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Water Extract of Djulis Husk Exerts Protective Effect Against Metabolic Syndrome

Liang-Yi Wu[1], Wei-Lun Hung[2], Ling-Yun Cheng[1], Zheng-Yuan Su[1], and Bo-Yang Hsu[3]

[1]Department of Bioscience Technology, Chung Yuan Christian University, Taoyuan City 320314, Taiwan
[2]School of Food Safety, Taipei Medical University, Taipei 11031, Taiwan
[3]Department of Food Science, National Ilan University, Yilan County 260007, Taiwan

ABSTRACT: Djulis (*Chenopodium formosanum* Koidz.) possesses various biological activities, including anti-oxidant, anti-hyperglycemic, anti-aging and hepatoprotective properties. Although djulis husk is typically considered agricultural waste, there is value in exploring ways to utilize it effectively. This study aimed to investigate the protective effects of the water extract of djulis husk (WDH) in rats with high-fructose-induced metabolic syndrome. The results showed that WDH significantly ameliorated the metabolic syndrome induced by a high-fructose diet in rats. Supplementation with low-dose WDH (0.5% of diet, w/w) significantly improved metabolic syndrome, including high blood pressure, hypertriglyceridemia, and insulin resistance. The protective effects of WDH against metabolic syndrome may be associated with increased expression of the genes encoding insulin receptor substrates-1 (IRS-1) and glucose transporter 4 (GLUT-4) in the epididymal fat. Thus, WDH is likely a functional food ingredient for the prevention of metabolic syndrome.

Keywords: *Chenopodium formosanum*, djulis, hyperglycemia, insulin resistance, metabolic syndrome

INTRODUCTION

Metabolic syndrome is characterized by a combination of metabolic abnormalities, such as overweight, hypertension, hyperglycemia, and hyperlipidemia (Grundy, 1999; Alberti et al., 2009). Also, several clinical features have been positively associated with metabolic syndrome, including hepatosteatosis, cardiovascular disease, diabetes, and Alzheimer's disease (Mameli et al., 2017; Więckowska-Gacek et al., 2021). While diabetes, a chronic metabolic disease, is characterized by high blood sugar, type 2 diabetes is associated with insulin resistance and lack of insulin. Interestingly, several insulin signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and mechanistic target of rapamycin (mTOR) pathways, are associated with diabetes. For example, the PI3K/Akt/glucose transporter type 4 (GLUT-4) signaling pathway is a major downstream pathway of insulin receptor substrates (IRS). Thus, the PI3K/Akt/GLUT-4 signaling pathway is critical in diabetes, and aberrations in this pathway likely lead to abnormal glucose and lipid metabolism (Roden and Shulman, 2019). There is a need to identify supplements that are effective and economical for treating metabolic syndrome.

Djulis, which has hypoglycemic activities, may be a source of such a supplement (Hsu et al., 2018; Li et al., 2021). *Chenopodium formosanum* Koidz. (djulis) contains abundant nutrients, such as protein, dietary fiber, and essential amino acids. In addition, various polyphenols, such as rutin and quercetin, are found in djulis (Hsu et al., 2017; Chyau et al., 2018). While the crude water extract of djulis exhibits hypoglycemic activity in 3T3-L1 adipocytes (Hsu et al., 2018), the ethanolic extract of djulis significantly decreased triglyceride levels in the same cells (Chyau et al., 2018). Additionally, consumption of djulis husk powder significantly decreases postprandial blood glucose levels in patients with type 2 diabetes compared with those who did not consume the same supplement (Li et al., 2021).

Since djulis husk is usually considered agricultural waste, finding ways to utilize it effectively is a valuable research topic. In addition, the hypoglycemic activity of djulis has been reported both *in vitro* and *in vivo*. Thus, this study aimed to investigate the protective effect of the water extract of djulis husk (WDH) against metabolic syndrome using a rat model of high-fructose-induced metabolic syndrome.

