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Research Article

Berberine Analogue Y53 has Improved Antioxidant and Anti-Inflammatory Activities in Diabetic C57BL/6J Mice with Liver Steatosis

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Abstract

This study was designed to investigate the antioxidant, anti-inflammatory and metabolism modulating activities of pseudoberberine (Y53), a berberine (BBR) analogue, in diabetic mice with fatty liver. Diabetes Mellitus (DM) of the C57BL/6J mice was induced by High Fat Diet (HFD)-feeding followed by single dose intraperitoneal injection of streptozotocin (STZ) (120 mg kg^{-1}). The animals were treated with saline, 50 mg kg^{-1} of BBR, 50 mg kg^{-1} of Y53 or 100 mg kg^{-1} of BBR, respective. The results showed that Y53 potently lowered serum lipids and glucose, increased serum insulin and pancreas weight and upregulated hepatic expression of Low-Density Lipoprotein Receptor (LDLR) and Insulin Receptor (InsR). The Y53 also suppressed liver steatosis, reduced fat accumulation, liver weight and index and restored liver function in the mice. The mice developed obvious oxidative stress and proinflammatory response after the onset of DM. Y53 significantly increased superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) level in the serum, liver and pancreas. On the other hand, Y53 greatly reduced the mRNA expression levels of proinflammatory cytokines like interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) but increased those of nuclear factor erythroid-2-related factor-2 (Nrf2), heme oxygenase-1 (HO-1) and NADPH quinine oxidoreductase-1 (NQO-1) in the liver and pancreas of the mice. The efficacies of Y53 were superior to those of BBR at the same dose and close to those of BBR at double dose. Results indicate that Y53 may be developed as a new oral hypoglycemic agent in the future.

Key words: Diabetes mellitus, fatty liver, oxidative stress, proinflammatory response, hypoglycemic effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, Diabetes Mellitus (DM) has become a public health problem world wide. Presently, chemical drugs such as biguanides, sulphonylureas and thiazolidinediones are widely used in clinic to lower blood glucose (Tiwari, 2015). In addition, numerous natural products are reported to have glucose-lowering activities; some of them are under development as novel oral hypoglycemic agents due to their good efficacy and low toxicity (Gao *et al*., 2015).

Berberine (BBR) is a natural compound isolated from plants such as *Rhizoma coptidis* (huanglian) and *Hydrastis canadensis* (goldenseal). Results from our laboratory and others proved that BBR could modulate glucose metabolism both in cultured cells and in various animal models (Kong *et al*., 2009; Pang *et al*., 2015). Notably, BBR was proved to be safe and effective in clinic to treat patients with type 2 DM (Lan *et al*., 2015).

One of the shortcomings of BBR was its poor absorption with an absolute oral bioavailability less than 1% in rats, which may have negative influence on its pharmacological activities (Chen *et al*., 2011). Pseudoberberine (Fig. 1, Y53) was an isomer of BBR. It was synthesized in our laboratory and was found to have enhanced Low-Density Lipoprotein Receptor (LDLR)-upregulating and lipid-lowering activities than BBR in our previous studies (Li *et al*., 2009). Furthermore, results showed that the glucose-lowering efficacy of Y53 was also superior to that of BBR in diabetic mice due to improved pharmacokinetic profiles (Shan *et al*., 2013).

In the pathogenesis of insulin resistance and DM, oxidative stress and proinflammatory response play important roles (Evans *et al*., 2005; Donath and Shoelson, 2011). A number of studies indicated that BBR could suppress oxidative stress and proinflammatory response in animals with DM (Li *et al*., 2014a). However, currently it is not clear whether Y53 has the same activities as BBR. In the present study, we use a diabetic mice model to observe the antioxidant and anti-inflammatory activities of Y53 and compare it with BBR.

