Songklanakarin J. Sci. Technol.
42 (6), 1239-1247, Nov. - Dec. 2020

http://www.sjst.psu.ac.th

Original Article

Antidiabetic activity of methoxyflavone-enriched extract of *Kaempferia parviflora* in streptozotocin-induced diabetic rats

Bungorn Sripanidkulchai[1*], Somsuda Somintara[2], Choowadee Pariwatthanakun[3], Kittisak Sripanidkulchai[2] and Vijittra Leardkamolkarn[4]

[1] *Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

[2] *Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

[3] *Department of Anatomy, Faculty of Medicine, Mahasarakham University, Mueang, Maha Sarakham, 44000 Thailand*

[4] *Department of Anatomy, Faculty of Science, Mahidol University, Ratchathewi, Bangkok, 10400 Thailand*

Received: 23 April 2019; Revised: 18 August 2019; Accepted: 24 August 2019

Abstract

Kaempferia parviflora has been traditionally used in folk medicine. This study investigates the hypoglycemic effects of the methoxyflavone-enriched ethanol extract of *K. parviflora* (MKE) in streptozotocin-induced diabetic rats. Compared to the control and vehicle groups, MKE at 150 and 300 mg/kg significantly decreased blood glucose, comparably to glibenclamide. The damage to the Islets of Langerhans was found to be ameliorated in the MKE-treated groups, with twice the increase in the cell count of the Islets. Immunohistological staining showed the loss of insulin as characterized by diffuse staining of the cells in the pancreas of the streptozotocin-induced diabetic rats. MKE-treated groups showed an increase in insulin density in the pancreas of the diabetic rats, similar to the glibenclamide-treated group. MKE at both doses and glibenclamide decreased the hepatic MDA content, but statistical significance was found only for the glibenclamide-treated group. The degeneration of renal tubules and cell necrosis in the kidneys decreased in the MKE-treated groups.

Keywords: *Kaempferia parviflora*, insulin, flavonoids, glibenclamide

1. Introduction

Diabetes mellitus is a chronic, metabolic disorder and a non-communicable disease characterized by hyperglycemia, resulting from an abnormality of metabolism of macronutrients, especially carbohydrates, proteins and lipids. It occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin. Type 2 diabetes accounts for the majority of people with diabetes worldwide (World Health Organization [WHO], 1999). The worldwide prevalence of diabetes in adults has continuously and rapidly increased over the past three decades from 108 million in 1980 to 422 million in 2014, especially in low- and middle-income populations (World Health Organization [WHO], 2016). As estimated by the International Diabetes Federation, the prevalence of diabetes will be up to 642 million in 2040. The disease brings about substantial economic losses to healthcare systems and increases national

*Corresponding author
Email address: bungorn@kku.ac.th

health expenditures. The many risk factors of diabetes involve lifestyle, physical exercise, and healthy weight, and these are targeted in disease prevention. Prevention has been difficult to attain, however, and patient compliance in the lifelong treatment of diabetes with insulin or with oral hypoglycemic drugs are also not always achievable because of undesirable side effects (Holman & Turner, 1991). Currently, medicinal plants are globally gaining acceptability due to their limited side effects and relatively low costs. Much attention has been paid to finding novel entities of natural plant-based antidiabetic drugs (Ghosh *et al*., 2012; Orhan, Aslan, Demirci, & Ergun, 2012; Wang, Wu, Zhou, Wang, & Chen, 2015).

