (Cat. No. 38057; Purinafeed, Seungnam, Korea) and water were supplied free to access. Adapt animals to HFD were selected at 1 week of adapt periods as six groups based on the body weights. All laboratory animals were treated according to the national regulations of the usage and welfare of laboratory animals, and approved by the Institutional Animal Care and Use Committee in Daegu Haany University (Gyeongsan, Gyeongbuk, Korea) prior to animal experiment [Approval No. DHU2018-009].

2. Preparations and administration of test substances

SPE (contains 18.18 μg/g of chlorogenic acid) were prepared and supplied by Aribio (Seungnam, Korea) as light beige powders pink, and stored at 4℃ in a refrigerator to protect from light and humidity until used. White powders of metformin hydrochloride (Wako, Osaka, Japan) were used as reference recommendation drugs. SPE were dissolved in distilled water as 40, 20 and 10 mg/ml concentration, and were orally administered once a day for 84 days in a volume of 10 ml/kg (equivalence to 400, 200 and 100 mg/kg), using

a stainless zonde attached to 1 ml syringe, from 1 weeks after HFD supply. In addition, metformin HCl was dissolved in distilled water as 25 mg/ml concentrations and also orally administered in a volume of 10 ml/kg, as equivalence to 250 mg/kg, once a day for 84 days from 1 week after initial HFD supply. In intact vehicle and HFD control mice, equal volumes of distilled water were also orally administered, instead of test substances to provide same restrain stresses from gastric gavages, respectively. The dosage of SPE was selected as 400, 200 and 100 mg/kg considering the results of previous efficacy test in ob/ob mice[22], and administration volume was decided to 10 ml/kg, the general dosing volume in mice[32], in the current experiment. The dose levels of metformin, 250 mg/kg were also selected based on previous animal studies[12-14].

3. HFD supply

Animals were supplied 45%/Kcal HFD (Cat. No. D12451; Research Diet, New Brunswick, NJ, USA) free to access after 7 days of acclimatization. In intact control mice, NFD (Cat. No. 38057; Purinafeed, Seungnam, Korea) was supplied free to access instead of HFD. Adapt animals to HFD were selected at 1 week of adapt periods as six groups (eight mice in each groups, total 40 HFD supplied mice and 8 normal diet supplied mice) based on the body weights, respectively.

4. Changes in body weights

Changes of body weight were measured at 8 days (at just immediately before start of HFD supply) and 1 day before initiation of administration, and at initial administration day, and then weekly with at a termination using an automatic electronic balance (XB320M, Precisa Instrument, Zuerich, Switzland). At initiation of administration and at a termination, all experimental animals were overnight fasted (water was not; about 12 hr) to reduce the differences from feeding. In addition, body weight gains were additionally calculated during adapt periods (from Day -8 to Day 0 of test article-administration) and administration periods (from Day 0 to Day 84 of test article-administration), as follow equation.

Body weight gains(g;during adapt periods,7 days) = Body weights at initiation of administration - body weights at initiation of HFD supply

Body weight gains (g;during administration periods, 84 days) = Body weights at a termination - body weights at initiation of administration

5. Mean daily food consumption measurements

Diets (150 g in each individual cage) were supplied, and reminder amounts of supplied diets were measured at 24 hrs after using an automatic electronic balance (XB320M, Precisa Instrument, Zuerich, Switzland). And then divided by reared animal numbers in same cages, these are regarded as individual mean daily food consumption of mice (g/day/mice) as [Amounts of supplied diets (150 g) - Amounts of remainder supplied diets after 24 hrs]/reared head of mice. These measurements were e conducted once a week for 84 days of administration periods according to previous reports[13,14].

6. Measurement of body fat density: Total and abdominal fat mass (%)

The mean fat densities on the total body and abdominal cavity regions of each mouse were detected by *in live* DEXA (InAlyzer; Medikors, Seungnam, Korea), once at end of 84 days continuous treatment of test substances, according to previous report[33].

7. Measurement of blood glucose level

At end of 84 days continuous treatment, bloods were collected from *vena cava*, under inhalation anesthesia with 2 to 3% isoflurane (Hana Pharm. Co., Hwasung, Korea) in the mixture of 70% N_2O and 28.5% O_2, using rodent inhalation anesthesia apparatus (Surgivet, Waukesha, WI, USA) and rodent ventilator (Model 687, Harvard Apparatus, Cambridge, UK), and deposited into NaF glucose vacuum tube (Becton Dickinson, Franklin Lakes, NJ, USA) and plasma were separated, and stored in a ultradeep freezer (MDF-1156, Sanyo, Tokyo, Japan) under −150 ℃ until analysis. Blood glucose levels were measured using automated blood analyzer (Dri-Chem NX500i; Fuji Medical System Co., Ltd., Tokyo, Japan).

8. Serum biochemistry

Some collected bloods from *vena cava* at 84 days after initial test substance treatment under inhalation anesthesia, were deposited into clotting activated serum tubes, and centrifuged at 15,000 rpm for 10 min under room temperature for separating the serum to AST, ALT, ALP, LDH, GGT BUN, creatinine, TC, TG, LDL and HDL measurement. Serum AST, ALT, ALP, LDH, GGT, BUN, creatinine, TC and TG levels were measured using automated blood analyzer (Dri-Chem NX500i; Fuji Medical System Co., Ltd., Tokyo, Japan), after stored in a ultradeep freezer (MDF-1156, Sanyo, Tokyo, Japan) under −150℃.

9. Measurement of serum insulin and blood HbA1c level

Blood HbA1c and serum insulin levels were determined using an automate HbA1c measuring equipment (Model Easya1c; Infopia, Anyang, Korea) and a mouse insulin ELISA kit (Cat. No. 80-INSMS-E01; Alpco Diagnostics, Windham, NH, USA), according to previously established methods[33,34] and manufacturer's protocol or instruction.

10. Organ weight measurements

At sacrifice, the weights of liver, pancreas, left kidney, left periovarian fat pads and abdominal wall deposited fat pads attached to the *muscularis quadratus lumborum* were measured at g levels, individually, and to reduce the differences from individual body weights, the relative weights (% of body weights) were also calculated using body weight at sacrifice and absolute weight as follow equation according to previously established methods with some modifications[13,14,33].

$$\text{Relative organ weights (\%)} = \frac{\text{Absolute organ weights}}{\text{Body weight at sacrifice}} \times 100$$

11. Measurement of lipid compositions in the feces

Lipid was extracted from feces collected at 8 hrs after last test substance administration, according to the method of Folch et al.[35]. The concentrations of fecal TC and TG measured by colorimetric assay using a commercial kit (TC colorimetric assay kit, Cat. No. 100102303, Cayman, Ann Arbor, MI, USA; Total Cholesterol Assay Kit (Colorimetric), Cat. No. STA-384, Cell Biolabs, San Diego, CA, USA) using a microplate reader (Sunrise, Tecan, Männedorf, Switzerland) according to previously established methods[12,33].

12. Liver lipid peroxidation and antioxidant defense systems

After measurements of organ weights, the MDA and GSH contents, CAT and SOD enzyme

activities in mouse hepatic tissues were assessed, respectively. Separated hepatic tissues were weighed and homogenized in ice-cold 0.01M Tris-HCl (pH 7.4) with bead beater (Taco™Pre, GeneResearch Biotechnology Corp., Taichung, Taiwan) and ultrasonic cell disruptor (KS-750, Madell Technology Corp., Ontario, CA, USA), and then centrifuged, at 12,000 × g for 15 min. Tissue homogenates were stored in an ultradeep freezer under −150 ℃ until analysis. The concentrations of liver lipid peroxidation was determined by estimating MDA using the thiobarbituric acid test and UV/Vis spectrophotometer (OPTIZEN POP, Mecasys, Daejeon, Korea) at absorbance 525 nm, as nM of MDA/mg protein. Contents of total protein were measured by previous method[36] using bovine serum albumin (Invitrogen, Carlsbad, CA, USA) as internal standard. Prepared hepatic homogenates were mixed with 0.1 ml of 25% trichloroacetic acid (Merck, West Point, CA, USA), and then centrifuged at 4,200 rpm for 40 min at 4 ℃. GSH contents were spectrophotometerically measured at absorbance 412 nm using 2-nitro-benzoic acid (Sigma-Aldrich, St. Louise, MO, USA). Decomposition of H_2O_2 in the presence of CAT was followed at 240 nm using spectrophotometer. CAT activity was defined as the amount of enzyme required to decompose 1 nM of H_2O_2 per minute, at 25 ℃ and pH 7.8. Results were expressed as U/mg protein. Measurements of SOD activities were made according to Sun et al.[37]. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitrotetrazolium blue to form formazan dye. SOD activity was then spectrophotometerically measured at 560 nm by the degree of inhibition of this reaction, and expressed as U/mg protein. One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitroblue tetrazolium by 50% during 1 min.