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Correspondence to Bo-Yang Hsu, E-mail: byhsu@niu.edu.tw

MATERIALS AND METHODS

Materials

The husk of djulis (*C. formosanum* Koidz.) was obtained from Dr. Pi-Jen Tsai, Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan. Acrylamide/bis-acrylamide solution (30%), D-(+)glucose, ethylenediaminetetraacetic acid, N, N, N', N'-tetramethyl-ethylenediamine, potassium phosphate monobasic, sodium phosphate and sodium dodecyl sulfate (SDS), rutin, and quercetin were purchased from Sigma Chemical Co. Potassium chloride and sodium chloride were purchased from Merck Co. Ammonium persulfate and glycine were purchased from Amresco Co. The cholesterol CHOD-PAP kit, high-density lipoprotein precipitant, low-density lipoprotein precipitant, and triglyceride GPO-RAP kit were purchased from Dia Sys Co. The rat insulin kit was purchased from Mercodia Co. The Bio-Rad protein assay kit was purchased from BIO-RAD Co. Mouse antiactin was purchased from Merck Millipore Co. The rabbit anti-insulin receptor β was purchased from BD Co. Rabbit anti-Akt1/2, rabbit anti-phospho-Akt1/2, rabbit anti-phospho-insulin receptor β, and rabbit anti-phospho-insulin receptor substrate-1 were purchased from Santa Cruz Biotechnology Co. Rabbit anti-insulin receptor substrate-1 and rabbit anti-glucose transporter 4 were purchased from Abcam Co. Goat antirabbit immunoglobulin (Ig) G and goat antimouse IgG were purchased from Jackson Immuno Research Laboratories Co. Apigenin and luteolin were purchased from ChemFaces Biochemical. Gallic acid, myricetin, and myricitrin were purchased from Tokyo Chemical Industry.

Extraction of djulis husk

The husks of djulis were dried with a freeze dryer. Next, the dried husks were mixed with deionized water at a ratio of 1:50 (g/mL) and extracted for 30 min. Then, the extraction solution was filtered, and the filtrate was freeze-dried and stored at 20°C.

HPLC analysis of the phenolic compounds

The phenolic compounds in the WDH were analyzed using a method based on Nour et al. (2013). An HPLC-UV/VIS system (JASCO) equipped with an Agilent C18 column (4.6×250 mm, 5 μm particle size) was used for the separation. The mobile phase consisted of (A) 1% acetic acid in water and (B) 100% methanol with a flow rate of 0.7 mL/min. The solvent gradient condition was 90% A and 10% B for 10 min, 65% A and 35% B at the 12th min, 60% A and 40% B at the 45th min, 30% A and 70% B at the 70th min, and returned to 90% A and 10% B at the 75th min. The column temperature was maintained at 30°C. A detection wavelength of 272 nm was employed for detection. Standard calibration curves for seven phenolic compounds (rutin, quercetin, myricitrin, gallic acid, myricetin, luteolin, and apigenin) were plotted individually by peak area against concentration. The linear regression equation of each standard was used to calculate the phenolic compound content.

Animals and the experimental design

Five-week-old male Sprague-Dawley rats were obtained from BioLASCO Taiwan Co., Ltd. The care of rats in this study complied with the Taiwan government's guidelines for the care and use of laboratory animals. The animal protocol was approved by the Institutional Animal Care and Use Committee of Chung-Yuan Christian University (no.10224). The rats were housed in a temperature- and humidity-controlled room at 22°C±2°C and 60% – 70% humidity with a 12-h light-dark cycle.

The rats were acclimated for one week and randomly divided into four groups (n=7 per group). The basal (B) group was fed a commercial rodent chow diet (Purina 5001). The control (C) group was fed a high-fructose (60%) diet. The low-dose WDH (L) group was fed a high-fructose (60%) diet with WDH (0.5% of diet, w/w). The high-dose WDH (H) group was fed a high-fructose (60%) diet with WDH (1% of diet, w/w). The experiment lasted for 13 weeks. Fasting plasma was collected every 2 weeks for biochemical analysis. Blood pressure was measured using the tail-cuff method. The oral glucose tolerance test (OGTT) was conducted in the 12th week. At the end of the experiment, the rats were sacrificed after fasting for 15 hours, and organs (including heart, liver, and kidney) and tissues (including epididymal fat pad, perirenal adipose tissue, mesenteric adipose tissue, abdominal adipose tissue and subcutaneous adipose tissue) were collected for analysis.