Kaempferia parviflora Wall. ex Baker (Zingiberaceae) is found in northeast Thailand and has been widely used in herbal medicines for centuries. The ethanol extract of the plant rhizome has been demonstrated to have several methoxyflavones as major constituents, such as 5,4-dimethoxyflavone (DMF), 5,7,4′-trimethoxyflavone (TMF) and 3,5,7,3′,4′-pentamethoxyflavone (PMF) (Mekjaruskul *et al*., 2013). Methoxyflavones of *K. parviflora* were reported to be demethylated by human intestinal bacteria (Kim, Kim & Han, 2014). The plant extracts showed very wide pharmacological activities including anti-inflammation (Sae-Wong *et al*., 2011; Sae-wong, Tansakul & Tewtrakul, 2009), aphrodisiac (Chaturapanich, Chaiyakul, Verawatnapakul, & Pholpramool, 2008; Wattanathorn *et al*., 2012), antipeptic ulcer (Rujjanawate, Kanjanapothi, Amornlerdpison, & Pojanagaroon, 2005), anti-allergic (Tewtrakul, Subhadhirasakul, & Kummee, 2008), antiobesity (Akase *et al*., 2011), anticancer (Banjerdpongchai, Suwannachot, Rattanapanone, & Sripanidkulchai, 2008; Leardkamolkarn, Tiamyuyen, & Sripanidkulchai, 2009), cardioprotectant (Malakul, Ingkaninan, Sawasdee, & Woodman, 2011a; Malakul, Thirawarapan, Ingkaninan, & Sawasdee, 2011b), antidepressant, improvement of learning and memory (Hawiset, Muchimapura, Wattanathorn, & Sripanidkulchai, 2011; Wattanathorn, Pangpookiew, Sripanidkulchai, Muchimapura, & Sripanidkuchai, 2007), antimutagenicity and α-glucosidase inhibitory effects (Azuma *et al*., 2011). In terms of antidiabetic activity, 5,7,3′,4′-tetramethoxyflavone, TMF and PMF constituents from *K. parviflora* showed *in vitro* inhibitory effects on α-glucosidase activity (Shimada *et al*., 2011). Powder and ethyl acetate extracts of *K. Parviflora* suppressed the glucose and lipid metabolism in Tsumura, Suzuki, Obese Diabetes (TSOD) mice (Akase *et al*., 2011; Shimada *et al*., 2011). The ethanol extract of *K. parviflora* also enhanced the energy production in myocytes through improved glucose, lactic acid and lipid metabolism (Toda *et al*., 2016b). In streptozotocin-induced diabetic rats, the treatment with ethanol extract of *K. parviflora* at doses up to 100 mg/kg for 4 weeks did not decrease blood glucose, but reduced vascular stress and improved endothelium-dependent relaxation of the aorta. The authors suggested a potential benefit of this plant in preventing vascular complications of diabetes (Malakul *et al*., 2011b). With several reports on the positive effects on glucose metabolism, this study investigates the *in vivo* hypoglycemic effects of the methoxyflavone-enriched ethanol extract of *K. parviflora* rhizome in streptozotocin-induced diabetic rats. The oral administration of MKE was conducted for 8 weeks; then the blood glucose levels were determined in conjunction with the histological analysis of the vital organs of the diabetic rats.

2. Materials and Methods

2.1 Chemicals

Streptozotocin, sodium carboxymethylcellulose (CMC) (Sigma Ltd, USA); glibenclamide (Siam Pharmaceutical Company, Thailand); and pentothal sodium (Abbott Laboratories, Italy) were obtained from local distributors. All the other chemicals used in the study were of analytical grade and were obtained commercially.

2.2 Preparation of the plant extract

To prepare the methoxyflavone-enriched extract from *Kaempferia parviflora* (MKE), the plant rhizome was purchased from the local farm in Loei province. Voucher specimen no. CRD-BS10 was identified by Professor Bungorn Sripanidkulchai and deposited at the Faculty of Pharmaceutical Sciences, Khon Kaen University. The ethanol extract was prepared following the previous procedure (Sripanidkulchai & Sutthanut, 2008). Briefly, the dried rhizome powder was macerated in 95% ethanol, filtered and dried using a rotary evaporator and freeze dryer, which provided a 5.71% yield. Then the extract was analyzed (Figure 1) for three major methoxyflavones (DMF, TMF and PMF) as previously described (Tuntiyasawasdikul, Limpongsa, Jaipakdee, & Sripanidkulchai, 2014). According to HPLC analysis, the yields for DMF, TMF and PMF were 13, 13 and 11%. For animal administration, MKE was suspended in 0.2% CMC before use.

