

Effects of guava leaf extract on glucose and lipid homeostasis in diet-induced insulin-resistant C57BL/6J mice

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Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT

In this study, the effects of aqueous guava leaf extract (GvEx) on insulin resistance were evaluated in diet induced insulin-resistant mice. Low (50 mg/kg), middle (150 mg/kg), and high (450 mg/kg) oral dose of GvEx were administrated to the high-fructose-high-fat fed insulin-resistant C57BL/6J mice. Our results showed that administration of high dose GvEx significantly enhanced the glucose tolerance, insulin sensitivity and increased the serum adiponectin content. Triglyceride and total cholesterol contents of blood and liver were all significantly decreased after treatment with GvEx. Further, western blot analysis revealed that high dose GvEx significantly enhanced the levels of peroxisome proliferator-activated receptor (PPAR)- γ and adiponectin in adipose tissue, as well as enhancing the phosphorylation of the AMP-activated protein kinase and PPARs in both liver and skeletal muscle tissues. In addition, the protein levels of phosphorylated protein kinase B and glucose transporter

were also induced in liver and skeletal muscle tissues. In conclusion, GvEx can improve the disturbed glucose and lipid homeostasis in diet-induced insulin-resistant C57BL/6J mice.

Keywords: metabolic syndrome, diabetes mellitus, insulin resistance, guava leaf extract

INTRODUCTION

Metabolic syndrome refers to the simultaneous presence of multiple metabolic abnormalities conditions, including abdominal obesity, impaired glucose tolerance, dyslipidemia, hypertension and other symptoms. These metabolic abnormalities are usually a sign of many chronic diseases, including type 2 diabetes, cardiovascular disease and hypertension. Metabolic syndrome of worldwide adult population is about 35%, which is 18.2% of Asian, 29.4% of Africa, 39.1% of Australia and 35% of American (Pudata and Konduru, 2011). According to the investigation of Taiwan Min-

istry of Health and Welfare showed that the prevalence of metabolic syndrome was 19.7% in adult population and increased with age. The results also noted that abdominal obesity will increase 50% chance of suffering from metabolic syndrome, while there are cases when coupled with abnormal blood pressure, the probability is as high as 75%. In addition, the subjects with metabolic syndrome will increase the risk of suffering from type 2 diabetes, hypertension, hyperlipidemia and cardiovascular disease for six times, four times, 3 times and 2 times, respectively (Ministry of Health and Welfare, Executive Yuan, Taiwan (R.O.C.), 2007).

Obesity is mainly because of excessive intake of energy resulting in excessive accumulation of body fat. As described by Bays *et al.*, persons with diabetes, dyslipidemia and hypertension are respectively 84%, 79% and 82% have overweight or obese phenomenon, indicating the close correlation between obesity and metabolic syndrome (Bays *et al.*, 2007). The formation mechanism of metabolic syndrome is not yet fully clarified, it is generally believed that may be associated with insulin resistance and abdominal obesity. The excessive free fatty acid inhibits insulin signaling pathways of muscle tissue, which resulting in the occurrence of insulin resistance and reducing glucose uptake in muscle cells (Eckel *et al.*, 2005). In addition, excess body fat can promote the secretion of interleukin-6 and tumor necrosis factor- α , which will increase inflammatory response, inhibit insulin signal transduction and further leading to insulin resistance occurs (Dandona *et al.*, 2004; Furukawa *et al.*, 2004; Lazar, 2005; Kasuga, 2006; Shoelson *et al.*, 2006). Based on the above, an excessive amount of body fat will lead to the occurrence of insulin resistance, indicating the importance of obesity amelioration for the prevention and/or improvement of metabolic syndrome.

Peroxisome proliferator-activated receptor γ (PPAR- γ), specifically and preferentially expressed in adipocytes, is mainly responsible for the regulation of insulin sensitivity, cell differentiation, inflammation and insulin sensitivity (Semple *et al.*, 2006; Yang *et al.*, 2008). PPAR- γ can be regulated by fatty acid, phytochemicals or drugs and affect the lipid and glucose metabolism related gene expression (Semple *et al.*, 2006; Wang *et al.*, 2014). Adiponectin is an adi-

pose-specific plasma protein which are decreased in obese and type 2 diabetic subjects with insulin resistance. Thiazolidinediones (TZDs), PPAR- γ synthetic ligands, is a new class of antidiabetic drugs that improve insulin action. The administration of TZDs significantly increased both the plasma adiponectin concentrations and adiponectin mRNA expression of adipose tissues in insulin resistant rodents (Maeda *et al.*, 2001). As described by Yoon *et al.*, adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase (AMPK), p38 Mitogen-activated protein kinase, and PPAR- α (Yoon *et al.*, 2006).

Guava leaf has traditionally been used as a traditional folk herb for diabetes patients in Oriental countries. In recent years, the anti-hyperglycemic and anti-metabolic syndrome activities of guava leaves extract has been reported in animal models and human clinical studies (Yoshitomi *et al.*, 2012a; Yoshitomi *et al.*, 2012b; Guo *et al.*, 2013; Khan *et al.*, 2013; Oriaifo *et al.*, 2014; Mathur *et al.*, 2015). However, the mechanisms of guava leaf extracts on diabetes and/or metabolic syndrome improvement are only partially discussed in these researches. Our previous study has confirmed the effects of aqueous guava leaf extract (GvEx) on insulin resistance improvement via modulation of the insulin signaling pathway in high glucose-induced insulin-resistant mouse FL83B cells (Liu *et al.*, 2015a). In the present study, the mechanisms of GvEx on metabolic syndrome improvement was comprehensively evaluated in hepatic, white adipose, and skeletal muscle tissues of high-fructose-high-fat fed insulin-resistant C57/BL6J mice.

2. Materials and methods

2.1. Plant material and GvEx preparation.

Plant material and GvEx preparation were described in our previous study (Liu *et al.*, 2014). Briefly, Jen Ju Pa leaves were collected at period between the initial appearance and the visible opening of flower buds. The plant materials were taxonomically identified and the data have been deposited at the Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute Council of Agriculture, Executive Yuan (FTHA000282). Guava leaf extraction were per-

formed with the optimal extraction conditions which derived from a response surface methodology. The freeze-dried GvEx was stored at -80°C and the quality of GvEx was evaluated by total phenolic content (26.12%) and chromatographic profile of the phenolic components including catechin, epicatechin, gallic acid, quercetin, chlorogenic acid, epigallocatechin gallate and caffeic acid (Liu *et al.*, 2014).

