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Research Paper

An herbal mixture containing ginseng, *Eucommia* leaf, and Chinese foxglove root may ameliorate insulin sensitivity in rats with metabolic syndrome.

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ABSTRACT

Metabolic syndrome (MetS) is a social problem worldwide. It has even been termed syndrome X and is considered prediabetes due to insulin resistance (IR). Management and/or prevention of MetS has become an important subject in public health. In addition to nutritional control and exercise, medicinal plants are recommended as an alternative method. In the present study, a commercial herbal mixture, containing ginseng, Du-Zhong (*Eucommia* leaf), Shu Di Huang (Chinese foxglove root), and other ingredient, was investigated using rats with MetS induced by fructose-rich chow. Acute treatment with a bolus intake of the herbal mixture may inhibit postprandial hyperglycemia in normal rats. This result seems to be consistent with the effect of metformin depending on the reduction in glucose absorption in the intestine. However, effective alleviation of metabolic disorders was only produced in the animal model receiving the repeated daily intake of herbal mixture for two weeks but was not observed in the animal model receiving acute treatment. The herbal mixture dose-dependently ameliorated the insulin sensitivity, which was evaluated in rats with MetS using HOMA-IR and insulin tolerance tests (ITTs). Lower mRNA levels of insulin receptor and its coupled signaling molecule IRS-1 in rats with MetS were also reversed by the herbal mixture. Additionally, the oral glucose tolerance test (OGTT) performed in rats with MetS supported the effectiveness of the herbal mixture in terms of both the changes in the shape of the glucose curve and the calculated area under the curve (AUC). Notably, the calculated incremental AUC (iAUC) was markedly modified in samples receiving repeated treatment, probably due to a marked reduction in the fasting blood glucose. Therefore, it does not seem suitable to apply the iAUC in case of chronic treatment. This is consistent with the criticism for overuse of the iAUC. Collectively, the present study suggested that an herbal mixture containing ginseng, Du-Zhong (*Eucommia* leaf), Shu Di Huang (Chinese foxglove root) and other ingredient is effective in ameliorating insulin sensitivity in the rats with MetS.

Key words: Metabolic syndrome, herbal mixture, insulin sensitivity, Ginseng, Du-Zhong (*Eucommia* leaf), Shu Di Huang (Chinese Foxglove Root), rats.

Abbreviations: MetS, metabolic syndrome; **TCM,** traditional Chinese medicine; **SD,** Sprague-Dawley; **AUC**, area under the curve; **OGTT,** oral glucose tolerance test; **T2DM,** type 2 diabetes; **FBG**, fasting blood glucose; **ITT,** Insulin tolerance test; **IR,** insulin receptor; **qRT-PCR**, quantitative reverse-transcription polymerase chain reaction; **TC,** cholesterol; **TG,** triglyceride; **GLUT4,** glucose transporter 4

INTRODUCTION

After the 20th century, the prevalence of metabolic syndrome (MetS) has widely increased in most countries. The main factors are a high socioeconomic status, decreased physical activity in daily life, genetics, smoking and high body mass index (Cameron et al., 2004). MetS has many definitions while glucose intolerance, hypertension, altered lipid profile and atherogenesis (Alberti and Zimmet 1998) were suggested by the World Health Organization (WHO) in 1999. However, the definition was modified by the International Diabetes Federation (IDF) in 2005

Table 1: Components of herbal mixture.

Component	(g/100 mL; W/W %)
Ginseng Radix	1.40 g (%)
Eucommiae Folium	1.70 g (%)
Rehmanniae Radix	0.70 g (%)
Jujubae Fructus	1.40 g (%)
Zingiberis Rhizoma	0.35 g (%)
D- Xylitol	5.00 g (%)
Citric Acid	0.02 g (%)

This product named "Ginseng-Du Zhong drink" in local market of Taiwan.

because MetS is a cluster of diseases, with biochemical, physiological and clinical factors that directly escalate the risk of diabetes (type 2) and cardiovascular diseases (Grundy et al., 2005;Wilson et al., 2005). MetS has been termed syndrome X, mainly due to its relationship with insulin tolerance (Reaven, 1988). An increase in the waist circumference of individuals by 10 cm may increase the MetS prevalence by approximately 80-90% within a short time (Palaniappan et al., 2004). Therefore, obesity research has widely focused on the strong relationship between arm fat and insulin resistance (IR). However, most patients with MetS also have hypertension (Gill et al., 2005). Overall, MetS is considered a complicated and progressive syndrome mainly due to IR. Management of MetS is important for l owering the risk of derived diseases (Wong, 2005). Weight control is most widely used in clinics because a 10% weight reduction in individuals may decrease the rate of MetS by approximately 20% (Duncan et al., 2003). A drop in the weight using Mediterranean diet is also positively linked with a reduction in lipids (Landaeta-Diaz et al., 2013) in addition to hypertension (Van Gaal et al., 1997) and IR (Wing et al., 1987). Anti-obesity drugs are also widely used in the clinic, although these drugs display side effects, including a decrease in tolerance by the body (Haddock et al., 2002). Therefore, phytochemicals and/or natural products are recommended (Ahsan, 2019) for use as an alternative method. Ginseng has been introduced as a valuable medicinal product for diabetic disorders not only in ancient Asia but also worldwide (Chen et al., 2019). Ginseng saponins, known as ginsenosides, are mentioned as being responsible for the antidiabetic effect of this medicinal plant (Gui et al., 2016).