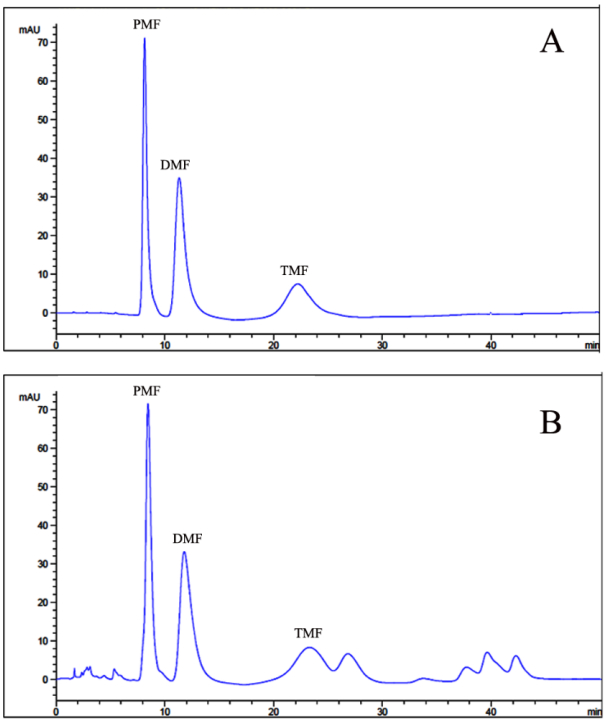

Figure 1. HPLC chromatograms of standard methoxyflavones (A) and MKE (B). The retention times of PMF, DMF and TMF were 8.2, 11.3 and 22.3 min, respectively.

2.3 Experimental animals

Male Wistar rats (200-300 g body weight) were purchased from the National Animal Center, Mahidol University, Thailand. They were housed in 290 x 330 x 250 mm suspended cages (local make, Thailand) at an ambient temperature of 22±2°C with 12 h light/dark cycle and were acclimatized for 7 days prior to the experiment. The animals received standard rat food (C.P. rat food 082S.W.T Co. Ltd, Samutprakan, Thailand) and water *ad libitum*. During housing the animals were monitored twice daily for health status. No adverse events were observed. All experiments were conducted under the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No 80-23) revised in 1996, and were approved by the Ethics Committee (Animal Care and Use Committee) of Khon Kaen University (Reference No AE 011/51). All sections of this report adhere to the ARRIVE Guidelines for reporting animal research (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

The rats were induced to be diabetic with a single dose of streptozotocin (45 mg/kg body weight, intraperitoneally) as described by Eidi, Eidi, and Esmaeili (2006). After 7 days of streptozotocin induction, the rats with a fasting blood glucose over 180 mg % were randomly divided into 5 groups of 8 animals each and treated orally daily for 8 weeks as follows. Group 1: diabetic control (control) received distilled water (0.7 mL); Group 2: vehicle control (vehicle) received 0.2% CMC (0.7 mL); Groups 3-4: *K. parviflora* extract treated groups (KD 150 or KD 300) received MKE extract at the doses of 150 and 300 mg/kg body weight; and Group 5: positive control with glibenclamide treatment received glibenclamide (5 mg/kg body weight). Another group of normal rats (Group 0, n = 4) was also used as a normal control for histological study. All animals were bi-weekly monitored for body weight and fasting blood glucose (Coulter Synchron CX4, Beckman, USA). At the final day of study, the animals were killed under deep anesthesia with sodium pentothal (45 mg/kg body weight, intraperitoneally) and then livers, kidneys and pancreases were collected for further analysis.

2.4 Histological analysis

Livers, kidneys and pancreases of diabetic rats were processed for histological examination with hematoxylin and eosin staining (Anderson & Gordon, 1996). The tissues were fixed in phosphate buffered 10% formalin (pH 7), embedded in paraffin, sectioned to 5 micron thickness and stained. Then the tissue morphology was investigated under light microscopy. For pancreas sections, the numbers of Islets of Langerhans and the number of cells in the islets in an area of 5,000 and 500 μm^2 were determined quantitatively, as described by Slavin, Zarow, Warden, and Fisler (2010), using a Nikon Eclipse 80i camera at a magnification of 20x and image analysis software (UTHSCSA Imagetool, version 3.00). For immunohistochemical examination, the paraffin sections were de-paraffinized in xylene and rehydrated in a graded ethanol series. To block endogenous peroxidase, the tissue sections were incubated with 1% hydrogen peroxide in 70% alcohol for 30 min, then treated for 20 min with 0.01 M sodium citrate buffer (pH 6.0) at 98°C for antigen retrieval and were allowed to cool at room temperature. The slides were then washed with 0.05 M phosphate buffered saline Tween (PBST) and incubated in blocking serum for 1 h. After washing with PBST, the slides were incubated with mouse anti-insulin (1:200) (Cell Signaling Technology, Beverly, MA, USA) for 2 h at room temperature. After twice washing with PBST for 5 min each time, dakocytomation envision Labeled Polymer-HRP anti-mouse was applied for 1 h at room temperature, then washed with PBST and the peroxidase reaction was developed using freshly prepared 3,3′-diaminobenzidine solutions. The tissue sections were counterstained with hematoxylin and then dehydrated, cleared, and cover slipped (Ding *et al*., 2013).

