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Article *in* Journal of Food Science · April 2019

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Mangosteen Vinegar Rind from *Garcinia mangostana* Prevents High-Fat Diet and Streptozotocin-Induced Type II Diabetes Nephropathy and Apoptosis

Naymul Karim, Atiar Rahman, Lanchakon Chanudom, Montakarn Thongsom, and Jitbanjong Tangpong

Abstract: Type II diabetes (T2D) nephropathy, a major cause of end-stage kidney disease, progresses and develops from oxidative stress. Natural polyphenols can protect the kidney from diabetic nephropathy exerting antioxidant activities. The present approach enumerates the reno-protective and anti-apoptotic effects of mangosteen vinegar rind (MVR, a phenolic aqueous extract) against high-fat diet (5 g/day up to five weeks)-/streptozotocin (single ip, dose 30 mg/kgBW)-induced T2D nephropathy of albino mice. *In vitro* total phenolic content, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity, and α-amylase inhibition activity as antidiabetic assay of MVR were performed. *In vivo* mice body weight, oral glucose, and maltose tolerance test, metabolic parameters (plasma glucose, insulin level, omeostasis model assessment-estimated insulin resistance), biochemical parameters (kidney hypertrophy, blood urea nitrogen, creatinine), oxidative stress parameters (malondialdehyde, superoxide dismutase, catalase) were estimated in an intervention study. Additionally, renal morphology and early apoptosis were observed following the H & E staining and TUNEL assay of the tissue frozen section. We found that the aqueous extract of MVR possesses potent *in vitro* antioxidative and antidiabetic activities. Animal intervention results showed that MVR 100, 200 mg/kgBW, and Glibenclamide 60 mg/kgBW treatments significantly improved ($P < 0.05$) the abovementioned parameters compared to the diabetic control group. Furthermore, treatments also significantly restored ($P < 0.05$) kidney histological alterations and reduced cellular apoptosis compared to the diabetic control group. These findings concluded that MVR treatments significantly modulated the glucose intolerance, metabolic alterations, and oxidative stress-induced pathological alterations and cellular apoptosis of diabetic kidney.

Keywords: anti-apoptosis, diabetic nephropathy, *Garcinia mangostana*, oxidative stress, renal protection

Practical Application: *Garcinia mangostana*, a polyphenol rich natural product, is obtained from the tropical rain forest area of Southeast Asian countries and processes diverse biological activities including antioxidant, anti-proliferative, anti-inflammatory, anti-carcinogenic, and so on. This research first time focuses on the nephro-protective and anti-apoptotic effects of mangosteen vinegar rind (MVR) from the mangosteen fruit pericarp. Our study provides the efficient data to prove the beneficial effect of MVR as a dietary supplement for the prevention and management of diabetic nephropathy.

Introduction

Diabetic nephropathy (DN), one of the most recognizable microvascular complications of diabetes mellitus (Aires Neto, Gomes, & Campos, 2013; Lin & Yin, 2012), leads to end stage of kidney disease (Guo, Han, Zhang, Xiao, & Yang, 2014; Powell et al., 2013; Vallon et al., 2012). Glomerular hypertrophy, glomerular hyperfiltration, increased urinary albumin secretion, basement membrane thickness, mesangial expansion, and extracellular matrix protein accumulation were recognized as pathological parameters of DN (Soetikno et al., 2011; Zelmanovitz et al., 2009). DN—causing hyperglycemia is vastly associated with increase reactive oxygen species (ROS) levels, that is mediated by increased polyol pathway flux, advanced glycation end (AGE) products formation, glucose autoxidation, xanthine oxidase activity, mitochondrial respiratory chain deficiencies, NAD(P)H oxidase, and nitric oxide synthase (Brownlee, 2001).

Apoptosis is morphologically distinct and program cell death is characterized by the membrane bleb formation, cell shrinkage, chromatin condensation, and DNA fragmentation due to the renal ischemia/reperfusion injury, unilateral ureteral obstruction, and chronic renal failure (Daemen et al., 1999; Morrissey & Klahr, 1999; Schelling & Cleveland, 1999). According to several *in vitro* studies, hyperglycemia causes glucose-induced various cell line apoptosis such as pancreatic β cell (Donath, Gross, Cerasi, & Kaiser, 1999), endothelial cell (Baumgartner-Parzer et al., 1995), and renal proximal tubule (Ishii et al., 1996). Thus, controlling of hyperglycemia-induced ROS generation might be the main strategy for reducing DN (Das & Sil, 2012; Marrazzo et al., 2014; Palsamy & Subramanian, 2011).

JFDS-2018-1909 Submitted 11/25/2018, Accepted 2/14/2019. *Authors Karim and Tangpong are with Biomedical Sciences, School of Allied Health Sciences, Walailak Univ., Nakhon Si Thammarat, 80161, Thailand. Author Rahman is with Dept. of Biochemistry and Molecular Biology, Univ. of Chittagong, Chittagong, 80280, Bangladesh. Authors Chanudom and Thongsom are with Biology Program, Faculty of Science and Technology, Nakhonsithammarat Rajabhat University, Nakhon Si Thammarat, Thailand. Direct inquiries to author Jitbanjong Tangpong, School of Allied Health Sciences, Walailak University, Nakhon-Si-Thammarat, 80161, Thailand (E-mail: njibjoy@yahoo.com).*