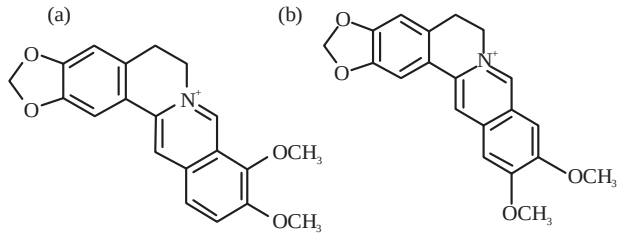

Fig. 1(a-b): Chemical structures of, (a) BBR and (b) Y53

MATERIALS AND METHODS

Chemicals and reagents: Berberine (BBR), streptozotocin (STZ) and sodium citrate were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.); Y53 was chemically synthesized in our laboratory as described previously. Superoxide dismutase (SOD) Activity Colorimetric Assay Kit and the Lipid Peroxidation Malondialdehyde (MDA) Assay Kit were from the Beyotime Institute of Biotechnology (Shanghai, China). Pierce™ Bicinchoninic Acid (BCA) Protein Assay Kit was from Thermo Fisher Scientific Inc. (Waltham, MA, U.S.A.). Commercially available kits for determination of serum cholesterol (CHO), LDL-cholesterol (LDL-c), triglyceride (TG), glucose, insulin, alanine aminotranferease (ALT) and aspartate aminotransferase (AST) were purchased from Nanjing Jiancheng Bioengineering institute (Nanjing, China). Isopropanol was from Beijing Baishun Chemical Technology Co., Ltd. (Beijing, China). Tissue Total Cholesterol Assay Kit and Tissue Triglyceride Assay Kit were from the Applygen Technologies Inc. (Beijing, China). SV Total RNA Isolation System, GoScript™ Reverse Transcription System and GoTaq® qPCR Master Mix were from Promega (Madison, WI, U.S.A.).

Animal experiment: The protocol of the animal experiment was reviewed and approved by the Research Committee of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences (CAMS); animals were cared for according to the institutional guidelines of CAMS.

Male C57BL/6J mice (20.0±1.50 g) were purchased from the Vital River Laboratories (Beijing, China) and housed in a room at 20-24°C with 50-60% humidity and 12 h light/dark cycle. After 3 days of accommodation period, some mice were fed with regular rodent diet as control group (n = 8), other mice were fed with a High-Fat Diet (HFD) (D12492, Research Diets, Inc., New Brunswick, NJ, U.S.A.) for 4 weeks.

Then after 12 h fasting, the HFD-fed mice were intraperitoneally injected with single dose of STZ (120 mg kg^{-1}) which was dissolved in 0.1 mol L^{-1} of cold sodium citrate (pH = 4.5) to induce diabetes. Seventy two hours later, about one third of the mice died; the rest of the mice were subjected to tail snipping after fasting, blood glucose levels were determined by a glucometer (Roche Applied Science, Indianapolis, IN, U.S.A.). The mice were considered as diabetic if their fasting blood glucose ≥ 11.1 mmol L^{-1}.

The diabetic mice were randomly divided into 4 groups with 10 mice each, which received saline (DM group), 50 mg kg^{-1} of BBR (DM+BBR50 group), 50 mg kg^{-1} of Y53 (DM+Y53-50 group) and 100 mg kg^{-1} of BBR (DM+BBR100 group), respectively. The BBR and Y53 were suspended in

saline and orally administered to the mice at 2 pm every day for 4 weeks. Body weights and food intakes of the mice were recorded every other day.

At the end of the experiment, mice were fasted for 12 h, blood samples were collected by retro-orbital puncture; serums were isolated for the measurement of CHO, LDL-c, TG, glucose, insulin, ALT and AST levels by commercially available kits. The mice were sacrificed by cervical dislocation; their livers and pancreases were harvested and weighed, liver index was calculated as liver weight/body weight (after experiment)×100. A portion of every liver tissue was fixed in 10% formaldehyde for hematoxylin and eosin (H and E) staining; the remaining liver and pancreas tissues were immediately frozen in liquid nitrogen.

Semiquantitative histopathological evaluation of liver sections: Liver sections were subjected to pathological scoring for steatosis (0~3) and lobular inflammation (0~3) as described in another report (Tang *et al*., 2015).

Determination of liver CHO and TG: About 0.1 g of liver tissue from each sample was homogenized in 1 mL of isopropanol for total lipid extraction as described before (Kong *et al*., 2008); CHO and TG contents were determined with commercially available kits and normalized to the weight of liver tissue.