Two hundred ginsenosides have been identified from ginseng plants and/or heat-processed ginseng products (Chen et al., 2020). Most studies have focused on the ginsenosides Rb1, Re, and Rg1, which were mentioned as the main components in ginseng. However, whether these ginsenosides are active saponins is still unclear (Chen et al., 2019). The gutta-percha tree, Eucommia bark, or "Du-Zhong" in Mandarin and 'To-chu' in Japanese, is another medicinal plant of *Eucommia ulmoides* Oliver (Eucommiaceae) used in the handling of diabetic disorders (Lee et al., 2005). The main hypoglycemic compounds that have been introduced are astragaloside, isoquercitrin, rutin, quercetin and other flavonoids (He et al., 2014). The main active compounds in the leaves and roots of this medicinal plant are indicated to be almost the same as those in the bark (Xing et al., 2019). Iridoids were also indicated as the active components of Du-Zhong, while some of them have been demonstrated to be ligand of GLP-1 receptors (Liu et al., 2007) in addition to being effective as natural glycation inhibitors (West et al., 2016). Both were beneficial in alleviating diabetic complications. Shu Di Huang (Chinese foxglove root) is the medicinal plant Di Huang (Rehmannia) steamed with water or alcohol, and this product is widely used to treat diabetic disorders in traditional Chinese medicine (TCM) (Poon et al., 2011). Generally, TCM is applied as an herbal mixture in clinics. Liu Wei Di Huang Wan is a six-ingredient pill containing five kinds of medicinal plants in addition to Di Huang, whose active principle is catalpol (Huang et al., 2010). Therefore, a mixture of the above three medicinal plants, including ginseng, Du-Zhong and Di Huang, may ameliorate metabolic disorders. However, variations in effectiveness between treatments with bolus intake (acute) and daily repeated (subacute) administration remain unclear. In the present study, one product containing these three medicinal plants in a mixture (Table 1) was investigated in rats with MetS induced by fructose-rich chow (Su et al., 2004). Additionally, we compared the variations in the alleviation of metabolic disorders by this mixture in rats between acute and subacute treatments that have not been performed before.

MATERIALS AND METHODS

Materials and chemicals

A herbal mixture of the product was sourced from the supplier (Bin-Han Pharmaceutics, Tainan, Taiwan). All other chemicals that were of analytical grade quality were

purchased from standard commercial suppliers.

Animals

Male Sprague-Dawley (SD) rats weighing 250 to 270 g were purchased from the National Laboratory Animal Center (Taipei, Taiwan). The animal experiments were approved (105122616) by the Institutional Animal Ethics Committee of Chi-Mei Medical Center. The animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and the guidelines of the Animal Welfare Act.

Postprandial glucose assay in normal rats

To evaluate the effect on glucose absorption, postprandial blood glucose was measured after a glucose load in normal rats with or without pretreatment with the test substance. The basal dose of the herbal mixture was calculated from the commercial dose of the product for human subjects. Additionally, twice the dose and 5-fold the dose were also applied as higher doses and run in parallel. The test substance at the indicated dose was administered to rats after 16 h of fasting by gavage. Forty-five minutes later, loading of food (containing 50% glucose) at 3 g/kg was performed. Blood glucose levels were measured at 30, 60, 120 and 180 min after food loading. Additionally, metformin was used as the positive reference. The area under the curve (AUC) for each group regarding the increase in glucose over baseline was calculated. Then, variations between the treated group and vehicle control group were compared.

Induction of the animal model in rats fed fructose- rich chow

Rats fed fructose-rich chow were used as the animal model. Fructose-rich chow (Teklad, Madison, WI) containing 60% fructose was available to the rats ad libitum. Approximately 4 weeks later, the formation of insulin resistance in fructose-rich chow-fed rats was characterized by the loss of the plasma glucose-lowering effect induced by intraperitoneal injection (IP) of tolbutamide (10 mg/kg), as described previously (Su et al., 2004). All rats became insulin resistant after 8 weeks of fructose-rich chow feeding. Another group of rats receiving standard chow (Purina Mills, Inc.) for the same period were used as the control.

Experimental design

Rats with MetS were divided into five groups: three groups received the herbal mixture orally at three individual doses, and one group received metformin (200 mg/kg, orally) as the positive control to compare with another group that received the same volume of vehicle as the negative control. One group of age-matched rats was used as the normal control in parallel. The basal dose of the herbal mixture (0.39 g/kg) was calculated from the commercial dose of the product for human subjects. Then, twice the dose (0.78 g/kg) and 5-fold the dose (1.95 g/kg) were also applied as the high dose and maximal dose and run in parallel. Acute treatment was obtained from the rats with MetS after oral intake of the test substance for 1 h. Blood samples of the rats under anesthesia with 2% isoflurane were then taken from the tail vein to determine the plasma biomarker levels. Subacute treatment of the herbal mixture was performed in rats with MetS through oral intake once daily at the same dose for two weeks. During the subacute treatment, the rats were fed fructose-rich chow except for the normal control group, which was fed standard chow as described above. Food and distilled water were provided ad libitum and all animals were observed daily for any disorders. Food consumption and body weight were also measured. After the end of the subacute treatment, all rats were anesthetized with 2% isoflurane after 16 h of fasting. Blood samples were taken from the tail vein to determine the plasma biomarker levels. Three days later, the animals were subjected to the oral glucose tolerance test (OGTT).

