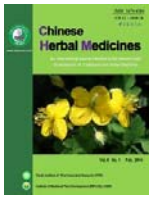

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Original article

Antidiabetic and Anti-oxidative Effects of Honokiol on Diabetic Rats Induced by High-fat Diet and Streptozotocin

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ABSTRACT

Objective To study the antidiabetic and anti-oxidative effects of honokiol (Hon) in *Magnolia officinalis* and its underlying molecular mechanism in diabetic rats induced by high-fat diet (HFD) and streptozotocin (STZ). **Methods** After ig administration with Hon [25, 50, and 100 mg/(kg·d)] to diabetic rats for consecutive 10 weeks, the levels of blood glucose (BG), oral glucose tolerance (OGT), blood lipids including total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C), hepatic oxidative stress including the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), methane dicarboxylic aldehyde (MDA), and cytochrome P4502E1 (CYP2E1) in diabetic rats were measured. **Results** Compared to the diabetic control rats, ig administration of Hon resulted in significant decrease in BG, TC, TG, and LDL-C levels in serum, as well as hepatic CYP2E1 activity and MDA content in diabetic rats, whereas the level of OGT and activities of hepatic CAT, SOD, and GSH-Px in diabetic rats were significantly increased. **Conclusion** Hon could alleviate hyperglycemia, hyperlipemia, hepatic oxidative damage, and insulin resistance in diabetic rats by inhibiting hepatic CYP2E1 activity.

Key words

antidiabetic; anti-oxidation; diabetic rats induced by high fat diet and Streptozotocin; honokiol

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1. Introduction

In recent years, there has been a growing interest to explore the relationship between liver injury and diabetes. Type 2 diabetes is an acknowledged risk factor for non-alcoholic fatty liver disease (NAFLD) and has a potent role in hepatic dysfunction (Cusi, 2009). Cytochrome P4502E1 (CYP2E1) could generate reactive oxygen species (ROS) (Cederbaum, 2006; Koop, 2006). The release of ROS by CYP2E1 has been proposed to increase oxidative stress, and thereby plays an important role in NAFLD and in the patho-physiology of diabetes (Kathirve et al, 2010; Chandrasekaran et al, 2012). It has been observed that CYP2E1 activity has been markedly increased in hyperglycemic and diabetic conditions (Arinc et al, 2007). The inhibition of CYP2E1 leads to decrease of advanced glycated end product formation in high glucose treated alcohol dehydrogenase and CYP2E1 over-expressing VL-17A cells (Swaminathan et al, 2013).

Magnolia officinalis Rehd. et Wils. has been used as folk medicines in China, Japan, and Korea for thousands of years. Honokiol (Hon) (Figure 1), as one of the important active compounds extracted from the stem barks of *M. abovata* and *M. officinalis*, has various bioactivities (Amblard et al, 2006; Greengerg et al, 2008; Chao et al, 2010; Wu et al, 2011; Lee et al, 2011). It has been reported that Hon possessed hepatoprotective activities (Chiu et al, 1997; Park et al, 2003; Hu et al, 2009) and anti-oxidative activities (Sheu et al, 2008; Dikalov et al, 2008; Tang et al, 2011; Vavilala et al, 2013). In addition,

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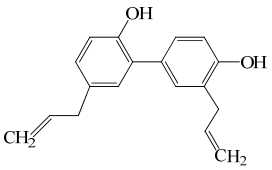

Figure 1 Chemical structure of Hon

angiopathy is a major complication of diabetes, Hon could inhibit the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) under oxidase-related oxidative stress in human umbilical vein endothelial cells (Sheu et al, 2008), stimulate adipocyte differentiation, and increase glucose uptake in insulin-sensitive and insulin-resistant murine and human adipocytes (Alonso-Castro et al, 2011). Although the above pharmacological effects of Hon are well-known, the antidiabetic and hepatoprotective effects in diabetic rats have not yet been established. In this study, we have examined the pharmacological effects of Hon on hyperglycemia, hyperlipidemia, hepatic oxidative stress, and CYP2E1 activity in diabetic rats induced by high-fat diet (HFD) and Streptozotocin (STZ).

