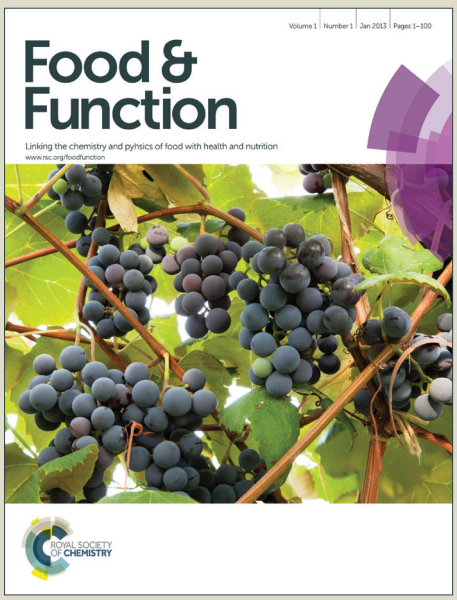
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Effects of puerarin in STZ-induced diabetes rats by oxidative stress and the TGF-β1/Smad2 pathway

ShaoYi She [1], WeiJuan Liu [2], Tong Li [3]* and YingKai Hong [1] *
1 Department of Urology, The First Affiliated Hospital of Shantou University Medical College, Shantou, PR China.
2 Department of gynecology, The First Affiliated Hospital of Shantou University Medical College, Shantou, PR China.
3 Department of Paediatrics, The First Affiliated Hospital of Shantou University Medical College, Shantou, PR China.

* corresponding authors: tonglistm@sohu.com (T. Li)

Abstract

The present study aimed to investigate the effects of pueraria on streptozotocine (STZ)-induced renal damage and its possible mechanisms. Wistar rats were randomly divided into five groups: normal control group, diabetes untreated model group, two-dosages (140 and 200 mg/kg bw/day) of puerarin treatment groups and a positive control group. Rats were studied 30 days after the STZ treatment, and the diabetes untreated model group presented with significantly higher kidney index, blood glucose, triglyceride (TG), total cholesterol (TC), malondialdehyde (MDA), Interferon-γ (IFN-γ), and IFN-γ/IL-4 levels, lower body weight, fasting blood insulin (FPI), IL-4, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and nitric oxide (NO) levels and worse renal function (higher blood urea nitrogen (BUN), serum creatinine (SCr), urine protein (UP) levels and glomerular extracellular matrix (relative area)) compared with the normal control group ($p < 0.05$). Furthermore, RT-FQ-PCR and western blot analysis showed that TGF-β1, Smad2, CTGF and FN protein and mRNA expression was significantly increased in the diabetes untreated model group compared with the normal control group. In constrast, the puerarin treatment dose-dependently significantly decreased kidney index, blood glucose, TG, TC, MDA, IFN-γ, and IFN-γ/IL-4 levels, increased body weight, FPI, IL-4, SOD, CAT, GSH-Px and NO levels and improved renal function (lower BUN, SCr, UP levels and glomerular extracellular matrix (relative area)) in puerarin treatment groups ($p < 0.05$). In addition, the mRNA and protein expression of TGF-β1, Smad2, CTGF and FN was downregulated. It can be concluded that puerarin exerted its anti-diabetes effect on the STZ-treated rats through the inhibition of the TGF-β1/Smad2 pathway.

Keywords: Streptozotocine, Diabetes, Puerarin, TGF-β1/Smad2 pathway, Kidney

1. Introduction

Diabetes mellitus is caused by the altered carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs like liver, adipocytes and skeletal muscles to insulin. It is a prevalent disease affecting citizens of both developed and developing countries. Under chronic hyperglycemic condition, the concentration gradient between the extra-and intra-cellular environment is enough to drive glucose into cells like erythrocytes. Subsequent increased sorbitol and the glycogenic pathways yield osmotic damage, while glycosylation reaction leads to formation of advanced glycation end products which cause diabetic complications through cell signaling [1]. The adverse effects of glycation and polyol accumulation leading to vascular diabetic complications are widely recognized [2,3].

Streptozotocin (STZ)-diabetes in adult rats has been shown to reduce androgen receptor content [4], uptake and retention of [3H]testosterone in accessory sex glands [5]. Earlier reports from the author's laboratory [6-8] and others [9, 10] have shown decreased synthesis and availability of testosterone in STZ-induced diabetic rats. Induction of diabetes causes a significant reduction in prostatic weight and serum testosterone level in rats.

Transforming growth factor-beta1 (TGF-β1) is a multifunctional cytokine that plays an important role in wound healing, cell proliferation, differentiation, apoptosis, and the immune response in several cells [11]. In particular, TGF-β1 is a key mediator of diabetic nephropathy and increases the ECM proteins, such as collagen I, IV, laminin, and fibronectin intheglomeruli [12]. In addition, TGF-β1 is known to mediate its fibrotic effects by activating the receptorassociated Smads, including Smad2 and Smad3 [13]. These effects are blocked by TGF- β1 neutralizing antibody or antisense strategies [14, 15]. Advanced glycation end products increase oxidative stress, causing induction of transcription factors such as nuclear transcription factor kappa B (NF-κB), which transcribe genes involved in glomerulosclerosis [16, 17].