13. Measurement of hepatic glucose-regulating enzyme activities

The hepatic enzyme source was prepared based on the method of Hulcher and Oleson[38]. A 0.3 g hepatic tissue was homogenized in buffer solution (0.1 M triethanolamine, 0.2 M EDTA, and 0.002 M dithiothreitol) and centrifuged at 1,000 × g for 15 min at 4 ℃. The supernatant was further centrifuged at 10,000 × g for 15 min at 4 ℃. The GK activity was measured according to the method described by Davidson and Arion[39] with slight modifications. A 0.98 ml of the reaction mixture (50 mM Hepes-NaGT (pH 7.4), 100 mM KCl, 7.5 mM MgCl2, 2.5 mM dithioerythritol, 10 mg/ml albumin, 10 mM glucose, 4 units of glucose-6-phosphate dehydrogenase, 50 mM NAD+, and 10 μl hepatic tissue homogenates) was pre-incubated at 37 ℃ for 10 min. The reaction was initiated with the addition of 10 μL of 5 mM ATP and the mixture was incubated at 37 ℃ for 10 min. The change in absorbance at 340 nm was recorded. The G6pase activity was measured based on the method of Alegre et al.[40]. The reaction mixture contained 765 μl of 131.58 mM Hepes-NaGT (pH 6.5), 100 μl of 18 mM EDTA (pH 6.5), 100 μl of 265 mM glucose-6-phosphate, 10 μl of 0.2 M NADP+, 0.6 IU/ml mutarotase, and 0.6 IU/ml glucose dehydrogenase. After pre-incubation at 37 ℃ for 3 min, the mixture was added with 5 μl hepatic tissue homogenates and incubated at 37 ℃ for 4 min. The change in absorbance at 340 nm was measured. The PEPCK activity was measured using the method of Bentle and Lardy[41]. The reaction mixture contained 72.92 mM sodium Hepes (pH 7.0), 10 mM dithiothreitol, 500 mM $NaHCO_3$, 10 mM $MnCl_2$, 25 mM NADH, 100 mM IDP, 200 mM PEP, 7.2 unit of malic dehydrogenase, and 10 μl hepatic tissue homogenates. The enzyme activity was determined based from the decrease in the absorbance of the mixture at 340 nm at 25 ℃

using a UV/Vis spectrophotometer (OPTIZEN POP, Mecasys, Daejeon, Korea). All chemicals and reagents used in this hepatic enzyme activity measurement was obtained from Sigma-Aldrich (St. Louise, MO, USA).

14. *Realtime* RT-PCR analysis

The ACC1, AMPKα1 and AMPKα2 mRNA expressions on the prepared hepatic tissues were determined by *realtime* RT-PCR with periovarian adipose tissue leptin, UCP2, adiponectin, C/EBPα, C/EBPβ and SREBP1c mRNA expressions, separately based on the previous reports[42]. Briefly, RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The RNA concentrations and quality was determined by CFX96™ Real-Time System (Bio-Rad, Hercules, CA, USA). To remove contaminating DNA, samples were treated with recombinant DNase I (DNA-free; Ambion, Austin, TX, USA). RNA was reverse transcribed using the reagent High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Analysis was carried out using ABI Step One Plus Sequence Detection System (Applied Biosystems, Foster City, CA, USA), and their expression levels were calculated as relative to vehicle control. The following thermal conditions were applied as 10 min at 94℃ and 39 cycles of 15 sec at 94 ℃, 20 sec at 57 ℃ and 30 sec at 72 ℃. The data were normalized by GAPDH mRNA expression, using comparative threshold cycle method[43]. The sequences of the PCR oligonucleotide primers were listed in Table 1.

Table 1. Oligonucleotides for *realtime* RT-PCR used in this study

Target	5' - 3'	Sequence	GenBank Accession Number
Leptin	Sense Antisense	CCAAAACCCTCATCAAGACC GTCCAACTGTTGAAGAATGTCCC	NM_008493
UCP2	Sense Antisense	CCGCATTGGCCTCTACGACTCT CCCCGAAGGCAGAAGTGAAGTG	NM_011671
Adiponectin	Sense Antisense	CCCAAGGGAACTTGTGCAGGTTGGATG GTTGGTATCATGGTAGAGAAGAAAGCC	NM_009605.4
C/EBPα	Sense Antisense	TGGACAAGAACAGCAACGAGTAC CGGTCATTGTCACTGGTCAACT	NM_001287523.1
C/EBPβ	Sense Antisense	AAGCTGAGCGACGAGTACAAGA GTCAGCTCCAGCACCTTGTG	NM_001287739.1
SREBP1c	Sense Antisense	AGCCTGGCCATCTGTGAGAA CAGACTGGTACGGGCCACAA	XM_006532714.2
ACC1	Sense Antisense	GCCATTGGTATTGGGGCTTAC CCCGACCAAGGACTTTGTTG	NM_133360.2
AMPKα1	Sense Antisense	AAGCCGACCCAATGACATCA CTTCCTTCGTACACGCAAAT	XM_011245321.1
AMPKα2	Sense Antisense	GATGATGAGGTGGTGGA GCCGAGGACAAAGTGC	NM_178143.2
GAPDH	Sense Antisense	CATCTTCCAGGAGCGAGACC TCCACCACCCTGTTGCTGTA	NM_008084

RT-PCR = reverse transcription polymerase chain reaction; UCP = Mitochondrial uncoupling protein; C/EBP = CCAAT-enhancer-binding protein; SREBP = Sterol regulatory element-binding protein; ACC1 = Acetyl-CoA carboxylase 1; AMPK = 5' adenosine monophosphate-activated protein kinase; GAPDH = Glyceraldehydes 3-phosphate dehydrogenase.

15. Histopathology

After measuring of organ weights, some parts of left lateral lobes of liver, left kidney, splenic lobes of pancreas, left periovarian fat pads and abdominal wall deposited fat pads attached to the *muscularis quadratus lumborum* were fixed in 10% neutral buffered formalin. After paraffin embedding using automated tissue processor (Shandon Citadel 2000, Thermo Scientific, Waltham, MA, USA) and embedding center (Shandon Histocentre 3, Thermo Scientific, Waltham, MA, USA), 3-4μm serial sections were prepared using microtome (RM2255, Leica Biosystems, Nussloch, Germany).

Representative sections were stained with hematoxylin and eosin (HE) for light microscopic examination (Eclipse 80i; Nikon, Tokyo, Japan). After that the histological profiles of individual organs were observed. Alternatively, portions of liver that had been dehydrated in 30% sucrose solutions were sectioned by cryostat for staining with oil red[12,33,44]. To observe more detail histopathological changes, the steatohepatitis regions and mean hepatocyte diameters (under HE staining) were calculated using an automated image analysis process (Model iSolution FL ver 9.1; IMT i-solution Inc., Vancouver, Quebec, Canada) on the restricted view fields according to previous method[12-14,33,44]. Steatohepatitis regions, the percentage of fatty deposited regions in hepatic parenchyma, were calculated as percentages of lipid deposited regions between restricted histological view field of liver (%/mm^2 of hepatic parenchyma) under cryostat and oil red staining, and mean diameters of hepatocytes were also calculated in restricted view fields on a computer monitor under paraffin embedding and HE staining using an automated image analysis process as μm; at least 10 hepatocytes per each view field of liver were considered. In addition, mean numbers of lipid droplet deposited vacuolated renal tubules were also calculated using an automated image analysis process among 100 tubules (number/100 tubules; at 1 field for sample), and mean diameters of white adipocytes in each fat fads were calculated in restricted view fields on a computer monitor using an automated image analysis process as μm; at least 10 white adipocytes per each fat pads were considered. Thicknesses of deposited periovarian and abdominal wall fat pads (mm), mean areas occupied by zymogen granules (%/mm^2 of pancreatic parenchyma), numbers of pancreatic islets (islets/10 mm^2 of pancreatic parenchyma) and diameters of pancreatic islets (μm) were also measured using an automated image analysis process according to our previous established methods[12-14,33,44]. The histopathologist was blinds to group distribution when this analysis was made.

16. Immunohistochemistry

Other prepared serial sectioned pancreas tissues were immunostained by an avidin-biotin-peroxidase (ABC) methods[12,33] using guinea pig polyclonal insulin (Cat. No. ab7842; Abcam, Cambridge, UK, dilution: 1:100) or rabbit polyclonal glucagon (Cat. No. ab133195; Abcam, Cambridge, UK, dilution: 1:100) antiserum. Briefly, endogenous peroxidase activity was blocked by incubated in methanol and 0.3% H_2O_2 for 30 minutes, and non-specific binding of immunoglobulin was blocked with normal horse serum blocking solution (Vector Lab., Burlingame, CA, USA. Dilution 1:100) for 1 hr in humidity chamber. Primary antiserum were treated for overnight at 4 ℃ in humidity chamber, and then incubated with biotinylated universal secondary antibody (Vector Lab., Burlingame, CA, USA, Dilution 1:50) and ABC reagents (Cat. No. PK-6200; Vectastain Elite ABC Kit, Vector Lab., Burlingame, CA, USA, Dilution 1:50) for 1 hr at room temperature in humidity chamber. Finally, reacted with

peroxidase substrate kit (Cat. No. SK-4100; Vector Lab., Burlingame, CA, USA, Dilution 1:50) for 3 min at room temperature. All sections were rinse in 0.01 M PBS for 3 times, between steps. The cells occupied by over 20% of immunoreactivities, the density, of each antiserum – for insulin and glucagon, as compared with other naïve cells, were regarded as positive, and the mean numbers of insulin- and glucagon-immunoreactive cells dispersed in the mm^2 of pancreatic parenchyma were counted using an automated image analysis process as establish methods[12,33] and insulin/glucagon cell ratios were additionally calculated as follow equation. The histopathologist was blinds to the group distribution when performing the analysis.

$$\text{Ratio} = \frac{\text{mean numbers of insulin immunoreactive cells}}{\text{mean numbers of glucagon immunoreactive cells}}$$

17. Statistical analyses

All numerical values are expressed mean ± standard deviation (SD) of eight mice. Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the obtain data were analyzed by one way ANOVA test followed by least-significant differences multi-comparison (LSD) test to determine which pairs of group comparison were significantly different. In case of significant deviations from variance homogeneity were observed at Levene test, a non-parametric comparison test, Kruskal-Wallis H test was conducted. When a significant difference is observed in the Kruskal-Wallis H test, the Mann-Whitney U (MW) test with Bonferroni correction was conducted to determine the specific pairs of group comparison, which are significantly different. Statistical analyses were conducted using SPSS for Windows (Release 14.0K, IBM-SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P < 0.05$. In addition, the percent changes as compared with HFD control were calculated to help the understanding of the efficacy of test substances, and the percent changes between intact and HFD control were also calculated to observe disease inductions as follow equations according to previous report[12,15].

