Ameliorative Effects of *Schizandra chinensis* Extracts and Their Soybean Powder Blends to Diabetes Mellitus

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**Abstract** *Schisandraceae refractilis* (Omija) is traditionally used as a food-based medicinal/pharmaceutical resources including antidiabetic agent in worldwide. In this study, the optimal combined formula of Omija extract and soybean mixture (OSM) were investigated for its effects on type 2 diabetes model expressed in *in Vitro* and *in Vivo* animal model. Using whole extracts, we confirmed inhibitory effects to α-glucosidase, α-amylase and the DPPH scavenging *in Vitro* and examined glucose tolerance in mice. The combined optimum formulation of OSM were significantly ameliorated type 2 diabetes-related. Furthermore, activation of p-Akt, p-AMPK, p-IRS and GLUT2 expression level is pivotal roles of in this anti-diabetic molecular mechanism on *in Vivo* without side effects. Therefore, these results suggest that OSM is good resources for improves of type 2 diabetes and its complications.

**Keywords:** Schisandraceae chinensis, type 2 diabetes, diabetes complication, db/db mice, antioxidant, combined mixture

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1. Introduction

*Schisandra chinensis* (Turcz.) Baill. is a genus of *S. chinensis* (Turcz.) Baill. It belongs to the deciduous creeping plant Magnoliaceae, *S. chinensis* Fructus is the red fruit of the Schi-sandra tree. Sweet, bitter, sour, spicy and salty taste that humans can feel. It got its name because it has a total of five flavors [1,2]. It is mainly distributed in the central region of Korea, and the flowering period is June-August, the fruit opening period is September-October, and the harvesting period is after frost [3]. *Omija (Schisandra chinensis)* is mainly distributed in Korea, Japan, Manchuria, and China. Soybean and its modified products are known to contain bioactive components such as proteins, oils, carbohydrates, minerals, vitamins, isoflavones, phytosterols, saponins and ferritins [4,5,6]. For several years, rigorous scientific and clinical research has revealed that most of the components of soybeans exhibit beneficial effects, showing preventive potential for several diseases. Many beneficial components of soybeans have been characterized, nutritionally and natural medicinally [7-11].

To date, several million Korean diabetic patients of the total population were reported, and 1 in 10 Koreans is diabetic, statistically [12]. By 2030, the number of diabetic patients is expected to nearly double. Recently, pediatric diabetes which appears in the age group of young ages were rapidly increased and the age range of diabetic patients was also showing a tendency to gradually decrease [12,13]. Diabetes mellitus is a disorder of the posterior pituitary gland and diencephalon which is responsible for regulating the diuretic effect. So, the pancreatic organs cannot produce the required amount of insulin or does not work properly on the target cells then cannot absorb energy into the body. Generally, diabetes is a disease in which the blood glucose level rises due to failure to do so [13] and divided to two types. Type 1 diabetes were mainly occurs in childhood which is cannot produce insulin at all due to destruction of β-cells in the pancreas due to genetic factors or autoimmune mechanisms [14]. Whereas, type 2 diabetes were mainly occurs in adulthood as non-insulin-dependent diabetes mellitus which results in insufficient insulin action due to insulin resistance or a relatively insufficient amount of insulin [15]. In diabetes, almost patients were drink a lot of water because of dehydration and elevated blood osmotic pressure due to high blood sugar [16]. In addition, lower levels of glucose utilization rate were induced loss of glucose through urine excretion and the body uses protein as an energy source instead of glucose and induces of body weight losses [17].
In addition, various diabetes-induced complications may also developed such as renal dysfunction, arteriosclerosis in blood vessels, decreased visual acuity by retinal hemorrhage, foot ulcers, and peripheral neuropathy [18]. Insulin administration is still the main treatment for type 1 diabetes, and pancreatic transplantation and pancreatic islet transplantation have been attempted [19]. Treatment of type 2 diabetes focused on reducing insulin resistance and reducing insulin requirements through restricted diet and the exercise therapy. In other hands, the treatment method for diabetes is prevention and management of various complications along with steady blood glucose management [20].