doi: 10.1111/1750-3841.14511

Journal of Food Science
A Publication of the Institute of Food Technologists

Garcinia mangostana is known as mangosteen plant and is belong to the family of Guttiferae. *G. mangostana* is obtained from the tropical rain forest area of Southeast Asian countries such as Thailand, Myanmar, Philippines, Sri Lanka, Indonesia, Malaysia. Mangosteen is famous for its dark purple/reddish color tasty fruit named as mangosteen fruit and it also defines as "queen of fruits" (Karim & Tangpong, 2018). Mangosteen possesses potent antioxidant, anti-proliferative, pro-apoptotic, anti-inflammatory, and anti-carcinogenic activities (Gutierrez-Orozco & Failla, 2013; Karim & Tangpong, 2018; Phyu & Tangpong, 2014; Zarena & Sankar, 2009). A variety of bioactive compounds are present in mangosteen fruits, xanthone is one of them (Gutierrez-Orozco & Failla, 2013; Karim, Chanudom, & Tangpong, 2018). Manimekalai and coworkers found that the epicarp of mangosteen fruit contains 160 aromatic compounds, whereas the endocarp contains 105 compounds, evaluated by gas chromatography–mass spectral analysis (Manimekalai, Sivakumari, Ashok, & Rajesh, 2016). Numerous *in vitro* researches revealed that *G. mangostana* exhibited antidiabetic activity by inhibiting α-amylase and α-glucosidase enzyme activities (Loo & Huang, 2007; Manaharan, Palanisamy, & Ming, 2012; Ryu et al., 2011). However, the comprehensive antidiabetic potential of *G. mangostana* is yet to be studied.

Therefore, the current study evaluated the protective effects of mangosteen vinegar rind (MVR) against high-fat diet (HFD)/streptozotocin (STZ)-induced type II diabetes (T2D) nephropathic mice.

Materials and Methods

Chemicals and reagents

Almost all analytical grade chemicals were used for analysis, and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), Merck & Co. (Darmstadt, Germany), and Millipore Corporation (Billerica, MA) except glibenclamide, which was purchased from the government pharmaceutical organization (GPO, Maha Nakhon, Thailand). In addition, STZ was purchased from Boehringer-Mannheim company (Mannheim, Germany).

Experimental sample collection

MVR from *G. mangostana* was purchased from Asia & Pacific Quality Trade Co., Ltd. (Bangkok, Thailand). The crude extract was rich in high percentage of xanthones. Isolated xanthone (100 mg) from MVR contained α-mangostin (69.01%), γ-mangostin (17.85%), gartanin (4.13%), 8-deoxygartanin (2.95%), garcinon E (2.84%), and other xanthones (3.22%), which was detected by HPLC analysis.

Crude extraction

Fruit pericarp was air-dried and ground in blender (Stainless steel jar blender, model no. BL-Y66S-1, GuangDong, China) to a fine powder. Then, 100 g powder was extracted with 500 mL boiling water for 15 min. Then the aqueous part was filtrated and freeze-dried under vacuum at −80 °C for 18 hr. After concentrating through freeze drying, the percentage of yield was calculated and stored at −30 °C for study. The percentage of MVR yield was 4.59 g%.

In vitro antioxidant activity of MVR

Total phenolic content of MVR was measured by the Folin Ciocalteau method (Kaisoon, Siriamornpun, Weerapreeyakul, & Meeso, 2011). Briefly, the blue color formed due to the polyphenol present in the aqueous extract was measured at 765 nm using a microplate reader and results were expressed as μg GAE/mg extract. The free radical scavenging activity of MVR was measured following the ABTS decolonization assay (Re et al., 1999). Here, the blue color $ABTS^+$ was produced from colorless ABTS by adding 7 mM ABTS in H_2O with 4.9 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 12 to 18 hr. Due to the presence of polyphenol in the aqueous extract, the $ABTS^+$ solution turns back to the natural colorless ABTS solution is quantified by a microplate reader at 734 nm and results were expressed as μM TEAC/mg extract. For DPPH radical scavenging assay (Zhu, Zhou, & Qian, 2006); succinctly, 0.1 mL different concentration of extract and standard solution were added with 0.1 mM DPPH in 95% ethanol, shaken well and the mixture was incubated at 37 °C for 1 hr at room temperature. The DPPH scavenging activity of MVR was measured at 517 nm using a microplate reader and calculated as IC_{50} values (mg/mL).

In vitro antidiabetic activity of MVR

The *in vitro* antidiabetic activity was analyzed by pancreatic alpha amylase kit protocol (Biocon. Diagnostik, Germany). This kit indicated alpha amylase and glucosidase inhibitory activity of MVR. This kinetic method denoted the inhibition of alpha amylase and glucosidase enzyme, that catalysis and hydrolysis of ethylidene-protected-substrate (4,6-ethylidene-G7-1.4-nitrophenyl-G1-α1D-maltoheptaoside) to formed *p*-nitro-phenol (color product, measured spectrophotometrically at 405 nm) and glucose (Junge, Troge, Klein, Poppe, & Gerber, 1989; Tietz, Huang, Rauh, & Shuey, 1986).

Animals and maintenance

Six-week-old ICR mice (25 to 30 g) were obtained from the National Laboratory Animal Center, University, Salaya district, Nakhon Pathom, Thailand. Animals were housed at room temperature (23 ± 2 °C) in a humidity-controlled environment (55 ± 10%) with a 12 hr light/dark cycle in the experimental lab of the Natural Product Utilization Unit of Walailak Univ. The mice were allowed to free excess of water and food. Guideline for Care and use of experimental laboratory animal and the protocol were approved by the Animal Care and Use Committee of Walailak University (No. 002 /2015).