2.5 Determination of liver lipid peroxide products

The rat liver was minced in three volumes of cold 1.15% potassium chloride solution by a motor-driven Teflon pestle in a glass homogenizing vessel in the ice bath at 2,000 cycles/min. The crude homogenate was centrifuged at 10,000 *g* for 10 min at 4°C; the supernatant was collected and further centrifuged at 104,000 *g* for 60 min at 4°C. The microsomal fraction obtained by resuspension of the sediment with cold distilled water was used for the determination of protein (Hartree, 1972) and of lipid peroxide products in terms of malondialdehyde (MDA) content using 1.1.3.3-tetramethoxypropane as the standard (Ohkawa, Ohishi, & Yagi, 1979).

2.6 Statistical analysis

All data are presented as mean ± S.E.M. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by *post hoc* Dunnett's multiple comparison test using SPSS program version 13.0. Significant differences required $p < 0.05$.

3. Results

This study demonstrated a significant hypoglycemic effect of the methoxyflavone-enriched ethanol extract of *K. parviflora* rhizome (MKE) in streptozotocin-induced diabetic rats. At 7 days after the intraperitoneal injection of streptozotocin, the animals showed characteristic features of diabetes, namely severe loss of body weight from 262.5±13.8 to 244.5±15.0 g or a 7% decrease, and a significant increase in blood glucose level from 78.6±8.3 to 258.8±5.0 mg % or a 229% increase. Treatments at both dose levels of MKE ameliorated the weight loss and significantly diminished blood glucose levels in these diabetic rats in a similar fashion as the positive drug glibenclamide did. The body weight gains of each complete group of experimental animals were not significantly different ($p > 0.05$) (Table 1). The diabetic rats gained approximately 3-13% of body weight during the 8-week period. The fasting blood glucose levels of both control and vehicle groups of diabetic rats continuously increased from 256.4±17.8 and 252.8±10.7 g at base line to 394.5±15.1 and 327.8±22.7 g at 8 weeks of duration. The hypoglycemic effect of MKE was detected after a 4-week period of administration, at which time the blood glucose levels were 351.3±13.0, 253.3±18.2, 236.4±14.9, 257.9±23.7 and 255.0± 16.5 g and continued to 394.5±15.1, 327.8±22.7, 239.5±5.3, 234.3±17.8 and 232.8±15.7 g at 8 weeks of treatment, for

Table 1. Body weights and fasting blood glucose levels of diabetic rats after treatment with methoxyflavone-enriched *K. parviflora* extract for 8 weeks.

Groups	Body weight (g)				
	Baseline	2-wk	4-wk	6-wk	8-wk
Control	248.13±4.30	254.00±2.97	247.88±5.91	248.13±5.85	270.00±7.17
Vehicle	246.00±4.69	243.75±7.27	256.50±9.29	269.63±9.60	294.50±9.19
KD150	245.25±5.58	244.50±9.03	250.63±8.94	267.25±10.33	284.88±9.18
KD300	230.75±7.05	240.00±5.16	238.75±5.85	244.63±8.12	271.38±10.22
Glibenclamide	259.88±6.06	255.63±8.65	247.00±11.34	263.38±11.94	282.63±13.47
Groups	Blood glucose levels (mg %)				
	Baseline	2-wk	4-wk	6-wk	8-wk
Control	256.38±17.76	307.75±19.02	351.25±13.01	360.50±14.33	394.50±15.12
Vehicle	252.75±10.71	214.88±17.50	253.25±18.17 [a]	319.50±25.40	327.75±22.68
KD150	264.13±31.54	264.63±11.94	236.38±14.92 [a]	250.50±12.36 [a,b]	239.50±5.33 [a,b]
KD300	262.88±22.45	289.50±20.10	257.88±23.75 [a]	237.00±20.68 [a,b]	234.25±17.76 [a,b]
Glibenclamide	260.38±12.47	262.75±8.61	255.00±16.55 [a,b]	241.25±9.67 [a,b]	232.75±15.65 [a,b]