Percent changes as compared with intact control (%)

$$= \frac{\text{Data of HFD control} - \text{Data of intact control}}{\text{Data of intact control}} \times 100$$

Percent changes as compared with HFD control (%)

$$= \frac{\text{Data of test substance administered mice} - \text{Data of HFD control}}{\text{Data of HFD control}} \times 100$$

III. Results

1. Effects on obesity

HFD control mice showed significant ($p<0.01$) increases of body weights as compared with intact mice from 1 week after HFD supply, and accordingly the body weight gains during 7 days of HFD adaption and 84 days of administration periods were also significantly ($p<0.01$) increased as compared with intact control, respectively. However, significant ($p<0.01$ or $p<0.05$) decreases of the body weights were detected in metformin 250 mg/kg treated mice from 35 days after start of administration, and from 14, 28 and 49 days after start of administration in SPE 400, 200 and 100 mg/kg treated mice as compared with HFD control, and accordingly the body weight gains during 84 days of administration were

also significantly ($p<0.01$) decreased in metformin 250 mg/kg, SPE 400, 200 and 100 mg/kg treated mice as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent decreases of body weights and body weight gains during 84 days of administration periods as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the present study (Table 2; Fig 1).

Table 2. Changes on body weight gains and mean daily food consumption in NFD or HFD supplied mice

Times / Groups	Body weights (g) at days after initial test substance treatment				Body weight gains during		Mean Daily Food Consumption (g)
	8 days before [A]	1 day before [B]	0 day* [C]	84 days* [D]	Adapt period [B–A]	Administration period [D–C]	
Controls							
Intact	28.45±1.08	28.95±1.13	25.91±1.12	31.33±2.27	0.50±0.11	5.41±1.60	5.49±0.26
HFD	28.46±1.16	32.85±1.54[a]	29.88±1.63[a]	47.88±3.05[a]	4.39±0.54[a]	18.00±1.66[a]	4.64±0.27[a]
Reference							
Metformin	28.39±1.00	32.70±1.22[a]	29.64±1.45[a]	39.20±1.97[ab]	4.31±0.37[a]	9.56±1.58[ab]	4.71±0.23[a]
Test material – SPE							
400 mg/kg	28.48±0.73	32.71±0.72[a]	29.66±0.66[a]	37.34±0.89[ab]	4.24±0.09[a]	7.68±0.97[ab]	4.74±0.24[a]
200 mg/kg	28.43±1.19	32.79±1.05[a]	29.85±1.20[a]	39.38±1.34[ab]	4.36±0.29[a]	9.51±0.98[ab]	4.70±0.27[a]
100 mg/kg	28.53±1.09	32.79±0.95[a]	29.76±0.97[a]	40.94±1.29[ab]	4.26±0.40[a]	11.18±1.46[ab]	4.71±0.23[a]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. * All animals were overnight fasted. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ as compared with HFD control.

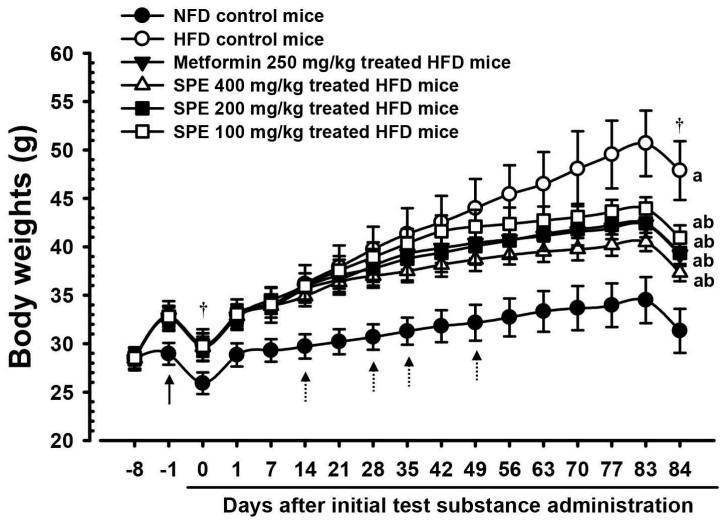

Figure 1. Body weight changes in NFD or HFD Supplied Mice.

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. All animals were overnight fasted before initial test substance administrations and sacrifice (†). [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ as compared with HFD control.

Although significant ($p<0.01$) decreases of mean daily food consumptions were detected in all HFD supplied mice as compared with intact control, no meaningful or significant changes on the mean daily food consumptions were detected in all test substance administered groups including SPE 400 mg/kg as compared with HFD control, in this study (Table 2).

Significant ($p<0.01$) increases of total body and abdominal fat densities at analysis of *in live* DEXA, periovarian deposited fat pad weights, abdominal wall deposited fat pad absolute and relative weights and periovarian and abdominal white adipocyte diameters and thicknesses of each deposited fat pads were detected in HFD control as compared with intact control, respectively. However, these increases of obesity related signs were significantly ($p<0.01$ or $p<0.05$) decreased by treatment of all test substances, respectively. Especially, all three different dosages of SPE

Table 3. Changes on absolute organ weights in NFD or HFD supplied mice

Organs / Groups	Absolute organ weights (g)				
	Liver	Kidney	Pancreas	Periovarian fat pads	Abdominal wall fat pads
Controls					
Intact	0.988±0.032	0.196±0.013	0.236±0.022	0.082±0.032	0.025±0.017
HFD	1.741±0.036[a]	0.305±0.017[a]	0.228±0.016	0.794±0.073[a]	0.588±0.093[a]
Reference					
Metformin	1.378±0.077[ab]	0.246±0.014[ab]	0.228±0.007	0.437±0.071[ab]	0.320±0.047[ab]
Test material – SPE					
400 mg/kg	1.309±0.058[ab]	0.242±0.038[ab]	0.238±0.009	0.209±0.039[ab]	0.172±0.037[ab]
200 mg/kg	1.387±0.057[ab]	0.243±0.009[ab]	0.230±0.008	0.433±0.083[ab]	0.324±0.057[ab]
100 mg/kg	1.498±0.076[ab]	0.267±0.014[ab]	0.223±0.010	0.580±0.077[ab]	0.393±0.033[ab]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ as compared with HFD control.

Table 4. Changes on relative organ weights in NFD or HFD supplied mice

Organs / Groups	Relative organ weights (*% of body weights*)				
	Liver	Kidney	Pancreas	Periovarian fat pads	Abdominal wall fat pads
Controls					
Intact	3.165±0.209	0.628±0.054	0.759±0.097	0.261±0.102	0.081±0.053
HFD	3.647±0.208[a]	0.639±0.051	0.479±0.051[a]	1.658±0.086[a]	1.234±0.221[a]
Reference					
Metformin	3.517±0.166[a]	0.628±0.056	0.583±0.036[ac]	1.112±0.156[ac]	0.821±0.152[ac]
Test material – SPE					
400 mg/kg	3.507±0.163[a]	0.647±0.095	0.637±0.029[bc]	0.559±0.104[ac]	0.461±0.102[ac]
200 mg/kg	3.528±0.176[a]	0.618±0.036	0.584±0.030[ac]	1.102±0.210[ac]	0.823±0.132[ac]
100 mg/kg	3.661±0.174[a]	0.653±0.037	0.546±0.024[ac]	1.417±0.178[ac]	0.960±0.070[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ as compared with HFD control.

Table 5. Changes on the histopathology-histomorphometry of the periovarian and abdominal wall deposited fat pads in NFD or HFD supplied mice

Items / Groups	Periovarian fat pads		Abdominal wall fat pads	
	Thickness (mm)	Adipocyte diameters (μm)	Thickness (mm)	Adipocyte diameters (μm)
Controls				
Intact	1.37±0.48	30.76±4.92	1.52±0.72	36.83±3.97
HFD	4.37±0.68[a]	89.01±14.47[a]	6.50±1.03[a]	100.26±18.46[a]
Reference				
Metformin	2.96±0.22[ac]	65.26±11.19[ac]	4.48±0.88[ac]	69.76±11.64[ac]
Test material - SPE				
400 mg/kg	2.33±0.39[ac]	44.62±12.40[bc]	3.75±0.77[ac]	54.97±12.36[ac]
200 mg/kg	3.02±0.43[ac]	61.06±10.24[ac]	4.34±1.00[ac]	67.35±10.37[ac]
100 mg/kg	3.47±0.50[ac]	68.48±10.23[ac]	5.09±0.67[ac]	78.50±11.01[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ as compared with HFD control.

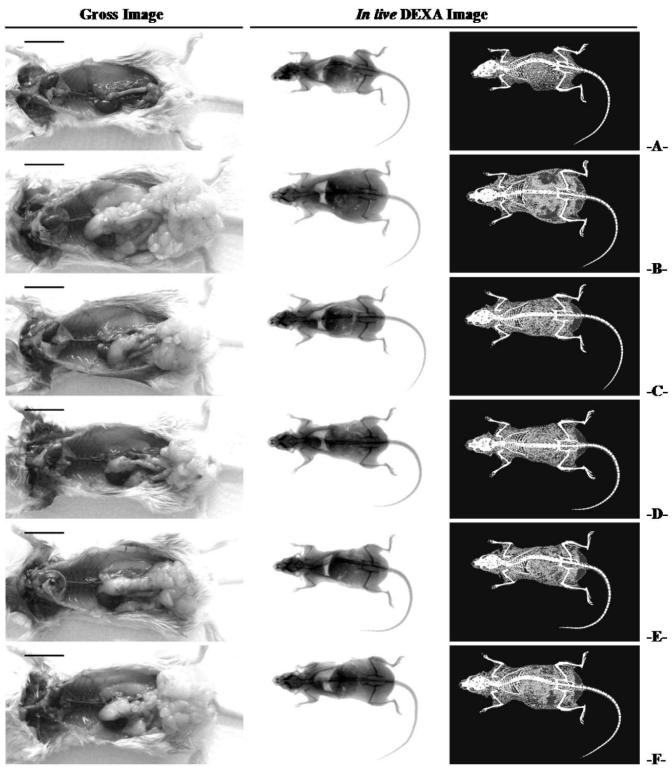

Figure 2. Representative gross body mass and abdominal fat pads with whole body DEXA images taken from NFD or HFD supplied mice.

