Original Article

Effects of Benjakul water extract on pancreas in high-fat fed rats

Kevalin Vongthoung*, Achiraya Kamchansuppasin*, Pornthep Temrangsee*, Narongsuk Munkong*, Noppamat Kaendee**, Nusiri Lerdvuthisopon***

Abstract

Introduction: Benjakul water extract (BWE) has been used as an adaptogenic drug. The decoction of Benjakul has been widely used to treat hyperglycemia in folk medicine in Thailand; however, no study has investigated its effect and mechanism of action on the pancreas. The aim of the present study was to verify the effects of BWE on the modulation of glucose tolerance and insulin resistance in high fat diet-induced obese rats.

Method: Male Sprague-Dawley rats were divided into six groups including the control group, high-fat diet alone (HF) group, high-fat diet co-fed with low-dose of BWE (HFB1) group, high-fat diet co-fed with high-dose of BWE (HFB10) group, high-fat diet co-fed with wild betel leaf bush water extract (HFW) group, and high-fat diet co-fed with metformin (HFM) group. After four weeks of the treatment period, oral glucose tolerance test (OGTT) and serum insulin levels were determined. The weight, histological features, beta-galactosidase immunohistochemistry, malondialdehyde (MDA) levels and target gene expression of the pancreas were also evaluated.

Result: In comparison with the HF group, the HFB1 and HFB10 treatments attenuated the OGTT, histopathological features, beta-galactosidase density and MDA contents of the pancreas. All treatment groups significantly increased insulin receptor substrate-2 (IRS-2) gene expression, whereas only HFW and HFM treatments were able to significantly increase glucose transporter-2 (GLUT-2) gene expression of the whole pancreas. Only the treatment in the HFW group could significantly increase the nuclear factor-kappa B p65 (NF-kappa B p65) gene expression.

Discussion and Conclusion: The BWE and wild betal leaf water extract (WWE) treatments could attenuate the glucose intolerance and histopathological features of the pancreases, induced by a high-fat diet. Moreover, both extracts could ameliorate oxidative stress and proliferation-induced pancreatic senescence by modulating the NF-kappa B p65 expression. In addition, all treatments could enhance insulin signaling through the up-regulation of the IRS-2 gene, whereas only the WWE treatment could improve glucose sensing through the up-regulation of the GLUT-2 gene. Thus, we provide evidence that BWE may be a suitable formula for balancing the glucose homeostasis.

Key words: Benjakul water extract, Metabolic syndrome, Glucose tolerance, Insulin resistance, Cellular senescence

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Introduction

Obesity has emerged as one of the most significant public health problems in western industrialized nations and developing countries including Thailand[1]. In 2014, more than 1.9 billion adults aged 18 years or more were found to be overweight and over 600 million were obese[2]. Obesity, particularly abdominal or visceral obesity, is a relevant predictor of non-communicable chronic diseases derived from abnormal energy metabolism such as increased risk for type 2 diabetes mellitus (T2DM)[3]. Visceral adipose tissue, in particular, can secrete adipokines that can induce insulin resistance and inflammation[4]. Abdominal obesity is the major determinant and the most prevalent manifestation of the metabolic syndrome[5]. Insulin resistance is defined as the inability of target organs, liver, muscle, and fat tissues to respond effectively to insulin stimulation[6]. Therefore, during the development of insulin resistance, the body compensates by increasing insulin secretion[6]. In order to maintain normoglycemia, due to increased body mass, the body compensates by hypersecretion of insulin and increased insulin synthesis, resulting in increased beta-cell mass[7]. High-fat feeding of rats, not genetically predisposed to developing diabetes, induces obesity and insulin resistance without the progression to T2DM[8].

Cellular senescence is the state of irreversible cell-cycle arrest[9]. It is involved in the development of many pathological features including the defects in pancreatic mass and insulin synthesis. Animal studies of metabolic syndrome have suggested that pancreatic senescence could occur as a consequence of an increase in reactive oxygen species (ROS) generation and/or islet cell proliferation[10]. In addition, excessive ROS levels can stimulate oxidative damage to many biomolecules, such as lipids. Malondialdehyde (MDA) is an important lipid peroxidative product that can be used to evaluate oxidative stress status in an animal model of metabolic syndrome[11]. Increased activity of redox-sensitive transcription factors, such as nuclear factor-kappa B (NF-kappa B), was found in the pancreatic tissues of rats fed a high-cholesterol diet[12]. Furthermore, a previous study has proposed that the activation of NF-kappa B signaling pathway was associated with the induction of senescent process[13].

Several studies have suggested that defective insulin signaling in the pancreas is an important event in the development of metabolic syndrome and diabetes that could be linked to the down-regulation of genes involved in insulin signaling, such as insulin receptor substrate-2 (IRS-2) gene[14]. IRS-2 is an important branch of insulin signaling pathway that has been suggested to play a major role in the regulation of pancreatic mass and insulin synthesis. Furthermore, the upregulation of IRS-2 in beta cells could prevent the progression of diabetes in mice[15]. Impaired glucose sensing in beta and alpha cells is also recognized as an adverse process that inhibits glucose-stimulated insulin secretion (GSIS), leading to impaired glucose tolerance[16]. It is well established that both glucose transporter-2 (GLUT-2) and glucokinase (GK) function as a glucose sensor for stimulating insulin secretion. Previous studies have shown the down-regulations of both these genes in the pancreatic tissues of animals fed a high-fat diet[17, 18].

Benjakul is composed of five constituents, namely *Piper retrofractum* Vahl. (Long pepper) fruit, *Piper sarmentosum* Roxb. (wild betel leaf bush) root, *Piper interruptum* Opiz. (pepper wood) stem, *Plumbago indica* Linn. (rose-color lead wood) root, and *Zingiber mekongense* Gagnep. (ginger) rhizome[19]. It has been used for practicing balanced health in Thai traditional medicines and as an adaptogen in the lists of the National Drug List of Herbal Medicinal Products A. D. 2006[20]. It has been widely used to be adaptogen for cancer treatment by folk doctors in the Southern region of Thailand[21]. It has been shown to have no toxicity, either acutely or chronically, in both experimental animals and humans[22, 23]. The pharmacokinetics of piperine, the major active component of Benjakul ethanol extract formulation, was dose-dependent[24]. However, the effects of Benjakul water extract (BWE) on pancreas have not yet been investigated in high-fat diet fed rats. Therefore, the main purpose of the present study was to verify the effect of BWE on the modulation of glucose tolerance and insulin resistance in high-fat diet-induced obese rats.