[a, b] are significantly different from control and vehicle groups at $p < 0.05$.

controls, vehicle, KD150, KD300 and glibenclamide-treated groups (Table 1). In the first 4-week period, the CMC or vehicle-treated group showed less increase in blood glucose than the control group. After that, the blood glucose levels of the vehicle group continuously increased and were significantly higher than those of the KD150, KD300 and glibenclamide-treated groups. Moreover, the determination of lipid peroxidation products in liver homogenate demonstrated the protective effects of MKE and glibenclamide in these diabetic rats. The levels of hepatic MDA were 18.6±1.2, 16.2±1.6 and 11.6±1.3 nmol/mg protein for KD150, KD300 and glibenclamide treated groups, respectively, which were significantly lower than those of the control group. However, the MDA level was significantly lower than in the vehicle group only with glibenclamide treatment (Table 2).

Table 2. Hepatic malondialdehyde (MDA) levels and numbers of cells in Islets of Langerhans in diabetic rats.

Group	MDA nmol/mg protein	Number of Islets /5000 μm^2	Number of Islet cell /500 μm^2
Control	26.93±0.89	1.75±0.25	35.04±4.83
Vehicle	20.72±2.88	1.63±0.26	36.08±4.92
KD150	18.59±1.19[a]	1.88±0.30	74.63±8.19[a,b]
KD300	16.22±1.59[a]	2.00±0.38	78.04±8.43[a,b]
Glibenclamide	11.61±1.31[a,b]	2.00±0.33	78.63±10.50[a,b]

[a,b] significant differences from control and vehicle at $p < 0.05$.

Histological analyses of three vital organs of these diabetic rats, namely liver, kidney and pancreas, demonstrated that MKE protected the organs against damage similarly as the standard drug glibenclamide. In general, the morphology of livers of normal control rats showed an arrangement of liver cells in plates or cords and radiating from the region of central venules and separated by sinusoidal capillaries (Figure 2A). The livers of diabetic rats showed small vacuoles in the cytoplasm of some hepatocytes and some dilatations of hepatic sinusoids. The other findings were statistically insignificant between groups (Figure 2B-F). The increase in hepatic lipid peroxidation levels coinciding with the histopathological liver lesions indicated small extent of liver damage in the diabetic rats in this experimental model. Treatment with either dose of the plant extract decreased the hepatic malondialdehyde levels of the diabetic rats. The histology of kidneys of normal rats revealed a normal pattern of glomerulus, proximal and distal convoluted tubules without any inflammatory changes (Figure 2G). In diabetic rats, degeneration of renal tubules was observed, especially in the distal convoluted tubules, which were characterized by numerous clear vacuoles in the cytoplasm and dilatation of renal tubules. Moreover, necrotic changes including nuclear pyknosis or clumping of chromosomes and shrinking of the nuclei, and/or karyorrhexis or fragmentation of the nuclei and breakup of the chromatin into unstructured granules, were also seen (Figure 2H-I). In contrast, MKE and glibenclamide ameliorated tubular cell necrosis, as characterized by a decrease of tubular vacuolization and improvement of tubular cell damage (Figure 2J-L). As compared to the architecture of the normal control rats, the cellular damage to Islets of Langerhans was evident in the diabetic rat pancreases (Figure 3A-C). The pancreatic beta cell necrosis and degeneration with numerous vacuoles together with atrophy or reduction of cell size and number were demonstrated. Acinar cells surrounding the Islets, however, appeared normal. Both, MKE and glibenclamide demonstrated improvements of cell damage, as characterized by partial restoration of the Islet cells, reduction of beta cell necrosis and vacuolization (Figure 3D-F). Furthermore, cell counting results revealed a significant increase in the number of Islets (Table 2). Finally, an immunohistological examination to assess the insulin expression in pancreatic islets was performed. In the normal controls, markedly diffuse staining with brown granules was observed (Figure 3G). In contrast, insulin localization in diabetic pancreases dramatically decreased as characterized by the depletion of brown granules (Figure 3H-I). The administration of MKE significantly

