

Tenebrio molitor (Mealworm) Extract Improves Insulin Sensitivity and Alleviates Hyperglycemia in C57BL/Ksj-db/db Mice

Seon Young Kim, Jae Eun Park and Ji Sook Han*

Department of Food Science and Nutrition, Pusan National University, Busan 46241, Korea

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Diabetes is one of the serious chronic metabolic diseases caused by Westernized eating habits, and the goal of diabetes treatment is to keep blood glucose at a normal level and prevent diabetic complications. This study was designed to investigate the anti-diabetic effects of a mealworm (*Tenebrio molitor* larva) extract (MWE) on hyperglycemia in an animal model with type 2 diabetes. Diabetic C57BL/Ksj-db/db mice were divided into three groups: diabetic control, rosiglitazone, and MWE. The mice supplemented with MWE showed significantly lower blood levels of glucose and glycosylated hemoglobin when compared with the diabetic control mice. The homeostatic index of insulin resistance was significantly lower in mice supplemented with MWE than in diabetic control mice. MWE supplementation significantly stimulated the phosphorylation of insulin receptor substrate-1 and Akt, and activation of phosphatidylinositol 3-kinase in insulin signaling pathway of skeletal muscles. Eventually, MWE increased the expression of the plasma membrane glucose transporter 4 (GLUT4) via PI3K/Akt activation. These findings demonstrate that the increase in plasma membrane GLUT4 expression by MWE promoted the uptake of blood glucose into cells and relieved hyperglycemia in skeletal muscles of diabetic C57BL/Ksj-db/db mice. Therefore, mealworms are expected to prove useful for the prevention and treatment of diabetes, and further studies are needed to improve type 2 diabetes in the future.

Key words : AMPK pathway, db/db-mice, hyperglycemia, insulin sensitivity, mealworm

Introduction

Diabetes is increasing worldwide, affecting about 415 million people to date. Among these, patients with type 2 diabetes specifically account for more than 90% of all diabetic patients. Type 2 diabetes is characterized by high blood glucose levels. Hyperglycemia is caused by an insufficient secretion of insulin by the pancreas [4] and insulin resistance mainly in skeletal muscles and adipose tissue [31]. Insulin resistance is a pathological condition in which the insulin sensitivity of tissues is reduced, and this condition is observed with inappropriate functioning of insulin [14]. Reduced insulin sensitivity is caused by a combination of genetic and environmental factors, and its pathophysiology involves complex signaling pathway that is activated by the insulin receptor [14, 28, 31].

Insulin sensitivity is associated with the activation of PI3K/Akt signaling pathway. In this pathway, insulin binds to the insulin receptor and activates the insulin receptor tyrosine kinase, leading to phosphorylation in insulin receptor substrate-1^{Tyr612} (IRS-1^{Tyr612}). Phosphorylated IRS-1^{Tyr612} activates phosphatidylinositol-3-kinase (PI3K) by binding to the SH2 (Src-homology-2) domain of the regulatory subunit p85 of PI3K. Continually, activated PI3K phosphorylates AKT^{Ser473} by converting phosphatidylinositol bisphosphate (PIP2) to phosphatidylinositol triphosphate (PIP3). Phosphorylation of Akt^{Ser473} enhances the translocation of glucose transporter 4 (GLUT4) from the cytoplasm to the plasma membrane in skeletal muscles [3, 11, 34]. Eventually, the activation of PI3K/Akt pathway, via translocation of GLUT4, absorbs blood glucose into the cell and thereby improves hyperglycemia.

Oral antidiabetic medications have been used to increase insulin sensitivity and alleviate hyperglycemia. Considerably, thiazolidinedione drugs (TZDs) are widely used to treat type 2 diabetes, which further increase the tissue sensitivity to insulin and delay the progression of diabetes [25]; however, these are synthetic drugs and may present undesirable side-effects such as hypoglycemia, poor control of post-

*Corresponding author

Tel : +82-51-510-2836, Fax : +82-51-583-3648

E-mail : hanjs@pusan.ac.kr

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prandial blood glucose level, and weight gain [13], which are of great concern in the treatment of diabetes. Thus, to reduce these side effects and effectively treat diabetes, the therapeutic use of natural products for treating diabetes has gained immense attention worldwide [33].

For centuries, people have consumed insects as food. The insects have abundant protein, fat, vitamins, and minerals [27], and have been used to treat various diseases [6]. As a representative edible insect, mealworm has been eaten as a traditional food in several countries. Mealworm is the larval form of the mealworm beetle, *Tenebrio molitor*, and it inhabits in stored grain [29]. Similar to other insects, mealworm is rich in nutrients, and in particular, contains a large amount of unsaturated fatty acids such as linoleic acid (C18:2) and oleic acid (C18:1) [26]. Thus, when consumed as a dietary supplement, mealworm prevents gout and hyperuricemia [6]. In addition, the beetle species belonging *T. molitor* have been used in traditional medicine to curb hepatofibrosis and diabetes [32]; however, no study has investigated the use of a mealworm to improve insulin sensitivity in type 2 diabetic mice. Therefore, we designed this study to examine the efficacy of the mealworm extract (MWE) on insulin sensitivity and hyperglycemia in C57BL/Ksj-db/db type 2 diabetic mice.