Experimental protocol

Mice were randomly separated into two groups, normal control group ($n = 6$) received standard laboratory diet and HFD-fed group ($n = 30$) received HFD composition (60% normal diet, 12% lard oil, 12% sugar, 8% yolk powder, 6% peanut powder, 1% milk powder, and 1% water) 5 g/day up to 5 weeks. Afterwards, diabetes was induced by a single dose of STZ 30 mg/kgBW (ip) (Boehringer-Mannheim, Mannheim, Germany) diluted in citrate buffer (10 mM, pH 4.5) to overnight fasted HFD-fed mice group (Karim, Jeenduang, & Tangpong, 2018b). Then oral glucose/maltose tolerance test (OGTT/OMTT) was performed by rendering glucose 2 and maltose 3 g/kgBW against MVR 400 mg/kgBW mice. MVR aqueous extract at the doses of MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW were orally administered at between 8:30 a.m. and 9:30 a.m. for one week. Mice body weights were recorded. The experiments were conducted by five independent groups ($n = 6$) as follows:

Group 1: Untreated normal control (received normal saline)

Group 2: Diabetic control (received normal saline)
Group 3: DM+ MVR (100 mg/kgBW)
Group 4: DM + MVR (200 mg/kgBW)
Group 5: DM+ Glibenclamide (60 mg/kgBW)

Animal sacrifice and tissue harvesting

Following seven days treatment with MVR and Glibenclamide, animals were fasted overnight and anesthetized using Sodium Nembutal (65 mg/kgBW). The blood sample was obtained via left ventricular puncture and perfuse with ice-cold saline, pH 7.4. The kidney and liver were discarded and kept in −30 °C for analysis. One kidney was homogenized in cold phosphate buffered saline (PBS) and 0.1% trichloroacetic acid (TCA) solution (pH 7.4; containing a mixture of protease inhibitors—leupeptin, pepstatin, and aprotinin) prior to centrifugation at 15,000 × *g* for 15 min. The resulting supernatant was collected for biochemical analysis while another kidney was kept for pathological evaluation and apoptotic cell determination.

Metabolic and biochemical parameters

Plasma glucose, creatinine (CREA), and blood urea nitrogen (BUN) levels were examined using commercially automated chemical purchased from Biosystems (Costa Brava, Barcelona) and Thermo Fisher Scientific (Middletown, VA). Additionally, the plasma insulin level was evaluated by insulin ELISA kit from Millipore Corporation (Billerica, MA). The kidney hypertrophy (KI) index was assessed by associating the kidney weight to the mice body weight. (KI—kidney weight /body weight) (Manaer, Yu, Zhang, Xiao, & Nabi, 2015). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = Blood glucose (mg/dL) × insulin (mU/L)/405 (Ahangarpour, Zamaneh, Jabari, Nia, & Heidari, 2014).

Measurements of oxidative stress and antioxidant marker

The plasma and kidney tissue malondialdehyde (MDA) levels were measured as a lipid peroxidation marker; following the protocols described by Ceci et al. (2014) and Goulart, Batoreu, Rodrigues, Laires, and Rueff (2005). The superoxide dismutase (SOD) and Catalase (CAT) were measured by pyrogallol autoxidation (Marklund & Marklund, 1974) and H_2O_2 decomposition method (Takahara et al., 1960); of kidney tissue as antioxidant enzyme defense system markers.

Kidney tissue histology

Tissue samples were washed with cold PBS solution and dehydrated with 95% ethanol, embedded in FSC 22 Mounting Media (Leica Biosystems, Maritime Square, Harbourfront Centre, Singapore). A 7-µm-thick kidney tissue section was obtained using Leica CM1950 Cryostat (Leica Microsystem, Wetzlar, Germany) and stained with hematoxylin and eosin for light microscopic morphological studies (Zeiss-Axio Scope. A1 light microscope, Jena, Germany). Images were captured at a 40× magnification using (Axiocam ERc 5s, Oberkochen, Germany) connected with the microscope. Histological evaluations were performed visually.

Kidney apoptosis (TUNEL assay)

Kidney tissue frozen sections were obtained according to above-mentioned procedure of kidney tissue histology. Kidney apoptotic cells from frozen section were quantified by commercially available "*in situ* Apoptosis Detection kit (Abcam, Cambridge, UK)." Apoptotic cells were counted using a light microscope (Zeiss-Axio Scope. A1 light microscope) at a 40× magnification and pictures were captured by (Axiocam ERc 5s).

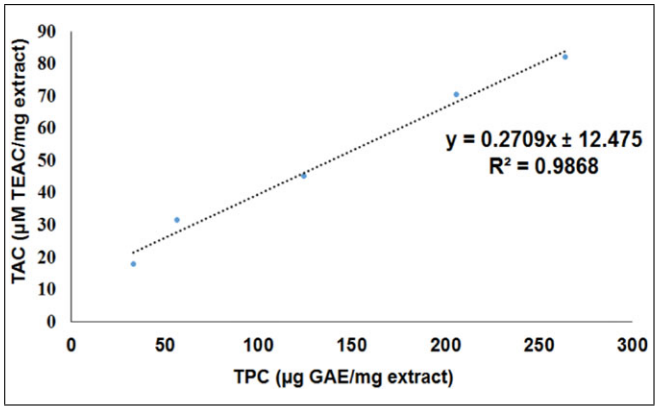

Figure 1—Correlation coefficient between total phenolic content (TPC; µg GAE/mg dw, X) and total antioxidant capacity (TAC; µM TEAC/mg dw, Y) of MVR extract.

