Hepatoprotective and Antioxidant Activities of the Essential Oil from Maqian (Zanthoxylum myriacanthum var. pubescens) in Streptozotocin-induced Diabetic Mice

Mahmoud Dahab\textsuperscript{1,2,a}, You-Kai XU\textsuperscript{1,b} and Ping ZHANG\textsuperscript{1,c,*}

\textsuperscript{1}Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna, Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, PR China
\textsuperscript{2}University of Chinese Academy of Sciences, Beijing 100049, PR China
\textsuperscript{a}mahmouddahab4@gmail.com, \textsuperscript{b}xyk@xtbg.ac.cn, \textsuperscript{c}zhangping@xtbg.org.cn

*Corresponding author

Keywords: Zanthoxylum myriacanthum var. pubescens, Essential oil, Hepatoprotective, Antioxidant.

Abstract. Background & objectives: Diabetes is a metabolic disorder and it can affect many organs and different types of antioxidant enzymes through increased production of reactive oxygen species (ROS). A few essential oils have been reported to have antioxidant and antidiabetic effects. The objective of our present study is to explore the anti-diabetic potential of Maqian essential oil (MQEO). Aim: The aim of this study was to evaluate the hepatoprotective and antioxidant effect of MQEO in streptozotocin (STZ)-induced diabetes in mice. Method: Diabetes was induced in male ICR mice with a single intraperitoneal injection of STZ (200 mg/kg body weight). MQEO was administered orally for 7 days. At the end of experiment liver was weighed and enzymatic and non-enzymatic oxidative stress parameters were measured, and part of liver was examined by hematoxylin/eosin staining. Results: MQEO treatment resulted in significantly decreased MDA level and increased antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in STZ-diabetic mice. Histology examination showed severe pathology changes in diabetic mice including congestion of sinusoids and focal necrosis in liver and MQEO restored tissue architecture almost as in the normal control mice. Conclusion: MQEO has pronounced antioxidant effect and could reverse liver damages in STZ-induced diabetic mice. It could be useful for controlling diabetic complications.

Introduction

Diabetes is characterized by high blood glucose levels as a result of insufficient insulin for the body’s needs. Streptozotocin (STZ) is widely used to induce experimental diabetes in animals and it is known to induce reactive oxygen free radicals and thereby damaging the pancreas and other organs including liver and kidney [1].

Many plants derived hypoglycemic therapies have the ability to restore the antioxidant defense systems. Herbal medicines have long been actively investigated in treating DM because they are considered to have little toxicity and thus suitable for long term use [2]. Their reported beneficial effects on diabetes are often multi-targets and thus may be better choices for precision medicine than combinations of various standard drugs. However, few studies on herbal therapy have investigated their effects on diabetic hepatopathy.
Maqian (*Zanthoxylum myriacanthum* var. pubescens Huang) is traditionally consumed by Dai ethnic villagers in Xishuangbanna Yunnan Province, China, and is used as indigenous remedy for healing against digestive disorders and relief swelling pain. Maqian is frequently used as a spice for roasting and salting meat, boiling fish and cooking vegetable [3-5]. Many *Zanthoxylum* species used as medicines and spices around the world have been reported to have antioxidant activity and hepatoprotective activity [6-8]. Therefore, the purpose of this study was to assess the antioxidant activity and hepatoprotective effect of MQEO in STZ-induced diabetic mice.

**Materials and Methods**

**Animals**

Male ICR mice, 4 to 5 week-old, were obtained from Vital River Laboratories-China. All mice were housed in pathogen-free conditions used individually ventilated cages (IVC) system, housed with temperature (22±2°C) and relative humidity (55 ± 5%) under 12 light/dark cycle. All experimental procedures were approved and performed in accordance with the guidelines of Institutional Animal Care and Use Committee of Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences.

**Plant Material, Preparation of the Essential Oil from Fruits of Maqian**

Fresh fruits of Maqian (*Zanthoxylum myriacanthum* var) were collected and air dried under ventilated condition. MQEO was extracted using the standard method and chemical analysis was performed by gas chromatography–mass spectrometry.

**Experimental Design**

After acclimatized period (7 days), diabetes was induced in the mice by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (200 mg/kg of body weight) and orally administered for 7 days. Animals were randomly divided into four groups: the normal group, STZ group, STZ+MQEO 150 mg kg\(^{-1}\) b.w., and STZ+saxaglipitin group. The blood glucose concentration in blood collected from the tail vein was measured by using a glucometer (Bayer Health Care LLC, Mishawaka, USA).

**Biochemical Assays**

After 7 days of treatment, liver was collected and stored at -80°C until further analysis. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione-S-transferase, the malondialdehyde and vitamin C levels in liver were measured with the commercial kits (Nanjing Jian Cheng Bioengineering Institute, China) according to the manufacturer’s instructions.

**Histological Analysis**

Liver tissues were fixed in 10% neutral formalin solution. Paraffin-embedded tissue sections and stained with hematoxylin and eosin (H&E).

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± SEM for six mice in each group. Differences in the mean values among different groups were analyzed by one-way analysis of
variance (ANOVA) followed by Tukey’s test, and those with a p value < 0.05 were considered statistically significant.

Results

Effect of MQEO on Body, Liver Weights and Glucose Level

Body weight significantly declined in STZ diabetic group compared to the normal control group on day 7 after the STZ injection. In contrast, no significant different of liver weight was observed in all groups (Table 1). The STZ induced diabetic mice showed increased glucose level, MQEO restored the glucose levels in diabetic mice to as in normal control (Fig. 1).