Materials and Methods

Preparation of materials

Mealworm was purchased from Yongin (Yongin, Gyeonggi-do, Korea). It was washed with distilled water, and then dried and ground into a powder (Shinhan Science & Technology Co., Kyunggi, Korea). For the extract, mealworm powder was appropriately dissolved in 80% ethanol and filtered using Whatman No. 1 filter paper at room temperature overnight. This process was repeated thrice. Later, the mealworm extract was concentrated in a rotary evaporator and freeze-dried into a powder (BUCHI Co., Flawil, Switzerland). The mealworm extract powder was preserved in a deep freezer (Samwon Freezing Engineering Co., Busan, Korea).

Animals and feeding diets

Male C57BL/Ksj-db/db mice were purchased from JOONG AH BIO (Suwon-si, Gyeonggi-do, Korea) and db/db mice were fed a pelletized commercial chow diet for 2 weeks after arrival. Before the experiment, the mice were randomly divided into three groups, $n=8$ per group. The db/db-control

mice group was supplemented with a standard semi-synthetic diet (AIN-93G), whereas the mice in the other two groups were fed a standard AIN-93G diet supplemented with either Rosiglitazone (RG, 0.005%, w/w, Sigma, St. Louis, MO, USA) or MWE (0.5%, w/w; Table 1). All mice were placed separately in a cage with controlled light (12 hr light/12 hr dark) and temperature conditions. Thereafter, the mice were given free access to food and water. At the end of the 6-week experiment, mice were anesthetized with ether after a 12 hr fasting period, and blood samples were collected from the inferior vena cava to determine the levels of plasma biomarker. All animal experiments were performed in accordance with guidelines of the Pusan National University for the care and use of laboratory animals (PNU-2018-1826).

Blood glucose and glycosylated hemoglobin levels

Every week, the glucose concentration in the venous blood from the mouse-tail vein was measured after a 12 hr fasting period using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Anticoagulated whole-blood samples were hemolyzed and the concentration of glycosylated hemoglobin (HbA1c) was measured by using immunoturbidimetry.

Table 1. Ingredient composition of the experimental diets supplemented to mice (%)

| | db/db | db/db-RG | db/db-MWE ¹⁾ |
|---------------------|---------|----------|-------------------------|
| Casein | 20 | 20 | 20 |
| Cornstarch | 39.7486 | 39.7486 | 39.7486 |
| Dextrose | 13.200 | 13.200 | 13.200 |
| Sucrose | 10 | 9.995 | 9.5 |
| Cellulose | 5 | 5 | 5 |
| Soybean oil | 7 | 7 | 7 |
| t-Butylhydroquinone | 0.0014 | 0.0014 | 0.0014 |
| Salt mix | 3.5 | 3.5 | 3.5 |
| Vitamin mix | 1 | 1 | 1 |
| L-Cystine | 0.3 | 0.3 | 0.3 |
| Choline bitartrate | 0.25 | 0.25 | 0.25 |
| Mealworm extract | — | — | 0.5 |
| Rosiglitazone | — | 0.005 | — |
| Total (%) | 100 | 100 | 100 |

¹⁾db/db (diabetes mellitus control), C57BL/Ksj-db/db mice receiving AIN-93G diet; db/db-RG, C57BL/Ksj-db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE, C57BL/Ksj-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). MWE: mealworm extract.

Plasma insulin level

All blood samples from the inferior vena cava were collected into heparin-coated tubes. After centrifugation at $1,000\times g$ for 15 min at 4°C , the plasma was cautiously removed from the sample. The levels of plasma insulin were measured via a radioimmunoassay with an enzyme-linked immunosorbent assay ELISA kit (Linco Research, Inc., Billerica, MA, USA).

Homeostatic index of insulin resistance

Homeostatic index of insulin resistance (HOMA-IR) was measured as an alternative method to determine insulin sensitivity. HOMA-IR was calculated using the homeostasis model with the following equation (Eq. (1)):

$$\text{HOMA-IR} = \{ \text{Fasting glucose (mmol/L)} \times \text{fasting insulin (IU/MI)} \} / 22.51 \quad (1)$$

Intraperitoneal glucose tolerance tests

At 5 weeks of MWE treatment, the intraperitoneal glucose tolerance test (IPGTT) was performed on all db/db mice after a 12 hr overnight fast. The mice were injected intraperitoneally with glucose (0.5 g/kg BW), and later their blood glucose levels were measured at 0, 30, 60, and 120 min by collecting blood from their tails. The IPITT was performed at 6 weeks of MWE treatment. After 12 hr overnight fasting, an insulin solution (2 U/kg of BW) was injected intraperitoneally in the db/db-mice, and the blood sample was collected at 0, 30, 60, and 120 min to check the glucose levels using a blood glucose meter (Roche Diagnostics GmbH, Germany).