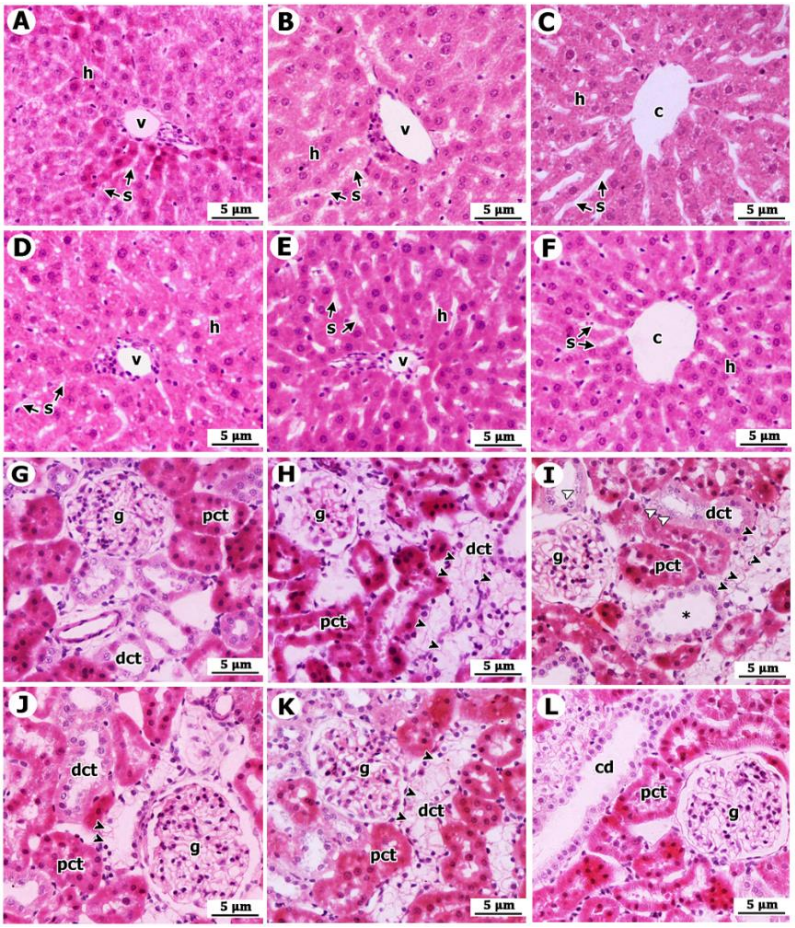

Figure 2. Photomicrographs of livers (A-F) and kidneys (G-L) of diabetic rats after treatment with methoxyflavone-enriched *K.parviflora* extract. (A, G = normal control, B, H = diabetic control, C, I = vehicle, D, J = KD150, E, K = KD300, F, L = glibenclamide, c = central vein, g = glomerulus, h = hepatic cord, s = hepatic sinusoid, v = hepatic venules, arrow = dilated sinusoids, pct = proximal convoluted tubule, dct = distal convoluted tubule, cd = collecting duct; black arrow heads indicate vacuolization and white arrow heads indicate cell necrosis, asterisks = dilated renal tubule.

improved the level of insulin expression and nearly achieved the same level as with the standard drug glibenclamide (Figure 3J-L).

4. Discussion

Kaempferia parviflora is a popularly used traditional medicine with several reported pharmacological activities. Phytochemical analysis showed numerous methoxyflavones as its active constituents (Sutthanut, Sripanidkulchai, & Yenjai, 2007). At least three major compounds including 5,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone and 3,5,7,3′,4′-pentamethoxyflavone were used as markers in the HPLC analysis of the extract (Tuntiyasawasdikul *et al*., 2014). In the current study the method of extraction gave a high methoxyflavone contents in the MKE of 11-13%. The MKE was demonstrated to have a significant hypoglycemic effect in streptozotocin-induced diabetic rats. The intraperi-toneal injection of streptozotocin caused characteristic features of diabetes: a severe loss in body weight and a significant increase in blood glucose levels of the rats, as previously reported (De, Chatterjee, Ali, Bera, & Ghosh, 2011; Junod, Lambert, Stauffacher, & Renold, 1969; Montano *et al*., 2010) suggesting that an appropriate diabetic model was used in this study. With the concerns on ethic care and welfare, the experiment was conducted under the National Centre of Replacement, Refinement and Reduction of Animals in Research (London, UK). Moreover, the number of used animal was minimized and the normal control rats (group 0) was used only in histological comparison (n = 4). This streptozotocin-induced diabetic model has been successfully used in several prior studies to evaluate hypoglycemic effects of medicinal plants (Gupta, Kumar, Chaudhary, Maithani, & Singh, 2012; Hassan *et al*., 2015; Mediani *et al*., 2016; Yuan, Gong, Meng, & He, 2013). Treatment with MKE for 8 weeks could ameliorate the weight loss and significantly diminished blood glucose levels in these diabetic rats. The hypoglycemic effect of MKE was detected at 4 weeks of administration. These findings conflict with a prior report (Malakul *et al*., 2011b). This disagreement may be explained by differences in