Recently, the efficacy of Schisandra is known to have the effects of nourishing, tonic, branching, and antitussive [21], strengthening the function of the central nervous system, improving blood circulation and lowering effect [22], restoring the liver function [23], antibacterial and antiulcer action [24], anticancer and antitumor effect [25], nitric oxide production promoting action [26], and oxidative, antibacterial, and nitrite scavenging properties of Schisandra seeds [27] have been reported. As the main pharmacological component of Schisandra, lignans such as shizandrin, shizandrol, shizandran, and gomisin are contained. It has been reported to have anticancer effects [28-30]. Furthermore, Omija has been used as a medicinal herb in oriental medicine and Chinese medicine, and has been recognized as an official medical treatment in Russia since the 1960s [31]. Even for over 40 years, clinical studies showing that it is effective in various diseases such as depression, neurosis, schizophrenia, alcoholism, vision disorders, hypotension, influenza infection, pneumonia, chronic sinusitis, allergic dermatitis, gastritis, and duodenal ulcer have been conducted for over 40 years [32]. Although the medicinal/pharmaceutical effect of omija is known through many studies, systematic research reports on the antidiabetic effect are insufficient. Therefore, in this study, the antidiabetic effect was verified using Schisandra extract, and based on this, a study was conducted to investigate the antidiabetic effect.

2. Materials and Methods

2.1. Extraction

Dried Omija (MunGyeong-Agricultural technology service center, Mungyeong, Korea) were extracted with 0%, 30%, and 50% ethanol (50 g material per 500 ml solvents, 3 hours, 70°C) and concentrated. To divide for two samples, deseed puree and no-seed puree processed and freeze-dried to powder. To get soybean powder, soybean were hydrated with tap water and removed soybean husks, steam boiled at 100°C, dried and homogenized. The OSM was freeze-dried to powder after mixed omija fruit ethanol extracts and soybean powder.

2.2. Animals

Six-week old ICR were purchased from Hanil Laboratory Animal (Jeonbuk, Korea). All animals were acclimatized to the laboratory environment for 1 week before the experiment. Mice were allowed free access to drinking water and food under constant room temperature (22 ± 2°C) and humidity (50% ± 10%) under an automatic 12 h light/12 h dark cycle. The mice were cared for and treated in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. The mice were randomly divided into 11 groups (10 mice per group) and tested glucose tolerances (Table 1). Each group was treated with drugs by oral administration once per day for 4 weeks.

Table 1. Sample definitions and code abbreviation

| Sample definitions | Codes of sample |
|--------------------|-----------------|
| Control group      | Control         |
| Omija fruit        | OF              |
| Omija pulp         | OP              |
| Omija fruit water extract | OFWE            |
| Omija fruit 30% ethanol extract | OF30EE         |
| Omija fruit 50% ethanol extract | OF50EE         |
| Omija pulp water extract | OPWE            |
| Omija pulp 30% ethanol extract | OF30EE         |
| Omija pulp 50% ethanol extract | OF50EE         |
| Soybean powder     | SB              |
| Metformin          | Positive        |

To perform optimum coformulation with soybean, mice were randomly divided into 5 groups (10 mice per group) and tested oral glucose tolerances (Table 2).

Table 2. Sample definitions and code abbreviation. S. chinensis extracts and their soybean powder blends

| Sample definitions | Codes of sample |
|--------------------|-----------------|
| Control group      | Control         |
| 100 mg/kg of omija fruit 50% ethanol extracts 1.5 : soybean 1 | O1.5:S1        |
| omija fruit 50% ethanol extracts 3 : soybean 1 | O3:1          |
| omija fruit 50% ethanol extracts 5 : soybean 1 | O5:1          |
| 10 mg/kg of acarbose | Acarbose       |

To find optimum dosages of omija extract and their soybean powder blends, mice were randomly divided into 5 groups (10 mice per group) and tested oral glucose tolerances (Table 3).

Table 3. Sample definitions and code abbreviation

| Sample definitions | Codes of sample |
|--------------------|-----------------|
| Control group      | Control         |
| 10 mg/kg of omija fruit 50% ethanol extracts 5 : soybean 1 mixture (low dose) | OSM 10         |
| 5 : soybean 1 mixture (middle dose) | OSM 30         |
| 100 mg/kg of omija fruit 50% ethanol extracts 5 : soybean 1 mixture (high dose) | OSM 100        |
| 200 mg/kg of metformin | Positive       |

During the experiment, body weights, food intake, water intake and blood glucose levels of the mice were measured once every week. After sacrifice, blood parameters such as fructosamine, VLDL, LDL, HDL, insulin, HOMA-IR and HOMA-β were measured by KPNT Inc. (Chungju, Korea). In addition, liver weight, pancreas weight, kidney weight and epidydimal fat weight were measured.