Method

Preparation of BWE and wild betal leaf water extract (WWE)

BWE contains unequal parts from five plants as follows: 2 parts of long pepper fruit, 16 parts of wild betel leaf bush root, 8 parts of pepper wood stem, 6 parts of rose-color lead wood root, and 4 parts of ginger rhizome. WWE, the single herb extract, contains only the root of one plant: *P. sarmentosum* Roxb. The plant materials were collected from Rayong Province, Thailand. The authenticity of the plant materials was verified by comparing them with the specimens deposited at the Herbarium of the Royal Forest Department, Bangkok, Thailand, where herbarium vouchers are kept. All plant were cleaned with water to remove extraneous material and then sliced and dried at 55 °C. Then they were grounded using an electric grinder to obtain a powder and extracted in a similar way as that practiced by the Thai traditional doctors. The powdered material from Benjakul with water (water:plant = 2:1 part) was decocted at 100 °C until only 1 part of water remained, and then it was filtered. The filtrates were evaporated using a freeze-drying (Lyolab LT, Lyophilization system, Inc. USA). The yield (% w/w) for extraction was calculated, and the dried extract was kept in the freezer at -20 °C until further use.

Preparation of high-fat diet

A high-fat diet, modified from that in Claret et al.[25], was used in this study. The major ingredients of the diet were pork belly, pork liver, margarine, sugar, wheat flour, standard chow diet, and hen egg yolk and white. The high-fat diet (5.12 kcal/g) was composed of 65% of calories derived from fat while the standard chow diet (3.04 kcal/g) was composed of 13% calories derived from fat. The standard chow diet (Charoen Pokphand Foods, Thailand) was purchased from the National Laboratory Animal Center, Mahidol University, Thailand. All other ingredients of a high-fat diet were obtained from Talaad Thai market, Thailand.

Animals care and feeding

All animal procedures were pre-approved by the Animal Ethics Committee at the Faculty of Medicine, Thammasat University (AE 001/2015). Male Sprague-Dawley rats (6–8 weeks old and body weight 180 - 220 g) were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The animals were housed individually in stainless-steel cages under regulated room temperature (24 ± 1 °C), relative humidity (60%) and light (12-hour light/dark cycle). After a week of acclimatization, the rats were divided into six groups (n = 8/group): control group, high-fat diet alone (HF) group, high-fat diet co-fed with low-dose of BWE (HFB1) (41.3 mg/kg BW/day) group, high-fat diet co-fed with high doses of BWE (HFB10) (413 mg/kg BW/day) group, high-fat diet co-fed with wild betel leaf bush water extract (HFW) (17 mg/kg BW/day) group, and high-fat diet co-fed with metformin (HFM) (9.5 and 19.1 mg/kg BW/day) group. The rats were fed using oral gavages. The body weights and dietary intake of the animals were recorded daily throughout the study period. At the end of the fourth week, the animals were sacrificed with an overdose of pentobarbital sodium (intraperitoneal injection), and their blood was collected by a cardiac puncture. After that, the pancreas was weighed and harvested for further analysis as described below.

Blood biochemical measurement

Determination of oral glucose tolerance test (OGTT)

At the end of the fourth week of the intervention, the blood glucose levels were determined with a glucometer (ACCU-CHEK Performa, Roche Diagnostics, Switzerland). All the rats were fasted for 16 hours and measured the resting level of blood glucose before 30 min of OGTT estimation. The rats were administered glucose (2 g/kg BW) by gavage administration and blood glucose was determined at 30, 60, 90, 120 and 150 minutes.

Determination of serum insulin concentrations

The animals were fasted for 16 hours, and the blood samples were collected by cardiac puncture. Clotted blood tubes were kept on ice until centrifugation at 1,000 ×g for 5 min. Serums were stored at -20 °C before its analysis. Serum insulin levels were analyzed by using the commercial ELISA kits (Mercodia Insulin ELISA, Sweden).

Hematoxylin and eosin (H & E) staining of pancreas

Tissue samples from the pancreas were taken from all animal groups. They were fixed in 10% buffer neutral formalin, dehydrated with alcohol and embedded in paraffin wax. Serial sections of 3 μm thickness were cut by using a microtome and subsequently stained with H & E. The serial sections were observed under a light microscope (Eclipse Ci-L microscope, Nikon, Japan) coupled with a digital microscope camera (DS-Fi2 microscope camera, Nikon, Japan). The number of islets was counted per cm^2 of pancreas tissue. The size of islets was calculated for each rat (n = 3/group) by AxioVision microscopy software (Carl Zeiss, Germany).

Immunohistochemistry (IHC) staining of pancreas

Senescence-associated beta-galactosidase (SA-beta-gal) is a hypothetical hydrolase enzyme that catalyzes the hydrolysis of beta-galactosides into monosaccharides only in senescent cells. SA-beta-gal was identified by immunostaining using anti-beta-galactosidase (polyclonal mouse anti-beta-galactosidase, Abnova, Jhouzih St., Taipei, Taiwan). Formalin fixed paraffin embedded tissues were cut at 3 μm and baked at 60 °C for 30 min prior to IHC. Antigens were retrieved using hyaluronidase. Peroxidase quenching was performed by using 3% H_2O_2 in methanol for 30 min at 37 °C. The non-specific binding site was blocked with 5% BSA in 0.1% Tween in 1×PBS. The section slides were incubated with 1:100 beta-galactosidase primary antibodies overnight at 4 °C. After rinsing, the sections were incubated at a dilution of 1:1000 horseradish peroxide donkey anti-rabbit IgG (BioLegend, Pacific Heights Blvd, San Diego, CA) for 30 min at 37 °C in the moisture chamber. Immunostaining was visualized using liquid diaminobenzidine tetrachloride (DAB) chromogen (Diagnostic BioSystems, Owens Drive, Pleasanton, CA). An insoluble brown precipitate of the DAB reaction developed at the beta-galactosidase antibody/antigen binding site. It was counterstained with Mayer's Hematoxylin (Invitrogen). After being air dried, the sections were mounted with Entellan® (Merck, Darmstadt, Germany) and cover slipped. The stained sections of pancreas were captured using the × 10, × 40 and × 100 objective attached to an Olympus BX60 light microscope equipped with a digital camera (Nikon, Japan) and interfaced with NIH Image 1.63 software. The mean density of SA-beta-gal in islet areas was analyzed using the NIH program.

Measurement of pancreatic MDA contents

MDA was used as a marker of oxidative stress. Pancreatic MDA contents (expressed as nM/mg protein of pancreatic tissue) were determined by the double heating method according to the previously published method with some modifications[26]. Briefly, the pancreatic supernatant was mixed with 10% trichloroacetic acid (MERCK, Germany) solution and then mixed with 0.67% thiobarbituric acid (Sigma-Aldrich, USA). The absorbance of the sample was then measured at 532 nm. 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, United States) was used as an MDA standard.