A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; DEXA = Dual-energy x-ray absorptionmetry; SPE = Standard Potato Protein Extracts. Scale bar = 17.5 mm.

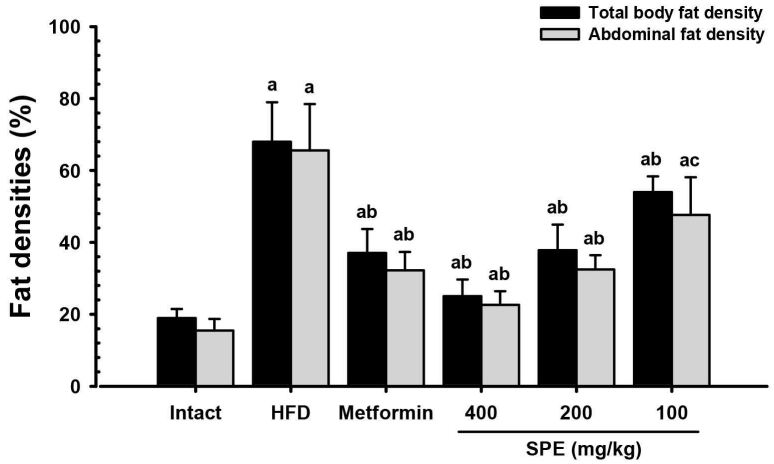

Figure 3. Total body and abdominal fat densities in NFD or HFD supplied mice.

Values are expressed mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; DEXA = Dual-energy x-ray absorptionmetry; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ and [c] $p<0.05$ as compared with HFD control.

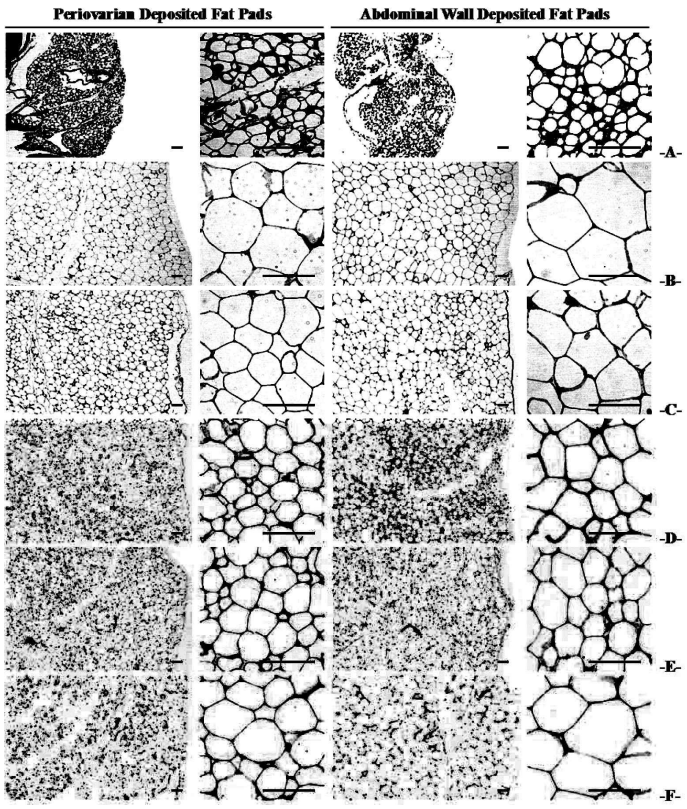

Figure 4. Representative histological images of the adipocytes, taken from NFD or HFD supplied mice periovarian and abdominal wall deposited fat pads.

A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. All Hematoxylin & Eosin stain. Scale bars = 100 μm.

400, 200 and 100 mg/kg treated mice also showed clear dose-dependent decreases of total body and abdominal fat masses as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current study (Table 3~5; Fig 2~4).

Significant ($p<0.01$) decreases of exocrine pancreas zymogen granule contents (the percentages of exocrine pancreas occupied by zymogen granules) were detected in HFD control as compared with intact control, result from release of zymogen granules. However, exocrine pancreas zymogen granule contents were significant ($p<0.01$ or $p<0.05$) increased in all test drug treated mice as compared with HFD control including SPE 200 mg/kg, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent increases of the percentage regions of exocrine pancreas occupied by zymogen granules as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in our experiment (Table 6; Fig 5).

2. Anti-diabetic hypoglycemic effects

Significant ($p<0.01$) increases of blood glucose levels, serum insulin levels and blood HbA1c contents were detected in HFD control as compared with intact control. However, these increments were significantly ($p<0.01$ or $p<0.05$) reduced by treatment of all four test articles including SPE 100 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed definitive dose-dependent decreases of the blood glucose levels as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in this experiment (Table 7; Fig 6).

Significant ($p<0.01$) decreases of pancreas relative weights were detected in HFD control mice as compared with intact control mice. However, significant ($p<0.01$) increases of pancreas relative weights were detected in metformin 250 mg/kg, SPE 400, 200 and 100 mg/kg treated mice as compared with HFD control mice, res-

Table 6. Changes on histopathology-histomorphometry of the pancreas in NFD or HFD supplied mice

Items / Groups	Zymogen granules (%/mm^2 of exocrine)	Mean islet numbers (numbers/ 10 mm^2)	Mean islet diameter (μm/islet)	Insulin-IR cells (cells/mm^2) [A]	Glucagon-IR cells (cells/mm^2) [B]	Insulin/ glucagon ratio [A/B]
Controls						
Intact	52.37±12.03	8.75±2.76	103.35±19.44	85.63±16.59	24.75±3.65	3.46±0.37
HFD	17.24±5.31[a]	30.88±3.68[a]	316.09±54.11[a]	873.00±134.85[a]	139.50±21.84[a]	6.26±0.25[a]
Reference						
Metformin	29.32±4.02[ab]	20.00±2.88[ab]	194.79±36.01[ab]	429.38±115.76[ab]	91.25±17.09[ab]	4.68±0.82[ab]
Test material - SPE						
400 mg/kg	49.03±11.72[b]	15.00±2.83[ab]	169.30±22.94[ab]	212.50±42.73[ab]	63.63±10.36[ab]	3.34±0.38[ab]
200 mg/kg	30.29±6.95[ab]	20.13±3.23[ab]	201.97±17.26[ab]	434.63±72.90[ab]	93.25±14.14[ab]	4.66±0.27[ab]
100 mg/kg	25.58±5.67[ac]	25.75±3.11[ab]	233.19±36.28[ab]	605.50±108.25[ab]	113.38±11.26[ab]	5.34±0.72[ab]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ and [c] $p<0.05$ as compared with HFD control.

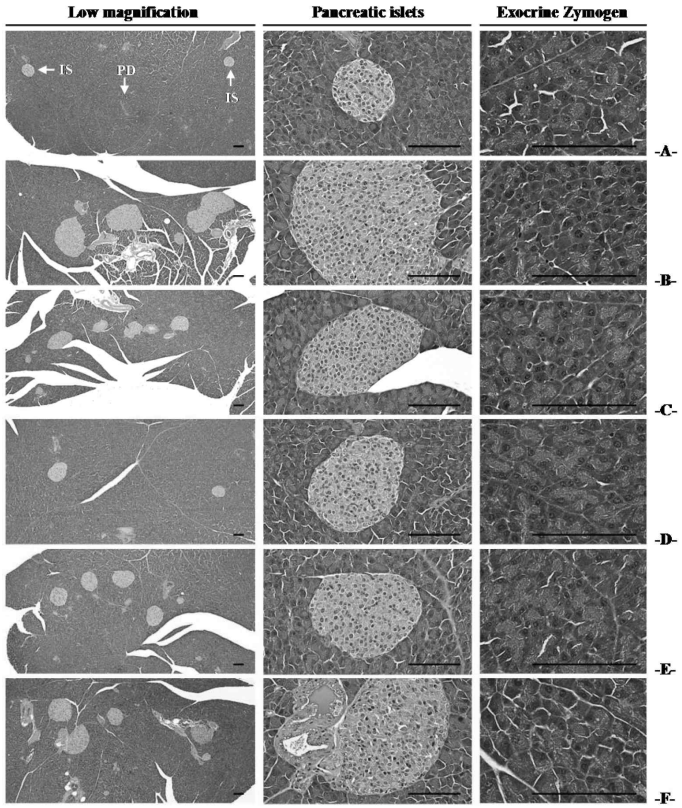

Figure 5. Representative general histological images of the pancreas, taken from NFD or HFD supplied mice. A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; IS = Pancreatic islet; PD = Pancreatic secretory duct; SPE = Standard Potato Protein Extracts. All Hematoxylin & Eosin stain. Scale bars = 100 μm.