2.2. Animals.

Ten-week-old male C57BL/6J mice were obtained from Taiwan NLAC (Taipei, Taiwan). All mice were housed in a climate-controlled room with 20-24°C temperature, 40-60% humidity, 12-hour light and dark cycle, and free access to normal chow and water at the agricultural college of Tunghai university. After one week, mice were randomly allotted to 6 groups including a normal control group (normal chow, not treated; N), an experimental control group (high-fructose-high-fat fed, not treated; C), a positive control group (high-fructose-high-fat fed, acarbose gavage administered as 61 mg/kg/day; P), a low dose group (high-fructose-high-fat fed, GvEx gavage administered as 50 mg/kg/day; TL), a middle dose group (high-fructose-high-fat fed, GvEx gavage administered as 150 mg/kg/day; TM) and a high dose group (high-fructose-high-fat fed, GvEx gavage administered as 450 mg/kg/day; TH). According to the result of our previous *in vitro* study, 200 and 400µg/ml GvEx (ED50 *in vitro*), respectively, showed significantly increased the rate of glucose uptake in both normal and insulin-resistant cells (Liu *et al.*, 2015). Therefore, the effective doses (ED50 *in vivo*) for animal model were 43.58 and 61.88 mg/kg, respectively, after calculation with the formula $\log(\text{ED50 } in vivo) = 0.506 \times \log(\text{ED50 } in vitro) + 0.475$ (Popiolkiewicz *et al.*, 2005; Kitagaki *et al.*, 2006). The low dose (50mg/kg) was the average of effective dosages. The middle dose (150mg/kg) was three times of low dose and high dose (450mg/kg) was nine times. The dose of acarbose was in accordance with the maximum effective dose of 61.88 mg/kg GvEx for animal model.

2.3. Induction of insulin resistance and GvEx treatment.

To induce insulin resistance, the C, P, TL, TM, TH groups were fed with high fat diet (fat content, 60 Kcal%, research diet) and 30% (w/v) fructose was added to the drinking water. These mice were gavage administrated with their relatively treatment at the beginning of high-fructose-high-fat feeding. The extract was dissolved in drinking water for the oral gavage. The N group was maintained on regular chow diet and plain water (untreated). At the end of the 10-week period, all animals were placed on a 14 h fast, sacrificed by cervical dislocation and exsanguination prior to sample collection. The tissues and serum were flash-frozen in liquid nitrogen and stored at -80 °C until further analyses were carried out.

2.4. Oral glucose tolerance test (OGTT).

After 14 h fasting, animals were subjected to OGTT on week 8. Briefly, individual mice were administered orally with 1 g/kg BW of glucose and their blood glucose levels were measured at 0, 30 60, 90, and 120 min post glucose challenge. The area under curve (AUC) over the blood glucose levels were calculated by the trapezoidal rule: $[(\text{gluc0min} + \text{gluc30min}) \times 30/2] + [(\text{gluc30min} + \text{gluc60min}) \times 30/2] + [(\text{gluc60min} + \text{gluc90min}) \times 30/2] + [(\text{gluc90min} + \text{gluc120min}) \times 30/2]$ (van Hoek *et al.*, 2009).

2.5. Biochemical measurement.

The concentrations of serum triglycerides (TG), cholesterol (CHOL), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) levels were determined by regular biochemistry assays using the corresponding commercial enzyme kit on a Biochemical Analyzer (F. Hoffmann-La Roche LTD., Basel, Switzerland). Serum insulin and adiponectin concentrations were measured using mouse insulin (Mercodia Inc., Uppsala, Sweden) and adiponectin (Abcam, Cambridge, United Kingdom) ELISA kits, respectively.

2.6. Homeostatic model assessment-insulin resistance (HOMA-IR).

The HOMA model is a method used to quantify insulin resistance and β -cell function from fasting serum glucose and insulin concentrations. The model has been widely used since it was first published (Wallace *et al.*, 2004). After 14 h fasting, the blood glucose and insulin concentrations of mice were obtained and used to HOMA-IR calculation. The formula was as follows: $\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/L)}] / 22.5$.

2.7. Hematoxylin-eosin (HE) stain

HE stain is the most widely used method in histology and histopathology analysis. It is a relatively simple method to demonstrate a wide range of cytoplasmic, nuclear, and extracellular matrix features on paraffin or frozen sections. Liver tissues were harvested and fixed in 10% neutral formalin, embedded in paraffin, cut into 6- μm -thick sections on slides, and stained with hematoxylin and eosin. Before immunostaining, deparaffinization was performed in xylene and graded ethanol to distilled water. After deparaffinization, slides were stained in hematoxylin for 1~3 min followed by a running water wash. After 15 mins wash, the slides were stained in eosin for 5 secs. Slides were dehydrated, cleared and mounted for examination by light microscopy.

2.8. Hepatic triglyceride (TG) and cholesterol (CHOL) analysis.

Liver tissues were homogenized in an ice-cold phosphate-buffered saline (PBS) solution. Hepatic lipids were extracted following an adaptation of published methods (Folch *et al.*, 1957). Briefly, homogenate was extracted with a 1:2 (v:v) mixture of methanol/chloroform, with vortexing to mix thoroughly. The samples were then spun at 4,200 g at 4°C for 10 min. Organic (lower) phase was dried under vacuum condition and the TG and CHOL levels were measured by regular biochemistry assays using the corresponding commercial enzyme kit on a Biochemical Analyzer (F. Hoffmann-La Roche LTD., Basel, Switzerland).