To elucidate effects of omija extracts mixture on genetic diabetic model, 7 weeks old male C57BL/KsJ db/db mice purchased DaoYeol Biotech (Seoul, Korea).
To investigate effects of omija extract mixture, mice were randomly divided into 6 groups (10 mice per group) and tested oral glucose tolerances (Table 4).

### Table 4. Sample definitions and code abbreviation

| Sample definitions                  | Codes of sample |
|-------------------------------------|-----------------|
| Normal group                        | Normal          |
| db/db mice control group            | Control         |
| db/db mice + OSM 10                 | OSM 10          |
| db/db mice + OSM 30                 | OSM 30          |
| db/db mice + OSM 100                | OSM 100         |
| db/db mice + 200 mg/kg of metformin | Positive        |

All animal experiment procedures conducted in accordance with guidelines and approval of the Institutional Animal Care and Use Committees (IACUC) of INVIVO Co. (IV-RA-06-1907-22).

2.3. Alpha-glucosidase Inhibitory Assay

Various omija extract mixtures were evaluated for α-glucosidase inhibitory activity according to the method given by Tibbot and Skadsen [33] with slight modifications. Extracts (50 μL) at varying concentrations was incubated with 10 μL of the α-glucosidase (Sigma-Aldrich, MO, USA) enzyme solution (1 U/ml) for 20 min at 37°C with an additional 125 μL of 0.1 M phosphate buffer (pH 6.8). After 20 min, the reaction was started with the addition of 20 μL of 1 M p-nitrophenyl-α-D-glucopyranoside (pNPG) and the mixture was incubated for 30 min. The reaction was terminated with the addition of 0.1 M Na2CO3 (50 μL) and final absorbance was measured at 405 nm using microplate reader (Infinite 200, TECAN Group Ltd, Switzerland). Acarbose was used as a positive control at varying concentrations (12.5-400 μg/ml). Inhibitory activity was calculated as:

\[
\text{Inhibitory activity} \% = \left( \frac{OD_{\text{blank}} - OD_{\text{sample}}}{OD_{\text{blank}}} \right) \times 100
\]

One unit of the enzyme can be defined as the amount of enzyme (α-glucosidase) required for the formation of one μmol of product (p-Nitrophenol) from the substrate (p-nitrophenyl-α-D-glucopyranoside) per minute. IC50 (concentration required to inhibit 50% of the enzyme activity) was calculated using regression equation obtained through plotting concentration in the range 12.5-400 μg/ml (x-axis) and % inhibition (y-axis) for different extracts and fractions.

2.4. Amylase Inhibitory Assay

This assay was carried out using a modified procedure of McCue and Shetty (2004) [34]. A total of 250 μL of extract (1.25-10 mg/ml) was placed in a tube and 250 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing amylase solution (0.5 mg/ml) was added. This solution was preincubated at 25°C for 10 min, after which 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500 μL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using multiwell plate reader. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition: Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

\[
\text{Inhibitory activity} \% = \left( \frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100
\]

2.5. DPPH Radical Scavenging Assay

Free radical scavenging ability of the various omija extract mixtures were tested by DPPH radical scavenging assay as described by Desmarchelier et al. (1997) [35]. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 μl of this solution was mixed with 1.6 ml of extract in methanol at different concentrations (12.5-150 μg/ml). The reaction mixture was thoroughly vortexed and left in the dark at room temp for 30 min. The absorbance of the mixture was spectrophotometrically measured at 517 nm. BHT was used as reference [35]. Percentage DPPH radical scavenging activity was calculated by the following equation:

\[
\text{DPPH radicals scavenging activity} \% = \left( \frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100
\]

Then % of inhibition was plotted against concentration. The experiment was repeated three times at each concentration.

2.6. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 5μm, deparaffinize, hydrated and rinsed in xylene, graded ethanol series and distilled water, and washed with PBS. IHC was performed using the avidin-biotin complex technique (DAKO, USA). Sections were incubated overnight at 4°C with primary antibody anti-insulin antibody (DAKO, USA). Immunolabeling was detected using biotinylated immunoglobulins followed by ABC complex (DAKO, USA). All slides were counterstained with methyl green. Quantitative IHC analysis was performed using the Image J program (NIH, USA). Randomly selected 5 sections in high resolution (x400 magnification) of slides from control group and test group were analyzed. They were presented as % signal area (normalized to the tissue mass of each sections) and analyzed. [36]

2.7. Western Blotting

The procedures for preparation of whole-cell protein lysates and Western blot analysis were previously described [37].
2.8. Statistical Analysis

All experimental results were calculated as mean±standard error (Mean±S.E.) using statistical programs (SPSS ver.12.0, SPSS Inc., Chicago, IL, USA). Statistical analysis according to the statistical significance test between each experimental group was post-tested with Duncan's multiple range test if there is significance (P<0.05) after one-way analysis of variance (ANOVA) was performed.