Puerarin lobata (daidzein 8-C-glucoside), the main isoflavone glycoside found in the root of *P. lobata*, has been used for various medicinal purposes in traditional oriental medicine for thousands of years. Recently, several studies have shown that puerarin, as an effective, natural free-radical scavenger, is capable of preventing the damage caused by free radicals and LPO [18, 19]. Puerarin increases the activities of SOD and catalase during oxidative stress, and strengthens the response capability of islet cells against oxidative stress, which leads to cell apoptosis [20]. It has also been reported that puerarin or *P. lobata* extract exhibit activity in the treatment of diabetes mellitus [21]. Many of Chinese medicines used for diabetes on market contain *P. lobata*. The toxicological information about puerarin is not sufficient, but life-threatening interaction between *P. lobata* extract and methotrexate in rats has been found [22, 23]. In addition, both verapamil and erythromycin lactobionate could inhibit the elimination of puerarin in rats [24, 25].

The present study aimed to provide a mechanistic insight into the potential effects of puerarin in an STZ-induced rats model.

2. Material and method

2.1. Material

Puerarin was purchased from xian xiaocao biotechnology Ltd, China. Its purity is 96%.

2.2. Animals

Male Wistar rats weighing 210–230 g were purchased from the Medical Laboratory Animal Center of Shantou University, China, and the quarantined and acclimated examinations were performed to confirm the normality. The research was conducted in accordance with the Shantou University guidelines for laboratory animal use and care.

2.3. Induction of diabetes mellitus in rats

Diabetes was induced by injecting STZ (Sigma, USA) at a dose of 40 mg/kg body weight (bw) in 0.1 M cold citrate buffer of pH4.5, interaperitoneally. STZ-injected animals exhibited severe gly-cosuria and hyperglycemia and rats were stabilized over a period of 7 days. Onset of diabetes was confirmed in the experimental rats by measuring blood glucose concentration at 96 h after injection of STZ. The rats with blood glucose level above 250 mg/dl were considered to be diabetic and were used for the experiment. Control rats were administrated with citrate buffer (pH 4.5) [26].

2.4. Experimental design

Animals were divided into five groups with 10 animals each. Group I served as a normal control; group II had STZ-treated surviving diabetic model control rats; group III had the STZ-induced diabetic rats treated with puerarin (140 mg/kg bw/day), group IV had the STZ-induced diabetic rats treated with puerarin (200 mg/kg bw/day), group V served as a positive control and received Valsartan (0.5 mg/kg/bw) for 30 day, orally administrated. Rats were fasted and sacrificed at the termination of the experiment i.e. on the 30th day the blood samples were collected to analyze the effect of puerarin on biochemical parameters.

2.5. Antioxidant enzymes activities and lipid peroxidation

GSH-Px activity was measured using the spectrophotometric method, as previously described by Paglia and Valentine (1967). MDA concentration, as a marker of lipid peroxide production, was measured spectrophotometrically by the method of Ohkawa et al. [27]. Catalase activity was determined using the method of Aebi [28]. Activity of SOD was investigated using the modified method of Marklund and Marklund [29]. The assay mixture contained 800 µl of Tris–HCl buffer (50 mM), 100 µl of pyrogallol (0.2 mM) and 100 µl tissue homogenate. Change of the absorbance was measured at 420 nm against enzyme blank at 10 s intervals for 1 min.

2.6. Blood NO, TG, TC, IFN-γ, IL-4 levels

Blood NO, TG, TC, IFN-γ, IL-4 levels were measured with commercially available kits according to the manufacturer's instructions.

2.7. Measurement of Blood urea nitrogen (BUN), creatinine and total protein

Creatinine and BUN were determined enzymatically using commercially available kits (Spinreact, Gerona, Spain). Total protein was determined using Diamond Diagnostic Kit (Egypt), according to the manufacturer's protocol. Serum levels of BUN, creatinine and total protein were determined by colorimetric methods using UV–visible spectrophotometer (UV-1601PC, Shimadzu, Japan).

2.8. Fasting blood glucose and fasting insulin levels

Fasting blood glucose and insulin levels were analyzed in all groups before and after the experiment. Blood glucose was measured using glucometer (Abott, USA) while Fasting plasma insulin was measured by ELISA assay (Linco, Millipore).

2.9. Protein lysate preparation and Western blot analysis of TGF-β1, Smad2, CTGF and FN

Total protein was extracted with a RIPA solution (radioimmuno-precipitation assay buffer) at −20 °C overnight. We used BSA (bovine serum albumin) as a protein standard to calculate equal total cellular protein amounts. Protein samples (30 μg) were resolved by denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using standard methods, and then were transferred to PVDF membranes by electroblotting and blocking with 1% BSA. The membranes were probed with the primary antibodies (TGF-β1, Smad2, CTGF, FN and β-actin) at 4 °C overnight, washed three times with PBST, and incubated for 1 h at 37 °C with horseradish peroxidase conjugated secondary antibodies. The membranes were washed three times and the immunoreactive proteins were detected by enhanced chemiluminescence (ECL) using hyperfilm and ECL reagent (Amersham International plc., Buckinghamshire, U.K.). The results of Western blot analysis were quantified by measuring the relative intensity compared to the control using Kodak Molecular Imaging Software and represented in the relative intensities.