Measurement of SOD activities and MDA levels: At the end of the experiment, SOD activities and MDA levels of serums, livers and pancreases were determined according to the suppliers' protocols after homogenization (for livers and pancreases). The SOD activities and MDA levels were normalized to protein concentrations in livers and pancreases.

RNA extraction and Real-Time reverse transcriptase-polymerase Chain Reaction (RT-PCR): Total RNAs were isolated from livers and pancreases and reversely transcribed into cDNAs as described previously (Kong *et al*., 2008). Real-time PCR was performed with gene specific primers (Table 1, Thermo Fisher Scientific Inc.) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The reactions were performed in an ABI Prism® 7900 High-Throughput Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.) with a condition same as our previous report (Kong *et al*., 2008). The comparative threshold cycle (C_T) method was used for relative quantification of target gene expression, which was plotted as fold of control.

Statistical analysis: Values are Mean±S.D. of 8-10 animals in each group. After validation of the test for homogeneity of variance, differences among study groups were examined by one-way ANOVA followed by the Newman-Keuls test for multiple comparisons. $p<0.05$ was considered to be statistically significant.

RESULTS

The Y53 improves metabolic disorders more effectively than BBR in diabetic C57BL/6J mice with liver steatosis. Diabetes Mellitus (DM) of the C57BL/6J mice was induced by HFD-feeding and STZ injection. The blood glucose levels were almost the same among studying groups of diabetic mice before drug administration.

As shown in Table 2 and 3, compared to those of the control group, serum glucose increased for about 4.09-fold ($p<0.001$) while serum insulin declined by about 78.8% ($p<0.001$) and pancreas weight declined by about 25.6% ($p<0.05$) in the DM group of mice. After 4 weeks of treatment, BBR at 50 mg kg^{-1} could reduce serum glucose and restore serum insulin level to some extents ($p<0.05$ vs. DM group). Notably, Y53 at the same dose manifested stronger efficacies in modulating serum glucose/insulin and restoring pancreas weight ($p<0.05$ vs. DM+BBR50 group). Four weeks' administration of Y53 at 50 mg kg^{-1} reduced serum glucose by about 52.8% ($p<0.01$ vs. DM group), increased serum insulin by about 2.97-fold ($p<0.01$ vs. DM group) and restored pancreas weight to nearly normal ($p<0.05$ vs. DM group). The efficacies of Y53 were nearly the same as those of BBR at double dose (Table 2 and 3).

The HFD-fed mice ate less food than the control mice which were fed with a regular rodent diet (Table 3), perhaps

Table 1: Primers for real-time PCR (5′ to 3′)

Genes	Forward primers	Reverse primers
TNF-α	CCAAAGGGATGAGAAGTTCC	CTCCACTTGGTGGTTTGCTA
IL-6	CCATCCAGTTGCCTTCTTGG	TGCAAGTGCATCATCGTTGT
Nrf2	AGCAGGACATGGAGCAAGTT	TTCTTTTTCCAGCGAGGAGA
HO-1	GAATGAACACTCTGGAGATGACAC	TGTGAGGGACTCTGGTCTTTG
NQO-1	AGCGTTCGGTATTACGATCC	AGTACAATCAGGGCTCTTCTCG
LDLR	GATGGCTATACCTACCCCTCAA	TGCTCATGCCACATCGTC
InsR	TCTTTCTTCAGGAAGCTACATCTG	TGTCCAAGGCATAAAAAGAATAGTT
GAPDH	CTCTGGAAAGCTGTGGCGTGATG	ATGCCAGTGAGCTTCCCGTTCAG

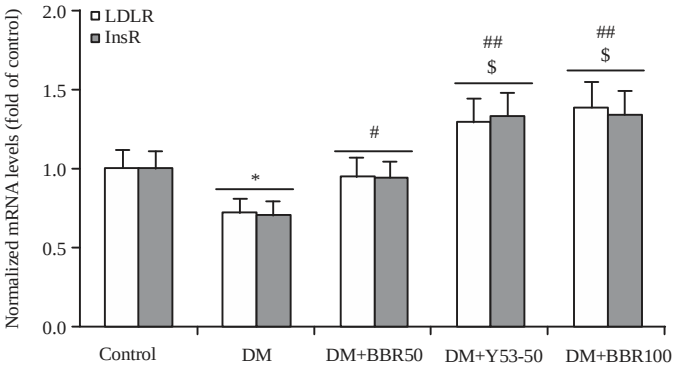

Fig. 2: Effects of Y53 and BBR on hepatic LDLR and InsR mRNA levels. Values are Mean±SD of 8 to 10 animals in each group. *p<0.05 vs. that of control group, #p<0.05, ##p<0.01 vs. that of DM group, $p<0.05 vs. that of DM+BBR50 group