2. Materials and methods

2.1 Chemicals and reagents

Streptozotocin (STZ) and metformin (Met) were purchased from Sigma (USA). Honokiol (Hon) and chlorzoxazone (Chl) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (China). Reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) was purchased from Roche Co. (Switzerland). 6-Hydroxy Chl was purchased from TRC (Canada). The test kits of blood glucose (BG), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), methane dicarboxylic aldehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute (China).

2.2 Type 2 diabetic rats

All procedures were approved by the Ethics Committee for the Use of Experimental Animals of Hubei University, and complied with health guidelines for the care and use of laboratory animals. Male Wistar rats weighing (200 ± 20) g, purchased from the Provincial Disease Prevention and Control Center of Hubei, were maintained in SPF animal room at (22 ± 2) ^{o}C, with the humidity of (60 ± 5)% and 12 h/12 h day/night cycle. The animals were provided with commercially available rat normal pellet diet (diet consisting of 5% fat, 53% carbohydrate, and 23% protein, with total calorific value of 25 kJ/kg) and water ad libitum for 1 week adaptation. Then the rats were randomly divided into two groups, the rats in normal control (NC) group were fed with normal diet, and the rats in diabetic control (DC) group were fed with HFD (composed of 10% lard oil, 10% white sugar, 5% yolk powder, 1% cholesterol, and 74% regular diet). The rats in DC group were ip injected with STZ (30 mg/kg, dissolved in citrate buffer, pH 4.4) 6 weeks later, and rats in NC group were injected with same volume of citrate buffer. After 1 week of STZ injection, the diabetic rats with glucose level ≥ 16.7 mmol/L were selected for the further study. Animals were kept on their respective diet till the end of the experiment.

2.3 Experimental design

In the present work, a total of 48 rats (8 normal and 40 diabetic rats) were divided into six groups (n = 8) as follows: NC (healthy rats treated with vehicle alone), DC (diabetic rats treated with vehicle alone), Met250 (diabetic rats treated with 250 mg/kg Met as positive control), Hon25, Hon50, and Hon100 (diabetic rats treated with 25, 50, and 100 mg/kg Hon, respectively) groups. The diabetic rats were ig administered with Hon (dissolved in CMC solution) once daily for continuous 10 weeks. At the end of experiment, blood samples collected from orbit and serum were separated by centrifugation at 3000 r/min for 10 min for the determination of blood glucose and lipid. The liver was immediately removed and stored at −80 ^{o}C for the determination of CAT, SOD, GSH-Px, MDA, and CYP2E1.

2.4 Biochemical assay

The levels of BG, TC, TG, HDL-C, LDL-C, and MDA, as well as the activities of CAT, SOD, and GSH-Px, were measured by commercial assay kits according to the manufacture's instruction (China). The hepatic CYP2E1 activity was determined and briefly stated as follows. The rat liver microsome (RLM) was prepared by $CaCl_2$ precipitation method (Peng et al, 2009). Chl was used as CYP2E1 probe substrate and the production of 6-hydroxy Chl in RLM incubation was used to evaluate the activity of hepatic CYP2E1. All incubations were performed at 37 ^{o}C for 60 min in 400 μL potassium phosphate buffer (0.1 mol/L, pH 7.4) containing 5 mmol/L $MgCl_2$, 1 mg/mL RLM, 1 mmol/L NADPH, and 75 μmol/L Chl (Guengerich, 2006). The incubation was started by adding NADPH and stopped by cooling on ice and adding 800 μL ethyl acetate. After the protein precipitation, the supernatant was evaporated at 40 ^{o}C and the residue was dissolved in 100 μL mobile phase (22% acetonitrile and 78% water). The content of 6-hydroxy Chl was determined by HPLC method (Kamdem et al, 2011) using Phenomenes-C_{18} column (150 mm × 4.6 mm, 5 μm) with a flow rate of 0.8 mL/min and a detection wavelength of 282 nm.

2.5 Oral glucose tolerance test

On the day before rats were sacrificed, the rats underwent an oral glucose tolerance (OGT) test after 16 h fasting with free access to water. After ig administration of glucose (2 g/kg), blood samples were drawn from caudal vein at time 0 (prior to the glucose load), 30, 60, 90, and 120 min. And BG was determined by commercial assay kits (China).