Plasma biomarker analyses

The blood collected in a heparin-coated tube was further centrifuged to obtain plasma samples. The concentration of plasma glucose was measured by the glucose oxidase method via an autoanalyzer (Quik-Lab, Ames, Miles Inc., Indiana, USA). The plasma insulin levels were determined using a commercial ELISA kit (Mercodia AB, Uppsala, Sweden). Plasma lipids, including cholesterol, triglyceride, and HDL levels, were measured using Cayman Chemical assay kits (Ann Arbor, MI, USA).

Oral glucose tolerance test (OGTT)

Rats were fasted for 18 h prior to the OGTT. In brief, D-glucose (2 g/kg body weight) was administered by oral gavage (t = 0 min). Blood samples (0.3 mL of whole blood) were collected from the tail vein at t = −30, 0, 30, 60, 90, 120, and 180 min. The AUC was calculated by the trapezoidal rule for changes in fasting blood glucose (FBG) during the OGTT. The incremental AUC (iAUC) was further calculated after correction for baseline values, as described previously (Carstensen et al., 2003).

Assay of insulin sensitivity

Homeostatic model assessment-insulin resistance (HOMA-

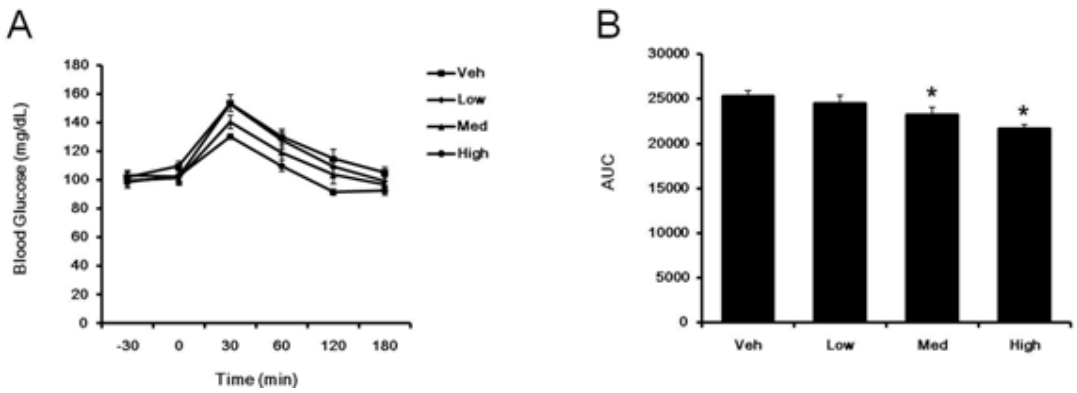

Figure 1: Acute effect of the herbal mixture on postprandial hyperglycemia in normal rats. Rats received oral intake of the herbal mixture at three doses; low dose (0.39 g/kg; Low), medium dose (0.78 g/kg; Med) and high dose (1.95 g/kg; High) to compare with that received same volume of vehicle (Veh). (A) Changes in fasting plasma glucose. (B) The calculated area under the curve (AUC) value for each group.

IR) scores were calculated using fasting serum insulin and FBG concentrations measured at the end of the experimental period according to the following formula:

HOMA-IR = Insulin (mU/L) × Fasting plasma glucose (mg/dL)/2430

The insulin tolerance test (ITT) is another way to evaluate changes in insulin sensitivity. At the end of treatment, ITTs were performed in each group. In brief, intravenous injection (IV) of 0.5 U/kg insulin (Novo Nordisk Pharma, Malov, Denmark) was administered to rats after a 12-h fast under anesthesia and the blood glucose level was measured 0, 15, 30, 45 and 60 min later. Additionally, the AUC was calculated for changes in FBG during the ITT.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from soleus muscle was isolated using TRIzol. The primers used in qRT-PCR were obtained from Roche (Roche Diagnostics GmbH, Germany). The concentration of each PCR product was calculated relative to a corresponding standard curve. Relative gene expression data were analyzed using real-time quantitative PCR and the 2-ΔΔCq method. This gene expression was shown as the ratio of the target gene level to that of β-actin, the housekeeping control, as described in our previous report (Niu et al., 2016). Each sample was run in duplicate. The primers for each signal are listed as follow:

Insulin Receptor F: 5'- GCCATCCCGAAAGCGAAGATC-3';
Insulin Receptor R: 5'- TCTGGGTCCTGATTGCAT-3';
IRS-1 F: 5'- GCCAATCTTCATCCAGTTGCT-3';
IRS-1 R: 5'- CATCGTGAAGAAGGCATAGGG-3';
Rat Akt F: 5'- GGAAGCCTTCAGTTTGGATCCCAA-3';
Rat Akt R: 5'- AGTGGAAATCCAGTTCCGAGCTTG-3';
GLUT4 F: 5'-AGTGGAAATCCAGTTCCGAGCTTG-3';
GLUT4 R: 5'-GGGCTGTGAGTGAGTGCTTTC-3';
β-actin F: 5′-CTAAGGCCAACCGTGAAAAG-3′;
β-actin R: 5′-GCCTGGATGGCTACGTACA-3′.