Table 7. Changes on blood glucose levels and serum lipid contents in NFD or HFD supplied mice

Items / Groups	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Low density lipoprotein (mg/dl)	High density lipoprotein (mg/dl)
Controls					
Intact	93.13±18.19	98.00±23.48	70.63±14.78	18.38±4.03	93.75±20.60
HFD	263.38±49.22[a]	268.75±32.27[a]	252.25±67.07[a]	79.38±10.91[a]	15.00±5.10[a]
Reference					
Metformin	175.00±22.38[ac]	170.75±17.77[ac]	149.00±25.35[ac]	55.13±13.60[ac]	44.63±12.21[ac]
Test material - SPE					
400 mg/kg	141.25±17.50[ac]	139.88±22.74[ac]	94.38±12.11[ac]	34.75±12.16[ac]	71.13±10.48[bc]
200 mg/kg	175.50±25.77[ac]	173.38±20.11[ac]	149.63±19.24[ac]	55.38±12.64[ac]	44.38±10.04[ac]
100 mg/kg	205.00±16.28[ad]	213.38±13.48[ac]	179.13±10.33[ac]	62.00±11.41[ac]	29.13±7.20[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ and [d] $p<0.05$ as compared with HFD control.

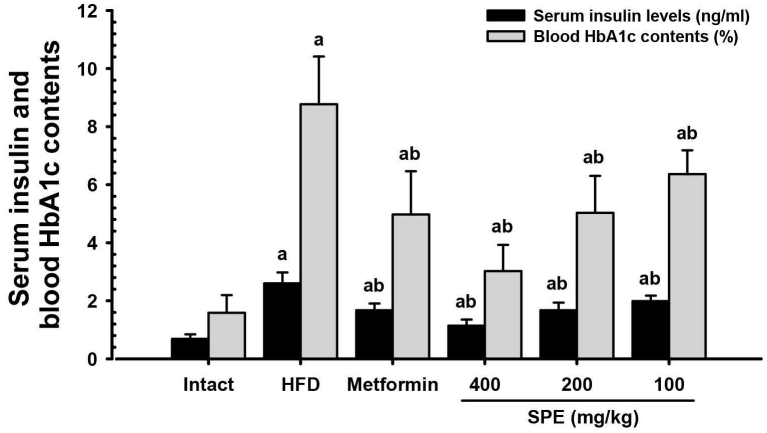

Figure 6. Serum insulin and blood HbA1c contents in NFD or HFD supplied mice.

Values are expressed mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; HbA1c = Glycated hemoglobin, hemoglobin A1c; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ as compared with HFD control.

pectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed clear dose-dependent increases of the pancreas relative weights as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in this result. Anyway, no meaningful changes on the absolute pancreatic weights were demonstrated in all experimental HFD mice including HFD control mice as compared with intact control, and also no significant changes on the absolute weights were noticed in all four test material treated mice as compared with those of HFD control mice, in our result (Table 3 and 4).

Significant ($p<0.01$) increases of pancreatic islet numbers and mean diameters were detected in HFD control as compared with intact control, results from marked hyperplasia of pancreatic islet itself or component endocrine cells, respectively. However, these hyperplasia and expansion of islets were significantly ($p<0.01$) reduced by treatment of all test substances including SPE 200 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent decreases of the pancreatic islet numbers and mean diameters as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the present measurement (Table 6; Fig 5).

Significant ($p<0.01$) increases of insulin and glucagon-immunoreactive cells, and also insulin/glucagon cells were detected in HFD control mice as compared with intact control, respectively. However, these abnormal increases of insulin and glucagon-immunostained cells and their ratio (insulin/glucagon cells) were significantly ($p<0.01$) normalized by treatment of all test substances including SPE 100 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed definitive dose-dependent decreases of the insulin- and glucagon-immunolabeled cell numbers, insulin/glucagon cell ratios as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current measurement (Table 6; Fig 7).

3. Effects on hyperlipidemia

Significant ($p<0.01$) increases of serum TC, TG, and LDL levels and significant ($p<0.01$) decreases of serum HDL levels were detected in HFD control as compared with intact control. However, the serum TC, TG and LDL levels were significantly

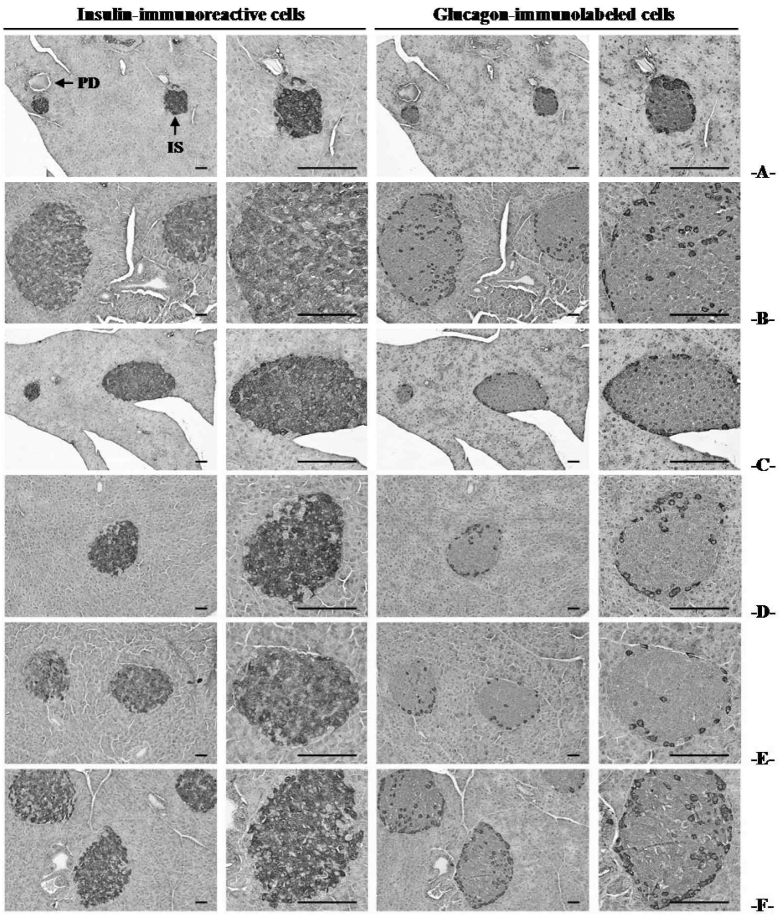

Figure 7. Representative histological images of the insulin- and glucagon-immunoreactive cells in the pancreas, taken from NFD or HFD supplied mice.

A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; IS = Pancreatic islet; PD = Pancreatic secretory duct; SPE = Standard Potato Protein Extracts. All immunostained by avidin-biotin-peroxidase complex. Scale bars = 100 μm.

($p<0.01$) decreased and the serum HDL levels were significantly ($p<0.01$) increased in all test substances including metformin 250 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed dose-dependent changes as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in this measurement (Table 7).

Although non-significant slight increases of fecal TC and TG contents were detected in HFD control as compared with intact control, the fecal TC and TG contents in all four test material treated mice including metformin 250 mg/kg were significantly ($p<0.01$ or $p<0.05$) elevated as compared with HFD control mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent increases of the fecal TC and TG contents as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current observation (Fig 8).

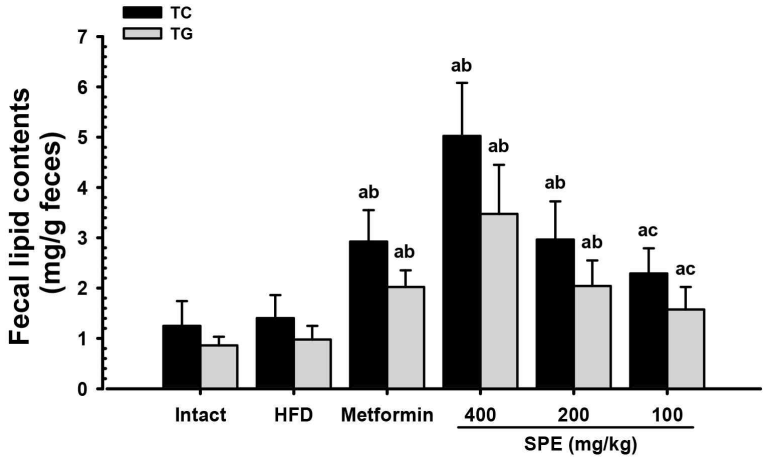

Figure 8. Fecal TC and TG content in NFD or HFD supplied mice.

Values are expressed mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; TC = Total cholesterol; TG = Triglyceride; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ and c $p<0.05$ as compared with HFD control.

4. Effects on hepatopathy

Significant ($p<0.01$) increases of liver absolute and relative weights were detected in HFD control as compared with intact control, respectively. However, these increases of absolute liver weights were significantly ($p<0.01$) normalized by treatment of all four test substances including SPE 400 mg/kg, as compared with HFD control mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed definitive dose-dependent decreases of the liver absolute weights as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in this observation. Anyway, no significant changes on the relative liver weights were noticed in all four test material treated mice as compared with those of HFD control mice, in our observation (Table 3 and 4).

Significant ($p<0.01$) increases of serum AST, ALT, ALP, LDH, and GGT levels were detected in HFD control as compared with intact control. However, these serum levels were significantly ($p<0.01$) decreased in all test substance administrated mice including SPE 200 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed dose-dependent decreases of these serum levels as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the present analysis (Table 8).

Significant ($p<0.01$) increases of steatohepatitis (percentages of fatty changed regions in liver parenchyma) and mean hepatocyte diameters (hypertrophy) were detected in HFD control as compared with intact control, result from severe hypertrophy of hepatocyte related to intracellular lipid depositions. However, these steatohepatitis and hepatocyte hypertrophies were significantly ($p<0.01$ or $p<0.05$) normalized by treatment of all four test substance, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed dose-dependent decreases of the steatohepatitis regions, the hepatocyte hypertrophies, and the mean diameters of hepatocytes as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current calculation (Table 9; Fig 9).