2.6 Statistical analysis

Data were expressed as $\bar{x} \pm s$. The significance of difference versus the control group was determined by Student's *t*-test. Statistical analysis was performed by One-way ANOVA using SAS 11.1. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of Hon on glucose tolerance in diabetic rats

The BG concentration-time curves and the area under curves ($AUC_{0\text{-}120\ min}$) of OGT are shown in Figure 2. After ig administration of glucose (2 g/kg), the BG level and $AUC_{0\text{-}120\ min}$ in DC group were significantly greater than those in NC group ($P < 0.01$). Compared with DC group, the $AUC_{0\text{-}120\ min}$ of Hon25, Hon50, and Hon100 groups were significantly decreased by 32.7%, 35.4%, and 27.9%, respectively. The results indicated that Hon could improve glucose tolerance of diabetic rats. However, the difference in glucose tolerance between treated and NC rats showed that Hon could not normalize it in diabetic rats. In addition, the effect of Hon on glucose tolerance in diabetic rats was not dose-dependent.

3.2 Hypoglycemic and hypolipidemic effects of Hon in diabetic rats

The effects of Hon on blood glucose and lipid in diabetic rats are shown in Table 1. The levels of BG and blood lipids in DC group were markedly higher than those in NC group ($P < 0.01$). Compared with the DC group, the ig administration of Hon (25, 50, and 100 mg/kg) for continuous 10 weeks resulted in the significant decrease by 29%−41% in BG, 38%−49% in TC, 74%−86% in TG, and 34%−43% in LDL-C ($P < 0.05$ or 0.01), and no obvious effect in the HDL-C of diabetic rats. Moreover, there was no significant difference between Hon- and Met-induced hypoglycemic and hypolipidemic effects, and the effect of Hon on BG and blood lipids was not dose dependent.

3.3 Effect of Hon on hepatic oxidative stress

The effect of Hon on hepatic oxidative stress is shown in Table 2. Compared with NC group, the activities of hepatic CAT, SOD, and GSH-Px were significantly decreased, whereas the level of hepatic MDA was significantly increased in DC group ($P < 0.01$). After ig administration of Hon (25, 50, and 100 mg/kg) to diabetic rats for continuous 10 weeks, the level of hepatic MDA was markedly decreased by 45%−53%, and the activities of hepatic CAT, SOD, and GSH-Px were significantly increased by 15%−29%, 12%−22%, and 30%−31%, respectively, as compared with DC rats. In addition, the results indicated that there were no significant difference in the activities of hepatic CAT, SOD, and GSH-Px after ig administration of Hon and Met to diabetic rats, and the effects of Hon on the activities of hepatic CAT, SOD, and GSH-Px were not dose dependent.

3.4 Effect of Hon on hepatic CYP2E1 activity

6-Hydroxy Chl in RLM incubation solution was

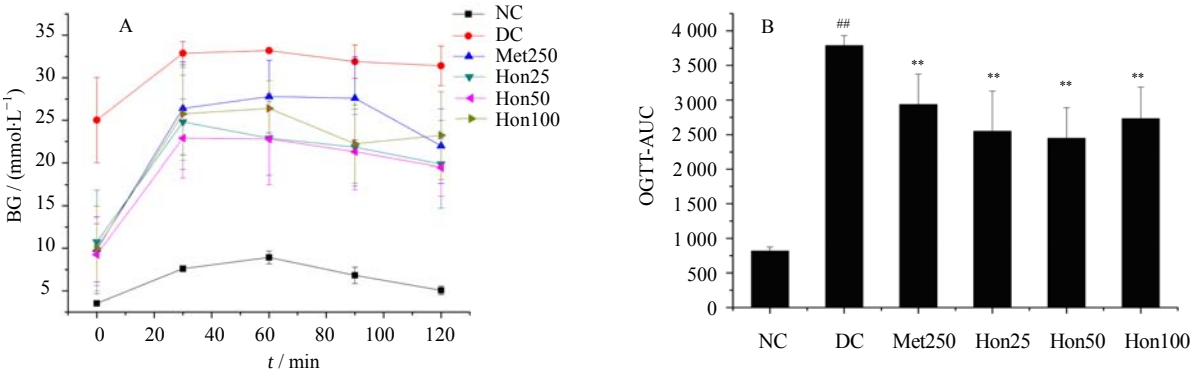

Figure 2 Concentration-time curves of OGT (A) and AUC of OGT (B) in serum ($\bar{x} \pm s$, $n = 8$)