Plasma membrane fraction of skeletal muscle

Muscle tissue was placed in a buffer (5 mM sodium azide, 0.25 M sucrose, 0.1 Mm phenylmethylsulfonyl fluoride, 10 Mm NaHCO_3 , pH 7.0) at 4°C . All reagents were purchased from sigma (Sigma, St. Louis, MO, USA). Subfractionation of muscle membrane was performed as mentioned in a study by Baron et al. [2], using procedures modified by Klip et al. [16]. Dissected skeletal muscle was homogenized and centrifuged at $1,000\times g$ for 10 min, and the supernatant was then collected and stored. The resulting pellet was resuspended in the buffer and rehomogenized in a glass homogenization tube. The supernatant was collected, combined with the first supernatant, and the combination was centrifuged at $9,000\times g$ for 10 min. The resulting supernatant was then centrifuged at $190,000 g$ for 60 min. The mem-

branes were subsequently applied to a discontinuous sucrose gradient comprising 25, 30, and 35% sucrose (wt/vol) solutions and were centrifuged at $190,000\times g$ for 16 hr. Membranes were collected from the top layer of each sucrose gradient, resuspended in the buffer, pelleted by centrifugation at $190,000\times g$ for 60 min, and resuspended in the buffer.

Western blot

Western blot analysis was performed on skeletal muscle tissue extract. Skeletal muscle tissues were homogenized in ice-cold lysis buffer, centrifuged at 14,000 rpm, 4°C , for 15 min, and the supernatant was collected. Protein concentrations in the supernatants were measured using a protein assay kit (Bio-Rad, Hercules, CA, USA). Next, 20 μg protein samples were separated on 12% resolving Tris-HCL gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline, 0.1% Tween-20 for 1 hr at room temperature. The blocked membranes were incubated with respective antibodies overnight at 4°C . Antibodies against IRS-1, PI3K, phospho-Akt^{Ser473}, Akt, and GLUT4 were purchased from Abcam (Cambridge, UK). Antibodies against phospho-IRS-1^{Tyr612} were purchased from Thermo Fisher Scientific (Rockford, IL, USA). The membranes were then washed and probed with a secondary antibody for 1 hr at room temperature. Each antigen - antibody complex was visualized using enhanced chemiluminescence western blotting detection reagents and detected via chemiluminescence with LAS-1000 plus (Fujifilm, Tokyo, Japan). Band densities were determined by an image analyzer (Multi Gauge V3.1; Fujifilm) and normalized to β -actin for the total protein content.

Statistical analyses

The data are presented as means \pm SD. Statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC, USA). Differences between the groups were evaluated for significance using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc tests.

Results

Body weight, food intake, and water intake

During the experiment, body weight, food intake, and water intake of the db/db-mice were observed every week. Fig. 1 presents the body weight change in mice for 6 weeks.

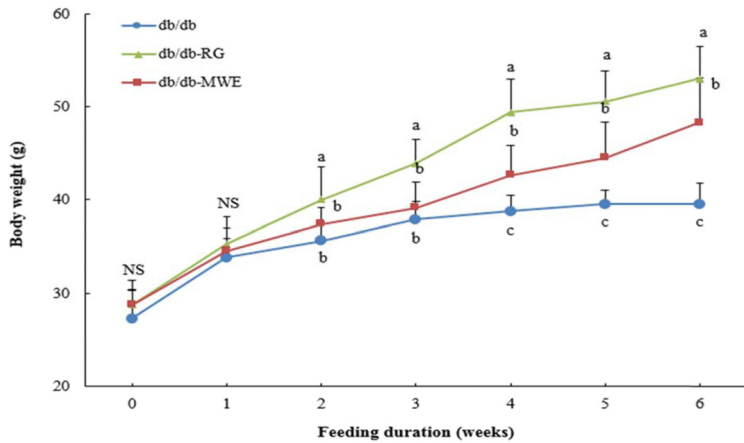


Fig. 1. Weekly changes in body weight in C57BL/KsJ-db/db mice supplemented with mealworm extract. db/db (diabetes mellitus control): C57BL/KsJ-db/db mice receiving AIN-93G diet; db/db-RG: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). Values are presented as means \pm SD, $n=8$ per group, ^{a-c}Mean values designed by different letters are significantly different between groups ($p<0.05$).

During the commencement of the experiment, body weights in db/db-control, db/db-RG, and db/db-MWE groups were not significantly different. As the experiment progressed, body weight gradually increased. At the end of the experiment, mice in the db/db - RG group displayed significantly higher body weight those in the db/db-control and db/db-MWE groups. Daily food intake and water intake of mice are listed in Table 2. The daily food intake did not differ significantly among the db/db-control, db/db-RG, and db/db-MWE groups; however, daily water intake was 14.48 ± 3.79 and 10.84 ± 2.83 in the db/db-control and db/db-MWE groups, respectively, which indicates that the water intake of db/db-control group was significantly higher than that of db/db-MWE group.