Determination of GK, IRS-2, GLUT-2 and NF-kappa B expression

Total RNA from pancreas was extracted by TRIzol reagent (Invitrogen, USA) according to the manufacturer's recommendations. Total RNA concentrations were determined using a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). Subsequently, RNA was reverse transcribed into cDNA using the cDNA synthesis kits (Applied Biosystems, USA) according to the manufacturer's procedures. Real-time PCR was evaluated using a TaqMan reagent kit and real-time PCR instrument (Applied Biosystems). The relative mRNA levels of GK (Assay ID Rn00561265_m1), IRS-2 (Assay ID Rn01482270_s1), GLUT-2 (Assay ID Rn00563565_m1), and NF-kappa Bp65 (Assay ID Rn01502266_m1) were analyzed by the $2^{-\Delta\Delta CT}$ method. The expression levels of beta-actin (Assay ID Rn00667869_m1) were used for normalization.

Statistical analysis

All values were statistically analyzed using SPSS (version 16.0) for Windows and reported as mean ± standard error of the mean (SEM) for each group. The results were analyzed using one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) test. A p-value less than 0.05 was considered to be statistically significant.

Result

Effects of BWE on glucose homeostatic parameters

Fasting blood glucose (FBG) showed a significant increase in HF, HFB1, and HFW-fed rats when compared to the control rats (Figure 1). There were no significant differences in blood glucose concentrations at 30 min of OGTT between the six groups. At 60 min, blood glucose concentrations were found to be significantly increased in HF, HFB1, HFB10, and HFM groups compared to the control group (Figure 1). At 90 min, blood glucose concentrations were found to be significantly increased in the HFB1 group compared to the control group (Figure 1). At 120 min, blood glucose concentrations of the HF group were higher than the other groups while the blood glucose concentrations of the HFB10 and HFM-fed rats showed a significant decrease when compared to the HF rats (Figure 1). At 150 min, blood glucose concentrations were significantly increased in the HF, HFB1, HFB10 and HFW groups compared to the control group. Thus, it can be summarized that the blood glucose concentrations showed a significant decrease in the HFB10 and HFM-fed rats when compared to the HF rats (Figure 1). However, there was no significant difference in the fasting serum insulin between the six groups (Table 1).

Effects of BWE on pancreas and relative pancreatic weights

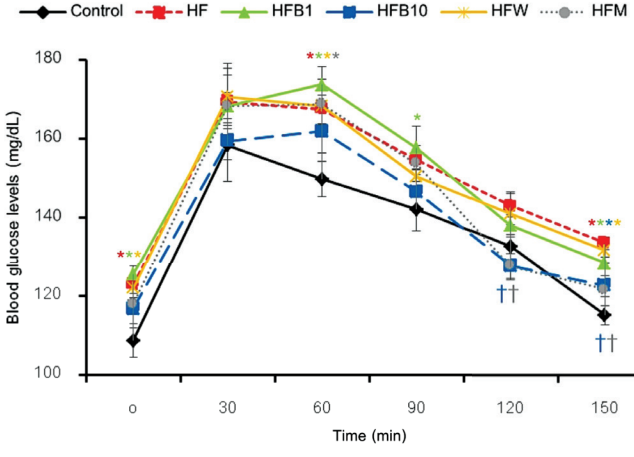
The pancreatic weights of all groups did not show any significant differences ($p < 0.05$) when compared to that of the control group (Table 1). The relative pancreatic weights of the HF, HFB1, and HFW groups were found to be significantly lower ($p < 0.05$) than the control group (Table 1). The relative pancreatic weight of the HFM group was significantly higher ($p < 0.05$) than that of the HF group (Table 1).

Table 1. Fasting serum insulin and pancreas weight of the experimental groups

Groups	Fasting serum insulin (mU/L)	Pancreas weight	
		Absolute weight (g)	Relative weight (g/100 g body weight)
Control	35.98 ± 3.65	2.05 ± 0.04	0.56 ± 0.01
HF	35.94 ± 8.57	1.87 ± 0.05	0.45 ± 0.02*
HFB1	35.22 ± 3.29	1.80 ± 0.03	0.44 ± 0.01*
HFB10	31.00 ± 4.19	1.91 ± 0.12	0.48 ± 0.03
HFW	45.06 ± 5.05	1.82 ± 0.10	0.43 ± 0.02*
HFM	35.53 ± 6.28	2.10 ± 0.15	0.53 ± 0.05†

Values are expressed as mean ± SEM (n = 8)

* $p < 0.05$ vs. control,† $p < 0.05$ vs. HF

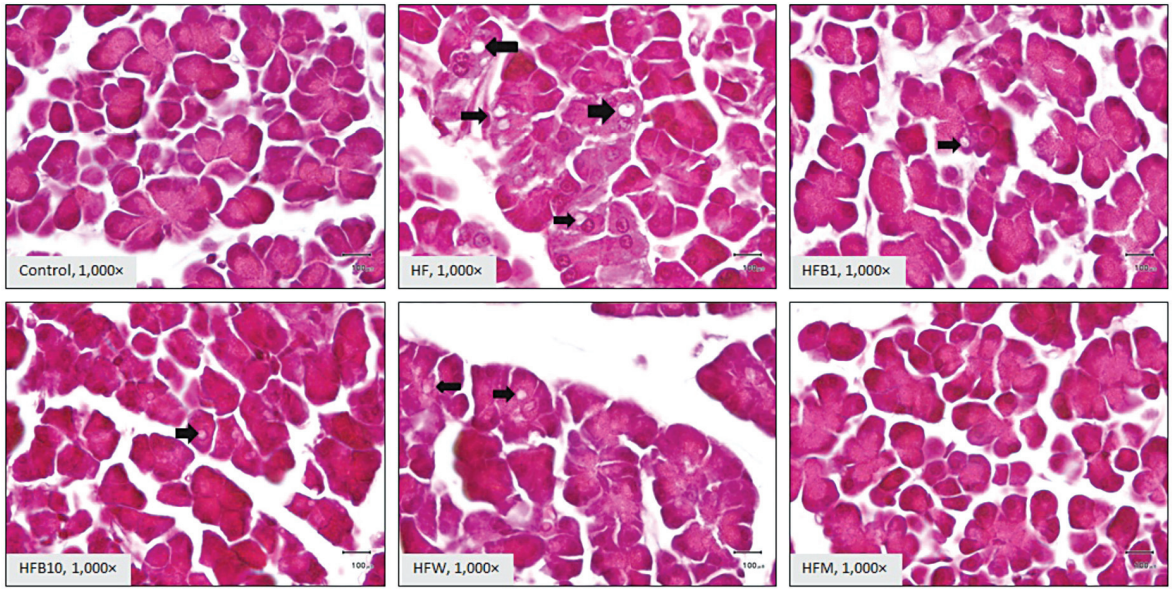


* $p < 0.05$ vs. control,† $p < 0.05$ vs. HF

Figure 1 Oral glucose tolerance test (OGTT) of the experimental groups. Values are expressed as mean ± SEM (n = 8)