3. Results and Discussion

3.1. Inhibitory Effects to α-glucosidase, α-amylase Activities and DPPH Scavenging Effects

To elucidate inhibitory effects of the various omija extracts to α-glucosidase and α-amylase activity, assays in an in Vitro test were carried out (Figure 1A and Figure 1B). The inhibitory effects to α-glucosidase activity was rapidly increased at the concentration of 3.0 mg/ml, and were elevated in a dose dependent manner (Figure 1A). Inhibition of α-amylase activity showed significant changes from the concentration of 10 μg/ml, and at the concentration of 10 mg/ml, the highest concentration of the sample, 86.61±1.80%, indicating that α-amylase activity was inhibited in a concentration-dependent manner (Figure 1B). Similarly, acarbose (positive control drug) was inhibits each enzyme activities in a dose dependent manner (Figure 1A and Figure 1B). To investigate antioxidative effects of various omija extract mixtures, we measured DPPH free radical scavenging activity (Figure 1C). Each to 62.83±1.06% at a concentration of 10 mg/ml. These results suggest that various omija extract mixtures were reduces oxidative stress and blocks absorption of carbohydrate-based nutrients.

3.2. Inhibitory Effects of Water and Ethanol Extracts of Omija Fruit (OF) and Pulp (OP) on Blood Glucose Level in ICR Mice.

Figure 1. Inhibition of (A) α-glucosidase, (B) α-amylase and (C) oxidative stress by omija extracts and soybean powder, and their blends. Within the same concentration, values not followed by the same capital letter are significantly different (p<0.05)
In order to compare the anti-glucose tolerance effect of samples for each extraction solvent of omija, the oral glucose tolerance test (OGTT) were performed and analyzed (Table 5). As a result of this experiment, blood glucose level at 30 min after glucose administration are in the control: 374.71±12.01 mg/dL, 100 mg/kg OF: 296.43±12.06 mg/dL, 100 mg/kg OP: 332.71±15.00 mg/dL, 100 mg/kg OFWE: 348.86±12.29 mg/dL, 100 mg/kg OPWE: 320.57±14.97 mg/dL, 100 mg/kg OF30EE: 303.71±15.29 mg/dL, 100 mg/kg OP30EE: 283.00±21.71 mg/dL, 100 mg/kg OF50EE: 235.29±11.27 mg/dL, 100 mg/kg OP50EE: 254.86±18.62 mg/dL. Significant differences showed in all test groups except the OFWE group and the OP group compared to the control group, and blood sugar in the OF50EE group was observed to be significantly lower.

### 3.3. Efficacy Comparisons of Combined-Formula of Omija and Soybean Extracts on Blood Glucose Level in ICR Mice

To investigate the anti-glucose tolerance effect in animals administered with various mixtures composition of omija and soybeans, OGTT was performed (Figure 2). As a result, there was no significant difference between the entire test group in fasting blood sugar (-30 min) before administration of the sample and blood sugar (0 min) after administration of the sample. Whereas, blood glucose level at 30 min after glucose administration are control: 461.29±13.93 mg/dL, 100 mg/kg O1.5:S1: 409.71±20.62 mg/dL, 100 mg/kg O3:S1: 371.14±17.50 mg/dL, 100 mg/kg O5:S1: 347.00±11.31 mg/dL, 10 mg/kg Acarbose: 357.43±19.56 mg/dL. Compared to the control group, blood glucose in all test groups was observed to be significantly lower. In particular, the O5:S1 group showed strong effects to the positive control group acarbose group (Figure 2B).

