Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2018, Vol. 68, No. 2, pp. 163–169
DOI: 10.1515/pjfns-2017-0018
http://journal.pan.olsztyn.pl

Original research article
Section: Food Chemistry

Anti-Glycemic and Anti-Hepatotoxic Effects of Mangosteen Vinegar Rind from *Garcinia mangostana* Against HFD/STZ-Induced Type II Diabetes in Mice

*Naymul Karim, Nutjaree Jeenduang, Jitbanjong Tangpong**

Biomedical Sciences, School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat 80161, Thailand

Key words: diabetes, high-fat diet, hepatotoxicity, MVR, oxidative damage

This study focuses on anti-glycemic and anti-hepatotoxic effects of mangosteen vinegar rind (MVR) on five weeks high-fat diet (HFD) / single dose streptozotocin (STZ) 30 mg/kg BW induced male ICR diabetic mice. Mice were randomly divided into five groups (n=6), normal control, diabetic control, and diabetic groups treated with MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW for one week. After the treatment, lipid profile, glycogen and bilirubin contents, oxidative damage (malondialdehyde, MDA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) were measured in plasma and/or liver tissues. MVR and glibenclamide treatment to HFD/STZ-induced diabetic mice significantly reduced their plasma glucose, plasma lipid profile, and hepatic lipid profile ($P<0.05$). Increased hepatic glycogen content indicates improvement of insulin sensitivity. Moreover, oxidative damage markers were ameliorated in MVR- and glibenclamide-treated groups compared to the diabetic control group. MVR with phenolic compounds content of 75 mg GAE/g dry weight and antioxidant potential of 303 mmol/L Trolox/g dry weight acted as a hepatoprotective agent against oxidative damage.

INTRODUCTION

Diabetes is the most common epidemic disease worldwide. In the world, approximately 422 million adult people (up to 2014) are living with diabetes mellitus. Type II diabetes is much more common than type I. Diabetes type II not only affects the adult population, but also children [WHO, 2016]. Diabetes is a metabolic disorder, which is correlated with abnormalities of glucose, lipid and protein homeostasis [Van den Berghe *et al.,* 2006]. High glucose levels generate reactive oxygen species (ROS) in the body *via* several pathways, such as glucose autoxidation, the polyol pathway and production of advanced glycation end products [Bonnefont-Rousselot, 2002]. Increased ROS production leads to cellular and organ damage including the liver, kidney and pancreas by lipid peroxidation and reduced antioxidant enzyme activity [Das & Sil, 2012]. The liver is a large organ of the body which plays a major role in lipid metabolism, glucose homeostasis and stores glucose as glycogen [Nguyen *et al*., 2008]. Increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are liver injury biomarkers and are higher in diabetics [Sivakrishnan & Kottaimuthu, 2014]. Liver plays a significant role in blood glucose homeostasis *via* uptaking elevated concentration of glucose from blood and its storage as glycogen; and additionally produces glucose for circulation by glycogenolysis under starvation conditions [Sherwin, 1980]. In type II diabetes, glucose uptake decreases *via* a glucose transporter due to insulin resistance, and glucose output is increased ultimately decreasing glycogen content [Wilcox, 2005]. There is an available medication for type II diabetics such as metformin which has been reported to have an adverse effect on liver disease [Miralles-Linares *et al.*, 2012]. It is important to find out which alternative therapeutic and natural herbs are the best and with fewest adverse effects [Pandey *et al.*, 2011]. Phenolic compounds have potent antioxidant capacity and can scavenge generated ROS from our body. Phenolic compounds are found in many natural herbs and are a source of alternative medicine for diabetes and other metabolic diseases [Pandey & Rizvi, 2009]. A previous study has proved that aqueous extract of mangosteen vinegar rind (MVR) from *Garcinia mangostana* is rich in polyphenolic compounds and possesses antioxidant activity [Phyu & Tangpong, 2014].

The aim of this study was to investigate the anti-glycemic and anti-hepatoprotective effects of MVR against HFD/STZ-induced diabetes in mice.

MATERIALS AND METHODS

Chemicals and reagents

Mangosteen vinegar rind (MVR), which is a one-year fermented pure rind extract from *Garcinia mangostana*, was supplied from Asia & Pacific Quality Trade Co., Ltd. (Bangkok Office), Thailand. MVR contains 69.01% alpha mangosteen, 17.85% gamma mangosteen, 4.13% gartanin, 2.95% 8-deoxygartanin, 2.84% garcinon E, and 3.22% other xanthones.