2.10. Real time fluorescence quantitative PCR analysis (RT-FQ-PCR)

Total RNA was isolated from liver tissue using RNeasy Mini Kit (Qiagen, Hilden, Dü sseldorf, Germany) as recommended by manufacturer. For cDNA synthesis, 1 μg of the total RNA was treated with DNase I and reverse-transcribed with RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, ON, Canada) using oligo(dT) primers. Then reverse transcription, amplification and detecting fluorescence signals were detected by the real time quantitative PCR instrument. To perform the qPCR reaction, 10 μl Maxima SYBR Green/ROX qPCR Master Mix (2×) were mixed with forward and reverse primers (10 μM each), cDNA template and nuclease-free water to reach a final volume of 20 μl. RT-qPCR amplification conditions included an initial step at 50 ^{o}C for 2 min, followed by a step of initial denaturation at 95 ^{o}C for 10 min and a subsequent 2-step program at 95 ^{o}C for 15 s and 58 ^{o}C for 60 s for 40 cycles. PCR reactions were carried out in triplicate. β-actin (ACTB) "housekeeping" genes were used for normalization and data were analyzed with $\Delta\Delta C_t$ (threshold cycle) method. Sequences of the primers (Invitrogen, Carlsbad, CA, USA) used in this study

are listed as followings.

TGF-β1: 5'-CCCGCATCCCAGGACCTCTCT-3' and 5'-CGGGGGACTGGCG AGCCTTAG-3';
Smad2: 5'-AGAAGTCAGCTGGTGGGTCTG-3' and 5'-TCATGATGACTGTGAAGATCAGG-3';
CTGF: 5'-CTGCAGGCTAGAGAAGCAGAG-3' and 5'-GATGCACTTTTTGCCCTTCT-3';
FN: 5'-GGACACTATGCGGGTCACTT-3' and 5'-TCAAAACCAGTTGGGGAGTC-3';
β-actin: 5'-GCTGCGTGTGGCCCCTGAG-3' and 5'-ACGCAGGATGGCATGAGGGA-3'.

2.11. Statistical analysis

All experiments were repeated at least three times and all values are represented as means±S.D. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze differences. Values of $P<0.05$ were considered statistically significant.

3. Result

The effects of puerarin treatment on body weight and kidney index are presented in Table 1. Rats in the untreated model control group had the lowest weight gain and kidney index. Puerarin treatment (140 or 200 mg/kg bw/day) significantly and dose-dependently enhanced body weight and kidney index, respectively compared to the untreated model control group ($p < 0.05$). Valsartan treatment (0.5 mg/kg/bw) also significantly enhanced body weight and kidney index, respectively compared to the untreated model control group ($p < 0.05$).

Table 1. Effect of puerarin treatment on rats' body weight and kidney index

Group	Body weight	Kidney index
I	360.1±13.8	5.05±0.35
II	255.7±18.4 **	9.78±0.72 **
III	306.3±22.7 ##	7.45±0.53 #
IV	342.5±24.1 ##	6.52±0.44 ##
V	335.1±26.3 ##	5.38±0.32 ##

Data are expressed as mean±SD (n = 10). **$P<0.01$, compared with normal control group (I); # $P<0.05$, ## $P<0.01$, compared with diabetes model control group (II).

The effects of puerarin treatment on blood glucose, FPI, TG and TC are presented in Table 2. Rats in the untreated model control group had the highest blood glucose, TG, TC and lowest FPI level. Puerarin treatment (140 or 200 mg/kg bw/day) caused an decrease in blood glucose, TG, TC with a concomitant increase in FPI values as shown in Table 3. In addition, valsartan treatment (0.5 mg/kg/bw) also caused an decrease in blood glucose, TG, TC with a concomitant increase in FPI values as

shown in Table 3.

Table 2. Effect of puerarin treatment on blood glucose, FPI, TG and TC levels in rats of different groups

Group	Blood glucose (mmol/L)	FPI (pmol/L)	TG (mmol/L)	TC (mmol/L)
I	10.38±0.98	42.63±3.97	1.12±0.11	1.08±0.09
II	20.71±1.31 **	21.08±1.54 **	1.86±0.13 **	2.14±0.13 **
III	18.03±1.19 #	28.06±2.81 #	1.54±0.1 ##	1.77±0.13 ##
IV	14.26±1.06 ##	35.31±2.42 ##	1.39±0.15 ##	1.48±0.11 ##
V	12.33±0.92 ##	39.66±1.87 ##	1.27±0.11 ##	1.22±0.08 ##

Data are expressed as mean±SD (n = 10). **P<0.01, compared with normal control group (I); #P<0.05, ##P<0.01, compared with diabetes model control group (II).

The activities of SOD, CAT and GSH-Px and the levels of MDA and NO in the rats' kidneys are summarized in Table 3. Results showed that the STZ negative control group significantly decreased the level of SOD, CAT and GSH-Px compared with the normal control group. The administration of low and high doses of puerarin successfully enhanced the SOD, CAT and GSH-Px levels. The valsartan positive control group registered a significantly higher SOD, CAT and GSH-Px levels. In terms of MDA and NO, the STZ negative control group expressed a significantly higher MDA and NO levels compared with the normal control group and low and high doses of puerarin treatment (140 or 200 mg/kg bw/day) treatments were able to resume the MDA and NO levels.

Table 3. Effect of puerarin treatment on serum MDA, SOD, CAT, GSH-Px and NO levels in rats of different groups

Group	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)	GSH-Px (U/mg)	NO (μmol/L)
I	3.12±0.18	206.4±15.73	36.52±2.55	62.81±3.09	38.17±1.63
II	7.81±0.63 **	89.3±5.3 **	15.71±1.21 **	27.85±1.37 **	14.38±1.07 **
III	5.95±0.33 ##	138.2±9.8 ##	23.74±1.75 ##	44.19±2.51 ##	23.11±1.26 ##
IV	4.68±0.25 ##	173.2±11.2 ##	29.93±1.99 ##	57.38±2.88 ##	29.61±1.84 ##
V	3.99±0.14 ##	109.7±8.5 ##	22.61±2.05 ##	40.61±2.73 ##	24.57±1.58 ##

Data are expressed as mean±SD (n = 10). **P<0.01, compared with normal control group (I); #P<0.05, ##P<0.01, compared with diabetes model control group (II).