2.9. Western blot analysis.

The liver, white adipocytes and skeletal muscle tissues were homogenized with ice cold lysis buffer (0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) at a ratio of 100 mg tissue/1 mL buffer, respectively. Homogenates were then centrifuged at 27,000 x g for 10 min at 4°C. The supernatant was isolated and immunoblotting and was performed as described previously (Liu *et al.*, 2015a). The supernatant was used for the determination of protein abundance, including phospho-tyrosine of AMPK (p-AMPK (Thr)), phospho-serine of protein kinase B (p-Akt (Ser)), PPAR- α , PPAR- γ , PPAR- δ , adiponectin, glucose transporter (Glut) 2, and Glut 4. β -Actin was used as an internal control to ensure adequate sample loading for all lanes. The bound antibodies were detected with horseradish peroxidase-conjugated secondary antibodies, followed by enhanced chemiluminescence substrates (Millipore, Billerica, MA).

2.10. Statistical assay.

All experiments were performed at least in triplicate, and the results are expressed as the mean \pm SD. Statistical analysis was done by one-way ANOVA with Tukey's *post hoc* test. A *p* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Supplementation of GvEx significantly decreased the body weights of high-fructose-high-fat fed mice. As shown in Figure 1, mean body weights were no difference between all groups at the beginning of the study. After ten weeks of treatment, mean body weights were significantly lowest in TH (30.8 ± 2.2 g), followed by TM (31.8 ± 2.7 g) and P (32.3 ± 1.9 g) as compared to C (34.4 ± 2.3 g), suggesting the beneficial effect of GvEx on lowering body weight (Figure 1). In addition, treated with high dose GvEx significantly decreased the weights of epididymal fat and white adipose tissue (sum of perirenal fat, abdominal fat, and epididymal fat) in high-fructose-high-fat fed mice (Table 1). A previous report indicated that in high-fat diet induced obese mice, treated with different dose of *Psidium guajava* leaves extract (LD: 50 mg/kg; MD:

100 mg/kg; HD: 200 mg/kg) decreased the body weight and visceral fat accumulation, and the lowering activity was dose-dependent (Li *et al.*, 2012). SHRSP.Z-Lep^{rfa}/Izm^{Dmcr} (SHRSP/ZF) rats, a metabolic syndrome animal model, which were administrated with 2 g/kg/day guava leaf extract for 6 weeks. The results indicated that administration of guava leaf extract sig-

nificantly reduced body weight and adipose weight as compared to control group (Yoshitomi *et al.*, 2012a). Based on these results, we propose that GvEx may be a purely nature component with anti-obesity and lipid lowering effects. Thus, extracts from guava leaves may be a purely nature component with anti-obesity and lipid lowering effects.

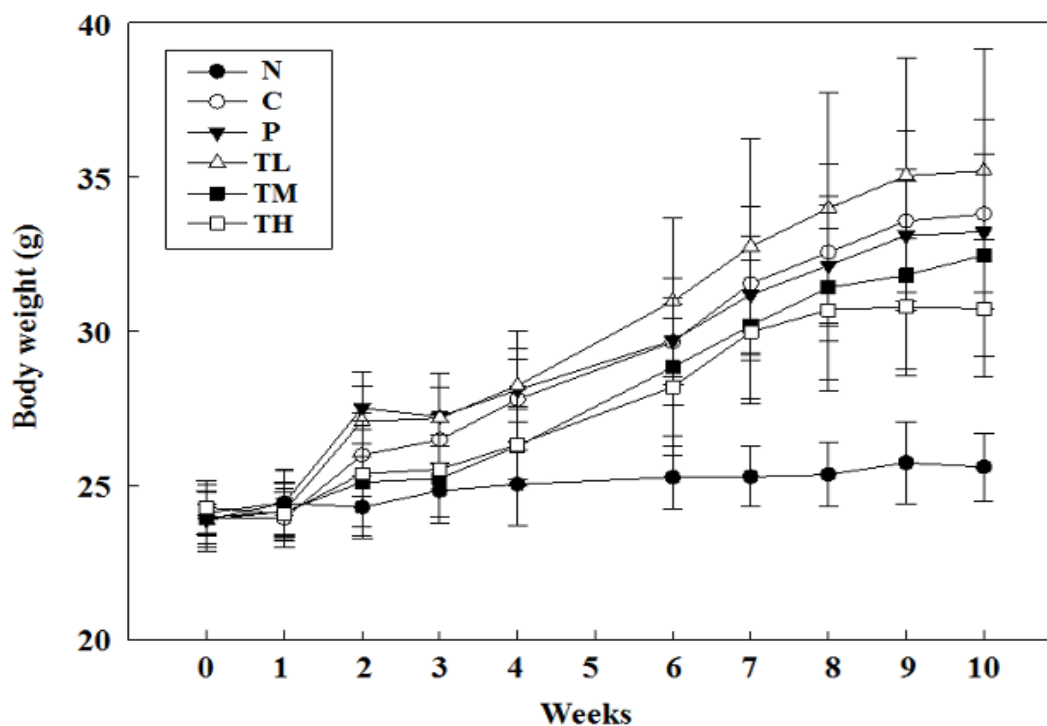


Figure 1: The effect of guava leaf extract on body weight in experimental animals. Mean body weights were significantly lowest in TH (30.8 ± 2.2 g), followed by TM (31.8 ± 3.1 g) and P (32.83 ± 2.0 g) as compared to C (34.2 ± 2.4 g) at week 10.

Table 1. The effect of guava leaf extract on body weight, liver weight, kidney weight, spleen weight, perirenal fat weight, abdominal fat weight, epididymal fat weight and total white adipose weight in animals of each group.

	N	C	P	TL	TM	TH
Body weight (g)	25.7±1.32 ^a	34.4±2.26 ^d	32.3±1.88 ^c	36.3±2.08 ^e	31.8±2.65 ^c	30.8±2.22 ^b
Liver (g)	0.94±0.07 ^a	1.06±0.11 ^b	1.06±0.06 ^{bc}	1.13±0.13 ^c	1.09±0.11 ^{bc}	1.01±0.09 ^{ab}
Kidney (g)	0.30±0.06	0.31±0.15	0.30±0.03	0.27±0.07	0.31±0.05	0.28±0.05
Spleen (g)	0.05±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.02	0.06±0.02
Perirenal fat (g)	0.15±0.19 ^a	0.69±0.22 ^b	0.62±0.29 ^b	0.80±0.25 ^b	0.57±0.29 ^b	0.53±0.23 ^b
Abdominal fat (g)	0.31±0.11 ^a	1.37±0.45 ^b	1.33±0.57 ^b	1.35±0.64 ^b	1.25±0.54 ^b	1.17±0.39 ^b
Epididymal fat (g)	0.45±0.37 ^a	1.70±0.43 ^c	1.32±0.49 ^{bc}	2.06±0.44 ^d	1.58±0.37 ^{bc}	1.27±0.36 ^b
White adipose tissue (g)	0.94±0.70 ^a	3.75±1.02 ^c	2.93±1.22 ^{bc}	4.45±1.02 ^d	3.42±0.97 ^{bc}	2.77±0.69 ^b

N: normal control, C: experimental control, P: positive control, TL: low dose, TM: middle dose, TH: high dose.