* Corresponding Author: E-mail: njibjoy@yahoo.com (J. Tanpong)

This product was freeze dried under vacuum at -80°C for 18 h using an evaporator and the % yield was calculated. Analytical grade chemicals were used for analysis which were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Merck & Co. (Germany) and Millipore Corporation (Billerica, MA, USA) while glibenclamide was purchased from the government pharmaceutical organization (GPO, Thailand).

Total phenolics content and total antioxidant capacity of MVR

Total phenolics content of MVR was evaluated by the Folin-Ciocalteu's method [Kaisoon *et al.,* 2011]. Briefly, 12.5 μL of MVR of different concentrations (0.1–1.0 mg/mL) and the control (water, instead of MVR) were added to a 96-well microplate followed by 12.5 μL of Folin-Ciocalteu's phenol reagent. After 5 min, 125 μL of a 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture, which was left for 30 min. Then, its absorbance was recorded at 765 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA). Gallic acid (0–100 mg/L) was used as a standard. The total phenolics content was calculated on the basis of response linear regression obtained from the curve of the standard and expressed as the gallic acid equivalents per g dry weight of MVR (mg GAE/g dry weight).

Total antioxidant capacity of MVR was determined by the ABTS assay [Re *el al.,* 1999]. $ABTS^{\bullet+}$ was produced by reacting 7 mmol/L ABTS in H_2O with 4.9 mmol/L potassium persulfate ($K_2S_2O_8$), stored in the dark for 12–18 h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750±0.025 at 734 nm. Briefly, 180 μL of the $ABTS^{\bullet+}$ solution was added to 20 μL of different concentrations of MVR (0.1–1.0 mg/mL). The absorbance was recorded at 734 nm and the extent of decolonization was calculated as a percentage reduction in absorbance. Different concentrations of Trolox were used for constructing the standard curve, and the total antioxidant capacity was expressed as mmol/L of Trolox equivalent per gram of dry weight (mmol/L Trolox/g dry weight).

Maintenance of animals

Fifty-four ICR adult male mice (6 weeks old, 25–30 g) were purchased from the National Laboratory Animal Center, University, Salaya district, Nakhon Pathom. The animals were allowed access to water and food *ad libitum* one week before the start of the treatment with a constant room temperature of 23±2°C, relative humidity of 55±10 %, ventilation and a 12 h light/dark cycle [Jarukamjorn *et al.*, 2011]. Experimental animal protocols were approved by the Animal Care and Use Committee of the Walailak University (No.002/2015).

Study design

Mice were divided into two groups, the control group (n=6) received a normal diet while the groups fed a high-fat diet (n=24) received high-fat food (60% normal diet, 12% lard oil, 12% sugar, 8% yolk powder, 6% peanut powder, 1% milk powder, and 1% water) for up to five weeks. After five weeks, diabetes was induced by intraperitoneal streptozotocin injection (STZ, 30 mg/kg BW) to overnight fasted high-fat fed mice (diabetic mice, DM). MVR at doses of 100, 200 mg/kg BW and glibenclamide at 60 mg/kg BW were orally administered at 8.00–9.00 am for one week. The experiments were broken into independent groups (n=6 per group) as follows:

- Group 1: Untreated normal control (received normal saline)
- Group 2: Diabetic control (received normal saline)
- Group 3: DM+ MVR (100 mg/kg BW)
- Group 4: DM + MVR (200 mg/kg BW)
- Group 5: DM+ glibenclamide (60 mg/kg BW).

Sample collection

After the treatment, mice from all groups were fasted overnight and anesthetized by sodium nembutal (65 mg/kg BW). Blood was obtained *via* a left ventricular puncture and perfused with ice-cold saline, pH 7.4. The liver was collected and preserved at -30°C for further analysis. The liver tissue was homogenized in cold 0.1% TCA solution, 10% $HClO_3$ and PBS, pH 7.4, using protease inhibitors (leupeptin, pepstatin, and aprotinin) prior to centrifugation at 13,500×g for 15 min at 4°C, and the supernatant was separated for analysis.