Statistical analysis

Results were expressed as mean ± SEM. Statistics software (IBM SPSS for Windows, v. 17.0, New York, NY) was used for data analysis. The statistical analysis of data was done by one-way analysis of variance followed by Tukey's *post hoc* test. The significant value was considered as $P < 0.05$.

Results

Evaluation of *in vitro* antioxidant activity of MVR

MVR polyphenols from *G. mangostana* showed the presence of total phenolic content and exerted the ABTS antioxidant capacity. In addition, DPPH scavenging activity confirmed that MVR showed potent free radical scavenging activity (Table 1). The correlation coefficient between total phenolic content and total antioxidant capacity of MVR extract is shown in Figure 1.

Evaluation of *in vitro* antidiabetic activity of MVR

In vitro antidiabetic activity of MVR was evaluated as α-amylase enzyme inhibition. The α-amylase inhibition of MVR was expressed as IC_{50} value (Table 1) and was compared with standard drug Acarbose (IC_{50} = 84.22 µg/mL, R^2 = 0.9932).

Effect of MVR on OGTT/OMTT

Induction of HFD for 5 weeks to normal mice significantly increased ($P < 0.05$) body weight compared with the normal control group (Figure 2a). The effects of MVR (400 mg/kgBW) on OGTT/OMTT are shown in Figure 2b,c. The MVR extract improved the glucose intolerance of the fasted HFD mice and the blood glucose level was significantly lower ($P < 0.05$) from 60 min compared with the HFD group (Figure 2b). Moreover, MVR extract enhanced the maltose intolerance and reduced blood glucose level significantly ($P < 0.05$) from 30 min compared with the HFD group (Figure 2c).

Effect of MVR on biochemical and renal function parameters

As OGTT/OMTT results are shown in Figure 2b,c, the diabetic control group exhibited significant increase ($P < 0.05$) in plasma glucose, HOMA-IR, and significant decrease ($P < 0.05$) in plasma insulin compared with the normal control group

Table 1–*In vitro* antioxidant and antidiabetic activity of MVR.

	In vitro antioxidant activity			*In vitro* antidiabetic activity
Extract	Phenolic content (µg GAE/mg dry weight)	Antioxidant capacity (µM TEAC/mg dry weight)	DPPH scavenging activity IC_{50} (µg/mL)	α-Amylase inhibitory activity IC_{50} (µg/mL)
MVR	264.02 ± 2.66	82.24 ± 0.81	0.753 ± 0.013	422.82 ± 7.83

Note. Values are expressed as mean ± SEM ($N = 3$).

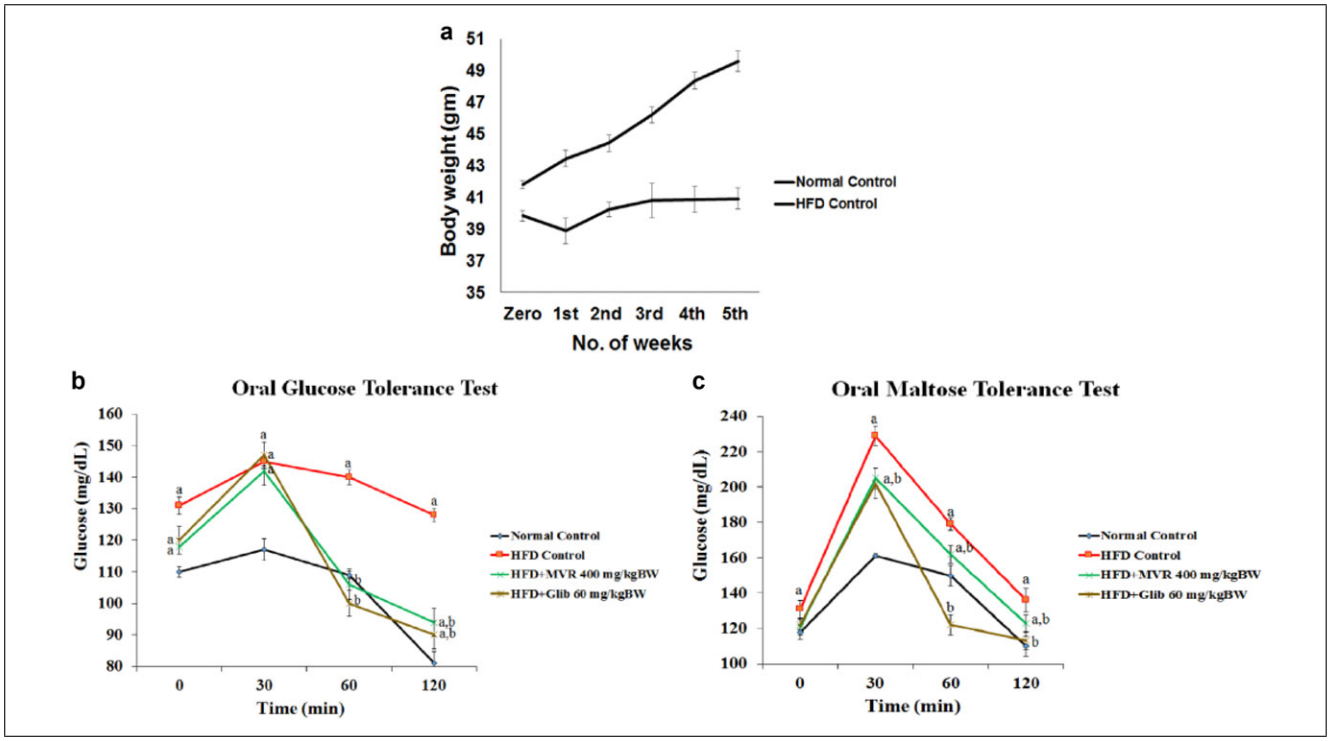

Figure 2–(a) Mice body weight change versus HFD intake; *$P < 0.05$ verses normal control. (b) Oral glucose tolerance test and (c) Oral maltose tolerance test of MVR; values are expressed as mean ± SEM ($n = 6$); were analyzed by one-way analysis of variance followed by the Tukey's *post hoc* test. [a]$P < 0.05$ versus normal control and [b]$P < 0.05$ versus HFD control.