Plasma IFN-γ level in the untreated model control group were significantly increased, whereas IL-4 level was decreased (Table 4). Puerarin treatment (140 or 200 mg/kg bw/day) restored the IFN-γ level and prevented the decreasing of IL-4 level

when compared to untreated model control group. Valsartan treatment (0.5 mg/kg/bw) also reduced the IFN-γ level and increased IL-4 level.

Table 4. Effect of puerarin treatment on serum IFN-γ, IL-4 and IFN-γ/IL-4 levels in rats of different groups

Group	IFN-γ (pg/ml)	IL-4 (pg/ml)	IFN-γ/IL-4
I	15.29±0.94	4.81±0.28	3.13±0.28
II	23.06±1.74 **	3.15±0.3 **	7.31±0.66 **
III	20.41±1.63 #	3.92±0.22	5.13±0.48 ##
IV	17.15±1.39 ##	4.49±0.29 #	3.79±0.31 ##
V	14.86±1.13 ##	4.73±0.5 #	3.15±0.29 ##

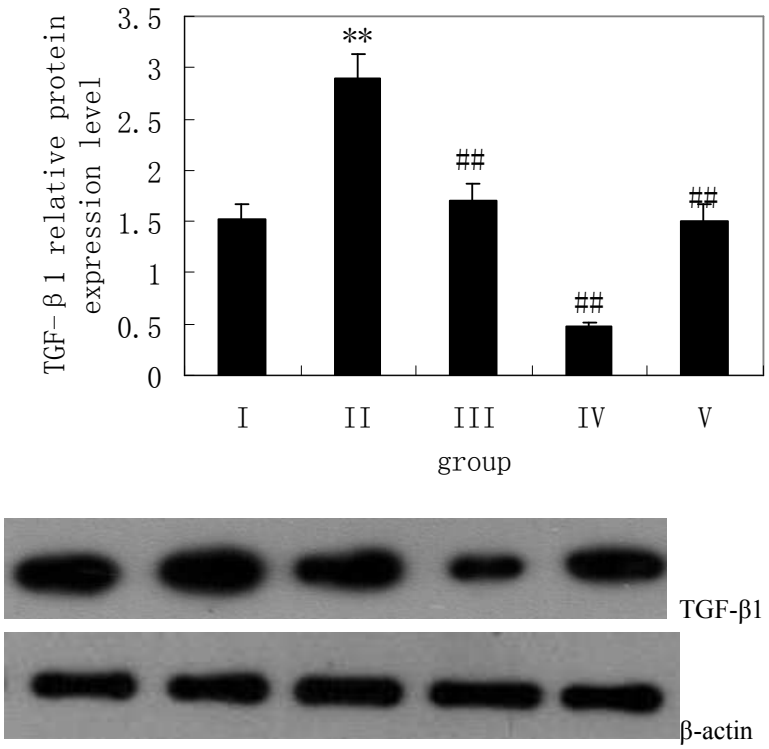
Data are expressed as mean±SD (n = 10). **P<0.01, compared with normal control group (I); # P<0.05, ## P<0.01, compared with diabetes model control group (II).

The values of BUN, SCr, UP and relative area of glomerular extracellular matrix were increased in untreated model control rats when compared to normal control rats. Treatment of rats with puerarin (140 or 200 mg/kg bw/day) significantly reduced BUN, SCr, UP and relative area of glomerular extracellular matrix in STZ-induced diabetic rats (Table 5). Likewise, BUN, SCr, UP and relative area of glomerular extracellular matrix was decreased in valsartan-treatment diabetic rats when compared with untreated model control group.

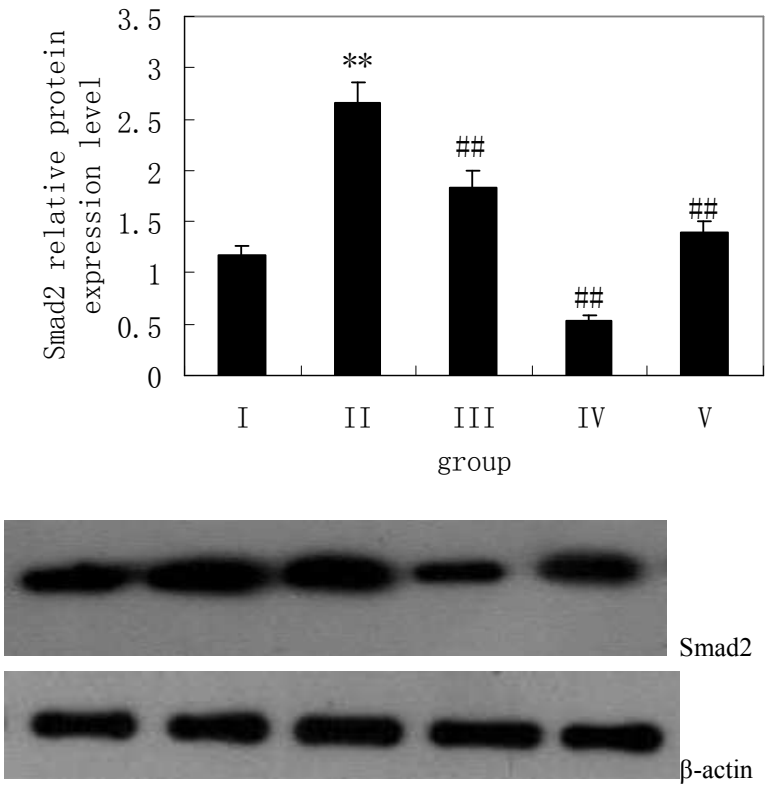
Table 5. Effect of puerarin treatment on BUN, SCr, UP levels and glomerular extracellular matrix (relative area) in rats of different groups