Table 2. The effects of supplementation with MWE on food consumption and drinking water intake of C57BL/KsJ-db/db mice

| | db/db | db/db-RG | db/db-MWE ¹⁾ |
|-----------------------|----------------------|-------------------|-------------------------|
| Water intake (ml/day) | 14.48 ± 3.79^a | 6.21 ± 1.88^c | 10.84 ± 2.83^b |
| Food intake (g/day) | 3.96 ± 0.68^{NS} | 3.50 ± 0.74 | 3.94 ± 0.66 |

¹⁾db/db (diabetes mellitus control): C57BL/KsJ-db/db mice receiving AIN-93G diet; db/db-RG: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). Values are presented as means \pm SD, $n=8$ per group, ^{a-c}Mean values designed by different letters are significantly different between groups ($p<0.05$). NS: not significant; MWE: mealworm extract.

Fasting blood glucose

Fig. 2 presents the effect of MWE supplement on fasting blood glucose levels. During the commencement of the experiment, blood glucose levels did not significantly differ among the groups; however, fasting blood glucose levels of mice in db/db-control group were elevated throughout the

experiment, presumably indicating the disease progression, whereas fasting blood glucose levels of mice in db/db-MWE group were slightly increased. Consequently, at the end of the experiment, fasting blood glucose was 525.63 ± 57.4 and

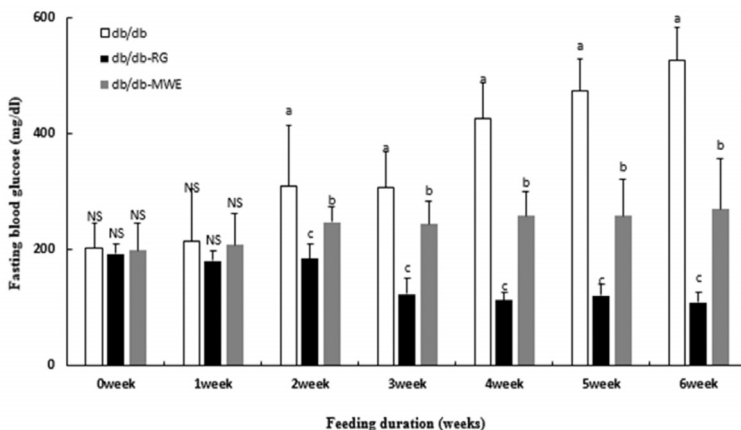


Fig. 2. Weekly changes in blood glucose levels in C57BL/KsJ-db/db mice supplemented with mealworm extract. db/db (diabetes mellitus control): C57BL/KsJ-db/db mice receiving AIN-93G diet; db/db-RG: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). Values are presented as means \pm SD, $n=8$ per group, ^{a-c}Mean values designed by different letters are significantly different between groups ($p<0.05$).

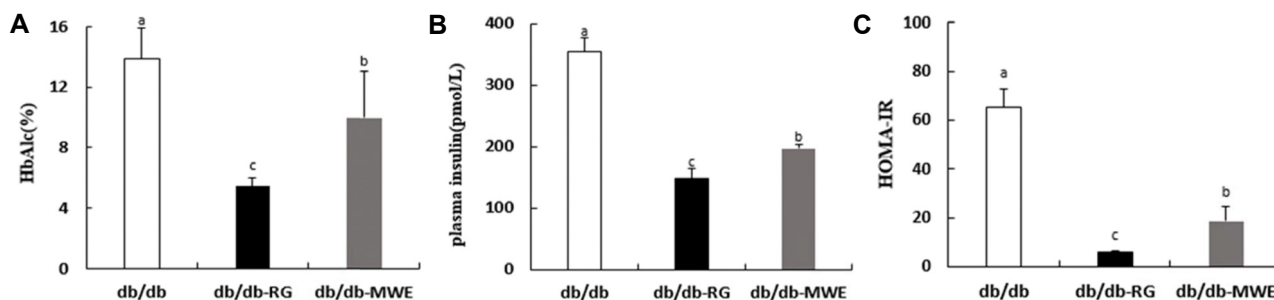


Fig. 3. The effects of supplementation with MWE on blood glycosylated hemoglobin levels and markers of insulin resistance in C57BL/KsJ-db/db mice. A. Blood glycosylated hemoglobin levels. B. Plasma insulin levels. C. Homeostatic index of insulin resistance in C57BL/KsJ-db/db mice. db/db (diabetes mellitus control): C57BL/KsJ-db/db mice receiving AIN-93G diet; db/db-RG: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). Values are presented as means \pm SD, $n=8$ per group, ^{a-c}Mean values designed by different letters are significantly different between groups ($p<0.05$). HbA1c: blood glycosylated hemoglobin; HOMA-IR: homeostatic index of insulin resistance.