Table 2–Effect of MVR on fasting plasma glucose levels, insulin, and HOMA-IR in HFD/STZ-induced diabetic mice.

Group	Glucose (mg/dL)	Insulin (ng/mL)	HOMA-IR
Normal control	81.31 ± 8.49	3.72 ± 0.34	4.37 ± 0.41
Diabetic control (DM)	220.00 ± 5.00[a]	0.52 ± 0.17[a]	1.67 ± 0.55[a]
DM+ MVR 100 mg/kgBW	118.76 ± 13.10[a,b]	1.79 ± 0.30[a]	3.06 ± 0.51
DM+MVR 200 mg/kgBW	105.39 ± 8.39[b]	2.17 ± 0.20[b]	3.31 ± 0.31[b]
DM+Glibenclamide 60 mg/kgBW	93.73 ± 4.36[b]	3.44 ± 0.55[b]	4.65 ± 0.75[b]

Note. Values are expressed as mean ± SEM ($n = 6$); were analyzed by one-way analysis of variance followed by the Tukey's *post hoc* test.
[a]$P < 0.05$ versus normal control.
[b]$P < 0.05$ versus diabetic control.

(Table 2). However, treatment with MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW to the diabetic mice group significant reduced ($P < 0.05$) the elevated plasma glucose, insulin resistance, and improved the plasma insulin levels compared with the diabetic control group (Table 2). Furthermore, diabetic mice exhibited significantly high ($P < 0.05$) KI, plasma BUN, and CREA values compared with the normal control group. MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW treatment to diabetic mice significantly modulated ($P < 0.05$) these renal parameters (KI, BUN, and CREA) compared with the diabetic control group (Table 3).

Effect of MVR on oxidative stress/antioxidant markers

The kidney tissue MDA level considered as oxidative stress marker were significantly elevated ($P < 0.05$) in the diabetic group compared with the normal control group. On the flip side, antioxidant markers such as kidney tissue SOD and CAT were significantly lessened ($P < 0.05$) in the diabetic group compared with the normal control group. One-week MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW treatments to diabetic mice were significantly enhanced ($P < 0.05$) kidney tissue antioxidant markers (SOD, CAT) and reduced elevated oxidative stress marker

Table 3–Effect of MVR on renal function parameters in HFD/STZ-induced diabetic mice.

Group	KI (10^{-3})	BUN (mg/dL)	CREA (mg/dL)
Normal control	5.45 ± 0.12	17.75 ± 0.85	0.39 ± 0.01
Diabetic control (DM)	7.23 ± 0.35[a]	25.50 ± 2.21[a]	0.50 ± 0.03[a]
DM + MVR 100 mg/kgBW	6.54 ± 0.38[a]	18.40 ± 1.46[b]	0.45 ± 0.02[a]
DM + MVR 200 mg/kgBW	5.58 ± 0.21[a,b]	17.60 ± 0.40[b]	0.43 ± 0.01[a,b]
DM + Glibenclamide 60 mg/kgBW	5.54 ± 1.12[a,b]	17.60 ± 0.97[b]	0.42 ± 0.01[a,b]

Note. Values are expressed as mean ± SEM ($n = 6$); were analyzed by one-way analysis of variance followed by the Tukey's *post hoc* test.
[a] $P < 0.05$ versus normal control.
[b] $P < 0.05$ versus diabetic control.
KI: kidney hypertrophy; BUN: blood urea nitrogen; CREA: creatinine.

Table 4–Effect of MVR on oxidative stress/antioxidant parameters in HFD/STZ-induced diabetic mice.

Group	Kidney MDA (nmol/g protein)	Kidney SOD (U/g protein)	Kidney CAT (K/mg protein)
Normal control	94.87 ± 7.3	170.39 ± 7.87	63.03 ± 6.43
Diabetic control (DM)	481.16 ± 2.80[a]	14.97 ± 3.30[a]	41.36 ± 1.98[a]
DM + MVR 100 mg/kgBW	331.02 ± 4.99[a,b]	40.62 ± 5.08[a]	42.62 ± 2.61[a]
DM + MVR 200 mg/kgBW	271.79 ± 7.70[a,b]	66.24 ± 6.34[a,b,c]	46.20 ± 3.11
DM + Glibenclamide 60 mg/kgBW	181.10 ± 22.06[a,b,c]	134.83 ± 9.62[b]	58.22 ± 4.82[b]

Note. Values are expressed as mean ± SEM ($n = 6$); were analyzed by one-way analysis of variance followed by the Tukey's *post hoc* test.
[a] $P < 0.05$ versus normal control.
[b] $P < 0.05$ versus diabetic control.
[c] $P < 0.05$ versus dose 100 mg.
MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase.

(MDA) in kidney tissue compared with the diabetic control group (Table 4).