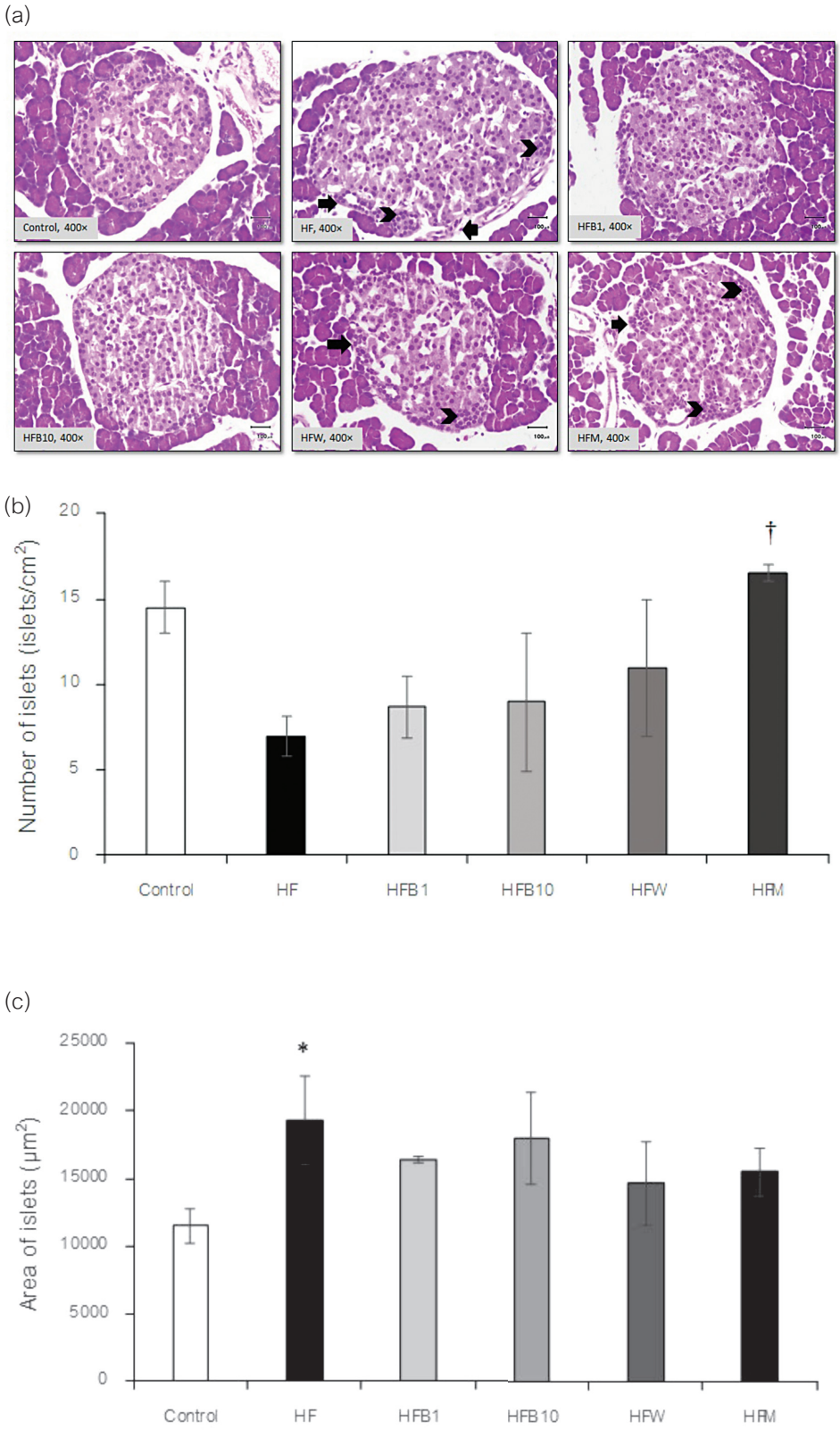
Effects of BWE on histology of pancreas

The histology of pancreatic acinar cells and islets of Langerhans is shown in Figure 2 and 3, respectively. The control group possessed the normal structure of acinar cell and islets of Langerhans (Figure 3a). The HF group had a higher number of clear vacuoles in acinar cells compared to the HFB1, HFB10, HFW and HFM groups (Figure 2). The HF rats also possessed a decreased number of islets (Figure 3b) and showed an enlarged islet area (Figure 3c). The number of islets in the HFM group was significantly higher ($p < 0.05$) than in the HF group. Likewise, there was a trend toward an increase in the number of islets in the HFB1, HFB10, and HFW-treated rats compared with the HF rat, but the differences were not significant. The area of the islets of the HF group was significantly higher ($p < 0.05$) than in the control group. There was a trend toward a decrease in this area of islets in the HFB1, HFB10, HFW and HFM-treated rats compared with the HF rats, but the differences were not significant.



* $p < 0.05$ vs. control,† $p < 0.05$ vs. HF

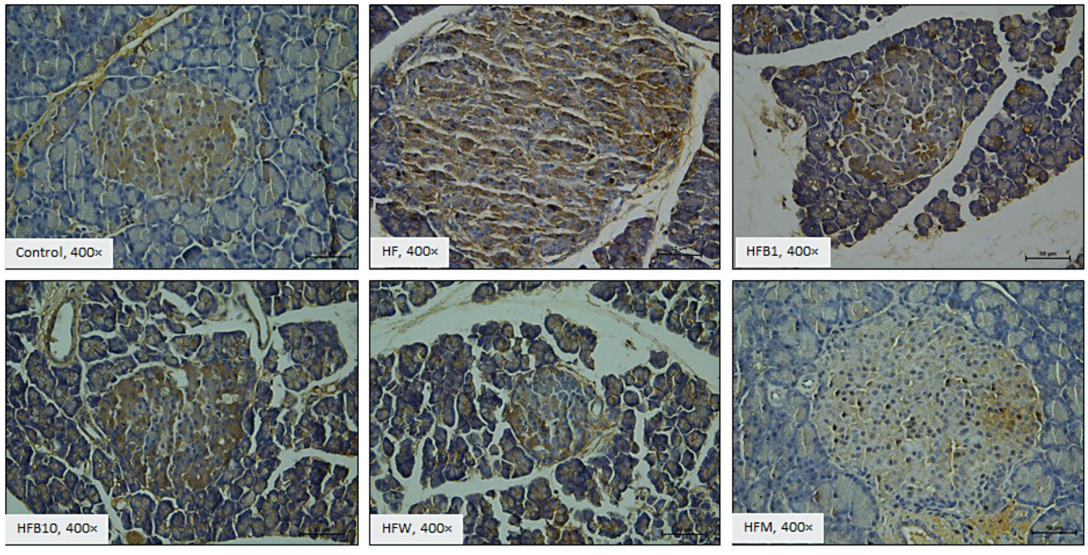
Figure 2 Pancreatic acinar cells histology of the experimental groups (H & E staining, scale bar = 100 μm), arrow represents clear vacuole in acinar cells. Values are expressed as mean ± SEM (n = 3)