269.50 \pm 87.47 in the db/db-control and db/db-MWE groups, respectively, that is, the fasting blood glucose level of mice in the db/db-control group was twice higher than that observed in the db/db-MWE group.

HbA1c levels, plasma insulin level, and HOMA-IR

As presented in Fig. 3, HbA1c values were 12.99 \pm 1.55 and 8.00 \pm 3.01 in the db/db-control and db/db-MWE groups, respectively. This indicated that the HbA1c levels, plasma insulin levels, and HOMA-IR values in the db/db-MWE group were significantly lower compared with those in the db/db-control group.

Intraperitoneal glucose and insulin tolerance tests

The effect of MWE supplement on glucose tolerance was estimated via IPGTT at 5 weeks of the experiment and the results are presented in Fig. 4. Blood glucose levels of mice in the db/db-control group were higher than those of mice in the db/db-MWE group at all time-points; however, blood glucose levels in the db/db-MWE group peaked at 30 min after glucose injection and recovered to levels close to the basal value at 120 min.

Effect of MWE on the activation of PI3K/Akt pathway

To clarify whether MWE supplement promotes the activation of insulin signaling pathway and leads to glucose uptake into cells of skeletal muscles, activation of PI3K/Akt pathway was investigated by observing the phosphorylation levels of IRS-1 and Akt, and by activation of PI3K. As presented in Fig. 5, the levels of IRS-1 phosphorylation and PI3K activation in the db/db-MWE group were significantly

increased compared to the db/db-control group. In the db/db-MWE groups, levels of IRS-1 phosphorylation were 1.7-fold higher than those in the db/db-control group. The levels of PI3K activation were 1.8-fold and 1.6-fold higher in the db/db-RG and db/db-MWE groups, respectively, than those in the db/db-control group. Moreover, the MWE supplementation stimulated the phosphorylation of Akt by

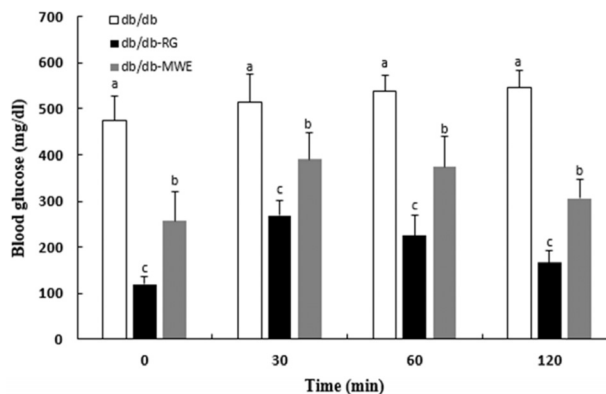


Fig. 4. The effects of supplementation with MWE on intraperitoneal glucose tolerance tests in C57 BL/KsJ- db/db mice. Blood glucose concentrations were measured at the indicated times. Glucose (0.5 g/kg of body weight) or insulin (2 units/kg of body weight) were injected in db/db-mice at time of zero. db/db (diabetes mellitus control): C57 BL/KsJ-db/db mice receiving AIN-93G diet; db/db-RG: C57BL/KsJ- db/db mice receiving AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-MWE: C57BL/KsJ-db/db mice receiving AIN-93G diet supplemented with mealworm extract (0.5 g/100 g diet). Values are presented as means \pm SD, $n=8$ per group. ^{a-c}Mean values designed by different letters are significantly different between groups ($p<0.05$). IPGTT: Intraperitoneal glucose tolerance tests.