Values with different letters on each column are significantly different ($p < 0.05$; $n \geq 8$).

3.2. Effects of GvEx on the improvement of glucose tolerance and insulin resistance in experimental animals.

While there was no significant difference in the dynamics of blood glucose levels following oral glucose loading on day 28 post treatment within high-fat-high-fructose fed groups, the levels of blood glucose in the TH group were significantly lower than that in the C group at 60 (219.1 ± 66.0 vs. 316.0 ± 85.5 mg/dL, $p = 0.006$), 90 (199.0 ± 57.5 vs. 247.7 ± 47.9 mg/dL, $p = 0.033$), and 120 minutes (191.0 ± 44.9 vs. 231.5 ± 45.9 mg/dL, $p = 0.039$) post glucose loading on day 56. In addition, the levels of blood glucose in the P group were significantly less than that of the C group at 60 minutes (316.0 ± 85.5 vs. 235.3 ± 34.3 mg/dL, $p = 0.004$) after oral glucose loading (Figure 2A). The area under the AUC is dependent on the rate of elimination of the administered glucose from the body. The AUC is inversely proportional to the clearance of the glucose. It can be used to estimate a change in the clearance of blood sugar in specific clinical conditions, such as metabolic syndrome and/or diabetes. As a result, the AUC values over the 120 minutes of glucose challenge in the TH group were significantly lower than that of the C group (27818 ± 7046 vs. 34075 ± 5190 , $p = 0.014$), suggesting the effects of GvEx on improvement of glucose tolerance (Figure 2B). HOMA is a method for evaluating IR and beta-cell function from blood glucose and insulin concentrations. The HOMA-IR is a widely used clinical and epidemiological tool for the assessment of insulin resistance; higher values are associated with higher incidence of metabolic syndrome, and lower insulin sensitivity (Qu *et al.*, 2011). High-fructose-high-fat feeding caused a significant elevation ($p < 0.05$) in fasting blood sugar levels (Figure 3A) and HOMA-IR (Figure 3C) and didn't affect serum insulin level (Figure 3B) as compared to N group. Oral administration of high dose GvEx in the last 10 weeks reduced serum insulin levels (Figure 3B) and HOMA-IR (Figure 3C), suggesting the effectiveness of GvEx that improve insulin sensitivity.

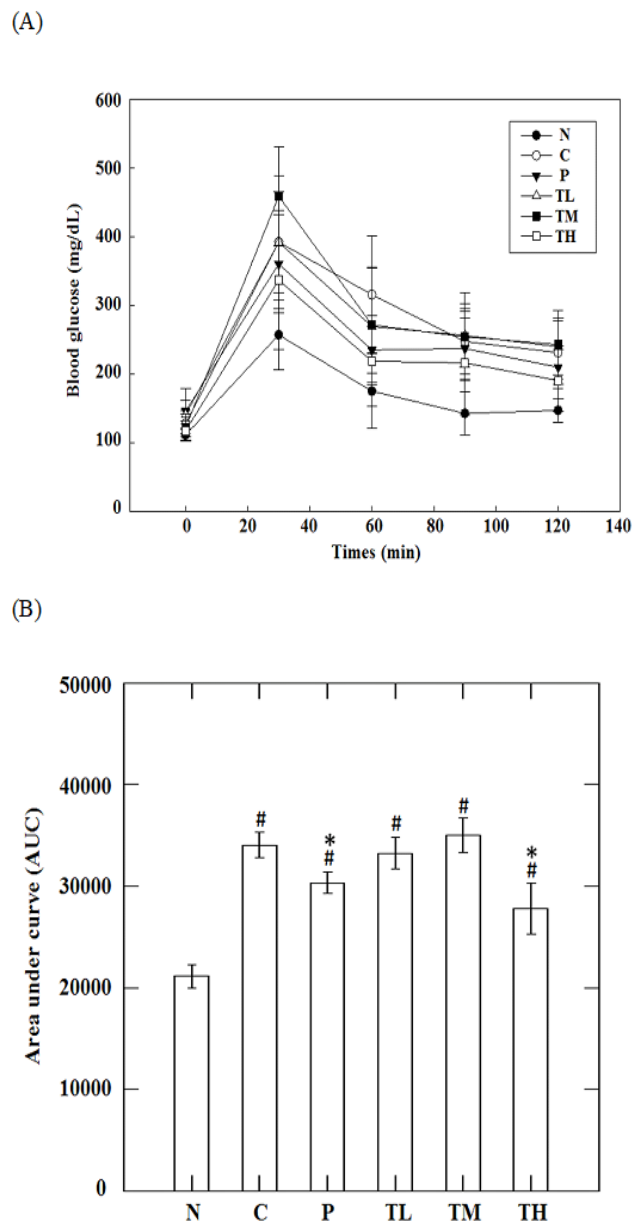


Figure 2 : (A) Oral glucose tolerance test curve and (B) averaged area under curve on serum glucose level in animals. #, significantly different ($p < 0.05$) from N group; *, significantly different ($p < 0.05$) from C group.