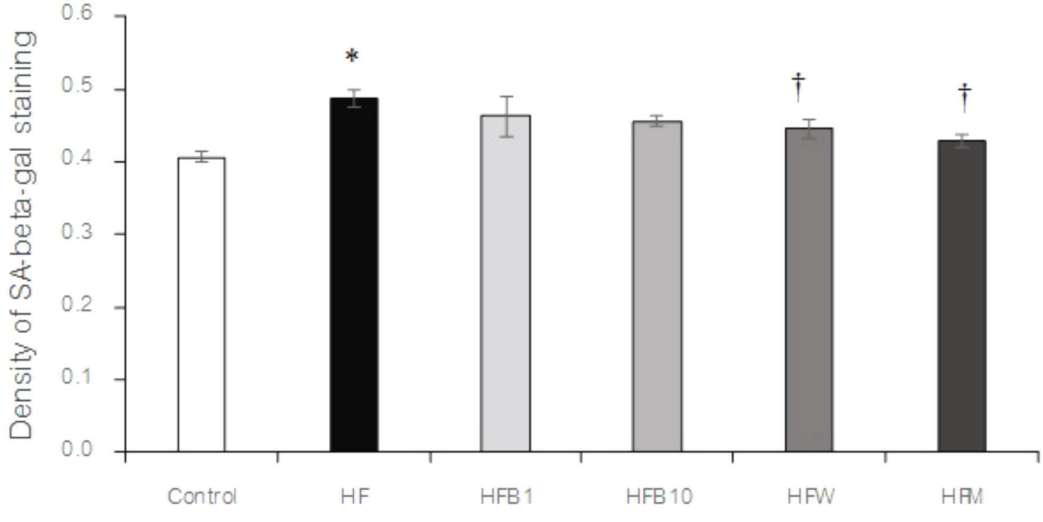
* $p < 0.05$ vs. control, † $p < 0.05$ vs. HF

Figure 3 Pancreatic islets of Langerhans histology (H & E staining, scale bar = 100 μm), arrow represents boundary of islets, arrowhead represents characteristic of cells inside islets (a), number of islets (b) and area of islets (c). Values are expressed as mean ± SEM (n = 3)

(a)



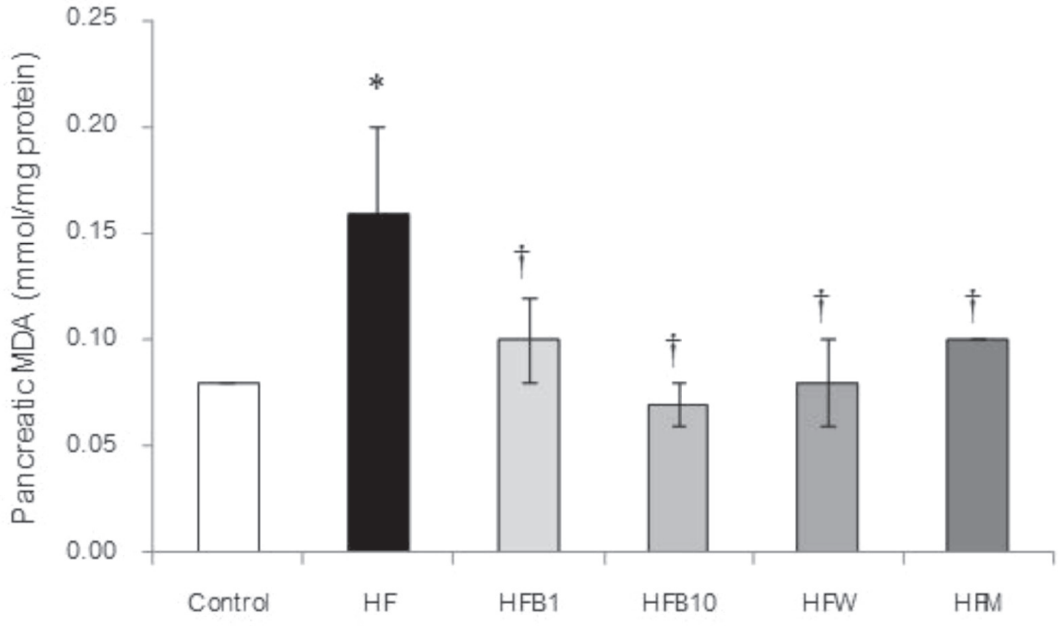
(b)



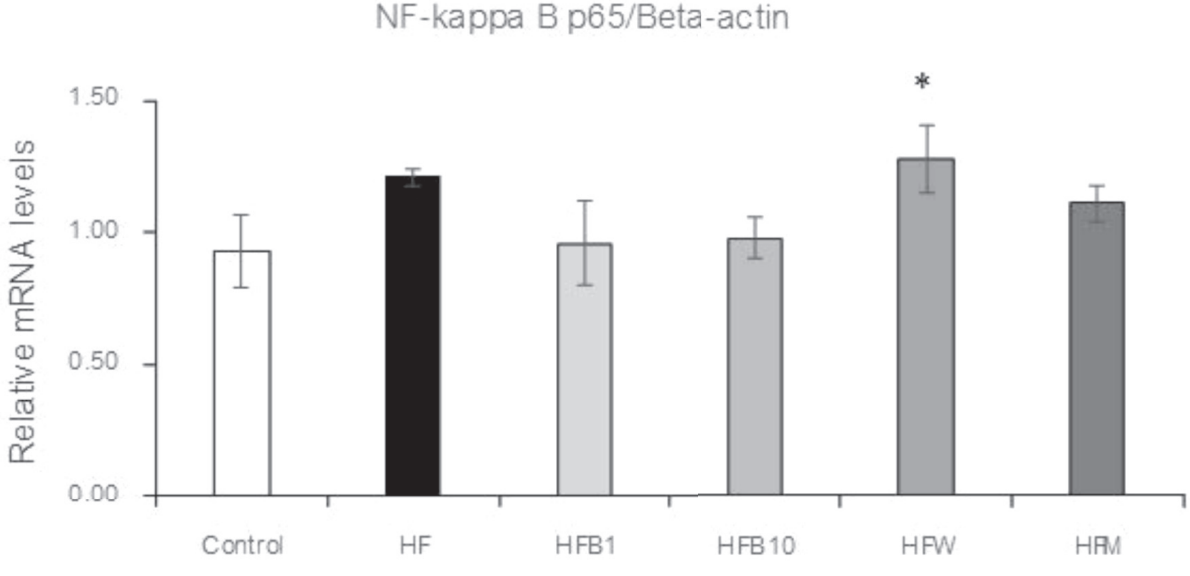
Effects of BWE on pancreatic senescence and oxidative stress markers of pancreas