Group	BUN (mmol/L)	SCr (μmol/L)	UP (mmol/L)	Glomerular extracellular matrix (relative area)
I	6.74±0.28	66.28±3.76	11.47±1.27	0.039±0.002
II	15.82±1.09 **	106.34±9.52 **	23.59±1.84 **	0.127±0.011 **
III	12.18±0.96 #	89.39±5.39 ##	18.83±1.47 ##	0.099±0.007 ##
IV	9.69±0.63 ##	74.15±5.03 ##	14.72±1.33 ##	0.081±0.005 ##
V	8.64±0.57 ##	69.49±4.11 ##	12.49±1.05 ##	0.058±0.004 ##

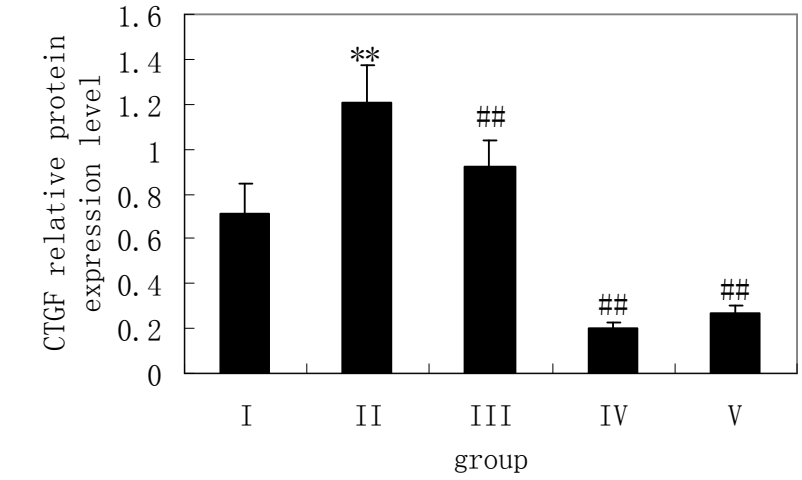
Data are expressed as mean±SD (n = 10). **P<0.01, compared with normal control group (I); # P<0.05, ## P<0.01, compared with diabetes model control group (II).

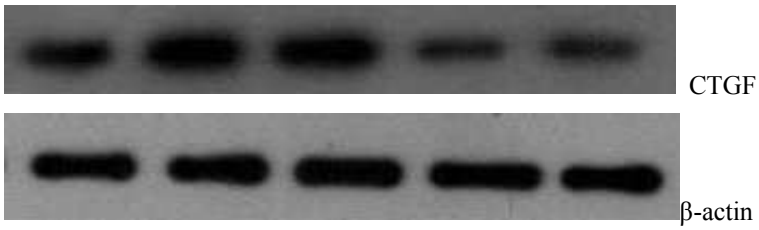


(1)

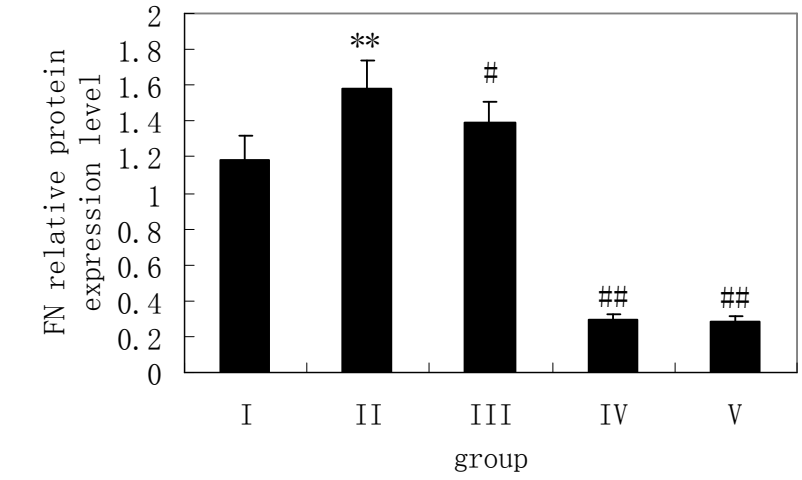


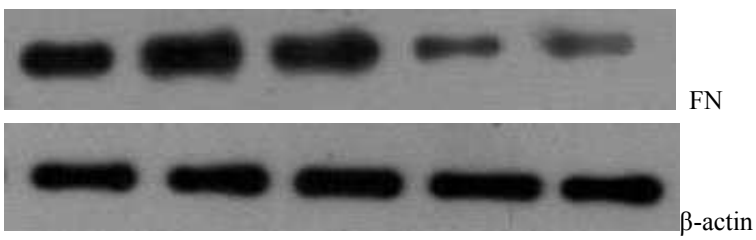
(2)





(3)