$^{\#\#}P < 0.01$ *vs* NC; $^{**}P < 0.01$ *vs* DC

Table 1 Effects of Hon on BG and blood lipids of diabetic rats ($n = 8$)

Groups	BG / (mmol·L^{-1})	TC / (mmol·L^{-1})	TG / (mmol·L^{-1})	LDL-C / (mmol·L^{-1})	HDL-C / (mmol·L^{-1})
NC	5.70 ± 0.32**	1.82 ± 0.39**	0.78 ± 0.22**	1.05 ± 0.17**	0.59 ± 0.06
DC	17.56 ± 4.26$^{\#\#}$	4.68 ± 1.20$^{\#\#}$	3.91 ± 0.96$^{\#\#}$	2.30 ± 0.40$^{\#\#}$	0.76 ± 0.08
Met250	9.14 ± 4.10$^{\#**}$	2.58 ± 0.29$^{\#\#**}$	1.12 ± 0.36**	1.42 ± 0.39$^{\#**}$	0.70 ± 0.17
Hon25	10.74 ± 5.04$^{\#**}$	2.37 ± 0.39$^{\#**}$	1.01 ± 0.29**	1.51 ± 0.32$^{\#\#**}$	0.69 ± 0.09
Hon50	10.34 ± 3.53$^{\#\#**}$	2.91 ± 0.39$^{\#\#**}$	0.53 ± 0.10$^{\#**}$	1.30 ± 0.29$^{\#\#**}$	0.85 ± 0.12
Hon100	12.46 ± 4.84$^{\#\#*}$	2.68 ± 0.33$^{\#\#*}$	0.84 ± 0.18**	1.49 ± 0.22$^{\#\#**}$	0.78 ± 0.20

$^{\#}P < 0.05$ $^{\#\#}P < 0.01$ *vs* NC; $^{*}P < 0.05$ $^{**}P < 0.01$ *vs* DC, same as below

separated perfectly with a retention time of 4.9 min under the HPLC conditions. No significant interference was observed in RLM incubation solution. The relative recovery, intra- and inter-day precision, and the equation of linear regression of 6-hydroxy Chl in RLM incubation solution determined by HPLC are shown in Table 3.

The effects of Hon on rat hepatic CYP2E1 activity are shown in Figure 3. The hepatic CYP2E1 activity of DC group was significantly higher than that of the NC group ($P < 0.01$). After ig administration of Hon (25, 50, and 100 mg/kg) for continuous 10 weeks, the hepatic CYP2E1 activities of diabetic rats were dose-independent reduced by 11.4% ($P > 0.05$), 51.3% ($P < 0.01$), and 11.2% ($P > 0.05$), respectively, as compared with DC group.

Table 2 Effects of honokiol on hepatic oxidative stress of diabetic rats (n = 8)

Groups	CAT / (U·mg^{-1})	SOD / (U·mg^{-1})	GSH-Px / (U·mg^{-1})	MDA / (nmol·mg^{-1})
NC	48.98 ± 7.73	21.50 ± 3.40	468.56 ± 72.74	0.06 ± 0.03
DC	37.91 ± 3.23$^{\#}$	14.29 ± 1.29$^{\#\#}$	260.68 ± 6.66$^{\#\#}$	2.40 ± 0.87$^{\#\#}$
Met250	48.29 ± 5.90**	15.62 ± 1.83$^{\#\#}$	369.88 ± 34.33$^{\#**}$	1.24 ± 0.09$^{\#\#*}$
Hon25	47.31 ± 4.86**	16.23 ± 1.69$^{\#\#*}$	338.55 ± 23.07$^{\#\#**}$	1.12 ± 0.10$^{\#\#*}$
Hon50	47.84 ± 2.53**	15.96 ± 1.41$^{\#\#*}$	341.29 ± 17.85$^{\#\#**}$	1.28 ± 0.18$^{\#\#*}$
Hon100	43.43 ± 8.43**	17.40 ± 2.33$^{\#\#**}$	342.58 ± 44.92$^{\#**}$	1.33 ± 0.12$^{\#\#*}$