The SA-beta-gal staining in islets is shown in Figure 4a. The density of SA-beta-gal staining was significantly higher ($p < 0.05$) in the HF group than in the control group (Figure 4b). In the HFW and HFM groups, the density of SA-beta-gal staining was significantly lower ($p < 0.05$) than in the HF group (Figure 4b). There was a trend toward a decrease in these densities in both BWE–treated rats, but the differences were not significant (Figure 4b). Pancreatic MDA levels were significantly increased ($p < 0.05$) in rats fed a high-fat diet compared to the controls (Figure 4c). In contrast to the HF group, all treated groups showed a significant reduction ($p < 0.005$) in the MDA levels when compared with the HF group (Figure 4c). Although there was no significant difference in the pancreatic NF-kappa B p65 mRNA expressions between the control and HF groups, the consumption of high-fat diet showed a tendency to increase in the mRNA levels when compared to the control group (Figure 4d). Treatment with both BWE and Metformin resulted in lower NF-kappa B p65 mRNA levels compared to the HF group (Figure 4d). However, only the HFW group showed a significant up-regulation ($p < 0.05$) in the NF-kappa B p65 mRNA levels when compared to the control group (Figure 4d).

(c)



(d)



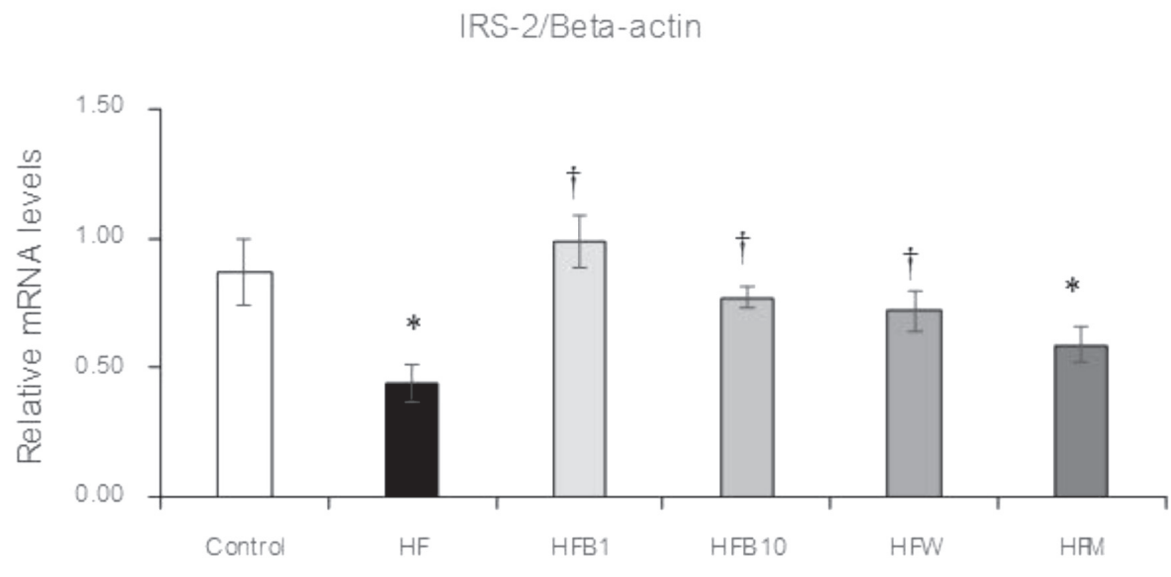
* $p < 0.05$ vs. control, † $p < 0.05$ vs. HF

Figure 4 SA-beta-gal staining in islets (scale bar = 50 μm) (a), density of SA-beta-gal staining (b), MDA levels (c) and NF-kappa B p65 mRNA expression (d) in pancreas of the experimental groups. Values are expressed as mean ± SEM (n = 3 for immunohistochemistry, n = 8 for MDA assay and n = 6 for mRNA expression).

Effects of BWE on IRS-2, GLUT-2 and GK expression

The HF and HFM groups showed a significant decrease in the IRS-2 mRNA level ($p < 0.05$) compared to the control group (Figure 5a). The HFB1, HFB10, and HFW groups showed a significant increase in the IRS-2 mRNA level ($p < 0.05$) compared with the HF group. The HF, HFB1, HFB10 and HFW groups showed a significant decrease in the GLUT-2 mRNA level ($p < 0.05$) when compared with the control group (Figure 5b). The HFW and HFM groups showed a significant increase in the GLUT-2 mRNA level ($p < 0.05$) compared to the HF group. The GK mRNA expressions in HF, HFB1, HFB10, HFW and HFM groups were significantly lower ($p < 0.05$) than in the control group (Figure 5c). In other words, the administration of the BWE, WWE, and metformin in HF-fed rats tend to increase the expression of GK mRNA.

(a)