Statistical analysis

The results are indicated as the mean ± SEM of the indicated sample number (n) in each group. Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by a Tukey's post-hoc comparison. The statistical analysis software used was SPSS 21. A P-value of 0.05 was defined as significant.

RESULTS

Effect of the herbal mixture on postprandial hyperglycemia in normal rats

In normal rats, oral intake of the herbal mixture did not modify the basal glucose level, regardless of fasting status. Notably, the increase in FBG in rats that received postprandial challenge was reduced by acute intake of the herbal mixture at 0.39 g/kg orally. The reduction in postprandial hyperglycemia by the herbal mixture (Figure 1a) was produced in a dose-dependent manner (Figure 1b). Additionally, the effectiveness of the herbal mixture at the maximal dose (1.95 g/kg) was similar to the influence of metformin (0.2 g/kg, orally) in normal rats (data not shown).

Variations in the effects induced by the herbal mixture between acute and repeated treatments in rats with metabolic syndrome

Rats that received fructose-rich chow may have induced

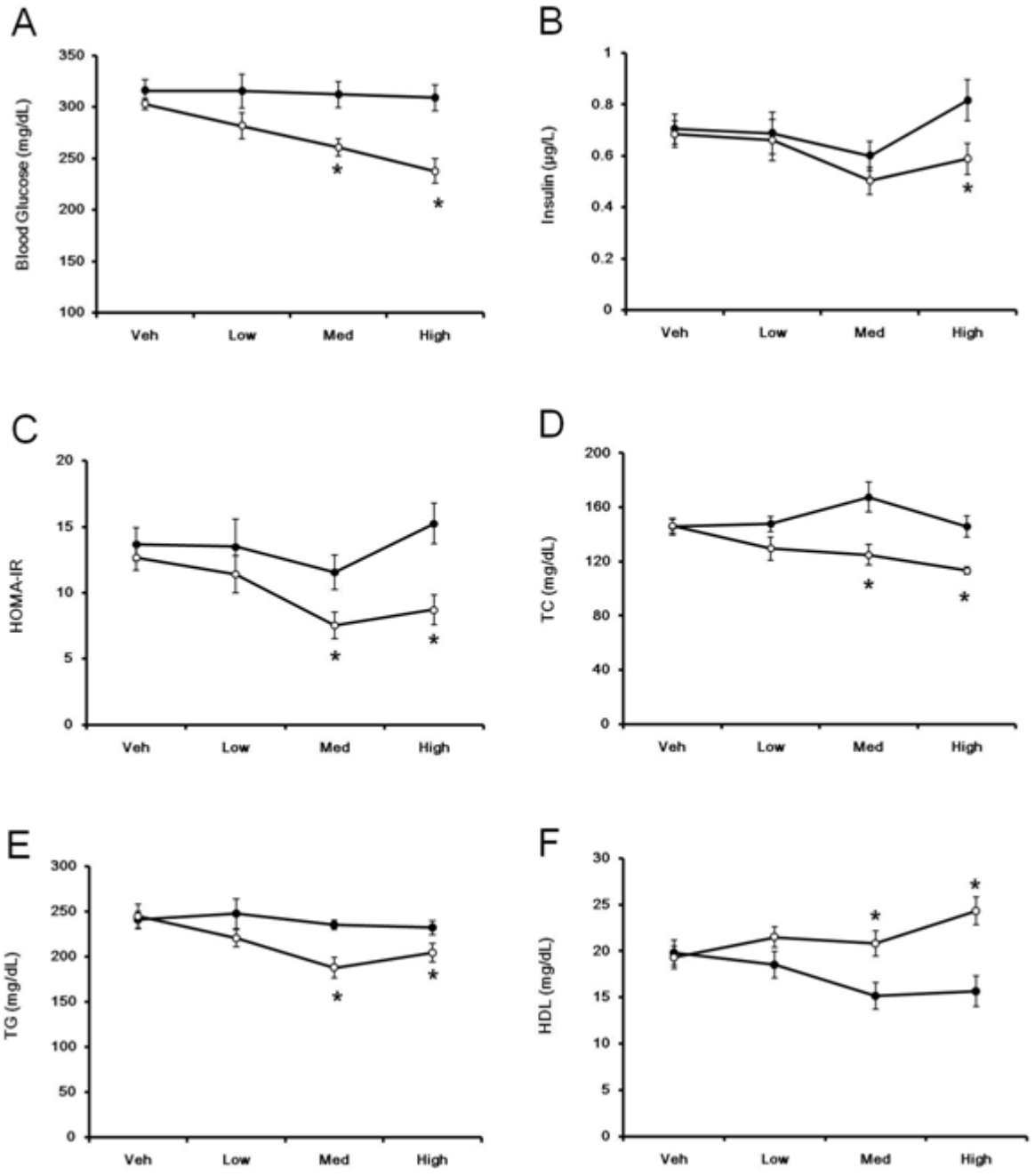

Figure 2: Differences in the changes in fasting plasma biomarkers in rats with metabolic syndrome receiving bolus intake compared with rats that received a repeated intake of the herbal mixture. (A) Changes in plasma glucose. (B) Changes in plasma insulin levels. (C) Changes in the calculated HOMA-IR. (D) Changes in plasma total cholesterol (TC). (E) Changes in plasma triglycerides (TG). (F) Changes in plasma HDL levels.