### 3.4. Dose Dependent Effects of OSM on Blood Glucose Tolerance Test in ICR Mice

Based on the Table 5 and Figure 2, O5:S1 (newly code name is OSM) were tested anti-glucose tolerance effects with various dose of OSM (Figure 3). As a result of this experiment, blood glucose level at 30 min after glucose administration is 378.17±23.35 mg/dL in the control group. Whereas, 10 mg/kg OSM (OSM 10): 340.67±13.76 mg/dL, 30 mg/kg OSM (OSM 30): 323.83±18.12 mg/dL, 100 mg/kg OSM (OSM 100): 265.33±9.00 mg/dL, 200 mg/kg metformin: 210.33±9.03 mg/dL. Compared to the control group, the low-dose group (OSM 10) showed a low correlative effects, whereas the medium-dose group (OSM 30), high-dose group (OSM 100), and positive control group were found to be significantly rescued (Figure 3B).
3.5. Dose and Time Dependent Efficacy of OSM on Blood Parameter in Diabetic Mice

In type 2 diabetes model, C57BL/KsJ db/db, we investigated the anti-diabetic effects of omija extraction mixtures for 4 weeks (Table 6 - Table 9). In the first week after OSM administration, blood glucose level in the test group was observed no significant differences compared to control group, whereas shows regulatory effects in the OSM administrated group than control group. At 4 weeks, blood glucose levels were normal: 115.5±1.38 mg/dL, control group: 691.5±12.69 mg/dL, OSM 10: 528.33±27.61 mg/dL, OSM 30: 470.00±15.18 mg/dL, OSM 100: 551.33±10.54 mg/dL, 200 mg/kg metformin: 548.83±14.59 mg/dL (Table 6). To check effects of OSM on body weight of type 2 diabetes model mice, we measured body weight once a week but no shown significant differences between all C57BL/KsJ db/db groups (Table 7). Moreover, OSM administration were shown no significant differences of food and water intake among the experimental C57BL/KsJ db/db groups for 4 weeks (Table 8 and Table 9, respectively).

Table 6. Effect of OSM administration on blood glucose in diabetic mice

| Group/Weeks | 0     | 1     | 2     | 3     | 4     |
|-------------|-------|-------|-------|-------|-------|
| Normal      | 64.4±2.7a | 116.5±7.0a | 117.8±5.2a | 135.0±8.0a | 115.5±1.4a |
| Control     | 389.5±12.3b | 448.6±45.3b | 644.0±46.9b | 665.2±28.7b | 691.5±12.7d |
| OSM 10      | 389.5±12.3b | 461.0±48.3b | 539.8±37.4bc | 556.7±26.2bc | 528.3±27.6c |
| OSM 30      | 389.5±12.4b | 489.7±62.5b | 502.2±39.5b | 473.8±25.2b | 470.0±15.2b |
| OSM 100     | 389.5±12.5b | 357.3±14.9b | 483.2±43.2b | 577.8±37.9bc | 551.3±10.5c |
| Positive    | 389.5±12.5b | 447.5±35.2b | 547.3±21.6bc | 562.2±11.2bc | 548.8±14.6c |

Within the same concentration, values not followed by the same capital letter are significantly different (p<0.05).

Table 7. Effect of OSM administration on body weight in diabetic mice

| Group/Weeks | 0     | 1     | 2     | 3     | 4     |
|-------------|-------|-------|-------|-------|-------|
| Normal      | 22.3±0.20 | 23.7±0.28 | 24.8±0.14 | 26.0±0.14 | 26.6±0.16 |
| Control     | 48.0±1.05 | 50.3±1.28 | 51.4±1.46 | 52.6±1.63 | 52.1±1.79 |
| OSM 10      | 46.7±0.90 | 48.8±0.83 | 49.3±0.68 | 50.3±0.69 | 49.6±0.81 |
| OSM 30      | 47.3±1.44 | 49.6±1.13 | 50.8±1.26 | 51.6±1.40 | 51.7±1.35 |
| OSM 100     | 46.2±0.53 | 48.2±0.48 | 49.7±0.76 | 50.3±0.76 | 51.0±1.01 |
| Positive    | 46.8±1.44 | 47.6±1.32 | 48.6±1.54 | 49.9±1.61 | 50.3±1.70 |