Plasma biochemical analysis

A blood sample was centrifuged at 17,700 *g* at 4°C for 12 min. The supernatant was collected and centrifuged at 12,290 *g* at 4°C for 10 min to obtain the plasma. The concentration of plasma glucose was measured using a blood glucose analyzer (Model 2300 STAT PLUS, YSI Co.). The concentration of plasma insulin was analyzed using an ELISA immunoassay kit (Mercodia). First, 25 μL of plasma sample and insulin standard solutions were placed into different wells in a 96-well plate. Next, 50 μL of peroxidase-conjugated mouse monoclonal anti-insulin antibody was added to each well and incubated for 2 h. After incubation, the wells were washed repeatedly with the washing solution. Then, 200 μL of 3,3',5,5'-tetramethylbenzidine was added to the wells to react in the dark for 15 min. Subsequently, 50 μL of 1 M sulfuric acid solution was added to terminate the reaction, and the samples were measured at 450 nm. The standard calibration

curve of insulin was plotted by absorbance against concentration, and linear regression was used to calculate the insulin content.

The plasma triacylglycerol concentration was measured using a glycerol phosphate oxidase-*p*-aminophenazone (GPO-PAP) kit. First, 5 μL of plasma sample and triacylglycerol standard solutions were added to different wells in a 96-well plate and incubated with 200 μL of reaction reagent at room temperature for 20 min. Then, the samples were measured at 500 nm. The standard calibration curve of triacylglycerol was plotted by absorbance against concentration, and linear regression was used to calculate the triacylglycerol content.

The plasma cholesterol concentration was measured using a cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) kit. First, 5 μL of plasma sample and cholesterol standard solutions were added to different wells in a 96-well plate and incubated with 200 μL of reaction reagent at room temperature for 20 min. Then, the samples were measured at 500 nm. The standard calibration curve of cholesterol was plotted by absorbance against concentration, and linear regression was used to calculate the cholesterol content.

Oral glucose tolerance test

The OGTT was conducted in the 12th week of the experiment. Prior to the test, the rats were fasted for 15 h, and blood samples were collected before administering the glucose solution (0 min sample). Next, the rats were orally administered a 1 g/kg glucose solution, and blood samples were collected at 0.5, 1.0, 1.5, and 2.0 h after administration. Lastly, plasma glucose and insulin concentrations were measured.

Protein levels in the epididymal fat pad

First, 1 g of epididymal fat pad sample was placed into a tube and mixed with lysis buffer. Then, the mixture was homogenized and centrifuged at 2,490 *g* at 4°C for 20 min. Next, the supernatant was obtained and preserved at 80°C for further analysis. Afterward, Western blot analysis was performed using a method modified by Pedersen et al. (1991). Namely, the sample solution was heated to 100°C for 10 min and centrifuged. Then, the supernatant reacted with 10 μL of sample buffer solution. Next, 10 μL of the mixture was subjected to electrophoresis using SDS-PAGE and transferred to a PVDF membrane. Afterward, the membrane was incubated with a blocking solution for 1 h. Subsequently, the membrane was incubated with primary antibodies against the insulin receptor (IR), IRS-1, Akt, and glucose transporter 4 at room temperature for 1 h, followed by washing with PBST for 42 min. Then, the membrane was incubated with a secondary antibody for 1 h and washed again with PBST for 52 min. Finally, protein levels were measured using a luminescent image analyzer (LAS-3000, Fujifilm).

Statistical analysis

The values are presented as means±SD. The results were statistically analyzed using Student's unpaired *t*-test. A significant difference was denoted when $P<0.05$.

RESULTS AND DISCUSSION

Effects of WDH on blood pressure

In the preliminary experiment, rats were fed a 60% high-fructose diet for 8 weeks to confirm their insulin resistance through an OGTT. Then, a short-term pre-experiment was conducted, during which the rats were administered WDH by gavage at 500 mg/kg by weight (bw) (low dose) or 1,000 mg/kg bw (high dose) for 7 days, followed by another OGTT. The results showed no significant differences in blood glucose levels, as measured by the area under the curve (AUC) and ΔAUC, among the control, low-dose WDH, and high-dose WDH groups ($P>0.05$) (data not shown). However, both WDH groups showed a significant reduction in insulin AUC and ΔAUC compared with the control group ($P<0.05$) (data not shown), indicating that WDH effectively enhanced insulin sensitivity. For the subsequent long-term study, a dose of 500 mg/kg bw was selected. During the pre-experiment, the rats' average body weight was approximately 500 g, corresponding to a daily WDH intake of 250 mg (500×0.5). The dosage, representing 1% of their average daily food intake (25 g), was designated as the high dose for the long-term study. Thus, a dose representing 0.5% of daily food intake was chosen as the low dose.