MetS with a high plasma insulin level but less change in FBG, as described previously (Su et al., 2004). We gave the herbal mixture to these rats in two ways: bolus oral intake (acute) and repeated intake once daily for two weeks (subacute). The difference in responses to the herbal mixture in rats with MetS was then compared between the two methods. As shown in Figure 2, the herbal mixture inhibited FBG in rats with MetS in a dose-dependent manner after subacute treatment. Similar effects were also observed in the changes in the lipid profile, including total cholesterol (TC), triglyceride (TG), and HDL. However, these plasma biomarkers were not modified by the herbal mixture during acute treatment. Therefore, repeated treatment seems to be required for the herbal mixture to

Table 2: Effects of herbal mixture on the feeding behaviors in diabetic rats.

	Vehicle	Low	Med	High
Body weight (g)				
Before	511.17 ± 30.54	511.00 ± 17.03	500.67 ± 30.90	491.33 ± 15.93
After	511.00 ± 23.21	492.50 ± 26.49	510.83 ± 33.33	501.33 ± 14.78
Food intake (g/day)				
Before	31.33 ± 1.20	33.17 ± 1.11	32.00 ± 0.82	33.17 ± 0.91
After	30.67 ± 1.09	31.00 ± 1.06	32.00 ± 1.39	32.50 ± 1.06
Water intake (mL/day)				
Before	42.83 ± 1.30	42.00 ± 1.71	43.50 ± 1.48	43.00 ± 2.83
After	43.33 ± 1.98	43.17 ± 1.19	45.33 ± 1.54	44.00 ± 1.55

Results shows the changes from basal (Before) to treatment (After) with the herbal mixture at three doses; low dose (0.39 g/kg; Low), medium dose (0.78 g/kg; Med) and high dose (1.95 g/kg; High). Then, they were compared to the vehicle-treated control (Vehicle). Each value (mean ± SEM) from the group (n =6) of rats with metabolic syndrome. No statistical difference obtained as compared with the values shown in vehicle-treated group.

modify metabolic homeostasis in animals. However, food intake and water consumption in addition to body weight were the same in rats with MetS received the repeated treatment of the herbal mixture (Table 2).

Effect of the herbal mixture on the oral glucose tolerance test in rats with metabolic syndrome

The OGTT has been widely applied for detecting prediabetes and type 2 diabetes (T2DM) in the clinic. In basic research, the glucose AUC, which is an index of glucose excursions, was suggested instead of the shape of the glucose curve in the intended meaning of the OGTT. Bolus intake of the herbal mixture failed to influence the blood biomarkers as described above and repeated treatments of the herbal mixture were investigated in rats with MetS during the OGTT in the present study. Additionally, repeated treatment with the herbal mixture may reduce FBG to influence the AUC calculation. Therefore, we also calculated the iAUC value in each group. The OGTT results are indicated in Figure 3. The effect on the OGTT of the herbal mixture after repeated treatment for two weeks on the OGTT (Figure 3a) was a marked reduction in the shape of the glucose curve, representing an improvement in glucose excursion. Similarly, a dose-dependent inhibition of plasma insulin induced by the herbal mixture was observed in these rats (Figure 3b). The calculated AUC values regarding the change in plasma glucose suggest a dose-dependent effect of the herbal mixture in rats with MetS (Figure 3c). However, it was quite different when comparing the calculated iAUC values (Figure 3d). Therefore, the iAUC seems to be unsuitable for application in samples that receive repeated treatment.

Effect of the herbal mixture on insulin sensitivity in rats with metabolic syndrome

HOMA-IR is a simple and particularly helpful tool in the assessment of insulin sensitivity (Wallace et al., 2004). Additionally, the insulin tolerance test (ITT) determines the sensitivity of insulin receptors in tissues by measuring the rate of decrease in blood glucose levels before and after intra-venous insulin administration (Gelding et al., 1994). ITT is considered a valid and well-established reproducible method for the assessment of insulin sensitivity in animals (Antunes et al., 2016). This decrease yields a curve over time, creating an area under the curve (AUC), which is used as the indicator of insulin sensitivity. The greater the AUC the lower the sensitivity to insulin would be. Therefore, both methods were applied in the present study to evaluate insulin sensitivity in rats with MetS. As shown in Figure 2, the plasma levels of glucose and insulin were both increased in rats with MetS. Therefore, the calculated HOMA-IR was also significantly ($P < 0.05$) increased. Notably, HOMA-IR was reduced by the herbal mixture in a dose-dependent manner after repeated treatment for 14 days (Figure 2C). This indicates the merits of the herbal mixture in the improvement in insulin sensitivity. Similarly, the maximal effect of the herbal mixture was close to but not higher than that induced by metformin regarding the reduction in HOMA-IR. In the ITT, as shown in Figure 4a, the lower insulin sensitivity in rats with MetS was markedly increased by the herbal mixture after repeated treatment for two weeks. This effect of the herbal mixture was