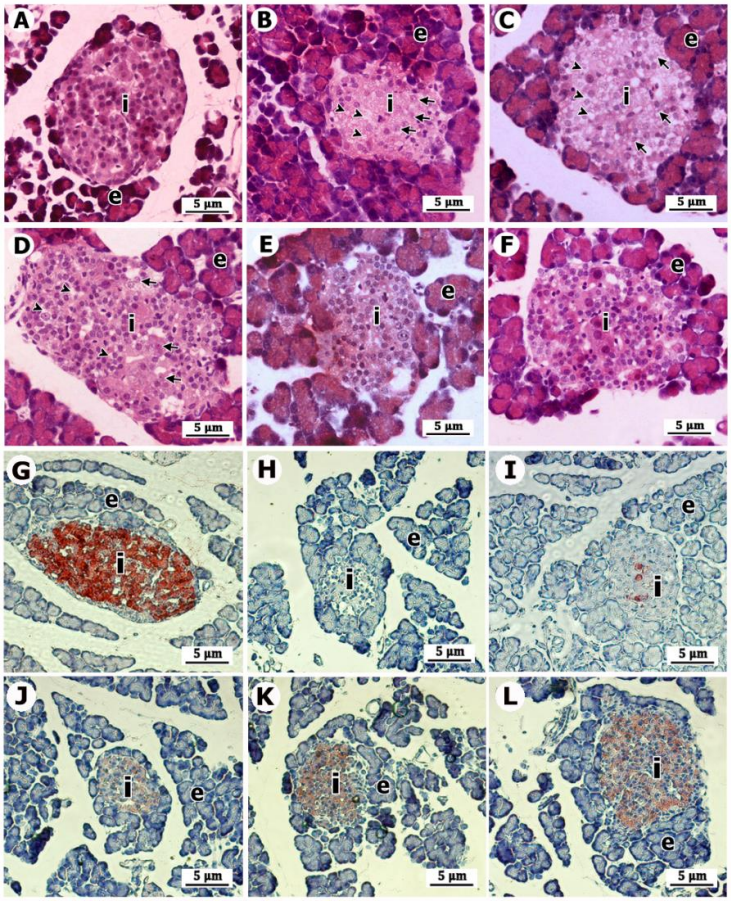

Figure 3. Photomicrographs of hematoxylin staining (A-F) and immunehisto-chemical staining of insulin (G-L) in pancreases of diabetic rats after treatment with methoxyflavone-enriched *K. parviflora* extract. (A, G= normal control, B, H = diabetic control, C, I = vehicle, D, J = KD150, E, K = KD300, F, L = glibenclamide, e = exocrine gland, i = Islet of Langerhans, arrow heads indicate vacuolization and arrows indicate cell necrosis).

dose level and duration of treatment. In this study, the higher doses of 150 and 300 mg/kg and the longer duration of 8 weeks of MKE were used. With 100 mg/kg and 4-week treatment in the previous study, the ethanol extract of *K. parviflora* had demonstrated a protective effect against endothelial dysfunction by restoring endothelium-dependent relaxation of aortic rings in diabetic rats in association with the reduction of vascular superoxide production and increasing nitric oxide bioavailability. Therefore, the present experimental design has demonstrated the *in vivo* hypo-glycemic effect of *K. parviflora* extract to support the aforementioned study. With the several types of methoxy-flavonoids contained in *K. parviflora* extract (Sutthanut *et al*., 2007), it is necessary to standardize the contents. The differences in content of methoxyflavones may be another factor to explain the different results of these two studies. Although MKE slightly decreased the hepatic MDA levels in comparison to the vehicle and control groups, this was without statistical significance, whereas the standard drug glibencla-mide significantly decreased the hepatic MDA level. The findings indicate the hepatoprotective effects of MKE, but at a lesser degree than of glibenclamide. This is in agreement with the results of histological studies. MKE also ameliorated the damage to renal tubules and the islets of Langerhans in the pancreases of diabetic rats.