Throughout the experiment, the systolic blood pressure in the control group was significantly higher than that in the basal group, indicating that a high-fructose diet elevated blood pressure ($P<0.05$). These findings are consistent with a previous study reporting that a high-fructose diet induces high blood pressure (Takagawa et al., 2001). Meanwhile, oral administration of WDH significantly reduced blood pressure compared with the control group ($P<0.05$) (Fig. 1).

Phenolic compounds are the primary bioactive compounds of djulis (Hsu et al., 2017; Chyau et al., 2018; Chen et al., 2019a; 2019b). Chen et al. (2019a) reported that 150 mg/g rutin was the most abundant phenolic compound in the water extract of djulis, and the extract also had smaller amounts of quercetin and kaempferol derivatives. Another study used 50% ethanol as a solvent to extract phenolic compounds from djulis. These results indicate that rutin-*O*-pentoside at 257.30±2.05 μg/g was the predominant flavonoid in the ethanolic extract, followed by quercetin-acetyl-rutinosidehexoside glu-

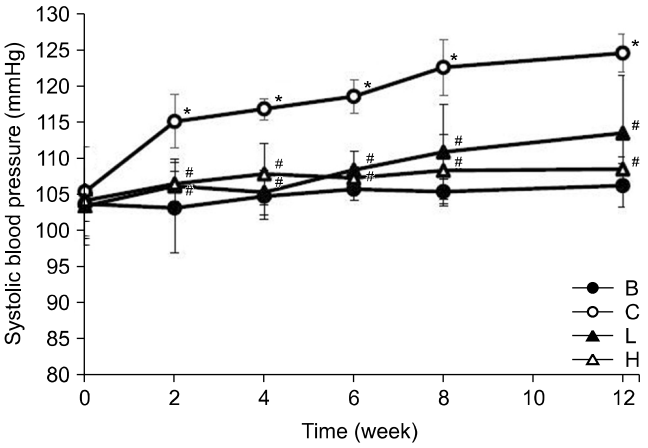

Fig. 1. Effects of the water extract of djulis husk (WDH) on the changes in systolic blood pressure of rats during the experimental period. B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group. Values are presented as mean±SD (n=7), and significant differences were analyzed by Student's *t*-test. $^{*}P<0.05$, compared with the basal group. $^{\#}P<0.05$, compared with the control group.

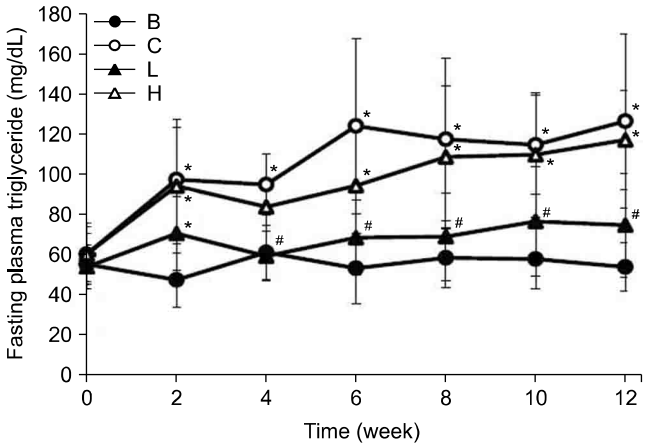

Fig. 2. Effects of the water extract of djulis husk (WDH) on the changes in the fasting plasma triglyceride levels of rats during the experimental period. B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group. Values are presented as mean±SD (n=7), and significant differences were analyzed by Student's *t*-test. $^{*}P<0.05$, compared with the basal group. $^{\#}P<0.05$, compared with the control group.

curonide at 72.78±7.92 μg/g (Hsu et al., 2017). In this study, the concentrations of rutin, myricitrin, and quercetin in the WDH were 4.69±0.78, 0.10±0.001, and 0.01±0.004 mg/g, respectively (Table 1). Meanwhile, the total phenolic content (TPC) and total flavonoid content (TFC) of WDH were 54.08±0.53 mg gallic acid equivalent (GAE)/g and 10.36±0.24 mg quercetin equivalent (QE)/g, respectively. These results suggest that WDH might contain unidentified phenolic compounds.