Table 8. Changes on serum AST, ALT, ALP, LDH, GGT, BUN and creatine levels in NFD or HFD supplied mice

Items / Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
Controls							
Intact	56.13±13.60	34.50±12.96	81.50±14.96	345.50±111.46	2.25±1.04	34.38±10.25	0.58±0.21
HFD	185.88±20.46[a]	148.00±14.23[a]	206.00±37.68[a]	2221.00±455.82[a]	10.25±1.67[a]	98.38±19.88[a]	2.31±0.54[a]
Reference							
Metformin	125.38±18.25[ac]	76.00±14.53[ac]	131.50±20.10[ac]	1220.88±171.93[ac]	6.57±1.04[ac]	60.50±12.21[ac]	1.35±0.19[ac]
Test material - SPE							
400 mg/kg	85.63±22.07[ac]	60.88±15.89[ac]	111.75±17.69[ac]	741.50±104.51[ac]	4.25±1.04[ac]	52.75±11.88[ac]	0.93±0.23[bc]
200 mg/kg	123.00±22.92[ac]	76.38±16.17[ac]	131.38±16.02[ac]	1221.25±142.86[ac]	6.63±1.30[ac]	59.75±10.54[ac]	1.38±0.13[ac]
100 mg/kg	151.63±23.94[ac]	120.38±18.92[ac]	159.88±18.07[ac]	1663.75±253.97[ac]	8.00±1.07[ac]	74.00±11.29[ac]	1.69±0.19[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; LDH = Lactate dehydrogenase; GGT = Gamma-glutamyltransferase; BUN = Blood urea nitrogen; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ as compared with HFD control.

Table 9. Changes on histopathology-histomorphometry of the liver and kidney in NFD or HFD supplied mice

Items / Groups	Liver steatosis (%/mm^2 of hepatic tissues)	Mean hepatocyte diameters (μm/cell)	Degenerative renal tubule numbers (%)
Controls			
Intact	7.10±3.32	17.61±3.87	4.88±2.23
HFD	76.46±10.13[a]	32.99±4.04[a]	70.50±11.02[a]
Reference			
Metformin	54.16±12.26[ac]	25.96±2.26[ac]	41.75±10.31[ac]
Test material - SPE			
400 mg/kg	43.22±10.53[ac]	22.49±1.69[bc]	23.00±7.28[ac]
200 mg/kg	53.08±11.85[ac]	25.57±2.69[ac]	39.63±11.43[ac]
100 mg/kg	61.40±9.33[ac]	28.31±1.67[ad]	54.25±10.25[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ and [d] $p<0.05$ as compared with HFD control.

5. Effects on nephropathy

Significant ($p<0.01$) increases of kidney absolute weights were detected in HFD control as compared with intact control, but they were significantly ($p<0.01$) normalized by treatment of all four test materials including SPE 400 mg/kg, constantly as compared with HFD mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent decreases of the kidney absolute weights as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in our calculation. Anyway, no meaningful changes on the relative kidney weights were demonstrated in all experimental HFD mice including HFD control mice as compared with intact control, and also no significant changes on the relative kidney weights were noticed in all four test material treated mice as compared with those of HFD control mice, in the present inspection (Table 3 and 4).

Significant ($p<0.01$) increases of serum BUN and creatinine levels were detected in HFD control

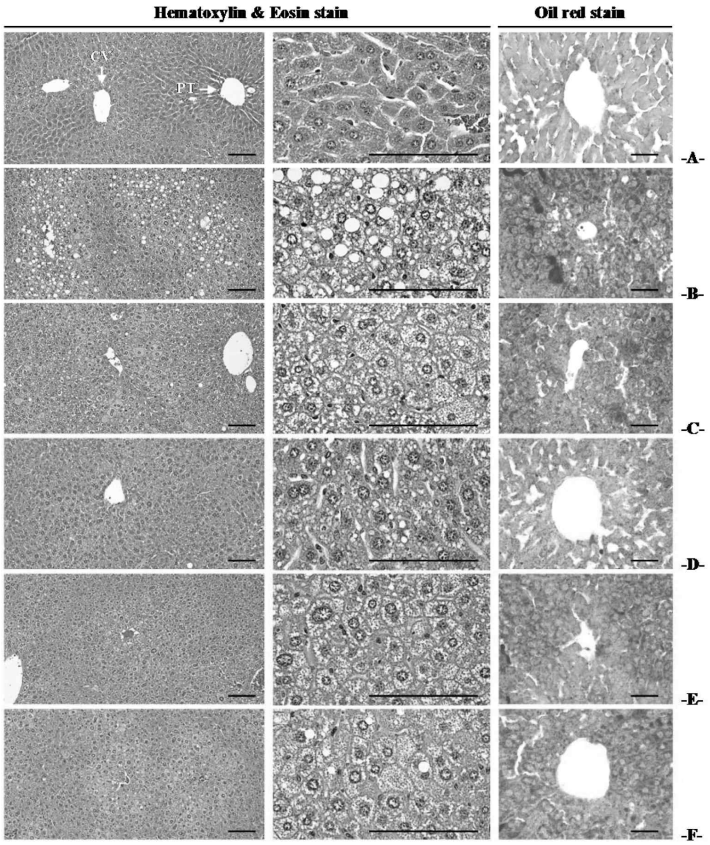

Figure 9. Representative histological images of the liver, taken from NFD or HFD supplied mice.

A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; CV = Central vein; PT = Portal triad; SPE = Standard Potato Protein Extracts. Scale bars = 100 μm.

as compared with intact control. However, the serum BUN and creatinine levels were significantly ($p<0.01$) decreased in all four test substance treated HFD mice including SPE 200 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed definitive dose-dependent decreases of the serum BUN and creatinine levels as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current inspection (Table 8).

Although, significant ($p<0.01$) increases of degenerative vacuolated renal tubules were detected in HFD control as compared with intact control, result from lipid droplet deposited diabetic nephropathies, but these diabetic nephropathies were significantly ($p<0.01$) normalized by treatment of all four test materials including metformin 250 mg/kg, as compared with HFD control, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed clear dose-dependent decreases of the numbers of vacuolated renal tubules as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the present observation (Table 9; Fig 10).

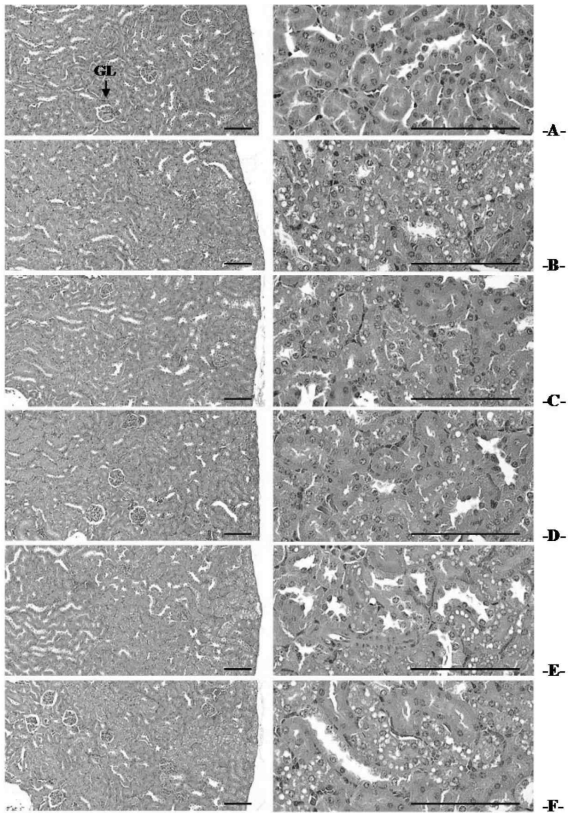

Figure 10. Representative histological images of the kidney, taken from NFD or HFD supplied mice.
A = Intact control: 10 ml/kg of distilled water orally administered mice with NFD supply; B = HFD control: 10 ml/kg of distilled water orally administered mice with HFD supply; C = Metformin: 250 mg/kg of metformin orally administered mice with HFD supply; D = SPE400: 400 mg/kg of SPE orally administered mice with HFD supply; E = SPE200: 200 mg/kg of SPE orally administered mice with HFD supply; F = SPE100: 100 mg/kg of SPE orally administered mice with HFD supply. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; GL = Glomerulus; SPE = Standard Potato Protein Extracts. All Hematoxylin & Eosin stain. Scale bars = 100 μm.

6. Effects on liver lipid peroxidation and antioxidant defense system

HFD control mice represented a significant ($p<0.01$) increment of liver lipid peroxidation, the hepatic MDA content elevations, and a significant ($p<0.01$) decrement of hepatic antioxidant defense system (GSH contents, CAT and SOD activity) as compared with intact control, but they were significantly ($p<0.01$ or $p<0.05$) normalized by treatment of all four test materials including SPE 400 mg/kg, as compared with HFD control mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent manner, as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current observation (Table 10).