Taken together, the mechanisms of the hypoglycemic effect of the plant extract may have involved multiple pathways. Not only the anti-glucosidase effect (Azuma *et al*., 2011) and suppression of glucose absorption (Shimizu *et al*., 1997), but other effects such as antioxidant, anti-inflammatory and modulation of glucose and lipid metabolism may also play roles. Lipid peroxidation as one characteristic of chronic diabetes that causes oxidative stress was also demonstrated in the present study. *Caesalpinia ferrea* C. Mart. (Fabaceae) leaf extract was also found to have a hypoglycemic effect via the decrease of oxidative stress in streptozotocin-induced diabetic rats (Hassan *et al*., 2015). Moreover, *K. parviflora* and its methoxyflavone constituents were shown to have strong anti-inflammatory effects through the inhibition of several pro-inflammatory genes (Horigome *et al*., 2017; Sae-wong *et al*., 2009, 2011), and the decrease in inflammation may also play a certain role in the protective effect of *K. parviflora* in the

diabetic rats. Furthermore, *K. parviflora* has previously shown an anti-obesity effect in spontaneously obese type II diabetic rats via the suppression and activation of many factors related to glucose and lipid metabolisms, including decreased fat accumulation, increased insulin secretion, lowered blood glucose in the glucose tolerance test, and decreased blood lipid profile (Akase *et al*., 2011).

Currently, these data have revealed cellular and molecular evidence of *K. parviflora* extract improving energy production via glucose and lipid metabolisms. In myocytes, *K. parviflora* extract enhanced the uptake of 2-deoxyglucose and lactic acid as well as their transporter mRNA expressions with enhanced ATP and mitochondrial biogenesis (Shimada *et al*., 2011). *K. Parviflora* and its active components methoxyflavones, enhanced lipolysis in mature adipocytes by activation of the transcription of the adipose triglyceride lipase and hormone-sensitive lipase, thereby preventing adipocyte hypertrophy (Okabe *et al*., 2014). *K. Parviflora* extract increased the physical performance and muscular endurance by reducing inflammation and improving energy metabolism as observed in the decrease in IL-6 and TNF mRNA expression and the increase in the mRNA expression of peroxisome proliferator-activated receptor g coactivator and glycogen synthase (Toda *et al*., 2016a). Clinical data have also supported the energy expenditure effect of *K. parviflora* as reportedly the plant extract improved physical fitness performance of athletes and elderly (Promthep, Eungpinichpong, Sripanidkulchai, Chatchawan, 2015; Wattanathorn *et al*., 2012), increased whole-body energy expenditure through activation of brown adipose tissue (Matsushita *et al*., 2015) and oxygen consumption (Yoshino, Awa, Ohto, & Morita, 2011). Overall, *K. parviflora* is a plant with potential to exert positive effects in metabolic disorder syndromes, not only diabetes, but also in obesity via mechanisms involving several pathways. It is necessary, however, to further elucidate the roles of each methoxyflavone constituent of the plant in each activity.

5. Conclusions

In conclusion, the methoxyflavone-enriched ethanol extract of *K. parviflora* at doses of 150 and 300 mg/kg, given for 8 weeks duration, exerts modulatory effects involved in the metabolism of glucose and lipids. The plant extract may beneficially be consumed to protect against or ameliorate various metabolic disorders.

List of abbreviations

CMC: sodium carboxymethylcellulose
DMF: 5,4-dimethoxyflavone
MDA: Malondialdehyde
MKE: Methoxyflavone-enriched ethanol extract of *K. parviflora* rhizome
PBST: phosphate buffered saline Tween
PMF: 3,5,7,3′,4′-pentamethoxyflavone
TMF: 5,7,4′-trimethoxyflavone
TSOD: Tsumura, Suzuki, Obese Diabetes

Acknowledgements

This work was supported by the research grant of the national Research Council of Thailand (NRCT). The authors would like to acknowledge Prof. James A. Will for editing the manuscript via Publication Clinic KKU, Thailand.

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