Although rutin and quercetin content in WDH are relatively low, djulis contains various rutin and quercetin derivatives (Hsu et al., 2017; Chen et al., 2019a). Therefore, the unknown phenolic compounds in WDH still need to be analyzed and identified. The water extract of djulis at 10, 50, and 100 mg/kg bw and its functional components, including rutin at 50 mg/kg bw and betanin at 50 mg/kg bw, possess antihypertensive activities (Chen et al., 2019a). These results suggest that the oral administration of a water extract of djulis, rutin, and betanin significantly decreased systolic and diastolic blood pressures by inhibiting angiotensin-converting enzyme activity and reducing the production of reactive oxygen species and peroxynitrite in spontaneously hypertensive rats. Thus, the antihypertensive effects of WDH may be attributed to rutin and its derivatives, as well as betanin and other unidentified phenolic compounds.

Effects of WDH on the changes in fasting plasma glucose, triacylglycerol, and total cholesterol

There were no significant changes in fasting plasma glucose levels among the four groups throughout the experiment (data not shown). However, the fasting plasma triacylglycerol level in the control group was significantly higher than that in the basal group ($P<0.05$). However, the fasting plasma triacylglycerol level of the low-dose WDH group was notably lower than that of the control group ($P<0.05$) (Fig. 2). These results indicate that the oral administration of low-dose WDH markedly ameliorated high-fructose diet-induced hypertriglyceridemia in rats. Additionally, the fasting plasma cholesterol level of the control group was significantly higher than that of the basal group (Fig. 3), and there was no significant difference in fasting plasma cholesterol levels between the low-dose WDH and basal groups. These results again suggest that WDH improved high-fructose diet-induced hypercholesterolemia.

Previously, Chen et al. (2019b) demonstrated that the ethanol extract of djulis (EECF) and its functional components, including rutin, quercetin and betanin, significantly reduced the plasma triacylglycerol and total cholesterol levels in mice with hyperlipidemia. The mice were administered EECF at 10, 25, or 50 mg/kg bw, ru-

Table 1. Contents of phenolic compounds in the djulis husk

Rutin (mg/g)	Quercetin (mg/g)	Myricitrin (mg/g)	Gallic acid (mg/g)	Myricetin (mg/g)	Luteolin (mg/g)	Apigenin (mg/g)	TPC (mg GAE/g)	TFC (mg QE/g)
4.69±0.78	0.01±0.004	0.10±0.001	N.D.	N.D.	N.D.	N.D.	54.08±0.53	10.36±0.24

Values are presented as mean±SD (n=3).
TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; QE, quercetin equivalent; N.D., not detected.

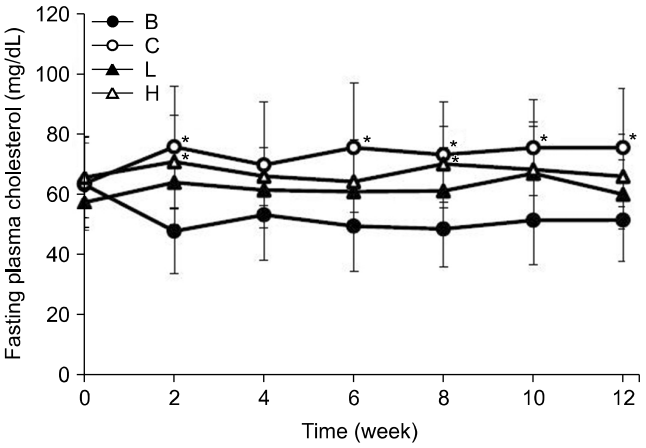

Fig. 3. Effects of the water extract of djulis husk (WDH) on the changes in fasting plasma cholesterol of rats during the experimental period. B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group. Values are presented as mean±SD (n=7), and significant differences were examined using Student's *t*-test. **P*<0.05, compared with the basal group.