Biochemical assay

Plasma glucose, lipid profile (triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), liver function test (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin) were examined using commercially automated chemicals from Biosystems (Costa Brava, Barcelona), Stanbio Laboratory (Boerne, USA) and Thermo Scientific (Waltham, MA USA) using photometric methods. Lipids were extracted from the liver tissue using the Bligh & Dyer extraction method [Bligh & Dyer, 1959]. Afterwards, 0.4 mL of extracted lipids were placed in a glass tube and 0.5 mL of 2% triton X-100 in chloroform was added. The mixture was evaporated in the dryer at 55–60°C and resolved in 0.5 mL of deionized water. Then, the sample was incubated at 37°C for 15 min using a shaking water bath. The contents of triglycerides and cholesterol with its fractions were measured using a Stanbio Laboratory (Boerne, USA) reagent kit [Carr *et al.*, 1993].

Determination of oxidative stress and antioxidant markers

The malondialdehyde (MDA) levels of plasma and liver tissue were measured as a lipid peroxidation marker according to previously described methods [Ceci *et al.*, 2014; Goulart *et al.*, 2005]. The antioxidant enzyme defense system markers, superoxide dismutase (SOD) and catalase (CAT), from the liver tissue were measured by the pyrogallol autoxidation [Marklund & Marklund, 1974] and H_2O_2 decomposition methods [Takahara *et al*., 1960].

Determination of liver tissue glycogen content

Liver tissue glycogen was extracted following Bennett *et al.* [2007] with a slight modification. Concisely, 100 mg of liver were homogenized in 1 mL of 10% $HClO_3$ and sonicated for 1 min. The mixture was centrifuged at 13,500×g for 15 min

and separated from the supernatant into new tubes. The resultant pellet was homogenized again in 1 mL of 10% $HClO_3$ using a sonicator for 1 min. After centrifugation at 13,500×g for 15 min, the supernatant was added to the previous supernatant. Then, 2.5 mL of ethanol was properly mixed with the supernatant and centrifuged at 3000×g for 15 min. The supernatant was carefully discarded and the glycogen was resolved in 1 mL of distilled water. The total glycogen content was measured following the phenolsulfuric acid method using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA) [Bennett *et al.*, 2007; Masuko *et al.*, 2005].

Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM); differences were considered to be statistically significant at $P<0.05$. Data were analyzed by one-way analysis of variance (One-way ANOVA) and multiple comparisons of groups were done by Tukey's post hoc test using a commercially-available statistic software package (SPSS for Windows, V. 17.0 Chicago, USA).

RESULTS

Total phenolic content and total antioxidant capacity of MVR

The content of total phenolics of MVR was 75±1.7 mg GAE/g dry weight. MVR was characterized by the scavenging activity against $ABTS^{\bullet+}$. Total antioxidant capacity of MVR was 303±20 mmol/L Trolox /g dry weight. The antioxidant capacity of rind extract is comparatively higher that of the other pants of mangosteen [Lim *et al*., 2013].

Effect of MVR on mice body weight and plasma glucose level

Mice were fed with HFD for five weeks and a single dose STZ (IP) 30 mg/kg BW led to significantly ($P<0.05$) increased mice body weight compared to the normal control. However, one-week treatments were able to reduce HFD-induced mice body weight (Table 1). Similarly, mice belonging to HFD/STZ 30 mg/kg BW group had a significantly ($P<0.05$) higher plasma glucose than the control group. MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW treatments led to the diabetic mice having significantly ($P<0.05$) reduced glucose levels as shown in Figure 1.

TABLE 1. Effect of mango vinegar rind (MVR) on mice body weight (BW) before and after treatment.

Group	Before	After	Weight gain (%)
Normal control	41±0.7	42±0.7	2.20
Diabetic control	51±2.9[a]	51±1.7[a]	1.50
DM+MVR 100 mg/kg BW	48±1.8[a]	48±1.2[a]	-0.42
DM+MVR 200 mg/kg BW	50±1.0[a]	50±1.1[a]	-0.04
DM+glibenclamide 60 mg/kg BW	50±2.0[a]	48±1.8[a]	-4.62

DM – diabetic mice. Data are expressed as mean ± SEM (n = 6). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Turkey's post hoc test. [a]$P<0.05$ *versus* Normal control.

Effect of MVR on glycogen content

Liver glycogen storage showed less tissue resistance. In this study, the diabetic control group had a significantly ($P<0.05$) lower glycogen content in their liver tissue compared to the normal control group. However, MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW treatments showed a significantly ($P<0.05$) improved glycogen content in the diabetic mice, which was indicative of improved insulin sensitivity compared to the diabetic control (Figure 2).