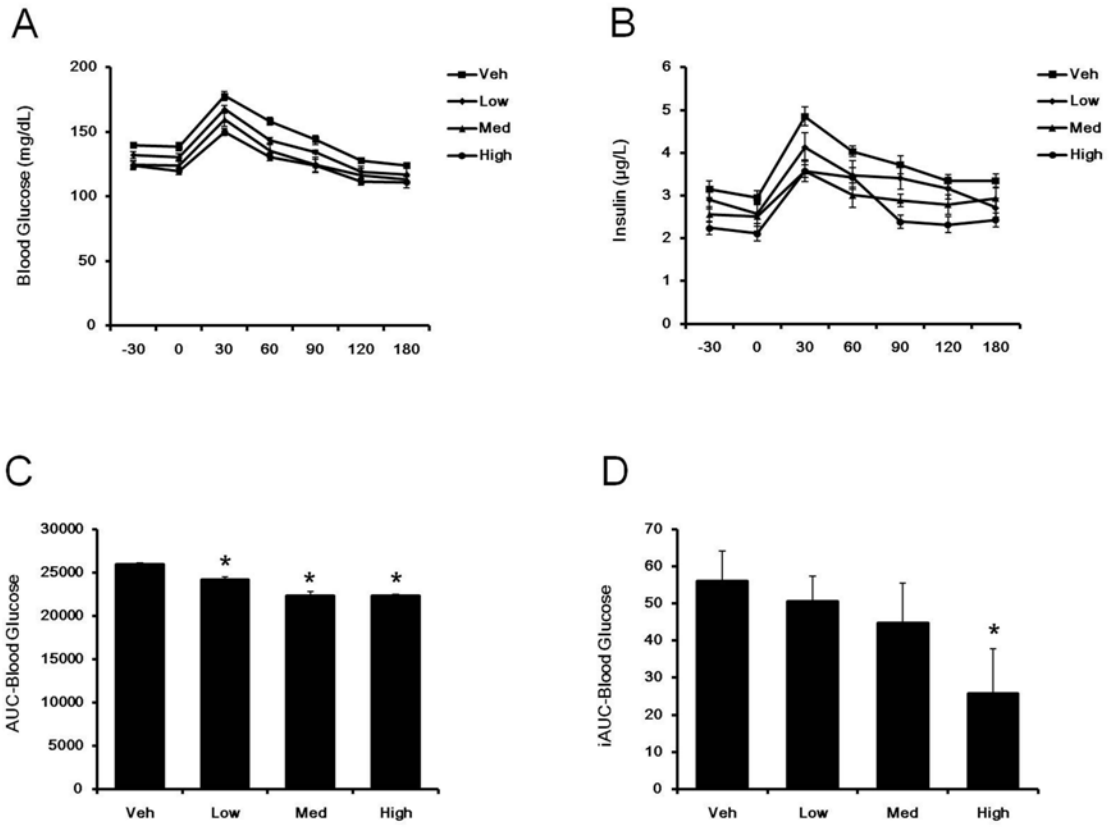

Figure 3: Changes in plasma biomarkers from rats with metabolic syndrome that received an oral glucose tolerance test (OGTT). (A) Changes in fasting plasma glucose after repeated intake of the herbal mixture for two weeks. (B) Changes in fasting insulin levels in these rats. (C) Calculated AUC from the changes in fasting plasma glucose. (D) Calculated iAUC from the AUC value.

produced in a dose-dependent manner (Figure 4b). However, neither HOMA-IR nor ITT was modified by the bolus intake of the herbal mixture (data not shown). Therefore, we investigated the potential signals related to an increase in insulin sensitivity induced by the herbal mixture using the soleus muscle. The mRNA levels of insulin receptor (IR) and its signal IRS-1 were both reduced in the soleus muscle of rats with MetS compared to normal rats, as shown in Figure 4c and Figure 4d. Additionally, the expression of the downstream signaling molecules protein kinase B (Akt) and glucose transporter 4 (GLUT4) were also changed in the same manner, as shown in Figure 4e and Figure 4f. Collectively, repeated treatment with the herbal mixture for two weeks may reverse the reduction in these signals in a dose-dependent manner. Therefore, an increase in insulin sensitivity by the herbal mixture seems to be produced through IRS-1 to enhance GLUT4 in rats with MetS.

DISCUSSION

In the present study, we found that a commercial herbal mixture (Table 1) containing ginseng, Du-Zhong and Shu Di Huang (Chinese foxglove root) may alleviate insulin resistance in rats with metabolic syndrome (MetS). Additionally, this effect was produced after repeated treatment for two weeks but not by bolus intake of the herbal mixture. It has been established that insulin resistance (IR), hyperinsulinemia and hypertriglyceridemia may develop in rats fed a high- fructose diet (Su et al., 2004; Mather 2009). Dietary fructose increases lipid accumulation mainly in the liver and it may affect the metabolism of skeletal muscle (Softic et al., 2016). Additionally, the metabolism of fructose by fructokinase results in the production of uric acid, which may feed back to regulate fructokinase and enhance the sensitivity of cells to the triglyceride-raising effects of fructose (Lanaspa et al., 2012). Higher fasting triglyceride is known to associate with IR (Hoffman 2006). Therefore, MetS induced by fructose-rich chow has widely been used as a representative model in animal research. In the present study, we also reproduced the same model in rats showing IR, hyperinsulinemia and other symptoms of MetS. Insulin sensitivity is important in the pathophysiology of MetS. Therefore, we used HOMA-IR and ITT to evaluate the