Effect of MVR on kidney morphology

As shown in Figure 3, the control subject (3a) showed normal kidney morphology. The glomerulus with mesangial cell, podocyte, Bowman's space, and renal tubules were clearly observed. Diabetic kidney (3b) showed structural damage of the glomerulus with no mesangial cell and podocyte, disappearance of Bowman's space, and renal tubules. MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW (Figure 3c,e) groups significantly ameliorated and restored kidney architectural damage compared with the diabetic control group (Figure 3 and Table 5).

Effect of MVR on kidney apoptosis

The effects of MVR on the kidneys apoptosis cells are shown in Figure 4(a–e). The TUNEL staining of kidney sections showed very less or no apoptosis cell in normal control (approximately 6 ± 2 apoptotic cell/kidney) compared with the diabetic control group (approximately 57 ± 5 apoptotic cell/kidney). However, acute treatments of MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW to the diabetic mice significantly reduced ($P < 0.05$) the apoptosis cell (brown color indicated by arrow sign) compared with the diabetic control group (Figure 4a,b).

Discussion

DN was considered as a major complication of diabetes in global population (Kelly et al., 2013) and its development, management, and prevention were regulated by nutrition and diet (Selcuk et al., 2012). Obesity- and/or diabetes-induced hyperglycemia is the main key factor for the development and progression of DN, which activates the various signaling pathways such as polyol, hexosamine, NF-kB, AGE, PKC, Bax/Bcl-2, and other pathways via ROS generation and lead to the insulin resistance associated with renal damage (Haq, Banday, & Bashir, 2010).

Till now, growing body of evidences published about protective effect of *G. mangostana* (fruit, rind, and isolated xanthones) against diabetes (Loo & Huang, 2007; Manaharan et al., 2012; Ryu et al., 2011; Taher, Tg Zakaria, Susanti, & Zakaria, 2016; Watanabe et al., 2018). But none of them focused on fermented extract of mangosteen rind against DN. In addition, our previous study focused the anti-glycemic and hepatoprotective effects MVR against HFD/STZ-induced T2D model (Karim, Jeenduang, & Tangpong, 2018a). We also found that isolated xanthone derivatives from *G. mangostana* treatment to HFD/STZ-induced T2D mice significantly reduced the elevated body weight, plasma glucose, KI (kidney damage indicator), kidney function parameters (BUN and CREA), and oxidative stress marker (plasma and kidney MDA) compared with diabetic control group (Karim et al., 2018b). We herein report the acute oral MVR treatments to HFD/STZ-induced T2D mice significantly ameliorated the T2D and attenuated the development of DN. In current study, MVR significantly reduced the KI, glucose level, HOMA-IR, BUN, CREA, MDA levels, and apoptotic cell with the improvement of the insulin, SOD, and CAT levels, which supported anti-DN effect.

In our *in vitro* study, aqueous extract of MVR possesses potent antioxidant activities evaluated by ABTS antioxidant capacity and DPPH scavenging assay due to the presence of phenolic compound (Gutierrez-Orozco & Failla, 2013; Zarena & Sankar, 2009). MVR from *G. mangostana* also revealed the potent *in vitro* antidiabetic activity measured by the enzymatic alpha amylase assay kit (using kinetic method). This result indicates the alpha amylase and glucosidase inhibitory activity. Because the 4,6-ethylidene-(G7)-1.4-nitrophenyl-(G1)-α1D-maltoheptaoside substrate present in alpha amylase assay kit were cleaved by alpha amylase and hydrolyzed by alpha glucosidase enzymes to form p-nitro-phenol and glucose (Junge et al., 1989), these enzymes were inhibited by MVR extract treatment.

Taher and his group found that administration of ethanolic extract of *G. mangostana* pericarp to normoglycaemic and STZ-induced diabetic rats significantly ameliorated blood glucose level, lipid profile, plasma lipid and kidney function parameters, and hepatic histological alterations; possibly via increasing the