Table 8. Effect of OSM administration on food intake in diabetic mice

| Group/Weeks | 0     | 1     | 2     | 3     | 4     |
|-------------|-------|-------|-------|-------|-------|
| Normal      | 2.7±0.01 | 3.7±0.46 | 3.6±0.14 | 3.8±0.10 | 3.4±0.09 |
| Control     | 8.9±0.33 | 8.2±0.21 | 7.4±0.39 | 8.6±0.32 | 8.2±0.41 |
| OSM 10      | 8.9±0.33 | 7.7±0.55 | 6.8±0.26 | 7.6±0.66 | 7.2±0.24 |
| OSM 30      | 8.9±0.33 | 7.9±0.15 | 7.2±0.92 | 7.7±0.63 | 7.3±0.12 |
| OSM 100     | 8.9±0.33 | 7.7±0.34 | 7.6±0.32 | 7.9±0.24 | 7.8±0.38 |
| Positive    | 8.9±0.33 | 6.9±0.60 | 6.7±0.59 | 7.7±0.54 | 7.6±0.60 |
Table 9. Effect of OSM administration on water intake in diabetic mice

| Group/Weeks | 0     | 1     | 2     | 3     | 4     |
|-------------|-------|-------|-------|-------|-------|
| Normal      | 3.83±0.02 | 4.43±0.35 | 5.24±0.5 | 6.09±1.37 | 4.97±0.14 |
| Control     | 34.84±0.06 | 30.35±2.27 | 28.04±3.97 | 37.76±2.78 | 38.53±3.92 |
| OSM 10      | 34.84±0.05 | 29.58±3.72 | 27.65±1.93 | 32.43±3.79 | 35.71±1.50 |
| OSM 30      | 34.84±0.07 | 27.09±0.75 | 28.95±4.71 | 32.96±3.71 | 32.56±2.80 |
| OSM 100     | 34.84±0.04 | 26.08±4.62 | 29.83±4.55 | 31.59±4.15 | 33.30±4.89 |
| Positive    | 34.84±0.05 | 24.26±3.99 | 27.83±3.37 | 32.31±1.81 | 35.12±3.77 |

Figure 4. Effect of OSM administration on organ weights of liver, pancreas, kidney and epidydimal fat in diabetic mice. After induction of diabetes, indicated dose of OSM or metformin were given daily. The untreated diabetic control group received equal volume water orally daily. Within the same concentration, values not followed by the same capital letter are significantly different (p<0.05)

3.6. Dose and Time Dependent Efficacy of OSM on Physio-organic Parameter in Diabetic Mice

After administration of the OSM for 4 weeks in db/db mice, the tissue weights were measured after an autopsy (Figure 4). In liver (Figure 4A) and pancreas (Figure 4B) tissue weight for each experimental group, were observed between the experimental C57BL/KsJ db/db groups. Interestingly, kidney (Figure 4C) and epidydimal fat (Figure 4D) weight were markedly increased in control db/db groups compared to normal group, OSM administration were reduced each tissue weights in a dose dependent manner. Kidney weight were significantly changed in OSM 100 group and epidydimal fat weights were reduced in OSM 30 and OSM 100 group. However, metformin (positive control) was not shown significant differences in all experimental model (Figure 4A-D).

3.7. Dose Dependent Efficacy of OSM on Glucose Resistant Parameter in Diabetic Mice

To investigate the regulatory effect of OSM in in vivo model, we compared insulin concentration, homeostasis model assessment-insulin resistance (HOMA-IR) and beta cell function (HOMA β-cell) in all experimental groups (Figure 5). As results, blood insulin levels were normal: 0.19±0.00 ng/ml, control: 0.59±0.07 ng/ml, OSM 10: 0.39±0.10 ng/ml, OSM 30: 0.35±0.02 ng/ml, OSM 100: 0.53±0.07 ng/ml, 200 mg/kg metformin: 0.46±0.08 ng/ml (Figure 5A). Mathematically, HOMA-IR calculated normal: 1.14±0.03, control: 20.98±2.65, OSM 10: 9.76±2.48, OSM 30: 7.95±0.69, OSM 100: 14.31±2.52, 200 mg/kg metformin: 13.07±2.84 (Figure 5B). All OSM and metformin administrated group shown significant reduction of HOMA-IR but didn’t shows dose dependent manner. In contrast, HOMA-β
index were not ameliorated by OSM and metformin administration (Figure 5C). These results suggest that OSM and/or metformin elevates insulin susceptibility with tiny acceleration of insulin secretion in \textit{in vivo} model.