Effect of MVR on plasma and hepatic lipid profile

In comparison with the normal control group, the HFD/STZ-induced diabetic groups showed significantly ($P<0.05$) higher TC, TG, LDL levels and lower HDL level. Treatment

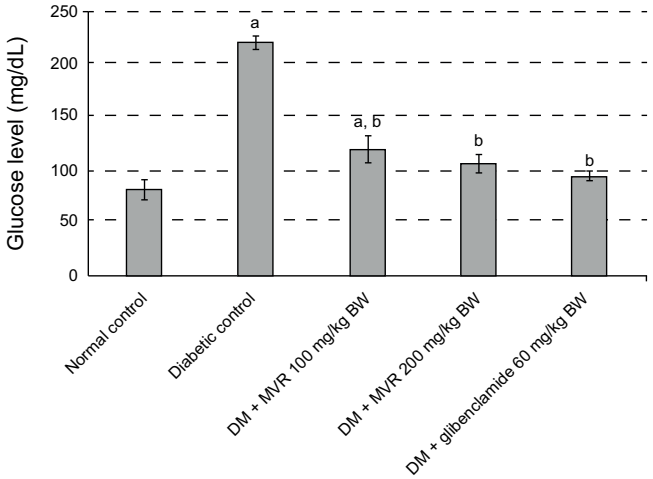

FIGURE 1. Glucose level in HFD/STZ induced type 2 diabetic mice (DM) model. Data are expressed as mean ± SEM (n = 6). [a]$P<0.05$ *versus* Normal control; [b]$P<0.05$ *versus* Diabetic control.

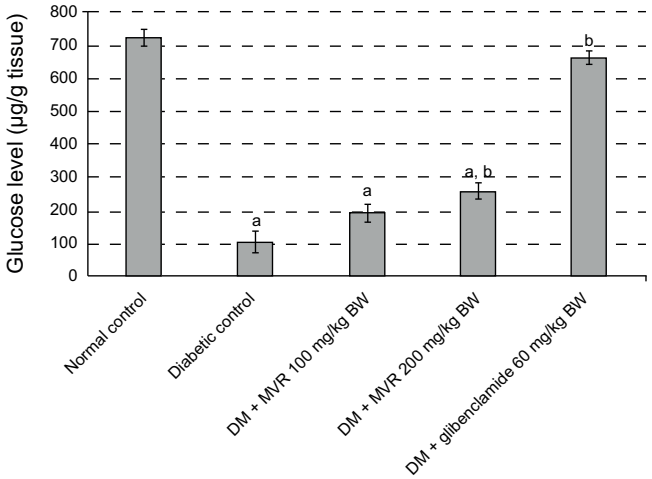

FIGURE 2. Glycogen content in diabetic mice (DM) treated with MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW. Data are expressed as mean ± SEM (n = 6). [a]$P<0.05$ *versus* Normal control; [b]$P<0.05$ *versus* Diabetic control.

TABLE 2. Effects of mangosteen vinegar rind (MVR) on plasma and hepatic lipid profile of mice model.

Group	Plasma				Liver tissue	
	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TC (mg/dL)	TG (mg/dL)
Normal control	120±8.3	48±3.3	34±2.2	74±4.5	175±5.8	67±7.8
Diabetic control	240±7.6[a]	94±2.2[a]	23±0.7[a]	198±7.3[a]	227±1.3[a]	202±9.4[a]
DM+MVR 100 mg/kg BW	225±3.6[a]	68±1.0[a,b]	25±0.7[a]	186±3.3[a]	222±5.2[a]	128±4.2[a,b]
DM+MVR 200 mg/kg BW	180±5.1[a,b,c]	60±4.7[b]	28±0.7[b]	140±5.5[a,b,c]	212±7.0[a,b]	103±4.6[a,b,c]
DM+glibenclamide 60 mg/kg BW	132±6.9[b]	53±3.7[b]	34±1.0[b]	88±7.3[b]	180±7.7[b]	74±4.7[b]

DM – diabetic mice; TC – total cholesterol; TG – total triglyceride; HDL – high density lipoprotein; LDL – low density lipoprotein. Data are expressed as mean ± SEM (n = 6). [a]P<0.05 *versus* Normal control; [b]P<0.05 *versus* Diabetic control; [c]P<0.05 *versus* MVR-treated at dose 100 mg/kg BW.

TABLE 3. Effect of mangosteen vinegar rind (MVR) on liver function markers of mice model.