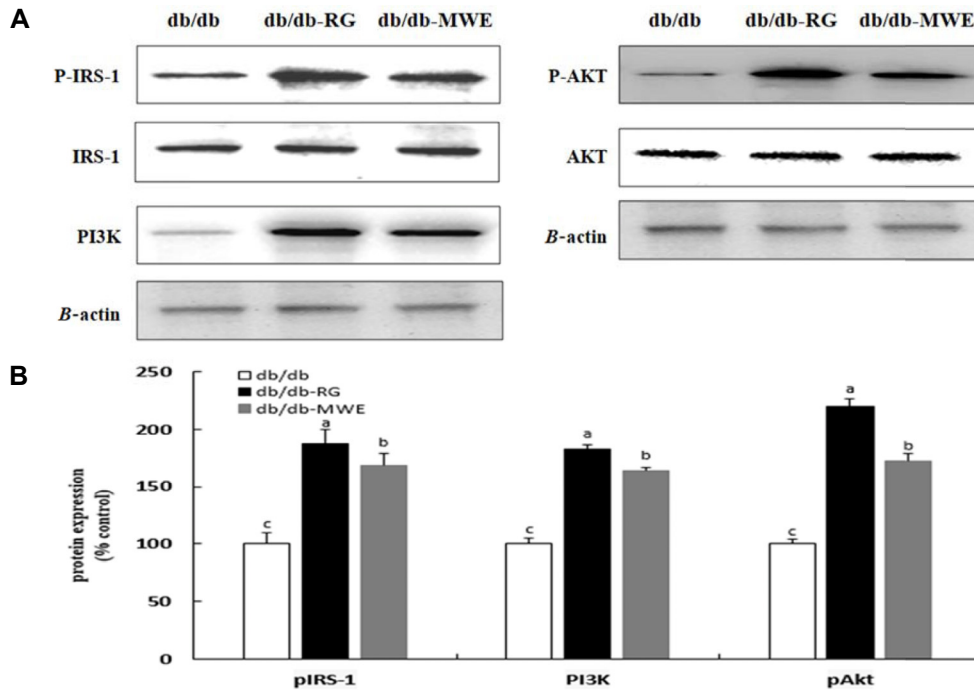


Fig. 5. The effect of MWE supplementation on pIRS-1, IRS-1, PI3K, pAkt, and Akt protein expression in skeletal muscle of C57BL/KsJ-db/db mice. Western blotting was performed, and signal intensities were determined by densitometric analysis using Multi Gauge V3.1 software. A. pIRS-1/IRS-1, PI3K, and pAkt/Akt protein expression, B. expression levels of pIRS-1/IRS-1, PI3K, pAkt/Akt. Each value is expressed as mean SD of experiments performed in triplicate. ^{a-c}Mean values designed by different letters are significantly different between groups ($p < 0.05$). pIRS-1: phosphorylated insulin receptor substrate-1; PI3K: phosphatidylinositol-3-kinase; pAkt: phosphorylated Akt.

1.7-fold compared with that in the db/db-control group. These observations suggested that MWE plays a pivotal role in the activation of PI3K/Akt pathway.

Effect of MWE on PM-GLUT4 expression

GLUT4 translocation from the cytoplasm to plasma mem-

brane is crucial in the absorption of glucose into the cell [22]. Thus, the effect of MWE supplementation on GLUT4 expression in plasma membrane was investigated. Fig. 6 presents the levels of PM-GLUT4 expression in the db/db-MWE group compared with those in the db/db-control group. In db/db-MWE group, the expression of PM-GLUT4

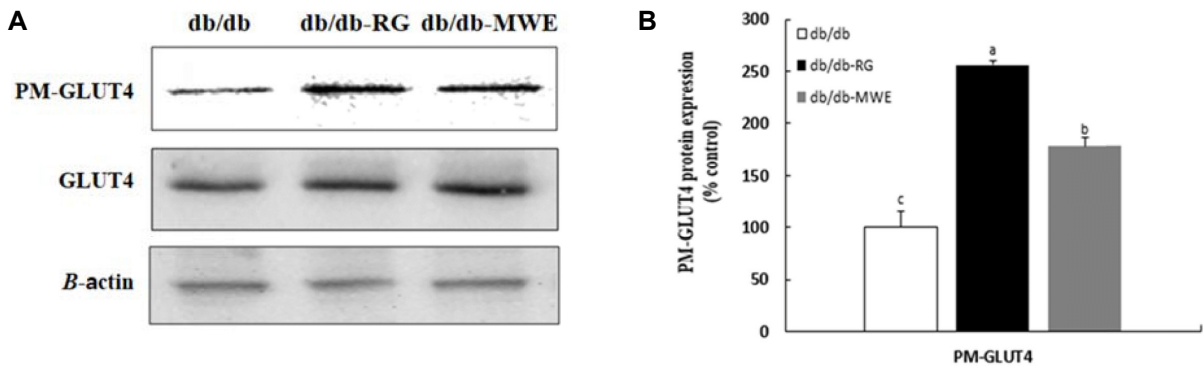


Fig. 6. The effect of MWE supplementation on PM-GLUT4 and GLUT4 protein expression in skeletal muscle of C57BL/KsJ-db/db mice. Western blotting was performed, and signal intensities were determined by densitometric analysis using Multi Gauge V3.1 software. A. PM-GLUT4 and GLUT4 protein expression, B. expression levels of PM-GLUT4 and GLUT4. Each value is expressed as mean SD of experiments performed in triplicate. ^{a-c}mean values designed by different letters are significantly different between groups ($p < 0.05$). PM-GLUT4: plasma membrane glucose transporter 4.

was significantly increased, and the levels were 1.8-fold higher than in the db/db-control group. This result indicates that MWE supplementation may stimulate the GLUT4 translocation to plasma membrane and, as a consequence, lead to increased glucose uptake into the cell.