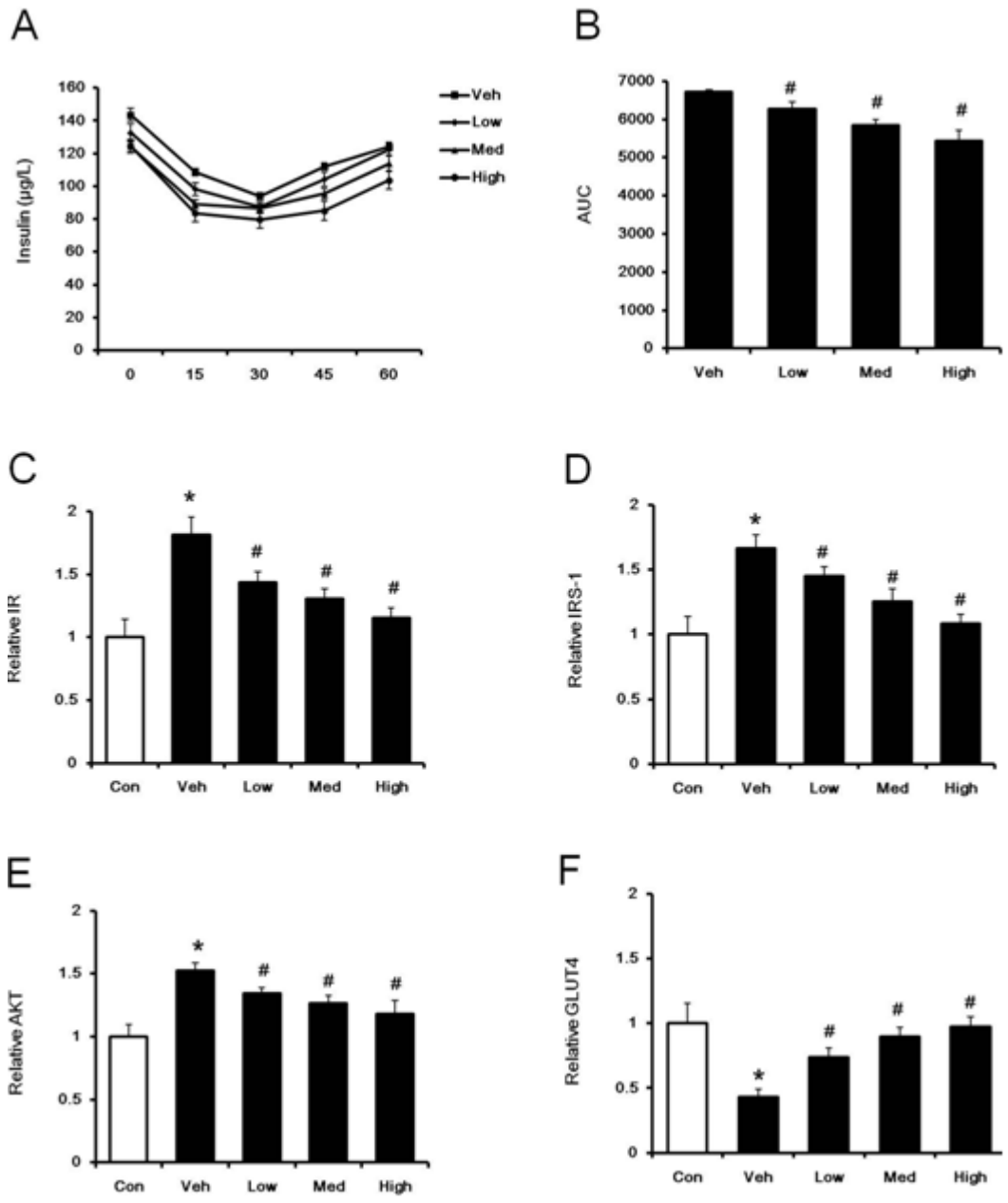

Figure 4: Changes in insulin sensitivity using the rats with metabolic syndrome that receiving repeated intake of the herbal mixture for two weeks. (A) Changes in fasting plasma glucose by the insulin tolerance test (ITT). (B) Calculated AUC in each group for ITT. (C) Changes in the mRNA level of insulin receptor (IR). (D) Changes in the mRNA level of IRS-1. (E) Changes in the mRNA level of protein kinase B (Akt). (F) Changes in the mRNA level of glucose transporter-4 (GLUT4). The mRNA level was determined by RT-qPCR in soleus muscle isolated from rats with metabolic syndrome.

changes in insulin sensitivity as described previously (Su et al., 2004; Antunes et al., 2016). Both indicators were changed in rats with MetS. Notably, the repeated intake of the herbal mixture may alleviate the metabolic disorders in a rat model induced by fructose-rich chow. Improvement of IR was identified in rats with MetS using the changes in HOMA-IR and ITT by the herbal mixture in the current study. Additionally, the lower mRNA levels of insulin receptor (IR) and IRS-1 were dose-dependently reversed by the herbal mixture in rats with MetS after a daily intake for two weeks. The downstream signals protein kinase B (Akt) and GLUT4 were also reversed in the same manner. In addition, fasting blood glucose and lipid profiles in rats with MetS were attenuated by the herbal mixture in a dose-dependent manner.