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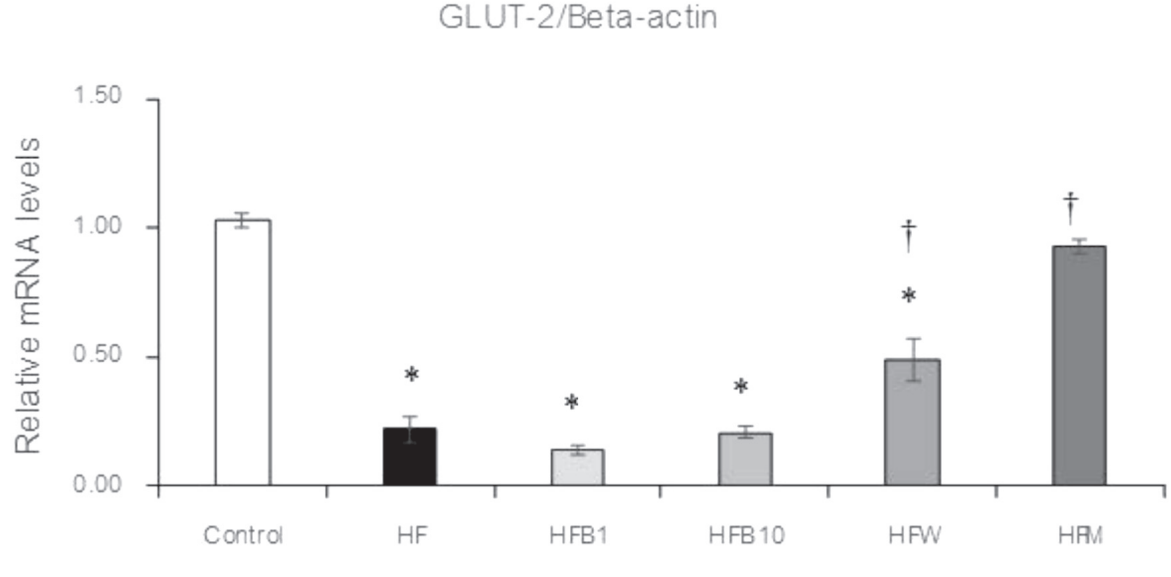
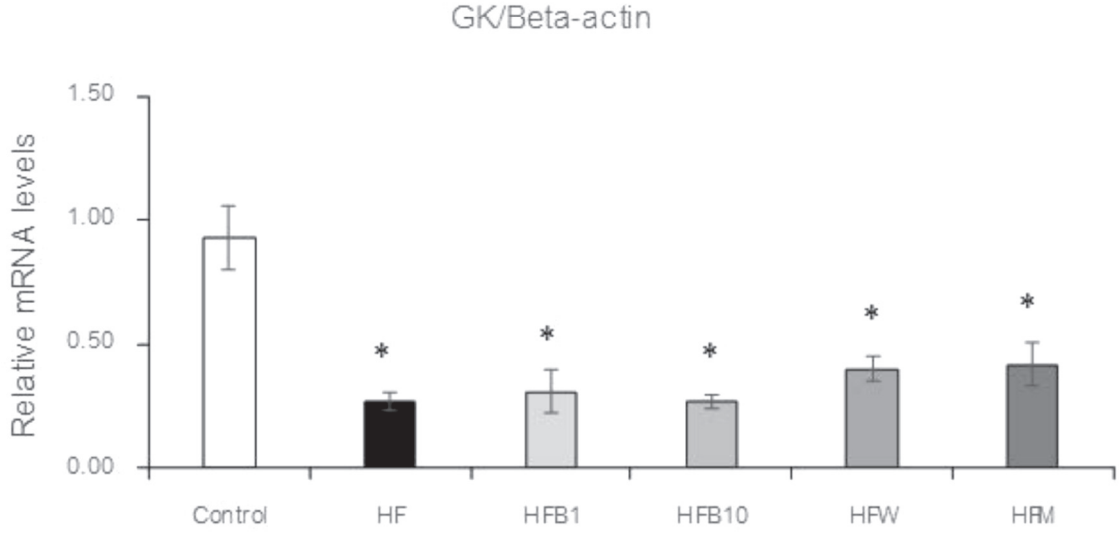


(c)



* $p < 0.05$ vs. control,† $p < 0.05$ vs. HF

Figure 5 Pancreatic IRS-2 (a), GLUT-2 (b) and GK (c) mRNA expression of the experimental groups. Values are expressed as mean ± SEM (n = 6)

Discussion and Conclusion

Although there was no significant difference in the serum levels of insulin between the groups, all HF-fed rats developed hyperglycemia and impaired OGTT compared with the controls. Our observations are concordant with those of Tanoue and colleagues[27]. Interestingly, both BWE2 and metformin treatments significantly protected impaired OGTT at 120 and 150 min. Moreover, there was a downward trend in blood glucose levels in both BWE1 and WWE treatments, especially at 120–150 min after glucose loading. The present results indicated that BWE and WWE treatment has a tendency to improve the impairment of glucose homeostasis. These observations are in agreement with those of Peungvicha et al., who reported that the WWE treatment caused a reduction in plasma glucose levels in diabetic rats[28].

The rats fed only with the high-fat diet also showed signs of ectopic fat accumulation in the acinar cells, as determined by clear vacuoles. In agreement with this feature, studies in obese Zucker diabetic fatty rodents have shown that fat vacuoles were observed in the acinar cells before the onset of hyperglycemia[29]. Recently, a study by Matsuda et al. also suggested that fat accumulation in the acinar cells was likely associated with the progression of pancreatic injury[30]. Compared to the HF group, all treatments could reduce the number of fat vacuoles in the acinar cells. Thus, these findings suggested that BWE and WWE might protect acinar cells from lipotoxicity. In support of these findings, our preliminary results showed that the BWE and WWE treatments could reduce hyperlipidemia and abdominal fat accumulation, which might be beneficial in the regulation of lipid metabolism in the pancreas (data not shown). Previous animal studies have also indicated the lowering of visceral fat accumulation by treatment with piperidine alkaloids from P. retrofractum and WWE[31, 32]. Furthermore, treatments with BWE and WWE tended to improve the irregular structure of pancreas induced by a high-fat diet by restoring the number and size of islets. In line with our results, a previous study found that treatments with WWE reduced islet cell death and normalized islet size in diabetic rats[33].

As mentioned above, the pathogenesis of pancreatic senescence is associated with the increases in ROS production and islet cell proliferation[10]. One important path-

way that induces cellular senescence is the up-regulation of NF-kappa B[13]. Consistent with a previous study, the present results showed that the increased levels of senescent marker were seen in the pancreas of the HF group[10]. These increases coincided with the elevation of the MDA levels, NF-kappa B p65 mRNA levels, and islet area. Thus, our data suggested that HF feeding could induce pancreatic senescence due to oxidative stress and increased islet cell proliferation. Consistent with a slight decrease in pancreatic senescence, the pancreatic MDA, NF-kappa B p65 mRNA and large islet area were improved after the treatment with BWE, suggesting that BWE might delay the onset of pancreatic senescence via the reduction of oxidative stress, NF-kappa B expression, and islet cell proliferation. The same trends were observed for WWE treatment. These results agree with previous observations on antioxidant effects mediated by the extracts of *P. sarmentosum, P. retrofractum, Z. mekongense, P. indica* and *P. interruptum*[34, 35, 36, 37, 38] In the present study, only the WWE treatment showed marked up-regulation of NF-kappa B p65 gene, indicating that some of the pancreatic side-effects could occur with its administration alone. However, a combination of WWE and other components of BWE could prevent this adverse effect. According to our preliminary studies, polyphenols and DPPH radical scavenging activities were found in both BWE and WEE (data not shown), thus indicating that their antioxidant activities might be attributed to the presence of polyphenols. However, further studies will be needed to determine the bioactive antioxidants associated with their antioxidant activities in both extracts.