tin at 5 mg/kg bw, quercetin at 5 mg/kg bw, and betanin at 5 mg/kg bw for 12 weeks. First, the mice treated with EECF had significantly lower levels of plasma triacylglycerol at 83.75±15.93 mg/dL (low-dose EECF), 82.40±15.27 mg/dL (medium-dose EECF), and 82.75±15.15 mg/dL (high-dose EECF) compared to the control group (139.00±18.77 mg/dL). Additionally, rutin, quercetin, and betanin significantly reduced plasma triacylglycerol levels to 67.67±3.18 mg/dL, 46.75±12.93 mg/dL, and 51.60±5.57 mg/dL, respectively. Also, the mice treated with EECF had significantly lower levels of total cholesterol at 152.80±4.22 mg/dL (low-dose EECF), 135.60±13.53 mg/dL (medium-dose EECF), and 139.20±4.24 mg/dL (high-dose EECF) compared to the control group (209.67±40.93 mg/dL). Similarly, rutin, quercetin and betanin significantly reduced total cholesterol levels to 140.00±25.54 mg/dL, 158.40±8.75 mg/dL, and 145.40±6.87 mg/dL, respectively. In addition, the ethanolic extract of djulis was found to reduce adipogenesis in 3T3-L1 adipocytes (Chyau et al., 2018), and the administration of rutin at 100 mg/kg bw over 6 weeks was observed to significantly improve plasma triacylglycerol, total cholesterol, and glucose intolerance in ovariectomized female Sprague-Dawley rats (Renuka et al., 2013). Similarly, Jung et al. (2013) found that quercetin supplementation (0.025% of the diet, w/w) for 9 weeks significantly reduced serum triacylglycerol and cholesterol levels in mice with high-fat diet-induced obesity.

In this study, the intake of rutin and quercetin by the rats through diet was 0.59 (mg/kg bw) and 0.00125 (mg/kg bw), respectively. Interestingly, low-dose WDH exerted stronger protective effects on hypertriglyceridemia and hypercholesterolemia than high-dose WDH (Fig. 2 and 3). These findings suggest that WDH might produce dose-independent responses in the animals.

Similar patterns were observed with other compounds, such as betanin and quercetin. For example, betanin at 10 and 20 mg/kg bw had more pronounced antidiabetic and antihyperlipidemic effects on streptozotocin-induced diabetic rats than betanin at 40 mg/kg bw (Abedimanesh et al., 2021). Similarly, quercetin at 50 mg/kg bw exhibited antidepressant-like activities in diabetic rats, but at 100 mg/kg bw, it was ineffective in improving depression-like behaviors (Demir et al., 2016). WDH may also contain unknown compounds that antagonize the components responsible for mitigating metabolic syndrome. In the high-dose WDH group, these antagonistic compounds could be present in higher concentrations and inhibit WDH's beneficial effects. Consequently, the high-dose WDH group exhibited less improvement in metabolic syndrome than the low-dose WDH group.

Effects of WDH on glucose tolerance

No significant changes in the plasma glucose levels were observed among the four groups (Fig. 4A). Additionally, the AUC of the plasma glucose showed no significant differences among the groups (data not shown). In contrast, the plasma insulin levels in the low-dose WDH group at 0 and 30 min were significantly lower than those in the control group. These results suggest that WDH improved insulin sensitivity in rats with high-fructose diet-induced metabolic syndrome ($P<0.05$) (Fig. 4B). Previously, a crude extract of djulis husk at 250 mg/kg bw was found to significantly reduce HOMA-IR in mice with high-fat diet-induced hyperglycemia (Tung et al., 2021). Additionally, ethanolic extracts of djulis at 10, 25, or 50 mg/kg bw and its functional components, including rutin at 5 mg/kg bw, quercetin at 5 mg/kg bw, and betanin at 5 mg/kg bw, were found to significantly improve glucose tolerance, insulin response, and HOMA-IR in mice fed with a high-fat diet (Chen et al., 2019b). Also, quercetin at 30 mg/kg bw was observed to significantly reduce the levels of serum glucose, insulin, and HOMA-IR in rats with high fat and high sucrose-induced obesity (Arias et al., 2014). Moreover, administering quercetin at 20 mg/kg bw for 8 weeks was reported to markedly reduce insulin resistance in diabetic rats (El-Baky, 2011).

Effects of WDH on the weights of adipose tissues in rats

At the end of the experiment, the weights of vital organs, including the heart, liver, and kidneys, were not statistically significantly different among the four groups (data not shown). However, the weight of adipose tissues, including the epididymal fat pad, perirenal adipose tissue, mesenteric adipose tissue, abdominal adipose tissue, and subcutaneous adipose tissue, were significantly higher in the control group than those in the basal