The herbal mixture inhibited the plasma TG level and

increased the fasting HDL level to result, resulting in a decreased ratio of TG/HDL, which is an indicator of IR in clinical practice except the African adults (Sumner et al., 2005). Therefore, the herbal mixture ameliorates metabolic alterations in rats with MetS due to alleviation of IR. The active principles of each medicinal plant contained in the herbal mixture are multiple and complicated. Many principles possess the ability to alleviate metabolic disorders, including hyperglycemia, hyperlipidemia and IR. For example, ginsenosides from ginseng were noted as being able to inhibit diabetic complications in a review article (Chen et al., 2019). Iridoids contained in Du-Zhong have been demonstrated to be ligands of the GLP-1 receptor (Liu et al., 2007) in addition to inhibition of glycation (West et al., 2016). Additionally, Shu Di Huang (Chinese foxglove root) is the main herb in one popular mixture that has been used to treat diabetic disorders a long time in TCM (Poon et al., 2011). The antidiabetic principle catalpol is known to be contained in both Du-Zhong and Di Huang (Huang et al., 2010) and catalpol has also been introduced as the ligand of GLP-1 receptor (Jia et al., 2015). Moreover, Di Huang also contained ursolic acid (Lo et al., 2017) and oleanoleic acid (Bala et al., 2014) that are known as the agonist of TGR5 which can stimulate GLP-1 secretion. Depending on these effective principles, this herbal mixture seems capable of ameliorating metabolic disorders. Additionally, the treated dose in animals was calculated from the dose suggested for human application. The effects of this herbal mixture were produced mainly by repeated intake for two weeks. Therefore, the merits of this herbal mixture in the alleviation and/or prevention of MetS can be identified in animal models. Otherwise, bolus intake of the herbal mixture may reduce postprandial hyperglycemia in normal rats; this acute effect was also observed in a dose-dependent manner. This response seems to be associated with the reduction in glucose absorption because similar results were observed in rats that received metformin, which was used as a positive reference.

This is consistent with a previous review showing that metformin may reduce glucose absorption in intestine (Horakova et al., 2019).The oral glucose tolerance test (OGTT) is an old tool that has been widely used to diagnose the impaired glucose tolerance (IGT) and/or type-2 DM (T2DM) in the clinic (Manco et al., 2017). The risk of transient postprandial hypoglycemia in patients with nonalcoholic fatty liver disease (NAFLD) has also been identified using OGTT (Morio et al., 2017). The shape of the glucose curve during the OGTT has been the focus (Abdul-Ghani et al., 2010) because the peak and decay in plasma glucose levels during the OGTT reflect the interplay between several factors, including the glucose intestinal absorption rate, insulin sensitivity, β-cell glucose sensitivity, the β-cell insulin sensitivity rate and enhancement factors, in addition to the secretion of gut hormones (Kim et al., 2016). Then, the area under the curve (AUC) was developed to quantify the total rise in blood glucose during an OGTT, while the AUC value was generally calculated using the trapezium rule, trapezoidal method, or composite trapezoidal method, as described previously (Allison et al., 1995). However, the baseline of fasting plasma glucose levels in each case during the OGTT was not the same. Therefore, the incremental area under the curve (iAUC) has been developed to solve this problem in diabetic clinics (Cheng et al., 2018). However, the iAUC is different from the value of ΔAUC, which has widely been used in pharmacokinetics (Purves, 1992). In the present study, repeated intake with of a herbal mixture showed a more marked reduction in the shape of the glucose curve during the OGTT, which was supported by the calculated AUC. However, the calculated iAUC values were markedly different, probably due to the changes in basal fasting glucose levels, which were dose-dependently attenuated by the herbal mixture.

This finding is consistent with a previous report (Cheng et al., 2018) that the calculated iAUC value is not suitable to apply in animals that received chronic or repeated treatment. In the clinic, the iAUC obtained from subtracting the baseline value of fasting plasma glucose has been challenged as being problematic (Sumner et al., 2005). Additionally, the same criticism was also reported in rats with T2DM using antidiabetic drugs (Liu et al., 2020). Our finding is fully consistent with these reports. However, unsuitable application of the calculated iAUC value in herbal products has not been mentioned before. The current study did not analyze the active principles in each medicinal plant contained in the herbal mixture. Therefore, which principle(s) are responsible for the acute effects and which induced the medicinal effects of repeated intake remain unknown. This is one of the limitations in of this report. However, we demonstrated that the herbal mixture is effective ameliorating metabolic disorders after a repeated intake in animals. Additionally, the present study provided a novel scientific evidence to support that the iAUC is not suitable for application in cases of repeated treatment with medicinal plants.

CONCLUSION

Taken together, a herbal mixture containing medicinal plants, including ginseng, Du-Zhong (Eucommia leaf), Shu Di Huang (Chinese foxglove root) and others, is effective in ameliorating the metabolic disorders in a rat model after repeated daily intake for two weeks. Improvement in insulin sensitivity is identified as the main mechanism for this influence in animals.

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