Our previous studies indicate that polyphenols of GvEx may effectively inhibit the activities of α -amylase and α -glucosidase (Liu *et al.*, 2014; Liu *et al.*, 2015b), so that the amount of glucose can be released slowly to avoid postprandial blood sugar rise sharply, thus preventing the excessive secretion of insulin. Furthermore, GvEx can also increase the sensitivity of

insulin receptor, including phosphorylated insulin receptor substrate 1, phosphoinositide 3-kinase, phosphorylated protein kinase B, etc., that will activate the insulin signaling pathways and promote glucose metabolism (Guo *et al.*, 2013; Liu *et al.*, 2015a). As described by Mathur *et al.*, to induce insulin resistance, the 4 weeks old male rats were provided with 15% (w/v) fructose as drinking water, while parallel treatment groups were administered with guava leaf extract for 8 weeks. After treatment period, the levels of blood glucose were significantly lower in the dose of 250 (PG1) and 500 (PG2) mg/kg/day guava leaf extract treated group than that in the control group at 120 minutes (112.6 ± 28.6 and 93.4 ± 18.2 vs. 152.2 ± 16.2 mg/dL) after oral glucose loading. The AUC of OGTT test in control, PG1 and PG2 groups was 36444 ± 1110 , 20208 ± 1023 and 18486 ± 1005 , respectively. The AUC was significantly less ($p < 0.001$) in PG1 and PG2 groups as compared to control group. In addition, fructose intake clearly led to over 3-fold increase in HOMA-IR value, and treatment with guava leaf extract showed a significant decrease in plasma insulin concentration, HOMA-IR values, and fasting blood sugar control group (Mathur *et al.*, 2015). In the Deguchi *et al.* (2000) study, 16 of subjects with pre-diabetes and mild diabetes were administrated with 190 ml of Guava Leaf Tea at every meal for 12 weeks. Results showed that the fasting blood sugar level were more pronounced decrease in the pre-diabetic subjects ($p = 0.06$, $n=7$). Moreover, the levels of blood insulin and HOMA-IR values were significantly decreased in all subjects (Deguchi *et al.*, 2000).

These results revealed that guava leaf extract can be used to alleviate glycemic response, including decreased blood sugar concentration and/or AUC. In addition, guava leaf extract that are effective in improving insulin resistance status of diabetic animals.

3.3. Effects of GvEx on lipid metabolism in experimental animals.

High fructose and/or high fat intake disturbs glucose metabolism and leads to a significantly increased rate of lipogenesis and TG synthesis in liver, and TG levels in circulation. These elevated TG levels can induce fatty liver, adipose accumulation, and decrease the

response to insulin thereby causing IR. Fructose and/or fat-induced insulin resistant statuses are commonly characterized by the metabolic dyslipidemia, including elevated levels of circulating free fatty acid, TG, or CHOL levels (Basciano *et al.*, 2005; Ginsberg *et al.*, 2005; Liu *et al.*, 2015c). In this study, high-fructose-high-fat fed mice showed insulin resistance and impaired glucose tolerance accompanied by increased TG and CHOL levels in circulation and liver, and accumulation of adipose tissue. High dose GvEx treatment significantly decreased the body weights (Figure 1) and adipose weights (Table 1) as compared to C group. In addition, administration of high dose GvEx indicated a significant decrease in the accumulation of TG and CHOL in the liver (Figure 4C, D) as well as in the circulation (Figure 4A, B).

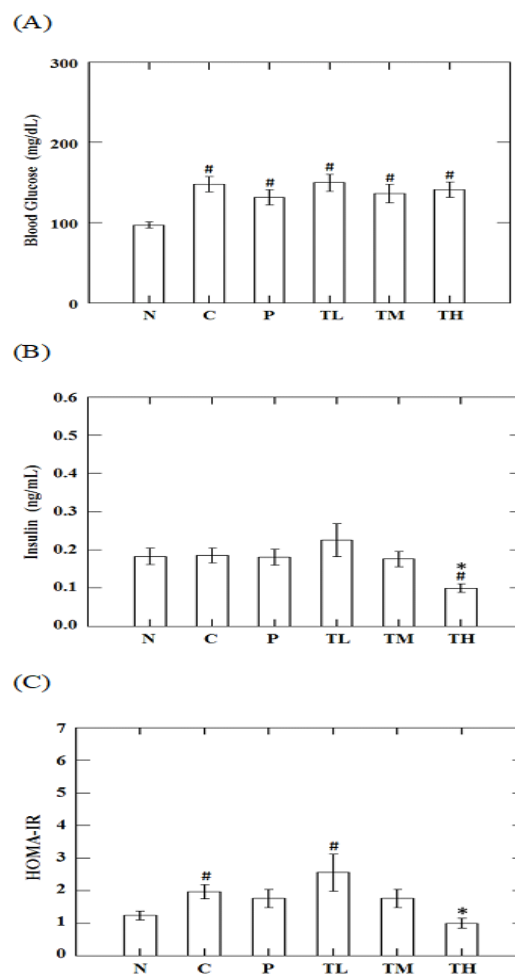


Figure 3: Effects of guava leaf extract on fasting serum (A) glucose, (B) insulin, and (C) insulin sensitivity of animals in each group. #, significantly different ($p < 0.05$) from N group; *, significantly different ($p < 0.05$) from C group.