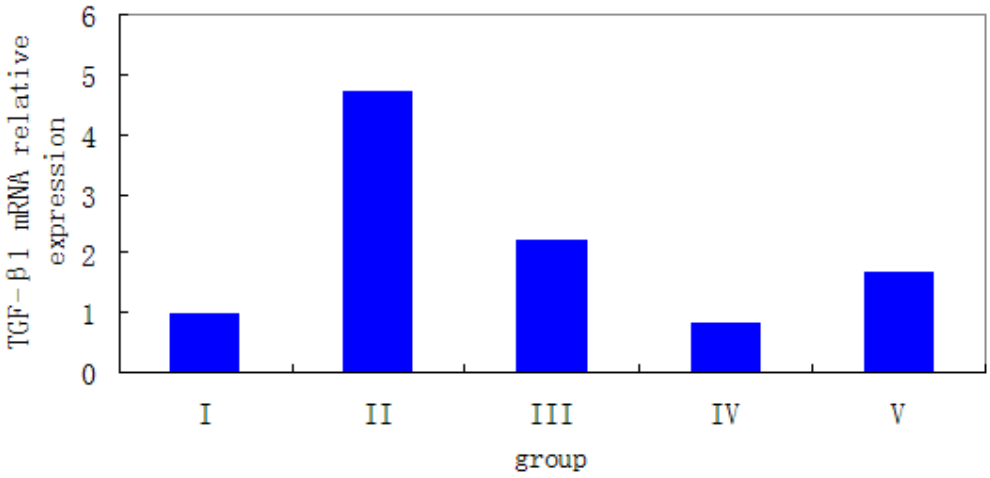




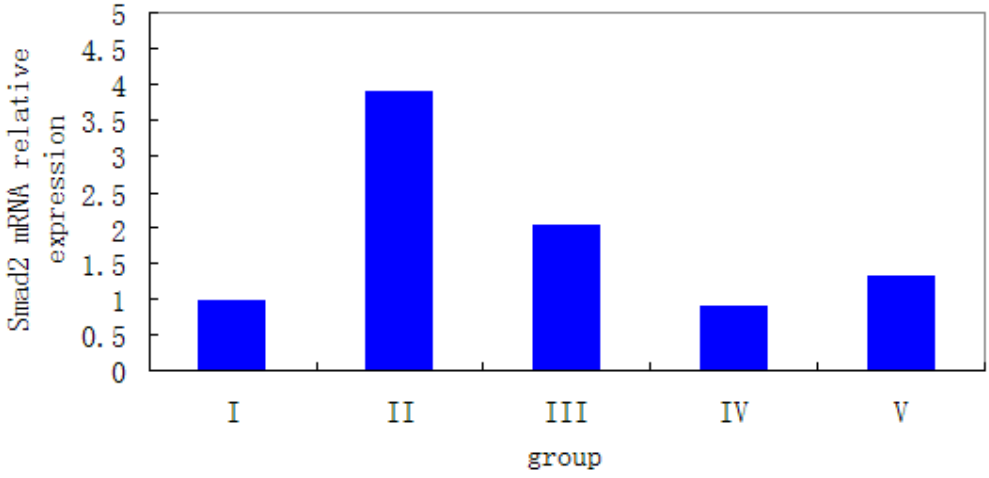
(4)

Fig. 1. Puerarin suppression on TGF-β1 (1), Smad2 (2), CTGF (3) and FN (4) protein expression in STZ-induced diabetes rats. The relative protein levels of TGF-β1, Smad2, CTGF and FN,

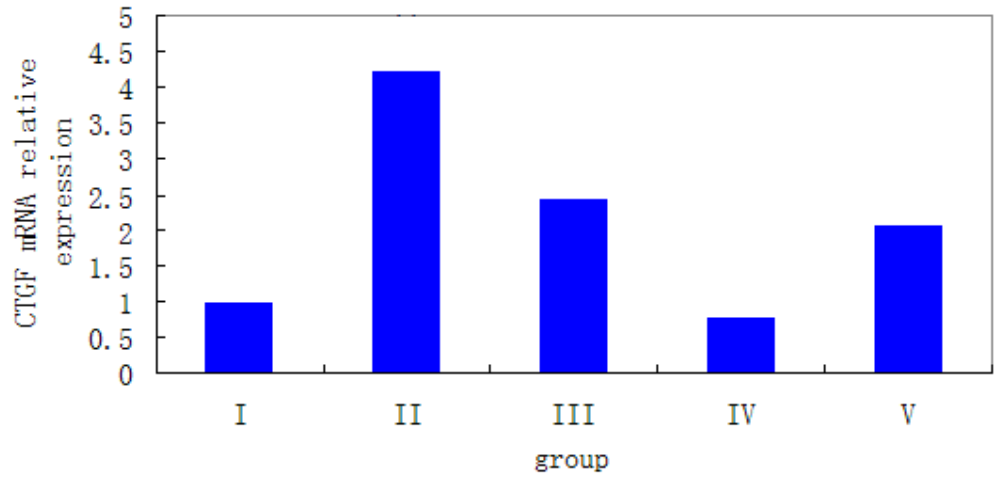
respectively, which were normalized to β-actin. Data are expressed as mean±SD (n = 3). **P<0.01, compared with normal control group (I); $^{\#}$ P<0.05, $^{\#\#}$$P$<0.01, compared with diabetes model control group (II).