Table 2: Effects of Y53 and BBR on serum biochemical parameters and liver lipid profiles in diabetic C57BL/6J mice with liver steatosis

Measurements	Control (n=8)	DM (n=10)	DM+BBR50 (n=10)	DM+Y53-50 (n=10)	DM+BBR100 (n=10)
Serum LDL-c (mmol L^{-1})	0.24±0.04	0.82±0.22***	0.64±0.11#	0.38±0.20##,$	0.33±0.10##,$
Serum CHO (mmol L^{-1})	2.92±0.25	6.24±1.20**	5.15±1.14#	3.27±1.02##,$	3.22±0.91##,$
Serum TG (mmol L^{-1})	0.66±0.11	1.54±0.38**	1.07±0.35#	0.61±0.27##,$	0.74±0.15##,$
Serum glucose (mmol L^{-1})	5.69±1.09	23.3±1.58***	18.1±1.66#	11.0±1.16##,$	11.9±2.19##,$
Serum insulin (ng mL^{-1})	1.70±0.21	0.36±0.07***	0.62±0.12#	1.07±0.16##,$	1.06±0.18##,$
Serum AST (U L^{-1})	217±41.9	224±39.3	210±49.8	195±29.3	195±38.1
Serum ALT (U L^{-1})	37.5±7.19	105±49.3***	80.2±55.0#	51.1±24.6##,$	49.4±17.3##,$
Liver TG (μmol g^{-1} liver)	30.8±4.83	67.0±8.21**	51.0±6.28#	34.8±5.21##,$	33.9±4.98##,$
Liver CHO (μmol g^{-1} liver)	38.1±5.22	78.3±8.23**	62.6±6.19#	45.6±5.57##,$	44.8±5.13##,$

Values are Mean±SD of 8 to 10 animals in each group. **p<0.01, ***p<0.001 vs. that of control group, #p<0.05, ##p<0.01 vs. that of DM group, $p<0.05 vs. that of DM+BBR50 group, DM: Diabetes mellitus, BBR: Berberine, Y53: Pseudoberberine, LDLC: Low-density lipoprotein-cholesterol, CHO: Cholesterol, TG: Triglyceride, AST: Aspartate aminotransferase, ALT: Alanine aminotranferease

Table 3: Effects of Y53 and BBR on food intake, body and organ weights in diabetic C57BL/6J mice with liver steatosis

Measurements	Control (n=8)	DM (n=10)	DM+BBR50 (n=10)	DM+Y53-50 (n=10)	DM+BBR100 (n=10)
Food intake (g per day)	5.92±0.81	3.98±0.67*	3.82±0.56*	3.91±0.65*	3.75±0.67*
Body weight (g)					
Before experiment	20.1±1.43	19.5±1.12	21.1±1.31	19.8±1.24	20.5±1.09
Before STZ	25.8±3.45	25.9±2.98	25.2±3.11	26.9±3.67	26.5±2.89
After experiment	30.6±4.38	23.9±3.77*	23.4±3.02*	23.8±3.26*	24.0±3.59*
Liver weight (g)	1.13±0.15	1.41±0.21*	1.35±0.15	1.10±0.17#,$	1.11±0.14#,$
Liver index (%)	3.69±0.55	5.90±0.87**	5.76±0.76	4.62±0.58#,$	4.63±0.55#,$
Pancreas weight (mg)	38.3±2.89	28.5±3.11*	28.6±3.35	36.5±3.11#,$	36.3±3.98#,$

Values are Mean±SD of 8 to 10 animals in each group. *p<0.05, **p<0.01 vs. that of control group, #p<0.05 vs. that of DM group, $p<0.05 vs. that of DM+BBR50 group, DM: Diabetes mellitus, BBR: Berberine, Y53: Pseudoberberine, STZ: Streptozotocin

due to the unpleasant smell and taste of the HFD. The body weights of the mice were not statistically different among groups before the experiment or before STZ injection. However, at the end of the experiment, due to STZ injection, the body weights of the mice with DM significantly declined compared to control mice (p<0.05). The BBR and Y53 had no influence on the body weight of the mice in this experiment (Table 3).