Discussion

Diabetes mellitus (DM) is a common metabolic disorder characterized by hyperglycemia, which is caused by impaired insulin secretion and resistance. During insulin resistance, the insulin does not function appropriately due to a decrease in the insulin sensitivity of tissues, thereby causing absorption of blood glucose into the cells and thus leading to hyperglycemia. Thus, improving insulin sensitivity to relieve hyperglycemia is essential in the treatment of diabetes [14, 31]. Effective antidiabetic drugs have been developed to treat type 2 diabetes. Thiazolidinedione (TZD) class comprises antidiabetic drugs that are widely used to improve insulin sensitivity and blood glucose control in diabetic patients [5]; however, these drugs may cause side effects such as headache, hypoglycemia, edema, and hypertension [18]. In particular, rosiglitazone, a member of the TZD class, increased the adipocyte differentiation, thereby leading to increased fat mass and appetite, and decreased physical activity. Due to these side effects of antidiabetic drugs, several studies have been conducted to improve the insulin sensitivity by using natural products with few side effects [17].

In natural products, insects have been used to treat various diseases. In particular, the mealworms have traditionally been considered as healthy food because they have abundant protein, lipids, vitamins, and minerals [27]. Mealworm is larva of *T. molitor* belonging to the beetle species that have been used in traditional antidiabetic medicines [32]. Nevertheless, no study is available on the use of mealworm as an antidiabetic in improving insulin sensitivity. Hence, this study aimed to research whether supplementation of the mealworm extract (MWE) to C57BL/KsJ-db/db type2 diabetic mice improves insulin sensitivity and reduces hyperglycemia. RG, an oral anti-diabetic agent, was used to compare the efficacy of MWE.

The daily food intake did not significantly differ among the db/db-control, db/db-RG, and db/db-MWE groups; however, the water intake was significantly higher in the db/db-control group than that in the db/db-MWE group. Polydipsia is one of the major symptoms of diabetes mellitus

[24]. A prominent increase of water intake in the db/db-control group reflects polydipsia. Conversely, the supplement of mealworm alleviated the symptoms of diabetes, proving that it can reduce water intake. The body weight of mice in the db/db-MWE group steadily increased for 6 weeks; however, the increase in the body weight of mice in the db/db-MWE group was significantly lower than that of mice in the db/db-RG group. Such body weight gain in the db/db-RG group is associated with increased adipocyte differentiation by activation of PPAR- γ as a side effect of rosiglitazone [17]. Fortunately, the supplementation of MWE did not reveal any side effect such as weight gain.

The progression of type 2 diabetes is related to diabetic complications. As hyperglycemia significantly affects the progression of type 2 diabetes, lowering the blood glucose levels can prevent diabetic complications [5, 19]. The fasting blood glucose levels were significantly lower in the db/db-MWE group than those in the db/db-control group from 2 weeks after the experiment. This phenomenon may be due to the omega-3 fatty acids or chitin and chitosan present in the mealworm. Omega-3 fatty acids have been extensively studied in relation to diabetes, and it is confirmed that omega-3 fatty acids are effective in reducing fasting blood glucose levels [10]. Recently, chitin and chitosan have also been extracted from mealworm and have been studied extensively in relation to diabetes [35]. Enzymatic digestion of chitosan produces low molecular weight chitosan oligosaccharides, and these have been proven effective in lowering the fasting blood glucose levels [12, 15]. HbA1c levels are indicative of the average blood glucose levels over the preceding 2-3 months and decreased HbA1c level can reduce the risk of developing macrovascular complications of type 2 diabetes [19]. In the db/db-MWE group, HbA1c levels were significantly decreased compared with those in the db/db-control group. These results suggest that long-term intake of MWE may reduce the blood glucose and further prevent diabetic complications.

Plasma insulin levels in the db/db-MWE and db/db-RG groups were significantly lower than in the db/db-control group. In the early stages of type 2 diabetes, hyperinsulinemia is observed to overcome insulin resistance in the peripheral tissues and maintain normal blood glucose levels [7]. In this study, the db/db-control group had hyperinsulinemia, whereas the db/db-MWE and db/db-RG groups did not. To assess the insulin resistance, HOMA-IR was calculated using fasting glucose and insulin concentrations.

HOMA-IR is homeostatic index of insulin resistance, which is decreased with increasing insulin sensitivity. In this study, HOMA-IR was significantly lower in mice of db/db-MWE group than those of the db/db-control group. These observations imply that MWE supplementation might contribute in improving insulin resistance.

Intraperitoneal glucose tolerance test was performed to assess the insulin sensitivity and utilization of excessive blood glucose [1, 21]. This study demonstrated that blood glucose levels were restored to levels close to the basal value after 120 min of glucose injection in db/db-MWE and db/db-RG groups compared with the db/db-control group. According to this result, supplementation of MWE increases the insulin sensitivity and effectively controls the blood glucose concentrations.