7. Effects on hepatic glucose-regulating enzyme activities

Significant ($p<0.01$) decreases of hepatic GK activities, one of blood glucose utilized hepatic enzyme, were detected in HFD control as compared with intact control. Although, significant ($p<0.01$) increases of hepatic G6pase and PEPCK activities, gluconeogenesis hepatic enzymes, were

Table 10. Changes on the liver lipid peroxidation and antioxidant defense systems in NFD or HFD supplied mice

Items / Groups	Lipid peroxidation	Antioxidant defense system		
	Malondialdehyde (nM/mg tissue)	Glutathione (μM/mg tissue)	Catalase (U/mg tissue)	SOD (U/mg tissue)
Controls				
Intact	5.62±2.36	71.78±14.66	64.00±11.82	7.69±1.58
HFD	72.28±13.11[a]	10.65±2.82[a]	12.38±3.80[a]	1.28±0.45[a]
Reference				
Metformin	47.08±11.05[ab]	32.11±11.54[ab]	31.89±10.63[ab]	3.21±1.10[ab]
Test material - SPE				
400 mg/kg	23.01±10.16[ab]	46.17±15.35[ab]	47.27±12.02[ab]	4.97±1.08[ab]
200 mg/kg	46.42±12.78[ab]	31.82±12.08[ab]	32.72±10.10[ab]	3.24±0.94[ab]
100 mg/kg	53.64±10.63[ac]	20.41±5.22[ab]	25.47±10.35[ac]	2.56±1.01[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; SOD = Superoxide dismutase; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ as compared with intact control; [b] $p<0.01$ and [c] $p<0.05$ as compared with HFD control.

Table 11. Changes on the hepatic glucose-regulating enzyme activities in NFD or HFD supplied mice

Items / Groups	Glucokinase (nM/min/mg protein)	Glucose-6-phosphatase (nM/min/mg protein)	PEPCK (nM/min/mg protein)
Controls			
Intact	5.65±1.66	121.88±28.70	1.52±0.32
HFD	1.03±0.38[a]	325.65±65.41[a]	7.04±1.42[a]
Reference			
Metformin	2.22±0.73[ac]	196.00±26.28[ac]	3.85±0.79[ac]
Test material - SPE			
400 mg/kg	3.62±0.43[ac]	154.70±22.37[bc]	2.54±0.72[ac]
200 mg/kg	2.24±0.52[ac]	199.43±37.36[ac]	3.88±0.73[ac]
100 mg/kg	1.76±0.29[ac]	239.46±41.94[ad]	5.14±0.81[ac]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; PEPCK = Phosphoenolpyruvate carboxykinase; SPE = Standard Potato Protein Extracts. [a] $p<0.01$ and [b] $p<0.05$ as compared with intact control; [c] $p<0.01$ and [d] $p<0.05$ as compared with HFD control.

observed in HFD control as compared with intact control. However, they were significantly ($p<0.01$) normalized by treatment of all four test materials as compared with HFD control mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed obvious dose-dependent manner of the hepatic glucose-regulating enzyme activities as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current detection (Table 11).

8. Effects on lipid metabolism-related gene expressions

We observed a significant ($p<0.01$) increment of hepatic ACC1, adipose tissue leptin, C/EBPα, C/EBPβ and SREBP1c mRNA expressions, and a significant ($p<0.01$) decrement of hepatic AMPKα1 and AMPKα2, adipose tissue UCP2 and adiponectin mRNA expressions by HFD supply as compared with intact control, but they were significantly ($p<0.01$) normalized by treatment of all four test

Table 12. Changes on lipid metabolism-related gene mRNA expressions in NFD or HFD supplied mice, *realtime* RT-PCR analysis

Groups / Items	Controls		Reference	Test material - SPE		
	Intact	HFD	Metformin	400 mg/kg	200 mg/kg	100 mg/kg
Hepatic tissue						
ACC1	1.00±0.10	4.51±1.55[a]	2.38±0.59[ab]	1.64±0.29[ab]	2.48±0.43[ab]	2.79±0.35[ab]
AMPKα1	0.98±0.10	0.30±0.08[a]	0.47±0.10[ab]	0.70±0.16[ab]	0.48±0.11[ab]	0.43±0.09[ac]
AMPKα2	1.00±0.07	0.40±0.08[a]	0.60±0.07[ab]	0.80±0.14[ab]	0.60±0.05[ab]	0.50±0.04[ac]
Adipose tissue						
Leptin	1.00±0.05	6.90±0.75[a]	3.74±0.90[ab]	2.23±0.64[ab]	3.73±0.77[ab]	4.97±1.22[ab]
UCP2	1.00±0.08	0.15±0.08[a]	0.34±0.12[ab]	0.52±0.14[ab]	0.35±0.09[ab]	0.25±0.05[ac]
Adiponectin	1.00±0.08	0.14±0.03[a]	0.37±0.09[ab]	0.56±0.18[ab]	0.35±0.10[ab]	0.22±0.07[ab]
C/EBPα	1.01±0.06	2.95±1.13[a]	1.56±0.16[ab]	1.30±0.16[ab]	1.57±0.20[ab]	1.79±0.12[ab]
C/EBPβ	1.00±0.07	4.06±0.78[a]	2.48±0.51[ab]	1.94±0.18[ab]	2.46±0.46[ab]	3.11±0.46[ac]
SREBP1c	1.00±0.12	3.35±0.83[a]	2.05±0.26[ab]	1.58±0.28[ab]	2.06±0.46[ab]	2.39±0.32[ab]

Values are expressed as Mean ± SD of eight mice. NFD = Normal pellet diet; HFD = 45%Kcal high fat diet; RT-PCR = reverse transcription polymerase chain reaction; UCP = Mitochondrial uncoupling protein; C/EBP = CCAAT-enhancer-binding protein; SREBP = Sterol regulatory element-binding protein; ACC1 = Acetyl-CoA carboxylase 1; AMPK = 5' adenosine monophosphate-activated protein kinase; GAPDH = Glyceraldehydes 3-phosphate dehydrogenase; SPE = Standard Potato Protein Extracts.
[a] $p<0.01$ as compared with intact control; [b] $p<0.01$ and [c] $p<0.05$ as compared with HFD control.

materials including metformin 250 mg/kg as compared with HFD control mice, respectively. Especially, all three different dosages of SPE 400, 200 and 100 mg/kg treated mice also showed definitive dose-dependent manner of the mRNA expressions normalization as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, in the current realtime RT-PCR analysis (Table 12).

IV. Discussion

As results of 91 days of continuous HFD supply, HFD control mice showed HFD supplement-induced AMPK down-regulation dependent dysregulation of glucose and lipid metabolism related insulin resistant obese type II diabetes, and oxidative stress related obesity, diabetic hepatopathy (Non-alcoholic fatty liver disease, NAFLD), nephropathy and hyperlipidemia in the present study. However, all of these diabetes and related complications including obesity were significantly inhibited by 84 days of continuous oral treatment of SPE 400, 200 and 100 mg/kg, dose-dependently, and they also dramatically normalized the hepatic lipid peroxidation and depletion of liver endogenous antioxidant defense system, the changes of the hepatic glucose-regulating enzyme activities, also changes of the lipid metabolism-related genes expressions including hepatic AMPKα1 and AMPKα2 mRNA expressions, dose-dependently. Especially, SPE 200 mg/kg constantly showed favorable inhibitory activities against type II diabetes and related complications as comparable to those of metformin 250 mg/kg in HFD mice, respectively.

The accumulation or increases of fat deposition in body is major characteristics of obesity, and cellular hypertrophy appeared to be the major mode of expansion of the intra-abdominal adipose tissue in rodents[12,14]. In obesity, the increases in the accumulation of adipose tissues are common features in obesity. Adipose tissue is currently known to work not simply as an organ for energy

storage, but also as an endocrine and secretory organ[45]. Adipose tissues secret adipokines and changes in the expression, secretion, and action of the adipokines in obesity are possibly implicated in the development of various diseases including insulin resistance[12,46]. In the present study, treatment of SPE 400, 200 and 100 mg/kg significantly and dose-dependently inhibited the accumulation of adipose tissues and adipocyte hypertrophy.

The decreases of mean daily food consumption detected in all HFD supplied mice as compared with NFD supplied intact mice, were considered as not critical problems in this study because the energy of HFD (4.73 kcal/g) used in the present study were relatively higher than that of NFD (4.00 kcal/g). Similar decreases of daily food consumption in HFD supplied mice were already reported in our previous studies[12,14,33]. In the present study, no meaningful or significant changes on the mean daily food consumptions were detected in all test substance administered groups including metformin 250 mg/kg as compared with HFD control, suggesting pharmacological effects of SPE detected in this study were difficult to consider as results from the inhibition of food consumption, at least in a condition of this experiment.

It is generally known that obesity develop pancreatic steatosis, acinar cell atrophy, and a diminution in the number of zymogen granules[12,47]. The increases of zymogen granules in exocrine pancreatic acinar cells mean the production of digestive enzymes especially for digestion of lipid and protein[48]. In the present study, the diminishments of zymogen depositions in exocrine pancreas induced by HFD supply were effectively inhibited by treatment of SPE 400, 200 and 100 mg/kg, dose-dependently. These findings are considered as direct evidences that SPE 400, 200 and 100 mg/kg have favorable anti-obese effects in HFD mice, may be mediated by inhibition of lipid digestions by decrease of pancreatic enzyme production or releases. Since it also could be completely excluded that SPE induced the increases of digestive tract motility, more detail mechanism studies should be proceeded in future to elucidate exact anti-obese mechanisms of SPE. Increased digestive tract motility also induced increases of fecal excretions, and consequently induced decreases of body weights[49], and dose-dependent marked and noticeable increases of fecal excretions with fecal TC and TG contents were dose-dependently observed by treatment of SPE 400, 200 and 100 mg/kg. Non-significant slight elevation of fecal TC and TG contents detected in HFD control mice of the present study is considered as secondary results from the considerable amounts of HFD intakes.