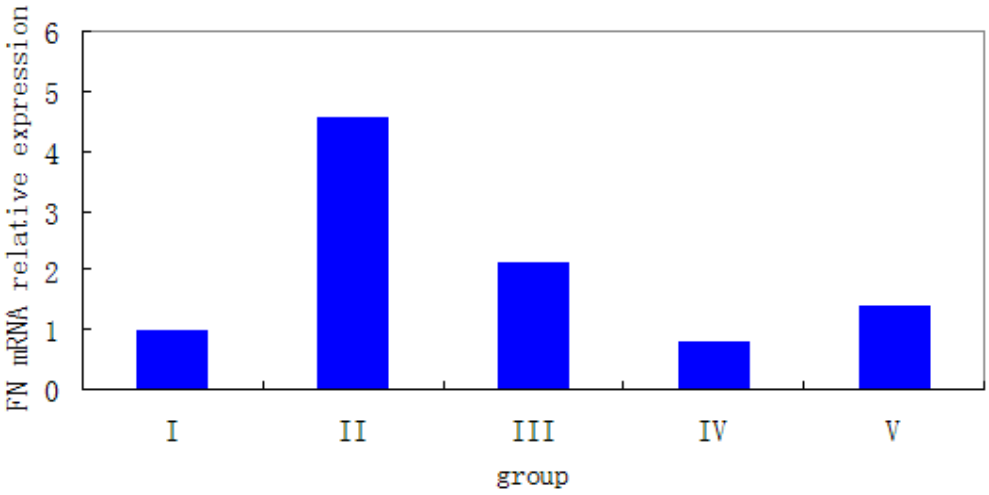
(1)



(2)



(3)



(4)

Fig. 2. Puerarin suppression on TGF-β1 (1), Smad2 (2), CTGF (3) and FN (4) mRNA expression in STZ-induced diabetes rats. The relative mRNA levels of TGF-β1, Smad2, CTGF and FN, respectively, which were normalized to β-actin.

Data of RT-PCR and Western blot showed that the expression of TGF-β1, Smad2, CTGF and FN mRNA and protein in the kidneys of the untreated model control rats was a significant up-regulated compared with in those of the normal control rats. The expression of TGF-β1, Smad2, CTGF and FN mRNA and protein in the kidneys were dramatically decreased in a dose-dependent manner after the administration of puerarin at the dose of 140 and 200 mg/kg. In addition, valsartan showed the similar regulation effect on the gene and protein expression of TGF-β1, Smad2, CTGF and FN (Fig 1 and 2).

4. Discussion

The present study shows the antidiabetogenic effect of puerarin on STZ-induced diabetic rats in a duration-dependent manner. STZ injection caused diabetes mellitus,

which may be due to destruction of β-cells of the islets of Langerhans of the pancreas [30]. STZ-induced diabetes is characterized by severe loss in BW [31] due to the degradation of structural proteins, which are responsible for the changes in BW.

In the present study, BW loss was significant in diabetic rats, but significantly improved BW gain after puerarin administration. In addition, puerarin administration decreased kidney index in diabetic rats. In this work, the antihyperglycemic effects of puerarin were validated in normal and STZ-induced diabetic rats. Puerarin can decrease blood glucose, TG, TC levels and increase FPI levels in a dose dependent manner. The levels of TC and TG have been decreased significantly in diabetic rat after puerarin supplementation. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis, which are under the control of insulin [32]. The puerarin supplementation also results to the significant attenuation in the level of serum HDL towards the control level, which again strengthens the hypolipidemic effect of this extract. There are reports that other medicinal plants have hypoglycemic and hypolipidemic effects that could prevent or be helpful in reducing the complications of lipid profile seen in some cases of diabetes in which hyperglycemia and hypercholes-terolemia coexist [32].

Oxidative stress induces the production of highly reactive oxygen radicals that are toxic to cells, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides [33]. However, endogenous antioxidant enzymes (e.g. SOD, CAT, and GSH-Px) are responsible for the detoxification of deleterious oxygen radicals [34]. ROS also interact with the lipid bilayer and produce lipid peroxides. Several lines of evidence suggest that increased lipid peroxidation constitutes a condition of increased oxidative stress in experimental diabetes [35]. Lipid peroxides such as MDA has already been suggested as an easily accessible biomarker of ROS damage. STZ-induced diabetic rats exhibited an enhanced susceptibility to lipid peroxidation, while antioxidants or puerarin could abrogate lipid peroxidation, which was in consistent with our data. On the contrary, antioxidant enzymes serve as a defense mechanism against ROS. SOD, GSH-Px and CAT are closely linked to oxidative stress. It was reported STZ caused a significant decrease of SOD, GSH-PX and CAT in diabetic rats [36], as well as in diabetic patients [37]. This has been confirmed by our present study. Our current results showed that puerarin had decreased kidney SOD, GSH-Px and CAT activities in a dose-dependent manner in diabetic rats.

In the present study, serum IFN-γ level and ratio of IFN-γ/IL-4 in diabetic model rats significantly increased compared to normal control rats. This showed cellular immune function disorder in diabetic model rats. Puerarin (140 or 200 mg/kg bw/day) treatment decreased serum IFN-γ level and ratio of IFN-γ/IL-4 in diabetic rats. This might be one of anti-diabetes mechanism of puerarin. In addition, puerarin (140 or 200 mg/kg bw/day) treatment decreased BUN, SCr, UP and relative area of glomerular extracellular matrix in STZ-induced diabetic rats, indicating that kidney function has been greatly improved. The suppressive effect of puerarin on levels of FN-γ, IFN-γ/IL-4, BUN, SCr, UP and relative area of glomerular extracellular matrix further suggests that the inhibitory effect of puerarin on STZ-induced kidney dysfunction is related to its antioxidant property.

