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

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**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, 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 Bergh et al., 2006]. High glucose levels generate reactive oxygen species (ROS) in the body via several pathways, such as glucose autoxidation, the polyl 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 diabetes 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.

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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 (Na2CO3) 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 et al., 1999]. ABTS+• was produced by reacting 7 mmol/L ABTS in H2O with 4.9 mmol/L potassium persulfate (K2S2O8), stored in the dark for 12–18 h. The ABTS+• solution was diluted to give an absorbance of 0.705±0.025 at 734 nm. Briefly, 180 μL of the ABTS+• solution was added to 20 μL of different concentrations of MVR (0.1–10.0 mg/mL). The absorbance was recorded at 734 nm and the extent of decolorization 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 experiment 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% HClO4, 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; Goulat 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 H2O2 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% HClO4, 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% HClO₃ 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⁺⁻. 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 parts 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.

**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). *P<0.05 versus Normal control; **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). *P<0.05 versus Normal control; **P<0.05 versus Diabetic control.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>Weight gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>41±0.7</td>
<td>42±0.7</td>
<td>2.20</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>51±2.9*</td>
<td>51±1.7*</td>
<td>1.50</td>
</tr>
<tr>
<td>DM + MVR 100 mg/kg BW</td>
<td>48±1.8*</td>
<td>48±1.2*</td>
<td>-0.42</td>
</tr>
<tr>
<td>DM + MVR 200 mg/kg BW</td>
<td>50±1.0*</td>
<td>50±1.1*</td>
<td>-0.04</td>
</tr>
<tr>
<td>DM + glibenclamide 60 mg/kg BW</td>
<td>50±2.0*</td>
<td>48±1.8*</td>
<td>-4.62</td>
</tr>
</tbody>
</table>

DM – diabetic mice. Data are expressed as mean ± SEM (n = 6). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey’s post hoc test. *P<0.05 versus Normal control.
TABLE 2. Effects of mangosteen vinegar rind (MVR) on plasma and hepatic lipid profile of mice model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Liver tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC (mg/dL)</td>
<td>TG (mg/dL)</td>
</tr>
<tr>
<td>Normal control</td>
<td>120±8.3</td>
<td>48±3.3</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>240±7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+MVR 100 mg/kg BW</td>
<td>225±3.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>68±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+MVR 200 mg/kg BW</td>
<td>180±5.1&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>60±4.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+glibenclamide 60 mg/kg BW</td>
<td>132±6.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53±3.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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). *P<0.05 versus Normal control; **P<0.05 versus Diabetic control; ***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.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>36±3.8</td>
<td>57±2.3</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>86±5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+MVR 100 mg/kg BW</td>
<td>56±3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83±6.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+MVR 200 mg/kg BW</td>
<td>51±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM+glibenclamide 60 mg/kg BW</td>
<td>39±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

DM – diabetic mice; ALT – alanine aminotransferase; AST – aspartate aminotransferase. Data are expressed as mean ± SEM (n = 6). *P<0.05 versus Normal control; **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; Sattayasaifai 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; Suriyaamoorthy et al., 2014]. HFD/STZ induction produces high glucose levels in diabetic mice [Li et al., 2014], leading to oxidative damage and induced hepatotoxicity [Berda 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Liver tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bilirubin (mg/dL)</td>
<td>MDA (nM/ml)</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.51±0.08</td>
<td>2±0.1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.22±0.02</td>
<td>7±0.3</td>
</tr>
<tr>
<td>DM+MVR 100 mg/kg BW</td>
<td>0.31±0.04</td>
<td>4±0.3</td>
</tr>
<tr>
<td>DM+MVR 200 mg/kg BW</td>
<td>0.33±0.03</td>
<td>3±0.2</td>
</tr>
<tr>
<td>DM+glibenclamide 60 mg/kg BW</td>
<td>0.46±0.04</td>
<td>2±0.2</td>
</tr>
</tbody>
</table>

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

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.

**CONCLUSION**

CONFLICT OF INTERESTS

All authors declare no conflict of interest.

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