Consistent with previous studies, rats fed the high-fat diet alone developed impairments in insulin signaling and glucose sensing in the pancreases, which were characterized by the down-regulated expression of IRS-2, GLUT-2, and GK compared with rats fed a standard chow diet[17, 18, 39]. In contrast, the current results showed that the BWE and WWE treatments could up-regulate IRS-2 gene in the pancreases of rats fed a high-fat diet, suggesting that BWE and WWE treatments might prevent the impairment of insulin signaling in the pancreas. However, only the WWE-fed rats showed a significant increase in the pancreatic GLUT-2 mRNA level. These results indicated that WWE might be the bioactive composition in the BWE formula for improving insulin signaling and glucose-sensing pathways.

In conclusion, our findings indicated that BWE might exert adaptogenic activities on glucose homeostasis in a model of metabolic syndrome. Our results also indicated that its mechanisms might be related to the improvement of pancreatic senescence via reduction of oxidative stress and islet cell proliferation, insulin signaling via transcription of IRS-2 gene and lipid deposition in the acinar cells. In addition, WWE might also exert adaptogenic effects against the impairment of glucose sensing in rat pancreas, which might be associated with its transcription of GLUT-2. Thus, our findings provided evidence that BWE might be useful as an adaptogenic formula for the modulation of the impaired glucose homeostasis and pancreatic abnormalities. Although BWE may protect against pancreatic changes, further studies are required to examine the pharmacokinetics and molecular basis for the pharmacological activity of BWE on insulin resistance and glucose handling in the management of diabetes mellitus. Long-term studies are required to confirm the present results and establish the durability of the improvements in glucose levels.

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บทคัดย่อ

ผลของสารสกัดน้ำของเบญจกูลต่อตับอ่อนของหนูที่ได้รับอาหารไขมันสูง

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บทนำ: สารสกัดน้ำของเบญจกูลเป็นยาปรับธาตุ การรักษาแบบพื้นบ้านในประเทศไทยใช้ยาต้มเบญจกูลซึ่งเป็นยาปรับธาตุเพื่อลดน้ำตาลในเลือด อย่างไรก็ตามยังไม่เคยมีการศึกษาเกี่ยวกับผลและกลไกของสารสกัดน้ำของเบญจกูลต่อตับอ่อน การศึกษาในครั้งนี้เพื่อศึกษาผลของสารสกัดน้ำของเบญจกูลต่อการควบคุมสมดุลของภาวะดื้อต่อกลูโคสและอินซูลินในตับอ่อนของหนูที่ถูกเหนี่ยวนำให้อ้วนด้วยอาหารไขมันสูง

วิธีการศึกษา: ศึกษาในหนูขาวเพศผู้สายพันธุ์ Sprague-Dawley ที่แบ่งออกเป็น ๖ กลุ่ม ได้แก่ กลุ่มควบคุมหรือกลุ่มที่ได้รับอาหารหนูปรกติ กลุ่มที่ได้รับอาหารไขมันสูง กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของเบญจกูลขนาดต่ำ กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของเบญจกูลขนาดสูง กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของข้าพลู และกลุ่มที่ได้รับอาหารไขมันสูงร่วมกับยา metformin เมื่อเลี้ยงหนูจนครบ ๔ สัปดาห์ จึงทำการทดสอบการตอบสนองของอินซูลินต่อระดับน้ำตาลในเลือด ระดับอินซูลินในซีรั่ม น้ำหนักของตับอ่อน ลักษณะทางจุลกายวิภาคศาสตร์ของเนื้อเยื่อตับอ่อน อิมมูโนพยาธิวิทยาของ beta-galactosidase ระดับของ malondialdehyde (MDA) และการแสดงออกของยีนเป้าหมายของตับอ่อน

ผลการศึกษา: หนูกลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของเบญจกูลขนาดต่ำและขนาดสูงมีระดับการตอบสนองของอินซูลินต่อระดับน้ำตาลในเลือดลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับหนูกลุ่มที่ได้รับอาหารไขมันสูงอย่างเดียว ลักษณะทางจุลกายวิภาคศาสตร์ของเนื้อเยื่อตับอ่อนดีขึ้น ลดความหนาแน่นของการติดสีของ beta-galactosidase ในเซลล์ไอส์เลตของตับอ่อนและลดปริมาณของ MDA ในตับอ่อน กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของเบญจกูลขนาดต่ำ กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของเบญจกูลขนาดสูงและกลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของข้าพลูสามารถเพิ่มการแสดงออกของยีน insulin receptor substrate-2 (IRS-2) และลดปริมาณ malondialdehyde (MDA) อย่างมีนัยสำคัญ ในขณะที่มีเพียงแค่กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของข้าพลูและกลุ่มที่ได้รับอาหารไขมันสูงร่วมกับยา metformin สามารถเพิ่มการแสดงออกของยีน glucose transporter-2 (GLUT-2) ของตับอ่อนทั้งหมดอย่างมีนัยสำคัญ มีแค่กลุ่มที่ได้รับอาหารไขมันสูงร่วมกับสารสกัดน้ำของข้าพลูเพียงกลุ่มเดียวที่มีการแสดงออกของยีน nuclear factor-kappa B p65 (NF-kappa B p65) เพิ่มขึ้นอย่างมีนัยสำคัญ

วิจารณ์ และสรุปผลการศึกษา: โดยสรุปผลการศึกษาพบว่า สารสกัดน้ำของเบญจกูลและสารสกัดน้ำของข้าพลูสามารถลดระดับน้ำตาลในเลือดและปรับปรุงโครงสร้างทางจุลกายวิภาคของเซลล์ไอส์เลตและเซลล์อะซินาร์ของตับอ่อนในหนูที่ได้รับอาหารไขมันสูงให้มีลักษณะที่ดีขึ้น นอกจากนี้สารสกัดทั้งสองชนิดสามารถทำให้ภาวะการอักเสบและการชราภาพของตับอ่อนในหนูที่ได้รับอาหารไขมันสูงดีขึ้นโดยการควบคุมการแสดงออกของยีน NF-kappa B p65 โดยรวมพบว่าสารสกัดทั้งสองชนิดสามารถเพิ่มการตอบสนองของอินซูลินผ่านการแสดงออกของยีน IRS-2 ในขณะที่มีเพียงแค่สารสกัดน้ำของข้าพลูที่สามารถปรับปรุงการรับสัญญาณของกลูโคสโดยการเพิ่มการแสดงออกของยีน GLUT-2 ดังนั้นแสดงให้เห็นว่าสารสกัดน้ำของเบญจกูลน่าจะเป็นตำรับที่เหมาะสมสำหรับการปรับสมดุลของกลูโคส

คำสำคัญ: สารสกัดน้ำของเบญจกูล, ภาวะอ้วนลงพุง, ความทนต่อกลูโคส, ภาวะดื้อต่ออินซูลิน, เซลล์แก่