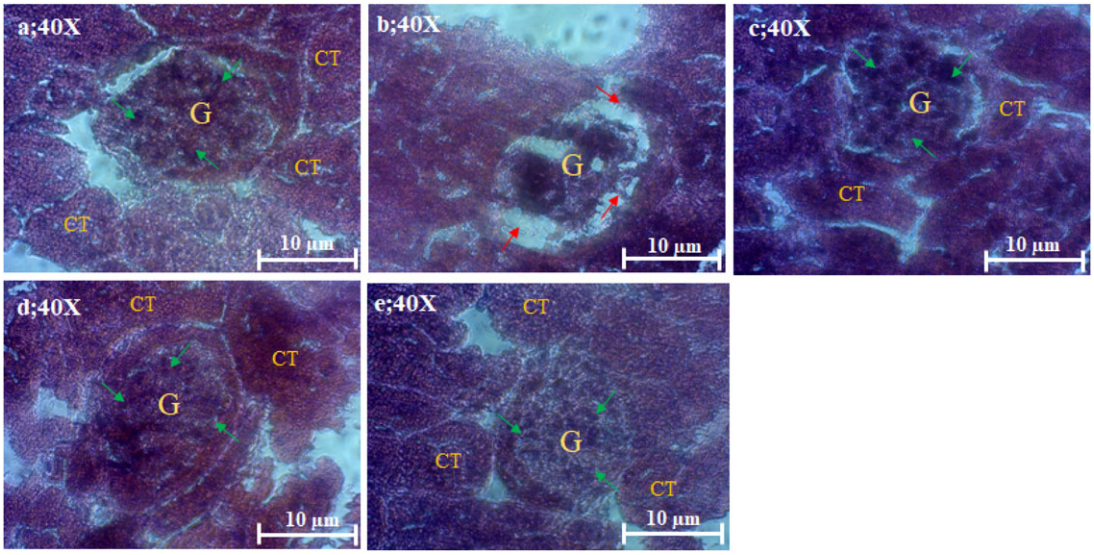

Figure 3–Histopathological changes in kidney of experimental mice. Kidney tissues were stained with H & E staining. Group I (a): Normal control, showing the typical histological structure of mice kidney. It shows normal glomerulus (G) structure, normal Bowman's space (no red arrow), normal mesangial cell and podocyte (green arrow), and normal convoluted tubules (CT); Group II (b): Diabetic control (DM), shows structural damage of kidney. It shows severe glomerulus (G) destruction, increased Bowman's space (red arrow), decreased mesangial cell and podocyte (no green arrow), and severe destruction of convoluted tubules (CT); Group III (c): DM+MVR extract (100 mg/kgBW), shows moderate improvement of glomerulus structure (G), increased mesangial cell and podocyte (green arrow), decreased Bowman's space (no red arrow), and convoluted tubules (CT) destruction; Group IV (d): DM+MVR extract (200 mg/kgBW), shows improvement of glomerulus structure (G), increased mesangial cell and podocyte (green arrow), decreased Bowman's space (no red arrow), and convoluted tubules (CT) destruction than group III (c); Group V (e): DM+Glibenclamide (60 mg/kgBW), shows normal structure of kidney. All magnification, 40×.

Table 5–Effect of MVR on kidney tissue histological evaluation in HFD/STZ-induced diabetic mice.

Group	Glomerulus structure	Mesangial cell and podocyte	Bowman's space	Convoluted tubules
Normal control	0	0	0	0
Diabetic control (DM)	+++	+++	++	+++
DM + MVR 100 mg/kgBW	++	++	+	+++
DM + MVR 200 mg/kgBW	+	+	0	+
DM + Glibenclamide 60 mg/kgBW	0	0	0	0

Note. Histological evaluation of kidney tissue ($n = 6$). Here, 0, +, ++, +++ are none, mild, moderate, severe damage of kidney organules.

number of insulin-producing β-cells (Taher et al., 2016). Tsai and team observed that α-mangostin (from mangosteen pericarp) treatment for 11 weeks significantly reduced HFD-induced hepatopathy through enhancing cellular antioxidant capacity, improving mitochondrial functions, and suppressing hepatocytes apoptosis (Tsai et al., 2016). It suggests that α-mangostin contained MVR, which may reduce the renal apoptosis through improving mitochondrial integrity and diminishing oxidative stress. A prospective randomized controlled pilot study revealed that mangosteen extract treatment to obese female patient for 26 weeks significantly improved the insulin sensitivity markers (insulin and HOMA-IR), inflammation marker (HsCRP and fibrinogen level), and lipid profile compared to control group (Watanabe et al., 2018). To the best of our knowledge, we used HFD (contain 40% fat) treatment for 5 weeks and single low dose STZ (30 mg/kgBW) to establish western T2D phenotype (Karim et al., 2018b). Chaudhari and his team used 2-weeks HFD treatment followed by single low dose STZ (35 mg/kgBW) to normal rat to induce the DN complications (Chaudhari, Bhandari, & Khanna, 2013). In consistence with previous studies, MVR extract treatments to HFD/STZ-induced diabetic mice also significantly ($P < 0.05$) reduced the OGTT/OMTT, plasma glucose, HOMA-IR, and renal markers (KI, BUN, CREA), with a concurrent increment of the insulin level compared to the diabetic control group. Zhang and team observed that hyperglycemia induced the ROS overproduction, which are associated with the decline of antioxidant defense system and increased lipid peroxidation in plasma and kidney tissue of the diabetic model (Zhang, Gao, Jin, Xu, & Chen, 2014). Acute treatment of MVR to diabetic mice significantly reduced ($P < 0.05$) the plasma and kidney tissue MDA levels with the simultaneous increase in renal antioxidant enzymes activity (SOD and CAT) compared with the diabetic control group (Sherif, 2014).

In association with biochemical data, the histological evaluation showed the severe glomerulus destruction, podocyte and mesangial cell loss, Bowman's space rupture, and convoluted tubules disappearance in diabetic control group compared with the normal control group; these results are in agreement with other studies (Elbe et al., 2015; Michael, Ganesh, & Viswanathan, 2012; Shiju, Rajesh, & Viswanathan, 2013). Oxidative stress plays a crucial role in the kidney cellular disruption and leads to vascular permeability and tissue damage (Brown, 2008). Treatments of MVR 100, 200 mg/kgBW and Glibenclamide 60 mg/kgBW significantly ameliorated the kidney architectural damage