Table 3 Relative recovery, precision, and equation of linear regression of 6-hydroxy Chl in RLM incubation solution (n = 6)

Equation of linear regression	QC samples / (μmol·L^{-1})	Inter-day precision / %	Intra-day precision / %	Relative recovery / %
$y = 19.75x - 1.3253$	1.0	7.17	3.25	109.6 ± 7.9
$r^2 = 0.9995$	4.0	1.57	4.66	95.8 ± 6.0
	8.0	0.31	8.23	98.2 ± 2.4

y: the peak area; x: 6-hydroxy Chl concentration

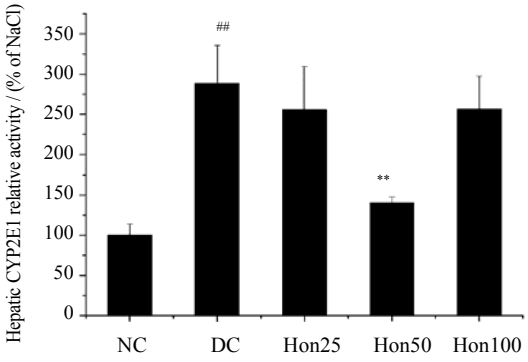

Figure 3 Effects of Hon on hepatic CYP2E1 activity ($\bar{x} \pm s$, n = 8)

4. Discussion

Both hyperglycemia and hyperlipidemia are related with diabetic mellitus (DM) and its complications (Sheetz and King, 2002). The inhibition of Hon on hyperglycemia and hyperlipidemia of diabetic rats induced by HFD and STZ suggested that Hon had the potential to be developed as a therapeutic agent for DM.

Oxidative stress and lipid peroxidation are critical factors involved in the progression of nonalcoholic steatohepatitis (NASH) (Angulo, 2002). CYP2F1 is of special interest because of its ability to metabolize and activate numerous hepatotoxic substrates in the liver such as ethanol, carbon tetrachloride, acetaminophen, and *N*-nitrosodimethyl-amine to more toxic products (Wang et al, 2000). Hepatic CYP2E1 was increased in human with NASH and in mouse model of NASH (Leclerq et al, 2000). CYP2E1 induction results in ROS generation through the reduction of molecular oxygen to water by NADH- and NADPH-dependent processes (Cederbaum, 2006; Schattenberg et al, 2004). Hepatocytes have anti-oxidative enzymes (including SOD, CAT, GSH-Px, hemeoxygenase-1, and so on) to inhibit oxidative damage. Hepatic specific CYP2E1 overexpression results in increased oxidative stress and nitrosative stress (Liu et al, 2005). The present work demonstrated that the ig administration of Hon significantly increased the activities of hepatic SOD, CAT, and GSH-Px, reduced the content of hepatic MDA in diabetic rats induced by HFD and STZ. The results indicated that Hon should be an effective component to treat hepatic oxidative damage in diabetic rats.

It is well known that diabetes could induce the up-expression of CYP2E1 in both mRNA and protein levels. The ROS produced by CYP2E1 is an important causative factor for insulin resistance in diabetes and related conditions (Rudich et al, 2005; Zhang et al, 2011). The oxidative stress induced by CYP2E1 impairs insulin signaling to glucose uptake in adipocytes, as well as insulin signaling to IRS-1, IRS-2, PI3-kinase, and Akt/PKB in hepatocytes, and thus leads to CYP2E1-dependent toxicity (Caro and Cederbaum, 2006; Kathirvel et al, 2009). CYP2E1 knockout could increase insulin sensitivity and protect from HFD-induced obesity and glucose intolerance of mice (Zong et al, 2012). Other studies have also strongly implicated oxidative stress in the development of insulin resistance. The present work has found for the first time that the ig administration of Hon could significantly decrease the hepatic CYP2E1 activity of HFD and STZ-induced diabetic rats. This important finding indicates that inhibition on hepatic CYP2E1 activity should be an important mechanism of Hon against insulin resistance of HFD and STZ-induced diabetic rats.

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