HbA1c is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time, and produced by high glucose exposed erythrocytes for long time[50]. Hyperglycemia is the main signs of diabetes, and hyperglycemia should be controlled to treat the diabetes[12,14]. As progression of insulin resistance Type II diabetes, marked elevations of blood insulin and Hb1Ac levels have been observed after long term HFD supply[34]. In addition, increased insulin secretion is in part related to pancreatic islet hyperplasia as progression of insulin-resistance by HFD supply[51]. Total pancreatic islet numbers and insulin-producing cells were increased after chronic consumption of a HFD, islets increased in area and number to secrete more insulin to try to maintain glucose homeostasis[52] with noticeable hypertrophy or hyperplasia of endocrine pancreas cells[51,52]. Also in this experiment, noticeable elevations of blood glucose, insulin and HbA1c contents were defected in HFD control mice as compared with intact control, and with increases of pancreatic islet numbers, expansions, insulin- and glucagon-immunoreactive cells and insulin/

glucagon cell ratios, suggesting insulin resistance type II diabetes at histopathological observations. However, 84 days continuous oral administration of SPE 400, 200 and 100 mg/kg dose-dependently inhibited these abnormal endocrine pancreas histopathological changes and elevations of blood glucose, insulin and HbA1c contents. These findings are considered as obvious evidences that SPE 400, 200 and 100 mg/kg have favorable hypoglycemic effects in HFD mice, may be through inhibition of pancreatic endocrine changes.

Since the most critical problem in hyperlipidemia is increases of serum LDL, TG and TC levels with decrease of HDL levels[13,14], the efficacy of hypolipidemic agents generally evaluated based on the decrease of serum LDL, TG and TC with increase of HDL levels[12,14]. In the present study, SPE 400, 200 and 100 mg/kg effectively and dose-dependently decreased the serum LDL, TG and TC levels, and increased the serum HDL levels. These results are considered as direct evidences that SPE 400, 200 and 100 mg/kg have favorable hypolipidemic effects in HFD mice, may be mediated by inhibition of lipid digestions by decrease of pancreatic enzyme production or releases. In addition, these favorable hypolipidemic effects of test substances detected in these HFD mice were also considered as results from the decreases of lipid absorptions and propulsion of lipids into feces, through the pancreatic digestive enzyme modulating effects as aforementioned.

As progression of diabetes, increases of liver weight due to the fibrosis or abnormal glycosylation relate hepatosteatosis and hepatocyte hypertrophic changes, due to lipid depositions in the cytoplasm were observed with elevation of serum AST, ALT, ALP, LDH and GGT levels[12,14]. This phenomenon has been regarded as diabetic hepatopathy, and it also has been accompanied in HFD supplied mice as NAFLD[14,53]. Improve of these abnormal changes have been considered as a direct evidences that improved the diabetic hepatopathies[54]. Elevation of serum AST and ALT levels are sensitive indicators of active live damage along with serum ALP, LDH and GGT increases, but does not indicate the cause or reversibility of the damage[55]. In this experiment, SPE treatments dose-dependently and effectively decreased the diabetic hepatopathies; they inhibited the increases of liver weights, serum AST, ALT, ALP, LDH and GGT elevations with steatohepatitis and related hepatocyte hypertrophic changes at histopathological observations.

In chronic diabetes, increases of kidney weights due to the swelling, inflammation and necrotic processes were observed with elevation of serum BUN and creatinine levels, so called diabetic nephropathy, and improve of these abnormal changes have been considered as a direct evidences that improved the diabetic nephropathies[12,14]. In the current analysis, HFD mice showed marked increases of the absolute kidney weights and elevations of serum BUN and creatinine levels with lipid droplet deposition related renal tubule vacuolation at histopathological observation, suggesting mild diabetic nephropathies, but they were normalized by treatment of SPE 400, 200 and 100 mg/kg, dose-dependently. These results are also considered as direct evidences that SPE 400, 200 and 100 mg/kg have favorable and dose-dependent nephroprotective effects on HFD-induced mild diabetic nephropathies.

There is considerable evidence on the role of free radicals in the etiology of diabetes and altered antioxidant defenses in diabetes[56]. Oxidative stress has been reported to play an important role in diabetes mellitus right from its genesis to the development of microvascular complications. Generation of free radicals by hyperglycemia is related to glucose auto-oxidation. Glucose auto-oxidation has been linked to non-enzymatic glycosylation and glycosylated proteins have been shown to be a source of free radicals,

ROS[12,57]. Oxidative stress in diabetes coexists with a decrease in the antioxidant status[58] which can increase the deleterious effects of free radicals. Generation of ROS related oxidative stress plays an important role in the etiology of diabetic complications[58]. Various toxic substances from lipid peroxidation destroy the surrounding tissues[59], and elevations of lipid peroxidation in the various organs were also demonstrated in HFD mice, and they were also acted as a potent redox cycler that generates harmful ROS and causes organ damages[60]. So the increased lipid peroxidation and decreases of endogenous anti-oxidant, the GSH contents, antioxidant active enzymes, the SOD and CAT activities are in the damaged liver tissue is secondarily important in terms of helping improve diabetes and related various complications[61], and marked elevation of hepatic lipid peroxidation, and depletion of GSH contents, decreases of SOD and CAT activities were noticed in HFD control of the present study, like other previous HFD mice studies[34,62]. In the present study, SPE 400, 200 and 100 mg/kg effectively and dose-dependently inhibited the deterioration of hepatic antioxidant defense system. These findings are considered as direct and obvious evidences that SPE 400, 200 and 100 mg/kg have favorable and dose-dependent antioxidant effects on HFD mice.

The hepatic enzyme GK is related to glucose homeostasis and its increased expression could cause an increase in blood glucose utilization for energy production or glycogen storage in the liver, leading to a reduction in the blood glucose level[63]. On the contrary, the enzymes G6pase and PEPCK are associated with gluconeogenesis and hepatic glucose output and their increased activities denote increased glucose level[64]. Generally, noticeable decreases of hepatic GK activities with increases of G6pase and PEPCK activities have been accompanied by HFD supply[34], and also in HFD control mice of the present study. Constantly, SPE 400, 200 and 100 mg/kg effectively and dose-dependently inhibited HFD-induced hepatic glucose-regulating enzyme activity changes. These findings are considered as reliable evidences that SPE 400, 200 and 100 mg/kg have favorable and dose-dependent hepatic glucose-regulating enzyme regulatory activities on HFD mice.

To elucidate the mechanisms by which SPE exert anti-diabetic and refinement activities on related complications including NAFLD, we investigated lipid metabolism and AMPK signaling in the hepatic and adipose tissues. Activation of AMPK in both types of tissue plays a major role in regulating glucose and lipid metabolism through the stimulation of fatty acid oxidation and inhi-bition of lipogenesis and glucose production[65]. Given the role of AMPK signaling pathway-related proteins in glucose and lipid metabolism, it is important to identify and analyze their mRNA and protein expression levels in adipose tissue and the liver. Thus, we investigated whether SPE affect mRNA expression of AMPK and AMPK signaling pathway-related-proteins in these tissues. Gene expression analyses showed that SPE 400, 200 and 100 mg/kg dose-dependently and significantly decreased the mRNA levels of lipogenic genes such as C/EBPα, C/EBPβ, SREBP1c, and leptin in the periovarian adipose tissue of HFD mice. SPE 400, 200 and 100 mg/kg also dose-dependently and significantly increased mRNA levels of the thermogenesis-related protein UCP2[66] in adipose tissue along with AMPKα1 and AMPKα2 in hepatic tissue. In addition, SPE 400, 200 and 100 mg/kg significantly and dose-dependently increased adiponectin mRNA expression in adipose tissue. The effect of fat cell-derived adiponectin on insulin sensitizing and fatty-acid-oxidizing actions is dependent on AMPK in the adipose tissue and liver[67]. In the present study, we demonstrated that mRNA of AMPKα decreased in the liver of HFD mice,

suggesting that alterations in AMPKα expression contribute to the pathogenesis of lipid accumulation, the NAFLD in the liver of HFD mice[42]. However, SPE 400, 200 and 100 mg/kg significantly and dose-dependently stimulated AMPK expressions, and inhibited ACC1 mRNA expressions in the hepatic, suggesting that SPE improve abnormal lipid metabolism by suppressing lipogenesis and promoting fatty acid oxidation via up-regulation of AMPK. In addition, upregulation of AMPK and AMPK signaling pathway constantly showed favorable modulatory activities on the body endogenous antioxidant defense systems[31] and glucose-regulatory enzyme activities[65]. Therefore, it was also considered that antioxidative effects and favorable modulatory activities on glucose-regulatory enzymes - GK, G6pase and PECK, aforementioned, were also mediated by upregulation of AMPK in SPE administered mice as similar to those of metformin, a well documented AMPK activator[31]. However, the effects on the obese induced by HFD supply are not completely elucidated in this study. Therefore, further studies are required to understand the mechanism of SPE against obese.

V. Conclusion

In the current study, oral administration of SPE at dose levels of 400, 200 and 100 mg/kg, HFD supplement-induced AMPK down-regulation dependent dysregulation of glucose and lipid metabolism related insulin resistant type II diabetes, and oxidative stress related obesity, diabetic hepatopathy (NAFLD), nephropathy and hyperlipidemia were significantly and dose-dependently inhibited, respectively. Especially, SPE 200 mg/kg constantly showed favorable inhibitory activities against type II diabetes and related complications as comparable to those of metformin 250 mg/kg in HFD mice, respectively. These findings are considered as clear and direct evidences that SPE 400, 200 and 100 mg/kg showed favorable and dose-dependent anti-diabetic and related complications including obesity refinement activities in HFD mice, through AMPK upregulation mediated hepatic glucose enzyme activity and lipid metabolism-related genes expression, antioxidant defense system and pancreatic lipid digestion enzyme modulatory activities, as comparable to those of metformin 250 mg/kg in SPE 200 mg/kg, at least, partially in a condition of this experiment. It, therefore, is expected that SPE will be promising as a new potent refinement agent or medicinal food for type II diabetes and related various complications including obesity, the metabolic syndrome, in future.

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