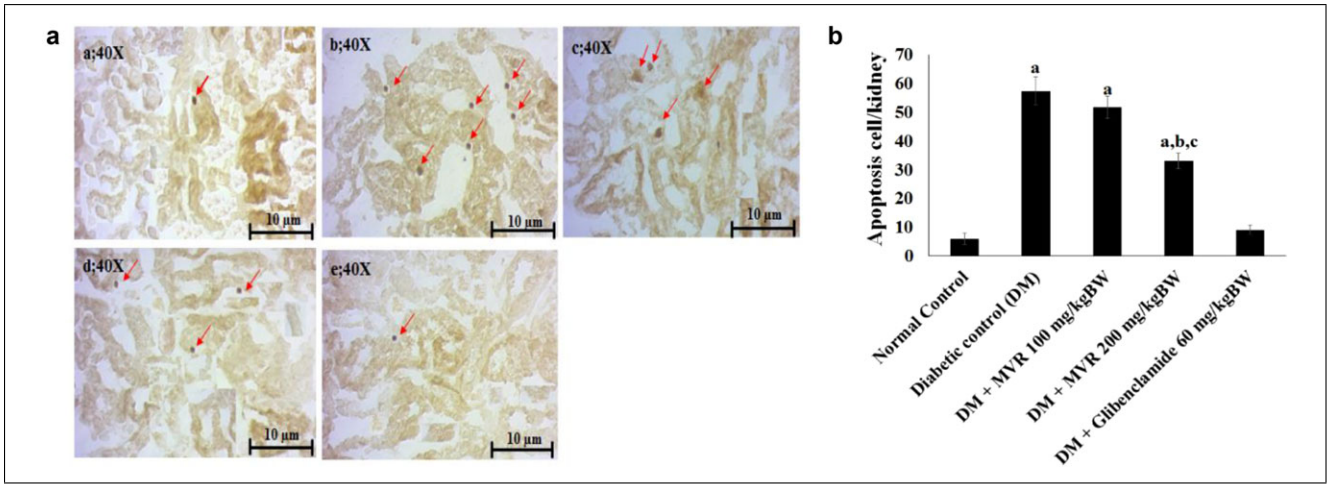

Figure 4–(a) Evaluation cellular apoptosis of experimental mice kidney by TUNEL assay. Group I (a): Normal control, showing the very few or no apoptotic cell of mice kidney; Group II (b): Diabetic control (DM), shows frequent apoptotic cell damage; Group III (c): DM+MVR extract (100 mg/kgBW), shows mild reduced of apoptotic cell; Group IV (d): DM+MVR extract (200 mg/kgBW), shows moderate reduced of apoptotic cell; Group V (e): DM+Glibenclamide (60 mg/kgBW), shows very few or no apoptotic cell of mice kidney. All magnification, 40x. (b) Number of apoptotic cell counting/kidney at 40× magnification of kidney tissue. The values were expressed as mean ± SEM ($n = 6$); and were analyzed by one-way analysis of variance followed by the Tukey's *post hoc* test. $^{a}P < 0.05$ versus normal control, $^{b}P < 0.05$ versus diabetic control, and $^{c}P < 0.05$ versus dose 100 mg.

compared with the diabetic control group in a dose-dependent fashion. Ayepola and his group revealed that isolated kolaviron biflavonoid from *Garcinia kola* seeds significantly suppressed the STZ-induced nephrotoxicity via ameliorating inflammatory cytokines (IL-1β) and renal apoptosis (TUNEL positive cell) compared to diabetic control (Ayepola, Cerf, Brooks, & Oguntibeju, 2014). MVR effects against HFD/STZ-induced DN was also confirmed by apoptosis assay (reduced the TUNEL positive cell). Diabetic group showed increased number of apoptosis nucleus (as brown staining, indicated by the arrow sign) compared with a normal control group. MVR treatments significantly ($P < 0.05$) ameliorated the HFD/STZ-induced kidney apoptosis compared with the diabetic control group either via inhibiting intrinsic or extrinsic pathways (Pal, Sinha, & Sil, 2014; Zhang et al., 2010).

Therefore, the search of new agent with minimum side effects and maximum preventive therapeutic outcome for the diabetics and associated complications is important. This is because established antidiabetic agents are associated with adverse effects (McCreight, Bailey, & Pearson, 2016; Miralles-Linares et al., 2012). Our result showed that MVR aqueous extract significantly reduced the HFD/STZ-induced hyperglycemia and renal toxicity complications of T2D mice. In addition, the dose of MVR 200 mg/kgBW showed similar effect to standard drug, Glibenclamide 60 mg/kgBW. Apart from this, MVR extract treatment also could be helpful for inflammation, allergy, bacteria, fungus, cancer, and other diseases as a result of rich polyphenolic compounds (Karim & Tangpong, 2018).

Conclusions

Taken together, acute treatment of MVR exhibited the renoprotective effect against T2D via decreasing oxidative stress and apoptosis. This protective activity could be attributed due to the presence of high percentage of polyphenolic compounds such as α-mangostin, γ-mangostin, gartanin, and so on, possibly through improving glucose level and lipid metabolism, enhancing mitochondrial integrity, diminishing oxidative stress, inhibiting lipid peroxidation process and inflammation, enhancing insulin sensitivity, and modulating several apoptosis pathways. Thus, the findings demonstrated that MVR could be a potential candidate for the management of DN owing its antioxidant and anti-hyperglycemic effects. Further studies are required to evaluate the specific antidiabetic mechanism of MVR extract. It is also required to isolate the abundant compounds from MVR extract and to perform the mechanistic studies on diabetics and associated complications.

Acknowledgments

This research was supported by the Science and Technology Research Grant 2015 (in part), Thailand Toray Science Foundation.

Authors' Contribution

Naymul Karim (NK) and Jitbanjong Tangpong (JT) together planned and designed the research. NK conducted the laboratory work in Natural Product Utilization Unit Lab, Walailak Univ. Thailand, under the supervision of JT. The manuscript preparation was done by NK, and reviewed by Md. Atiar Rahman (MAR), Lanchakon Chanudom (LC), and Montakarn Thongsom (MT).

Conflict of Interest

The authors declare no conflict of interest in this paper.

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