The HFD-feeding resulted in hyperlipidemia in diabetic mice (Table 2), Y53 lowered serum LDL-c, CHO and TG more effectively than BBR at the same dose (p<0.05), which was in agree with our previous results (Li *et al*., 2009). We also measured the mRNA expression levels of LDLR and Insulin Receptor (InsR) in the liver, which were proved to be upregulated by BBR in our previous studies (Kong *et al*., 2004, 2009; Li *et al*., 2009). As shown in Fig. 2, LDLR and InsR mRNA levels declined averagely by about 30% in diabetic mice fed with a HFD (p<0.05 vs. control), which might reflect compromises of the lipid and glucose metabolism in the animals. Y53 at 50 mg kg^{-1} increased hepatic LDLR and InsR mRNA levels by 1.79 and 1.87-fold (p<0.01 vs. DM group), respectively, which were close to those of BBR at 100 mg kg^{-1}.

The mice developed severe fatty liver after HFD-feeding. As shown in Fig. 3, H and E staining of the liver sections illustrated macro- and microvesicular steatosis as well as inflammatory cell infiltration in diabetic mice. Their liver

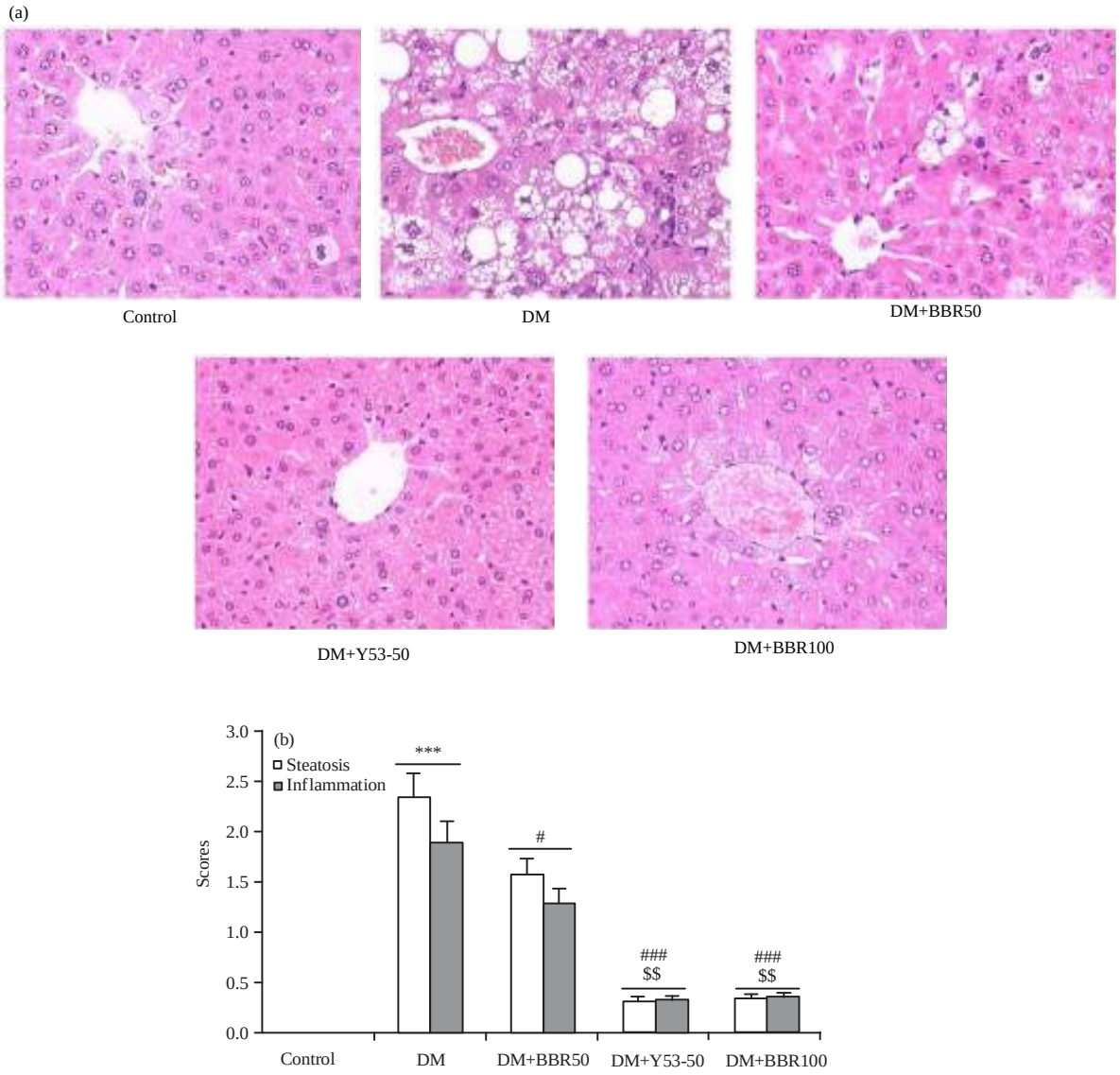

Fig. 3(a-b): Effects of Y53 and BBR on the pathological changes in the livers of C57BL/6J mice. Livers were harvested and fixed in 10% formaldehyde for H and E staining, (a) Representative images of each group (×400) and (b) Liver sections were subjected to pathological scoring for steatosis and lobular