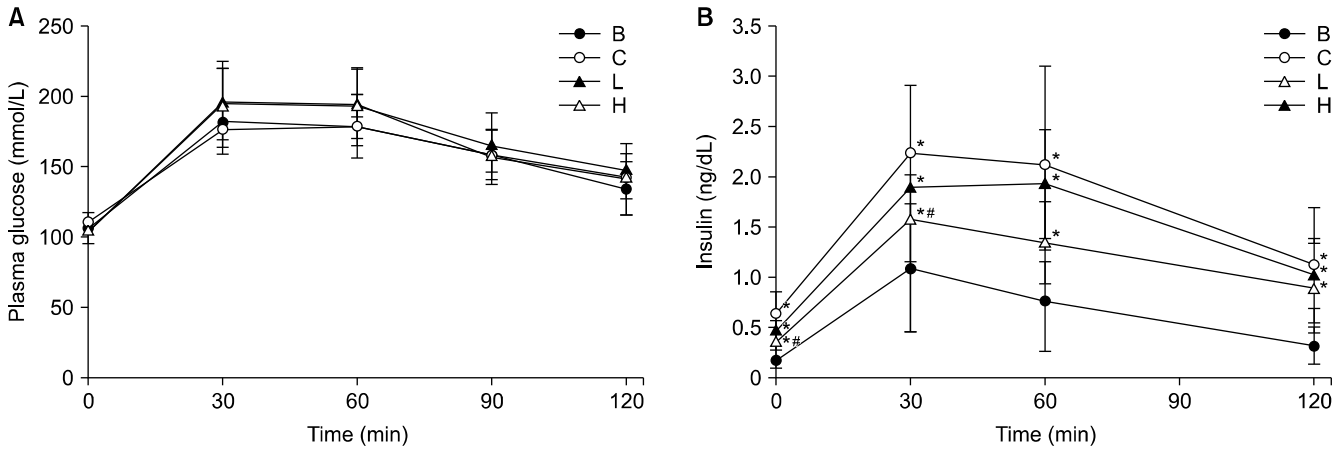

Fig. 4. Effects of the water extract of djulis husk (WDH) on plasma glucose and insulin levels in rats during an oral glucose tolerance test. (A) Plasma glucose. (B) Insulin levels. B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group. Values are presented as mean±SD (n=7), and significant differences were detected using Student's *t*-test. $^{*}P<0.05$, compared with the basal group. $^{\#}P<0.05$, compared with the control group.

Table 2. The weight of adipose tissues in rats at the end of the experiment

Group	B	C	L	H
Body weight(g)	467.2±45.7	476.0±13.9	463.6±46.9	458.9±46.5
Epididymal fat pad weight (g)	5.61±1.73	8.01±2.14*	6.27±1.29	8.14±1.48*
% total BW	1.20±0.34	1.69±0.47*	1.36±0.29	1.77±0.18*
Perirenal fat tissue weight (g)	6.86±2.69	11.42±3.35*	9.18±2.39	10.32±2.98
% total BW	1.46±0.52	2.41±0.74*	1.99±0.49	2.23±0.50
Mesenteric fat tissue weight (g)	4.27±1.28	6.06±1.53*	4.66±0.78	5.47±1.84
% total BW	0.91±0.26	1.28±0.34*	1.01±0.18	1.18±0.30
Abdominal fat tissue weight (g)	14.00±4.63	21.71±5.82*	17.09±3.58	19.98±5.31*
% total BW	2.98±0.89	4.58±1.29*	3.70±0.75	4.32±0.81*
Subcutaneous fat tissue weight (g)	11.22±3.65	18.54±7.99*	13.17±2.63	14.82±4.29
% total BW	2.39±0.67	3.90±1.71*	2.84±0.41	3.19±0.71
Total fat weight (g)	25.22±7.99	40.25±11.63*	30.26±5.51	34.80±9.04
% total BW	5.37±1.49	8.48±2.54*	6.54±1.03	7.51±1.36

Values are presented as mean±SD (n=7).
*$P<0.05$, compared with the basal group.
B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group.

group ($P<0.05$) (Table 2). These results indicate that a high-fructose diet promoted fat accumulation in these adipose tissues. In contrast, no significant differences in adipose tissue weights were observed between the low-dose WDH group and the basal group, suggesting that WDH might alleviate fat accumulation in rats with high-fructose diet-induced metabolic syndrome. Although the weights of the adipose tissues in the low-dose WDH group were lower than those in the control group, there was no significant difference between the groups ($P>0.05$), likely due to individual variability among the animals that resulted in a large SD. It was found that supplementation with rutin at 200 and 400 mg/day in humans significantly decreased abdominal visceral fat (Hashizume and Tandia, 2020). Additionally, quercetin at 0.8 g/kg bw was shown to markedly reduce abdominal fat in rats with metabolic syndrome (Panchal et al., 2012). Furthermore, administering quercetin at 17 mg/kg bw also reduced adipose tissue expansion in obese mice (Forney et al., 2018).