Table 1. Effect of MQEO on body and liver weights of mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight(g)</th>
<th>% change in B/W of initial B/W(g)</th>
<th>Liver/BW %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>23.4 ± 0.69</td>
<td>27.6 ± 1.06</td>
<td>5.5 ± 0.11</td>
</tr>
<tr>
<td>STZ</td>
<td>22.8 ± 0.79</td>
<td>15.9 ± 1.3*</td>
<td>6.6 ± 0.27</td>
</tr>
<tr>
<td>STZ + MQEO 150 mg kg(^{-1})</td>
<td>24.1 ± 0.4</td>
<td>22.8 ± 0.95*</td>
<td>6.5 ± 0.14</td>
</tr>
<tr>
<td>STZ + saxagliptin</td>
<td>26.8 ± 0.46</td>
<td>27.07 ± 1.9*</td>
<td>6.5 ± 0.26</td>
</tr>
</tbody>
</table>

Note: values are represented as mean ± SEM, n=5. * p<0.05 Vs Normal, #p<0.05 Vs STZ

Effect of MQEO on Oxidative Stress Parameters in Liver

Changes in enzymatic oxidative stress parameters in liver tissues are presented in Table 2. The activities of hepatic SOD, CAT, GPx and GST in experimental mice enzymatic antioxidants significantly decreased in the diabetic mice when compared to those of the
normal group, whereas they were restored with the treatment with MQEO or saxagliptin when compared to the diabetic group. The diabetic group of mice showed significant reduction in the levels of vitamin C in liver tissues in comparison to normal control. Conversely, treatment of diabetic mice with MQEO significantly increased vitamin C levels. The MDA content increased significantly in the STZ diabetic group compared to the normal control group. Treatment of diabetic mice with MQEO for 7 days resulted in a marked decrease in tissue MDA (Fig. 2). MQEO was as effective as saxagliptin in restoring oxidative stress parameters in liver.

![Figure 2.](image)

Note: Fig. 2, Effect of MQEO on TBARS (MDA) in liver of normal and diabetic mice. Each group (n=5) represents mean ± SEM. Data was analyzed by using one-way ANOVA and Kruskal al-walis followed by independent-samples T test. * p < 0.05 Vs Normal, ** p < 0.01 Vs Normal, #p < 0.05 Vs STZ.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>STZ</th>
<th>STZ + MQEO 150 mg/kg</th>
<th>STZ + saxagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD mmol/mg prot</td>
<td>67.28 ± 1.3</td>
<td>37.1 ± 1.7**</td>
<td>58.45 ± 1.9#</td>
<td>58.21 ± 2##</td>
</tr>
<tr>
<td>CAT U/mg prot</td>
<td>41.69 ± 3.8</td>
<td>19.78 ± 1.54**</td>
<td>35.25 ± 5.38#</td>
<td>28.95 ± 3.52#</td>
</tr>
<tr>
<td>GPx U/mg prot</td>
<td>208.8 ± 11.51</td>
<td>147.8 ± 5.89*</td>
<td>190.2 ± 6.33#</td>
<td>210.7 ± 8.47##</td>
</tr>
<tr>
<td>GST U/mg prot</td>
<td>1.177 ± 0.17</td>
<td>0.57 ± 0.05 ***</td>
<td>0.89 ± 0.1##</td>
<td>1.04 ± 0.1###</td>
</tr>
<tr>
<td>Vitamin C µg/ml</td>
<td>2.21 ± 0.07</td>
<td>0.8 ± 0.11 ***</td>
<td>1.49 ± 0.29#</td>
<td>1.16 ± 0.13###</td>
</tr>
</tbody>
</table>

Note: values are represented as mean ± SEM, n=5. * p<0.05 vs Normal, ** p<0.01 vs Normal, *** p<0.001 vs Normal, #p<0.05 vs STZ, ##p<0.01 vs STZ, ###p<0.001 vs STZ.
Effect of MQEO on Histopathological Changes in Liver

Representative liver images are shown in Fig. 3. The normal mice showed the hepatocytes with normal central vein and sinusoidal cords. In contrast, the livers of STZ-induced diabetic mice showed the congestion of sinusoids and focal necrosis. Livers from MQEO treated diabetic mice revealed similar structure as in the normal mice. Saxagliptin produced similar improvement in liver histopathological changes as MQEO.

![Figure 3.](image)

Note: Fig. 3, Effect of MQEO on liver histology. Representative hematoxylin and eosin (H&E)-stained liver sections of different groups of mice.

Discussion

Diabetic hepatopathy is characterized by changes in liver architecture including congestion of sinusoids and focal necrosis. Our results showed that the severity of the liver injuries was markedly reduced in MQEO treated mice.

Oxidative stress plays a major role in the pathogenesis of T1DM and reactive oxygen species (ROS) which could play a pivotal role in the development and progression of DM [9]. Many natural products have been shown to decrease oxidative stress in diabetic animals. We hypothesized that the improved liver morphology maybe related to reduce oxidative stress.

MQEO treatment reverted changes in markers of oxidative stress including MDA level and antioxidant enzymes in liver diabetic mice. The restored antioxidant enzymes by MQEO include SOD, CAT, GST, and GPx. This result suggests that besides glycemic control, MQEO may also be used to treat liver dysfunction in diabetes. Further studies are needed to elucidate the mechanisms of how MQEO restores diabetes-related liver damages.

Acknowledgement

This work was supported financially by the Hundred Talents Program of the Chinese Academy of Sciences (Y3ZK101B01). The authors want to thank the Central Laboratory of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences for technical support.
References