inflammation. Values are Mean±S.D. of 8 to 10 animals in each group. ***p<0.001 vs. that of control group, $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 vs. that of DM group, $^{\$\$}$p<0.01 vs. that of DM+BBR50 group

weight and index (Table 3), liver TG and CHO contents (Table 2) increased greatly as well (p<0.05 or p<0.01 vs. control). As a result, serum ALT level of the diabetic mice elevated dramatically (p<0.001 vs. control). Four weeks' administration of Y53 significantly ameliorated liver steatosis (Fig. 3a-b), reduced liver TG and CHO contents (Table 2), decreased liver weight and index (Table 3) and restored liver function (Table 2) in diabetic mice. The efficacies of Y53 were close to those of BBR at 100 mg kg^{-1} and superior to BBR at 50 mg kg^{-1} (p<0.05 or p<0.01). The Y53 ameliorates oxidative stress and proinflammatory response more potently than BBR in diabetic C57BL/6J mice with liver steatosis.

Next, the antioxidant and anti-inflammatory activities of Y53 were determined in the animals. The mice developed obvious oxidative stress after the onset of DM. As shown in Table 4, the SOD activities declined while the MDA levels increased greatly in the serum, liver and pancreas of the diabetic mice (p<0.01 vs. control). Four weeks' administration of Y53 at 50 mg kg^{-1} restored SOD activities and reduced MDA contents in the serum, liver and pancreas to near baseline