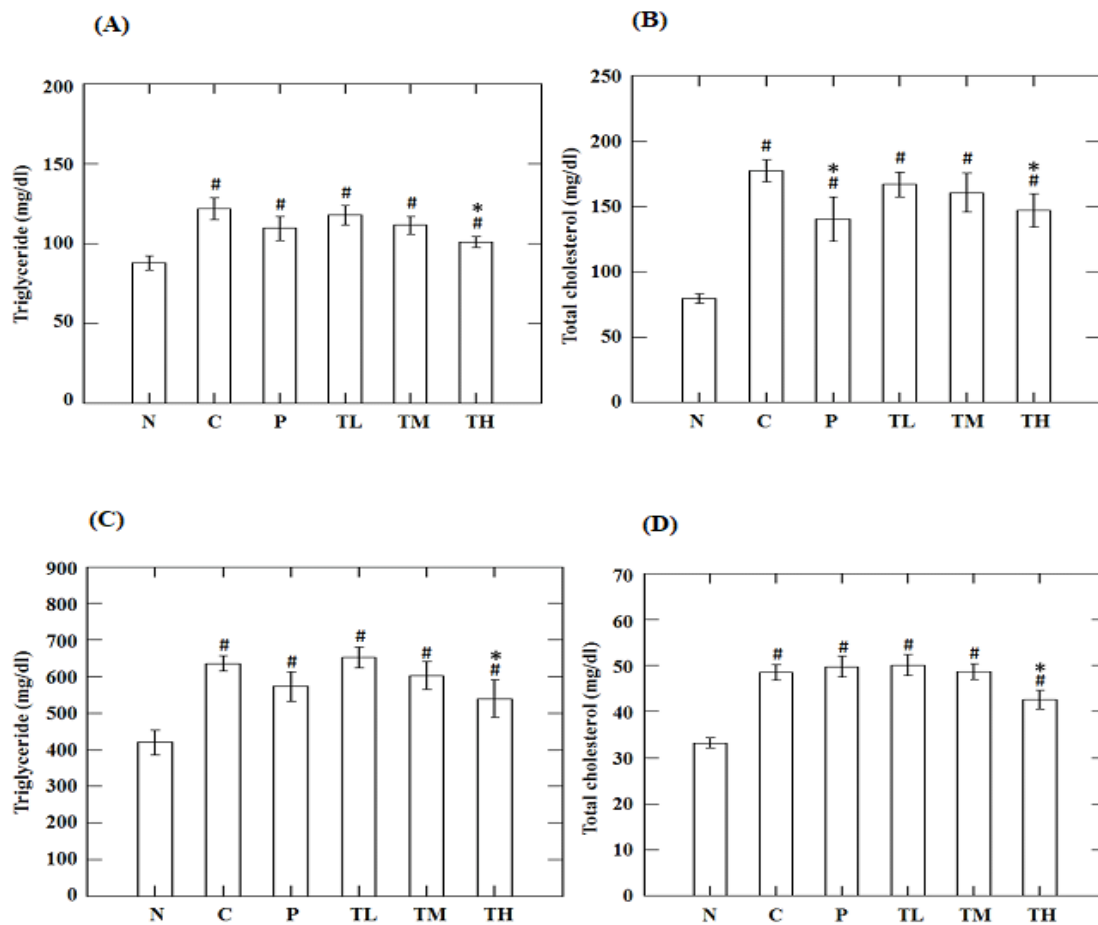


Figure 4: Effects of guava leaf extract on serum (A) triglyceride, (B) total cholesterol and liver (C) triglyceride, (D) total cholesterol in animals of each group. #, significantly different ($p < 0.05$) from N group; *, significantly different ($p < 0.05$) from C group.

PPAR- γ and adiponectin are the factors that regulate pathways of lipid and carbohydrate metabolism (Yamauchi *et al.*, 2001; Kota *et al.*, 2005). In our study, the protein levels of PPAR- γ and adiponectin were analyzed in epididymal fat tissue. The results showed a significant reduction in PPAR- γ and adiponectin levels of high-fructose-high-fat fed mice as compared to the levels in N group. Administration of high dose GvEx significantly elevated the PPAR- γ and downstream adiponectin levels (Figure 7A). Adiponectin, an insulin sensitizer that secreted by white adipose tissue, has been shown to activate fatty acid oxidation and enhance insulin sensitivity through the stimulation of AMPK in the peripheral tissues (Kadowaki and Yamauchi, 2005). Biochemical analysis of blood serum from the experimental animals showed that the concentrations of adiponectin, which was low under insulin-resistant state, were enhanced

by high dose treatment with GvEx (Figure 6). Adiponectin downstream effectors, including AMPK and PPARs, were assayed in liver and skeletal muscle tissues. As shown in Figure 7B, high-fructose-high-fat diet reduced the pAMPK and PPAR- α levels in liver, and supplemented with middle and high dose of GvEx will ameliorate it. Similar results were also observed in skeletal muscle tissue that protein levels of pAMPK and PPAR- δ were restored by GvEx (Figure 7C). According to the assays on adiponectin downstream effectors, data suggest that the adiponectin signaling is intact up to pAMPK and PPARs, thereby may stimulate the activation of fatty acid oxidation and decrease tissue TG content. Moreover, histological analysis of liver sections from experimental animals also showed that the accumulation of lipid droplets was drastically decreased in TH group as compared to C group (Figure 5).

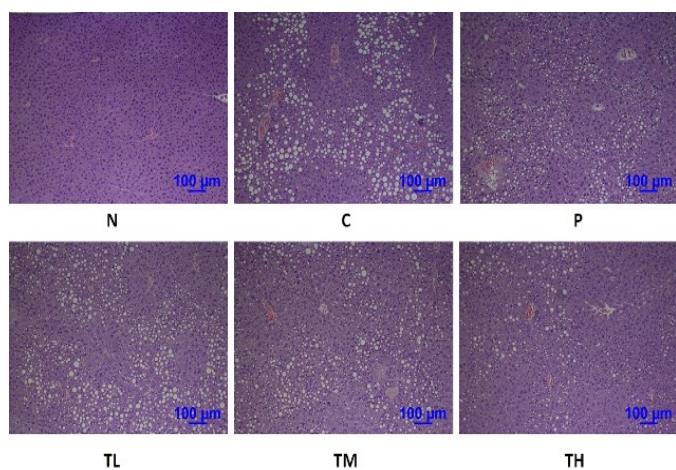


Figure 5: Hematoxylin-eosin staining of liver sections from mice with or without GvEx treatment. The accumulation of lipid droplets was drastically decreased in TH group as compared to C group.