Group	ALT (U/L)	AST (U/L)
Normal control	36±3.8	57±2.3
Diabetic control	86±5.8[a]	96±3.1[a]
DM+MVR 100 mg/kg BW	56±3.1[a,b]	83±6.9[a]
DM+MVR 200 mg/kg BW	51±1.7[b]	69±1.7[b]
DM+glibenclamide 60 mg/kg BW	39±2.5[b]	61±0.9[b]

DM – diabetic mice; ALT – alanine aminotransferase; AST – aspartate aminotransferase. Data are expressed as mean ± SEM (n = 6). [a]P<0.05 *versus* Normal control; [b]P<0.05 *versus* Diabetic control.

with MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW to the diabetic mice significantly (P<0.05) improved the lipid profile in plasma and liver tissue (Table 2).

Effect of MVR on plasma liver function test

The liver function markers (ALT and AST) of male ICR mouse were consistently significantly (P<0.05) higher than in the normal control group (Table 3). When compared with the HFD/STZ-induced diabetic group, MVR 100, 200 mg/kg BW and glibenclamide 60 mg/kg BW treatments significantly (P<0.05) attenuated the hepatocellular damage markers ALT and AST.

Effect of MVR on oxidative stress marker and antioxidant enzymes activity

The lipid peroxidation of plasma and liver tissue reported as the malondialdehyde content (MDA) was significantly (P<0.05) higher in the diabetic group compared to the normal control group. Moreover, the bilirubin level in plasma and SOD and CAT activities in liver tissue were significantly (P<0.05) lower in the diabetic group compared to the normal control group. However, MVR and glibenclamide significantly (P<0.05) improved both antioxidant enzymes activity and MDA levels in plasma and liver tissue compared to the diabetic control group as shown in Table 4.

DISCUSSION

The present study provides evidence that MVR shows remarkable anti-glycemic and anti-hepatotoxic effects by improving plasma glucose levels, lipid metabolism, hepatic glycogen content and hepatic antioxidant systems in HFD/STZ-induced type II diabetic mice. These mice had impaired insulin sensitivity with greater insulin secretion to compensate for elevated blood glucose levels similar to the obese human phenotypic condition. A low dose of streptozotocin partially destroys the pancreatic beta cell and reduces insulin secretion. Both HFD and STZ produced non-genetic and a comparatively less expensive type II diabetic model, giving effects similar to type II diabetes in human patients [Gilbert *et al.*, 2011].

In vitro and *in vivo* studies have proven that *Garcinia mangostana* is characterized by antioxidative and cytoprotective activities due to the presence of phenolic compounds [Phyu & Tangpong, 2014; Sattayasai *et al.,* 2013]. Several studies found that the polyphenolic compounds found in *Aegle marmelos*, *Commiphora mukul*, green tea, cinnamon and ginger have hepatoprotective properties in different animal models [Elgawish *et al.*, 2015; Ismail, 2014; Ramesh *et al.*, 2015; Suriyamoorthy *et al.*, 2014]. HFD/STZ induction produces high glucose levels in diabetic mice [Li *et al.*, 2014], leading to oxidative damage and induced hepatotoxicity [Berdja *et al.,* 2016; Cordero-Herrera *et al.*, 2015]. In this study, HFD/STZ-induced diabetic model had significantly (P<0.05) higher glucose levels than the normal control group. However, the MVR is able to reduce plasma glucose significantly (P<0.05) compared to the diabetic control group (Figure 1) due to the presence of phenolics which show free radical scavenging activity. Morin, a flavonoid was shown to display the antioxidant activity against high-glucose-induced oxidative stress by mediating apoptosis in primary rat hepatocytes [Kapoor & Kakkar, 2012].

Additionally, long term high-fat feeding initiated dyslipidemia and ROS generation in the HFD/STZ-induced diabetic mouse model. Phenolic compounds of MVR, likewise in the previous study concerning vanillic acid [Chang *et al.*, 2015] and phenolic-rich extract of white ginseng [Lee *et al.*, 2013], showed hypolipidemic and antioxidant

TABLE 4. Effect of mangosteen vinegar rind (MVR) on oxidative stress markers and antioxidant levels of mice model.