The increase in insulin sensitivity promotes glucose uptake into cells via insulin signaling pathway and thereby reduces hyperglycemia. To examine the levels of gene expression associated with insulin sensitivity in the insulin signaling pathway, western blotting was conducted. In the insulin signaling pathway, when insulin binds to the insulin receptor, IRS-1^{Tyr612} is phosphorylated. The phosphorylated IRS-1^{Tyr612} binds to the domain of PI3K and activates PI3K, which in turn phosphorylates Akt^{Ser473}. As a result, GLUT4 is transferred to the plasma membrane to absorb blood glucose into the cell [3, 11, 34]. In this study, the supplementation of mealworm extracts significantly increased the levels of gene expression in insulin signaling pathway. MWE significantly increased phosphorylation of IRS-1^{Tyr612} and Akt^{Ser473}, and activation of PI3K in skeletal muscles. In addition, the levels of GLUT4 expression were significantly increased in the plasma membrane of skeletal muscles after MWE supplementation.

Omega-3 fatty acids have been found to be associated with the upregulation of genes involved in insulin receptor signaling-1(IRS-1) and GLUT4 [8]. The fatty acids present in the mealworm comprise 77.74% unsaturated fatty acids. Among these, omega-3 fatty acids account for 46.1% of the unsaturated fatty acids [26]. Recently, published reports revealed that α -linolenic acid (C18:3, *n*-3), one of the omega-3 fatty acids, restored the decrease in palmitic acid-induced glucose uptake, and glucose uptake occurred via mechanisms of the insulin signaling pathway including Akt activation in skeletal muscles [23]. In addition to omega-3 fatty acids, chitin and chitosan are involved in lowering blood glucose via insulin signaling pathway [9]. Both high- and

low-molecular weight chitosan could effectively phosphorylate Akt and increase the translocation of GLUT4 in skeletal muscles [20]. The isolation of the chitin from the exuvium and whole body of mealworm, the rates were 18.1% and 4.92%, respectively. The relative average yield of chitosan from whole body was 3.65%[30]. According to these results, omega-3 fatty acids or chitin and chitosan in MWE may contribute, at least in part, to stimulate insulin signaling pathway in the db/db mice.

In conclusion, MWE supplementation effectively decreased hyperglycemia in type 2 diabetic db/db-mice. This was because MWE enhanced insulin sensitivity via activation of PI3K/Akt pathway, increased GLUT4 translocation in plasma membrane, and facilitated glucose uptake in the skeletal muscle cells. Therefore, this study suggests that MWE can possess a potential for improving insulin sensitivity and alleviating hyperglycemia via insulin signaling pathway in skeletal muscles of db/db-mice.

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초록 : C57BL/Ksj-db/db 제 2형 당뇨병모델을 이용한 갈색거저리 유충(밀웜) 추출물의 인슐린 감수성 및 혈당개선효과

김선영 · 박재은 · 한지숙*
(부산대학교 식품영양학과)

당뇨병은 서구화된 식습관으로 발생하는 심각한 만성대사질환의 하나이며, 당뇨병의 치료는 혈당을 정상적인 수준으로 유지하며 당뇨 합병증을 예방하는 것이다. 따라서 본 연구는 당뇨병 및 인슐린 저항성에 대한 유전적 분석에 널리 이용되는 C57BL/Ksj-db/db 당뇨동물모델을 이용하여 갈색거저리 유충(밀웜) 추출물의 6주간 섭취가 혈당개선에 미치는 영향에 대해 조사하고 이에 인슐린 민감성 개선과 당대사 조절을 통한 항당뇨 효과를 규명하고자 하였다. 제 2형 당뇨동물모델 실험 결과, db/db-MWE군(식이 0.5%)이 db/db-control군에 비해 유의적($p < 0.05$)으로 혈당이 감소하였다. 약물군인 db/db-RG군(식이 0.05%)은 부작용에 의해 눈에 띄게 체중이 증가하였으나, db/db-MWE군에서는 약물군에서의 체중증가와 같은 큰 부작용 없이 혈당 감소효과를 나타내었다. HbA1c와 혈장인슐린 농도의 경우 db/db-control군에 비해 db/db-MWE군이 유의적($p < 0.05$)으로 낮았다. 또한 골격근에서 p-IRS, p-AKT, PM-GLUT4의 발현을 확인한 결과, db/db-MWE군에서 db/db-control군에 비해 p-IRS, p-AKT, PM-GLUT4의 발현이 증가된 것을 알 수 있었다. 이는 밀웜 추출물의 섭취가 골격근 내로 당이 원활히 유입되도록 도와주어 인슐린 민감성을 개선시키며, 고혈당 증상을 개선시킨 것으로 사료된다. 밀웜 추출물을 식이에 0.5% 첨가하여 6주간 C57BL/Ksj-db/db 당뇨동물모델에 제공한 결과, 공복혈당과 HbA1c의 감소 및 인슐린 저항성을 개선시켰다. 이는 인슐린 민감성을 증가시키고, 당 대사 조절을 통해 고혈당 증상의 완화에 기인한 것으로 보인다. 따라서 밀웜은 당뇨병의 예방과 치료에 유용한 소재가 될 것으로 기대되며, 향후 제 2형 당뇨병 개선을 위해 더욱 다양한 연구가 이루어져야 할 것으로 사료된다.