3.4. Effects of GvEx on the improvement of metabolic syndrome.

Several studies have indicated that high fructose and/or high fat diet can induce insulin resistance, hyperinsulinemia, hyperglycemia, and even the diabetes. The main reasons which cause the occurrence of these symptoms include: (1) Diet-induced increase in blood triglyceride levels would decrease the number of activated insulin receptors, thereby reducing insulin sensitivity. (2) Chronic inflammation and high oxidative stress status caused by high fructose and/or high fat diet would reduce the levels of phosphorylated insulin receptor, and decrease the amount of adiponectin expression in adipose tissue, thus lowering insulin sensitivity and disturbing homeostasis of glucose and lipid metabolism (Qin *et al.*, 2003; Wellen and Hotamisligil, 2005; Ye *et al.*, 2007). Furthermore, visceral fat tissue is known to actively release substantial amounts of free fatty acid than the subcutaneous fat tissue, that can lead to insulin resistance and metabolic abnormalities, and thus resulting in high blood sugar levels and other risk factors for metabolic syndrome (Schetman *et al.*, 1989; Mann *et al.*, 1997; Mori *et al.*, 1997). Therefore, to reduce the concentration of triglycerides in the blood, increasing the degree of phosphorylated insulin receptor, activating insulin signal transduction, enhancing adiponectin abundance

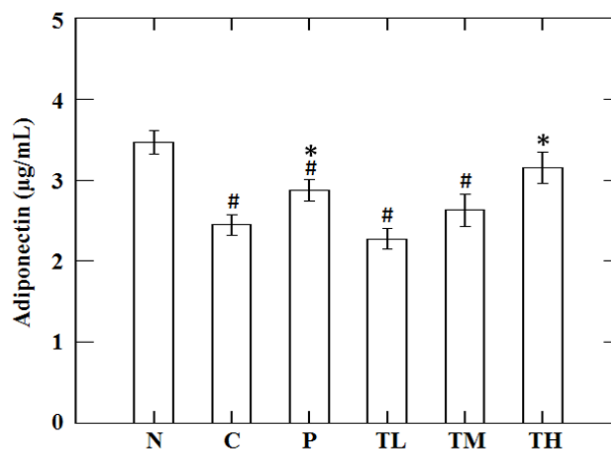


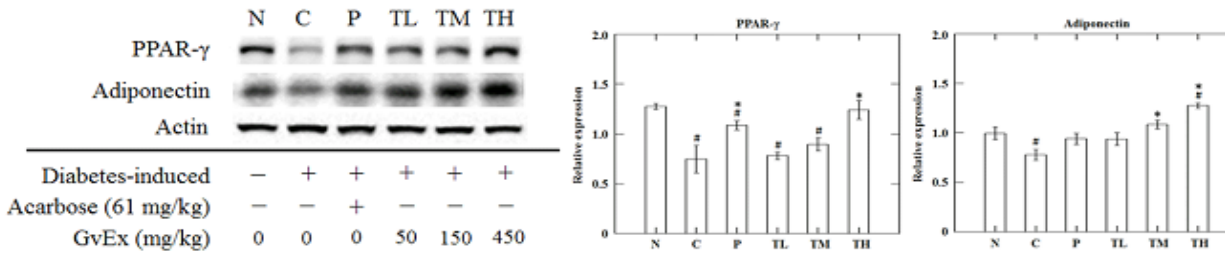
Figure 6: Effect of guava leaf extract on serum adiponectin in animals of each group. #, significantly different ($p < 0.05$) from N group; *, significantly different ($p < 0.05$) from C group

in adipose tissue, and reducing the amount of visceral fat would help prevent or ameliorate the metabolic syndrome status.

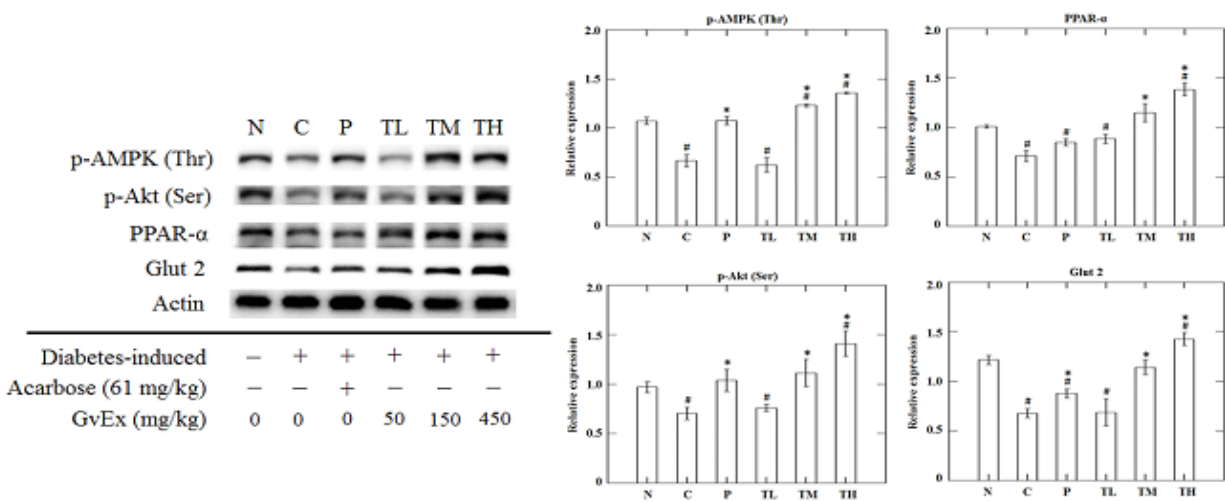
In our study, high-fructose-high-fat fed animals showed insulin resistance and impaired glucose tolerance accompanied by dyslipidemia. In addition, the adiponectin concentrations in circulation and the abundances of glucose and lipid metabolism-related signals in adipose, liver and skeletal muscle tissues were all drastically decreased. After 10-week treatment, the metabolic syndrome status of high-fructose-high-fat fed mice were ameliorated by high dose GvEx. Our previous study has demonstrated that GvEx could improve high-glucose induced insulin resistant conditions in mouse FL83B cells via enhancing the levels of signals involved in insulin transduction, including phospho-tyrosine of insulin receptor, phospho-tyrosine of insulin receptor substrate, p85 regulatory subunit of phospho-inositide 3 kinase, p-Akt (Ser), Glut 2, and glycogen synthase (Liu *et al.*, 2015a). These results were also confirmed in the present study by a diet-induced insulin resistant animal models. Data from experimental animals showed that the protein levels of p-Akt (Ser) and Glut were all significantly induced in both liver and skeletal muscle tissues as compared to C group (Figure 7B, C). According to the above results, we suggested that GvEx could improve the high-fructose-high-fat diet induced

metabolic syndrome status via activating lipid and glucose metabolism-related signaling in adipose, liver and skeletal muscle tissues (Figure 8).