Group	Plasma		Liver tissue		
	Bilirubin (mg/dL)	MDA (nM/ml)	MDA (nM/g protein)	SOD (U/g Protein)	CAT (K/mg protein)
Normal control	0.51±0.08	2±0.1	35±2.8	64±8.6	85±5.4
Diabetic control	0.22±0.02[a]	7±0.3[a]	183±3.6[a]	22±2.5[a]	27±3.4[a]
DM+MVR 100 mg/kg BW	0.31±0.04	4±0.3[a,b]	118±8.3[a,b]	26±7.2[a]	51±4.2[a,b]
DM+MVR 200 mg/kg BW	0.33±0.03[b]	3±0.2[a,b,c]	74±8.7[a,b,c]	40±4.2[b]	67±2.8[b,c]
DM+glibenclamide 60 mg/kg BW	0.46±0.04[b]	2±0.2[b]	56±7.6[a,b]	55±5.0[b]	77±1.5[b]

DM – diabetic mice; MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase. Data are expressed as mean ± SEM ($n = 6$). [a]$P<0.05$ *versus* Normal control; [b]$P<0.05$ *versus* Diabetic control; [c]$P<0.05$ *versus* MVR-treated at dose 100 mg/kg BW.

properties and were reducing hyperlipidemic markers (TC, TG, LDL), and increasing plasma HDL levels. Moreover, the oxidative stress marker (MDA) and activity of antioxidant enzymes SOD and CAT were improved. Consequently, we found that MVR significantly ($P<0.05$) ameliorated the plasma and hepatic lipid profile (Table 2) and oxidative damage (Table 4) in the HFD/STZ-induced diabetic type II model ($P<0.05$) compared to the untreated diabetic control group. We also found that MVR increased the glycogen content in the liver tissue of HFD/STZ diabetic mice indicating increased insulin sensitivity. High glucose levels were stored in the liver and muscle tissue with the help of insulin using a glucose transporter. As a result, the hepatic glycogen content in the HFD/STZ-induced diabetic control group significantly reduced ($P<0.05$) compared to the normal control group, which was indicative of tissue insulin resistance [Bhandari *et al.*, 2013]. Treatment with MVR and glibenclamide would be able to restore glycogen content indicating tissue glucose uptake and hepatic glucose production [Moore *et al.*, 2012].

Furthermore, AST and ALT, the enzymatic markers of liver cell damage, showed increased activity under these type II diabetic conditions compared to the normal control group. Furthermore, the plasma total bilirubin followed the same pattern with the hepatotoxic enzymatic markers (AST and ALT) and antioxidant enzyme markers (SOD, CAT); though all the values were within the normal range (Table 4). MVR-treated HFD/STZ diabetic mice showed higher bilirubin levels than the non-treated HFD/STZ diabetic mice. Recently, bilirubin levels have been reported to increase insulin sensitivity by regulating metabolism and reducing cholesterol level [Liu *et al.*, 2015]. Increased bilirubin levels are associated with the antioxidant enzyme hemeoxygenase-1 activity and may explain the versatile cellular protection [McCarty *et al.*, 2013]. Taken together, MVR may be able to improve liver function by maintaining the AST and ALT levels, and improving bilirubin levels with complicated type II diabetic patients and our HFD/STZ-induced diabetes type II model [Idris *et al.*, 2011]. The present study provides evidence that MVR acts as a hepatoprotective agent against oxidative damage, which is at least partly due to its phenolic compounds and antioxidant capacity.

CONCLUSION

In the present study we evaluated the anti-glycemic and anti-hepatotoxic effects of MVR. We used glibenclamide as a standard to compare the anti-glycemic and anti-hepatotoxic effects of MVR on diabetic mice. MVR from *Garcinia mangostana* and glibenclamide treatments improved the levels of glucose, hepatic glycogen, lipid profile, oxidative stress, antioxidant enzyme activity and liver function biomarkers of HFD/STZ-induced type II diabetic mouse models compared to untreated diabetic control group. Besides this, MVR high dose showed comparatively potent effects than the MVR low dose. The presence of phenolic compound in MVR extract exhibited antioxidant capacity, evaluated by *in vitro* study. It can protect tissues from cellular oxidative damage by scavenging hyperglycemia-induced free radicals and improve tissue glucose uptake. MVR may be a potential dietary supplement for hyperglycemia and hyperlipidemia patients. Further studies are still needed to clarify the underlying mechanisms.

RESEARCH FUNDING

This study was supported by the Thailand Toray Science Foundation (TTSF) Research Grants and in part by the Walailak University, Thailand.

CONFLICT OF INTERESTS

All authors declare no conflict of interest.

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Submitted: 6 October 2016. Revised: 13 February and 17 March 2017. Accepted: 27 April 2017. Published on-line: 6 November 2017.