Effects of WDH on the level of proteins of the insulin signal transduction pathway

The level of the proteins in the insulin signal transduction pathway, including IR, IRS-1, GLUT-4, and Akt, was analyzed. The levels of IRS-1 and Akt in the control group were significantly lower than those in the basal group ($P<0.05$) (Fig. 5). Additionally, the level of GLUT-4 in the control group was lower than that in the basal group. These findings suggest that a high-fructose diet reduced the level of the proteins in the insulin signal transduction pathway in rats. In contrast, IRS-1 and GLUT-4 levels in the low-dose WDH group were significantly higher than those in the control group ($P<0.05$), suggesting that low-dose WDH markedly increased the levels of the protein in the insulin signal transduction

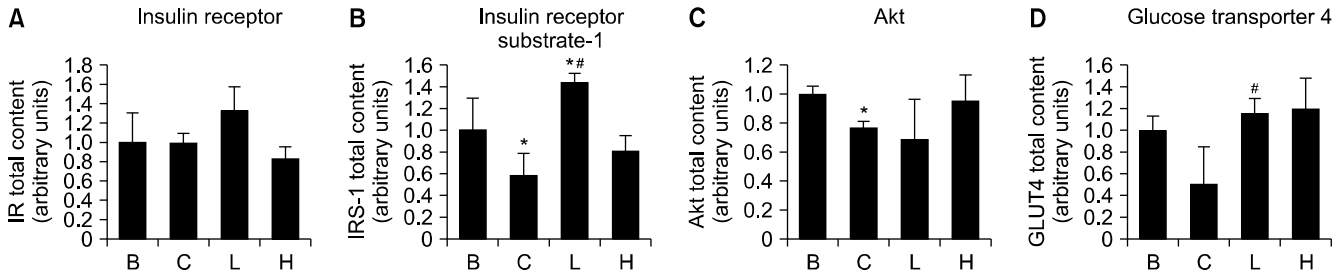

Fig. 5. Effects of the water extract of djulis husk (WDH) on the level of proteins of the insulin signal transduction pathway in the epididymal fat. (A) Insulin receptor. (B) Insulin receptor substrate-1. (C) Akt. (D) Glucose transporter 4. B, basal group; C, control group; L, low-dose WDH treatment group; H, high-dose WDH treatment group. Values are presented as mean±SD (n=3), and significant differences were determined using Student's *t*-test. $^{*}P<0.05$, compared with the basal group. $^{\#}P<0.05$, compared with the control group.

pathway in rats with high-fructose diet-induced metabolic syndrome. The crude extract of djulis husk at 250 mg/kg bw was found to increase the phosphorylation of IR substrate-1 (pIRS-1) and GLUT-4 in mice with high-fat diet-induced hyperglycemia (Tung et al., 2021). Additionally, rutin at 10 or 20 mg/kg bw significantly reduced HOMA-IR by regulating IR and IRS-1 in mice with high-fat diet-induced insulin resistance (Yu et al., 2018). Sandeep and Nandini (2017) reported that quercetin (0.1% of diet, w/w) notably improved insulin-mediated glucose uptake by increasing the level of GLUT-4 in rats with streptozotocin-induced diabetes. Furthermore, rutin was observed to significantly reduce fasting blood glucose, insulin levels, and HOMA-IR by increasing the pIRS-1 and Akt (Li et al., 2016).

Supplementation with WDH (0.5% of the diet, w/w) significantly improved high blood pressure, hypertriglyceridemia, and insulin resistance in rats with high-fructose diet-induced metabolic syndrome, likely in association with increased levels of IRS-1 and GLUT-4. This study demonstrated the protective effects of WDH against metabolic syndrome. WDH may be an effective food ingredient for improving metabolic syndrome. The protective effects of WDH against metabolic syndrome may be attributed not only to rutin and its derivatives but also to betanin and other unidentified phenolic compounds. Further investigation is required to analyze and identify the unknown bioactive compounds in WDH.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: LYW. Analysis and interpretation: LYW, LYC, BYH. Data collection: LYW, LYC, ZYS. Writing the article: WLH, BYH. Critical revision of the article: LYW, WLH, BYH. Final approval of the article: all authors. Statistical analysis: LYW, LYC. Obtained funding: LYW. Overall responsibility: LYW, BYH.

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