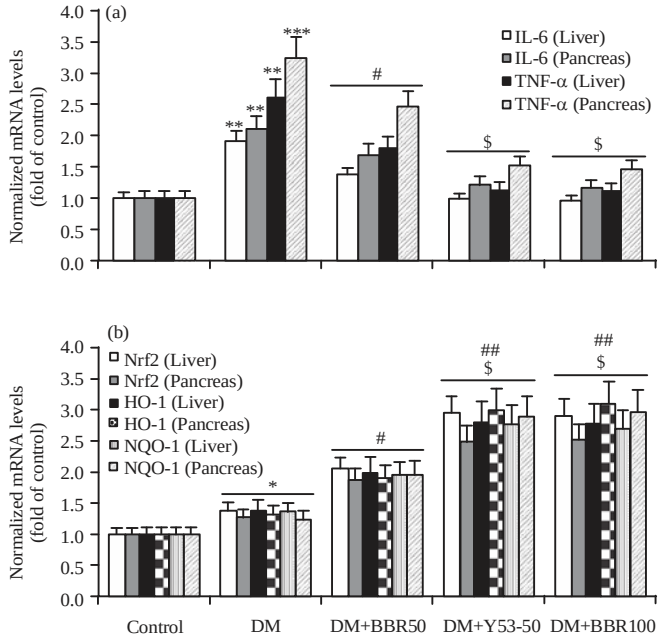

Fig. 4(a-b): Effects of Y53 and BBR on the mRNA expression levels of proinflammatory cytokines and key genes of the Nrf2 antioxidant pathway. Livers and pancreases were harvested for, (a) Real-time RT-PCR analysis of the mRNA levels of IL-6/TNF-α and (b) Nrf2, HO-1 and NQO-1. The expression levels of target genes were normalized to that of GAPDH and plotted as fold of control, which was designated as 1. Values are Mean±SD of 8 to 10 animals in each group. *p<0.05, **p<0.01, ***p<0.001 vs. that of control group, #p<0.05, ##p<0.01 vs. that of DM group, $p<0.05 vs. that of DM+BBR50 group

Table 4: Effects of Y53 and BBR on SOD activity and MDA level in the serum, liver and pancreas of diabetic C57BL/6J mice with liver steatosis

Measurements	Control (n=8)	DM (n=10)	DM+BBR50 (n=10)	DM+Y53-50 (n=10)	DM+BBR100 (n=10)
Serum					
SOD activity (U mL^{-1})	0.94±0.06	0.48±0.07**	0.66±0.09#	0.88±0.11##,$	0.89±0.11##,$
MDA (nmol mL^{-1})	4.18±0.27	6.82±1.12**	5.33±0.89#	4.28±0.76##,$	4.33±0.91##,$
Liver					
SOD activity (U mg^{-1} protein)	0.68±0.04	0.36±0.06**	0.52±0.08#	0.66±0.09##,$	0.73±0.10##,$
MDA (nmol mg^{-1} protein)	2.39±0.17	5.78±0.79**	4.23±0.68#	2.87±0.54##,$	3.08±0.71##,$
Pancreas					
SOD activity (U mg^{-1} protein)	0.81±0.06	0.39±0.07**	0.52±0.08#	0.72±0.09##,$	0.76±0.10##,$
MDA (nmol mg^{-1} protein)	3.51±0.27	6.88±1.66**	5.02±1.05#	3.79±0.78##,$	3.65±0.62##,$

Values are Mean± SD of 8 to 10 animals in each group, **p<0.01 vs. that of control group, #p<0.05, ##p<0.01 vs. that of DM group, $p<0.05 vs. that of DM+BBR50 group, DM: Diabetes mellitus, BBR: Berberine, Y53: Pseudoberberine, SOD: Superoxide dismutase, MDA: Malondialdehyde

levels (p<0.01 vs. DM group). The antioxidant activity of Y53 was similar to that of BBR at 100 mg kg^{-1} and superior to BBR at 50 mg kg^{-1} (p<0.05).

The mRNA expression levels of proinflammatory cytokines like interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) elevated significantly in the liver and pancreas of the diabetic mice (Fig. 4a) (p<0.01 or p<0.001 vs. control). The Y53 at 50 mg kg^{-1} largely suppressed the over-expression of IL-6 and TNF-α in the liver and pancreas (Fig. 4a) and inhibited inflammatory cell infiltration in the liver (Fig. 3a-b).

As BBR was reported to upregulate the expression of nuclear factor erythroid-2-related factor-2 (Nrf2) and stimulate the Nrf2/heme oxygenase-1 (HO-1) antioxidant pathway in fatty liver and DM (Li *et al*., 2014a; Yuan *et al*., 2015), we examined the efficacies of Y53 on this pathway in the present study. As shown in Fig. 4b, the expression levels of Nrf2 and its target genes like HO-1 and NADPH quinine oxidoreductase-1 (NQO-1) increased in the DM group of mice (p<0.05 vs. control). Similar results which might due to an adaptive response were observed in another report (Wang *et al*., 2011). When the diabetic mice were treated with 50 mg kg^{-1} of Y53,

the levels of Nrf2, HO-1 and NQO-1 mRNAs elevated by about 1.94 to 2.32-fold in the liver and pancreas ($p<0.01$ vs. DM group). The activities of Y53 in reducing proinflammatory response and upregulating Nrf2/HO-1/NQO-1 were similar to those of BBR at 100 mg kg^{-1} and superior to BBR at 50 mg kg^{-1} ($p<0.05$ or $p<0.01$).