(A) Adipose tissue



(B) Hepatic tissue



(C) Skeletal muscle

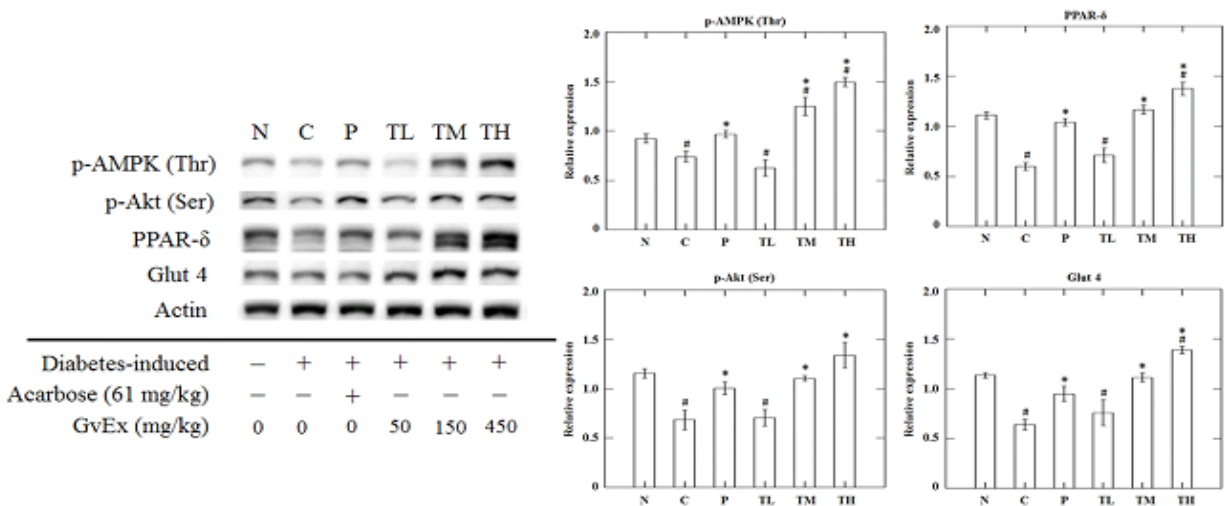


Figure 7: Effects of guava leaf extract on glucose and fat metabolism related signal transduction mechanism in (A) adipose tissue, (B) hepatic tissue, and (C) skeletal muscle of diet induced insulin resistant mice. #, significantly different ($p < 0.05$) from N group; *, significantly different ($p < 0.05$) from C group.

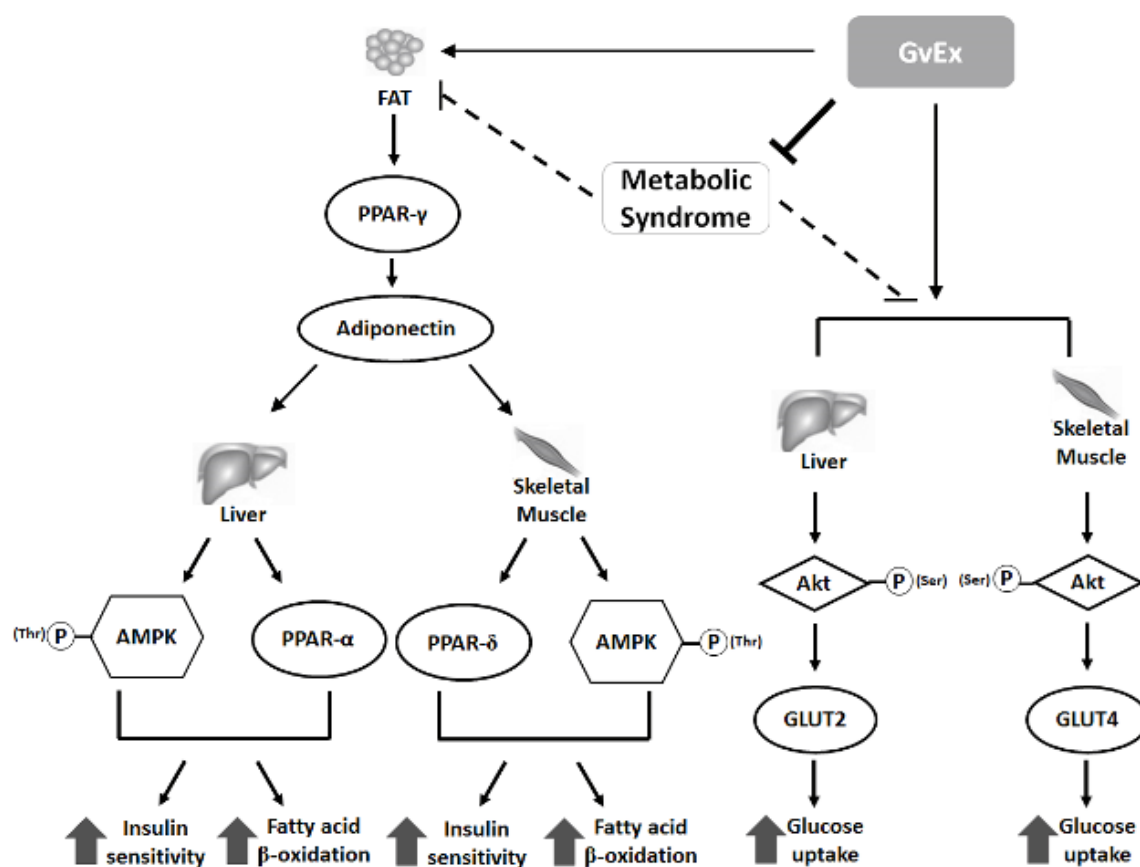


Figure 8: Schematic model of GvEx on modulating glucose and lipid homeostasis in diet-induced insulin-resistant C57BL/6J mice.

CONCLUSIONS

In conclusion, we evaluated the effects of GvEx on improvement of metabolic syndrome status in high-fructose-high-fat diet-induced insulin-resistant C57BL/6J mice. Our results showed that administration of high dose GvEx had preventive effects against the accumulation of lipid and ameliorated insulin resistance. The mechanism for this action was by the enhancing of glucose and lipid metabolism-related signals in adipose, liver and skeletal muscle tissues.

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