![Figure 5](image)

**Figure 5.** Effect of OSM administration on blood insulin levels, calculative HOMA-IR and HOMA-β cell index in diabetic mice. After induction of diabetes, indicated dose of OSM or metformin were given daily. The untreated diabetic control group orally daily received same volume water. Within the same concentration, values not followed by the same capital letter are significantly different (p<0.05)

![Figure 6](image)

**Figure 6.** Diabetic mice pancreatic tissues stained by immunohistochemistry for insulin using anti-insulin antibody than counter stained H&E methods. (A) normal mice, (B) control mice, (C) OSM 10, (D) OSM 30, (E) OSM 100, (F) metformin and (G) statistical analysis of β-cell area following Materials and Methods. Scale bar = 100 μm. **Values not followed by the same capital letter are significantly different (p<0.05)**

### 3.8. Effects of OSM in Insulin Secretory β-cell Function and Histologic Architecture

In order to confirm the effect of OSM on the β-cell area of the diabetes C57BL/KsJ db/db model, the β-cell area for each experimental group was analyzed using an immunohistochemical staining method (Figure 6) and we calculated β-cell area from Figure 6A-F and statistically displayed in Figure 6E. As results, β-cell area were completely reduced in control group with low signal,
whereas the area were increased with positive signals in OSM and metformin administrated group in a dose dependent manner. Therefore, these results suggest that OSM and/or metformin donate rescue of β-cell regeneration with anti-oxidative mechanisms.

3.9. Molecular Mechanism of OSM in Liver Tissue of Diabetic Mice

Finally, we analyzed PI3K/Akt/AMPK and IRS pathway from liver tissue in diabetic model mice (Figure 7). GLUT2 expression levels were markedly reduced in control group than normal group whereas all dose of OSM rescued. AMPK was not shown any significant differences all experimental groups including metformin group, however p-AMPK was significantly decreased in control group and completely recovered in OSM and metformin administrated groups. Similarly, remarkable reduction of p-Akt levels in control group but increased OSM administrated groups in a dose dependent manner. Moreover, p-IRS were reduced in diabetic control group but successfully recovered in OSM administrated group. Metformin, positive control group also shown similar function in these experimental condition. These results suggest that OSM and metformin-induced antidiabetic effects were mediated by increases of glucose susceptibility with elevation of GLUT2 expression and phosphorylation of AMPK, Akt and IRS in liver.

![Figure 7. Effect of OSM on AMPK, AKT phosphorylation and GLUT2 expression on liver tissue in diabetic mice. A Western blot and densitometry analysis of (B) p-AMPK/AMPK ratio, (C) p-Akt/Akt ratio and (D) GLUT2/β-actin ratio. Representative immunoblots are shown. a,b,c Values not followed by the same capital letter are significantly different (p<0.05). N: normal, C: control, L: OSM 10, M: OSM 30, H: OSM 100 and P: positive](image)

To confirm the antidiabetic properties of OSM, these studies conducted that inhibitory effects to alpha-amylase and alpha-glucosidase, and anti-oxidative effects in in vitro model. In addition, we observed antidiabetic effects by glucose tolerance test on normal and db/db mice with physiological parameters in in vivo model.

Diabetes mellitus is a complicated metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Type 1) and a cellular-mediated autoimmune destruction of the pancreatic β-cells, insulin resistance (Type 2). Usually, diabetes causes the onset of chronic complications including diabetic neuropathy [38], nephrosis [39], peripheral necrosis [40], retinopathy [41], cardiovascular disease (CVD) [42] and paradoxically risk of hypoglycemia [43] were most inducible complications.

In current, many antidiabetic drugs are approved in many countries and the choice should be made on the basis of the characteristics of the various drugs [44]. Metformin, class of biguanides, was the first antidiabetic drugs to become available which acts by decreasing hepatic glucose output and consequently lowering fasting glycaemia values [45]. In addition, sulfonylureas, glinides, thiazolidinediones and glucagon-like peptide-1 agonists (GLP-1), carbohydrate-related digestive enzyme inhibitors and dypeptidyl peptidase-4 (DPP-4) also well identified for commercial antidiabetic medicines [44].

Finally, OSM and/or individual materials ameliorated blood glucose through specific signaling pathway and dietary OSM attenuated obesity- and diabetes-caused increase of whole body risk. These effects of OSM may contribute to improvements in energy consumption and glucose utilization. Our results indicate that OSM has the potential to reduce the risk of diabetes. In conclusion, *S. chinensis* is traditionally used as a food-based medicinal/pharmaceutical resources including antidiabetic agent in worldwide. The optimal combined formula of *S. chinensis* and soybean powder were available to type 2 diabetes patients.

**Conflict Interests**

The authors have no financial or non-financial competing interests to declare.

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