DISCUSSION

Natural product BBR is an ancient drug but may become a promising candidate for the development of novel oral hypoglycemic agents in the near future. Here we study the efficacies of compound Y53, a BBR analogue, in diabetic mice with liver steatosis.

Our results proved that Y53 lowered serum lipids and glucose stronger than BBR at the same dose, which were in agreement with our previous reports (Li *et al*., 2009; Shan *et al*., 2013). We also found that in the mice, Y53 could upregulate hepatic expression of LDLR and InsR more potently than BBR. Berberine modulated LDLR and InsR mRNA expression through different mechanisms. In detail, LDLR mRNA was upregulated by BBR through the AMP-activated protein kinase (AMPK)/Raf-1/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway (Kong *et al*., 2004; Li *et al*., 2014b) while InsR expression was increased by BBR in a Protein Kinase D (PKD)-dependent but ERK-independent manner (Kong *et al*., 2009; Zhang *et al*., 2010).

Interestingly, in the *in vitro* experiments, the LDLR-upregulating activity of Y53 was superior to that of BBR (Li *et al*., 2009) but its activity on InsR expression was equal to that of BBR (Shan *et al*., 2013), which might suggest that Y53 itself had stronger bioactivity in modulating LDLR but not InsR. Our previous results also demonstrated that the pharmacokinetic profiles of Y53 such as the maximum plasma concentration (C_{max}) and area under concentration-time curve (AUC_{0-24}) were superior to those BBR, which might be attributed to the avoidance of P-glycoprotein (P-gp)-mediated cellular efflux of Y53 (Shan *et al*., 2013). The improved pharmacokinetic profiles of Y53 could explain its enhanced activity on InsR expression *in vivo*, which was crucial for insulin signaling and glucose homeostasis (Kong *et al*., 2009).

The stimulating activity of Y53 on the AMPK pathway was similar to that of BBR in cultured cells (Shan *et al*., 2013). However, Y53 promoted LDLR mRNA expression more effectively than BBR (Li *et al*., 2009), which implied that some unknown mechanisms might be involved in the activity of Y53 in modulating LDLR. In liver cells, in addition to the ERK pathway (Kong *et al*., 2004), BBR could upregulate LDLR expression through proprotein convertase subtilis in/kexin type 9 (PCSK9) down regulation (Dong *et al*., 2015). It is of scientific importance to study the efficacy of Y53 on PCSK9, a post-translational inhibitor of LDLR (Dong *et al*., 2015), to clarify its improved activity on LDLR expression.

Due to the improved pharmacokinetic profiles, it could be inferred that Y53 might stimulate the AMPK pathway more potently than BBR *in vivo*. The AMPK is a key molecule that controls energy balance and lipid/glucose metabolism in organisms (Coughlan *et al*., 2014). The improved efficacies of Y53 on TG metabolism and liver steatosis in the current study were in agreement with our inference.

One of the new findings of this study was that Y53 had improved activities in suppressing oxidative stress and proinflammatory response than BBR in various tissues in diabetic mice. Oxidative stress and proinflammatory response were critical for the onset of DM as excessive Reactive Oxygen Species (ROS) and proinflammatory cytokines might damage the insulin signaling pathway and have negative influence on islet function (Evans *et al*., 2005; Donath and Shoelson, 2011). Our results indicated that Y53 significantly restored pancreas weight and serum insulin level in diabetic mice. The detailed mechanisms for Y53 to protect the islet function in DM merit further investigation in the future.

In addition to the antioxidant efficacy, the Nrf2 pathway also had anti-inflammatory and cytoprotection activities in tissues (Jimenez-Osorio *et al*., 2015; Li *et al*., 2014a). Now Nrf2 is considered as a useful target to treat DM and its complications (Jimenez-Osorio *et al*., 2015; Li *et al*., 2014a). Results showed that Y53 had strong activities in upregulating Nrf2 and its target genes, which were in agreement with its potent antioxidant and anti-inflammatory activities.

CONCLUSION

Results of the present study indicate that compound Y53, a BBR analogue, ameliorates oxidative stress and proinflammatory response and improves metabolic disorders more potently than BBR when administered at the same dose in a mice model of DM and fatty liver. Further research work should focus on the activities of Y53 on the islet function as well as complications in DM. The Y53 may be developed as a useful new anti-diabetic agent in the future.

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