TGF- β1 is one of profibrotic growth factors. High level of blood glucose can induce wide expression of TGF- β1 gene and protein in kidney. It plays an important role in diabetes fibrosis [38]. Excessive activation of TGF- β1/Smad2 signal pathway results in the extracellular matrix (ECM) accumulation in kidney. This directly or indirectly results in CTGF and FN production. CTGF and FN are one of the important marks of diagnosis of diabetes [39]. Expression of TGF-β1, Smad2, CTGF, FN mRNA and proteins in diabetes model rats are significantly increased compared to normal control rats. Puerarin (140 or 200 mg/kg bw/day) treatment decreased expression of TGF-β1, Smad2, CTGF, FN mRNA and proteins in diabetes rats. This indicates that puerarin plays its anti-diabetes action by suppressing TGF- β1/Smad2 signal pathway.

Our experiment results show that puerarin can reverse STZ-induced increased blood glucose, TC, TG levels, enhance FBI level and decrease STZ-induced oxidative damage in kidney in diabetic rats. In addition, puerarin may improve kidney function by enhancing immunity indexs, BUN, SCr, UP levels and decreasing glomerular extracellular matrix in diabetic rats. In summary, our study demonstrates that renal-protective effect of puerarin may be related with its antioxidant effects, as demonstrated by its ability to inhibit ROS generation, kidney dysfunction, and activation of TGF- β1/Smad2 signals.

Reference

1 R. Ramasamy, S.F. Yan, A.M. Schmidt, The diverse ligand repertoire of the receptor for advanced glycation endproducts and pathways to the complications of diabetes. Vascul Pharmacol. 2012, 57, 160–167.

2 D. Neves, Advanced glycation end-products: a common pathway in diabetes and age-related erectile dysfunction. Free Radic. Res. 2013, 47, 49–69.

3 A. Simm, 2013. Protein glycation during aging and in cardiovascular disease. J. Proteomics, doi: 10.1016/j.jprot.2013.05.012. [Epub ahead of print].

4 M. Tesone, R.M. Oliveira-Filho, L.B. Valle, J.C. Calvo, J.L. Baranao, V.G. Foglia, E.H. Charreau. Androgen receptors in the diabetic rat. Diabetologia 1980, 18, 385-390.

5 A. Oksanen, P. Tuohimaa. Impaired 3H-testosterone uptake and metabolism in the accessory sex glands of diabetic castrated male rats. Horm Res 1975, 6, 157-168.

6 S. Sudha, V. Ravisankar, P.M. Julie, J. Arunakaran, P. Govindarajulu, K. Balasubramanian. Influence of streptozotocin-induced diabetes and insulin replacement on the pituitary-testicular axis during sexual maturation in rats. Exp Clin Endocrinol Diab 2000, 108, 14-20.

7 S. Sudha, V. Ravisankar, G. Valli, P. Govindarajulu, K. Balasubramanian. Streptozotocin-induced impairs prolactin binding to Leydig cells in prepubertal and pubertal rats. Horm Metab Res 1999, 31, 538-586.

8 S. Soudamani, S. Yuvaraj, T. Malini, K. Balasubramanian. Experimental diabetes has adverse effects on the differentiation of ventral prostate during sexual maturation of rats. Anat Rec 2005, 287A, 1281-1289.

9 F.T. Murray, D.F. Cameron, J.M. Orth. Gonadal dysfunction in the spontaneously diabetic BB rat. Metabolism 1983, 32, 141-147.

10 K. Ikeda, Y. Wada, H.E. Foster Jr, Z. Wang, R.M. Weiss, J. Latifpour. Experimental diabetes-induced regression of the rat prostate is associated with an increased expression of transforming growth factor-β. J Urol 2000, 164, 180-185.
11 J.P. Grande, Role of transforming growth factor-beta in tissue injury and repair. Proc. Soc. Exp. Biol. Med. 1997, 214, 27–40.
12 A.B. Roberts, B.K. Mccune, M.B. Sporn, TGF-beta: regulation of extracellular matrix. Kidney Int. 1992, 41, 557–559.
13 Zimmerman, C.M., Padgett, R.W., 2000. Transforming growth factor B signaling mediators and regulators. Gene 249, 17–30.
14 K. Sharma, Y. Jin, J. Guo, F.N. Ziyadeh, Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. Diabetes 1996, 45, 522–530.
15 F.N. Ziyadeh, B.B. Hoffman, D.C. Han, L. Iglesias-De, M.C. Cruz, S.W. Hong, M. Isono, S. Chen, T.A. McGowan, K. Sharma,. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal anti-transforming growth factor-beta antibody in db/db diabetic mice. Proc. Natl Acad. Sci. USA 2000, 97, 8015–8020.
16 S. Ohga, K. Shikata, K. Yozai, D. Okada, H. Usui, J. Wada, Y. Shikata, H. Makino,. Thiazolidinedione ameliorates renal injury in experimental diabetic rats through anti-inflammatory effects mediated by inhibition of NF-kappaB activation. Am. J. Physiol. Ren. Physiol. 2007, 292, F1141–F1150.
17 G. Wolf. Newinsights into the pathophysiology of diabetic nephropathy: from haemodynamics to molecular pathology. Eur. J. Clin. Investig. 2004, 34, 785–796.
18 F.L. Xiong, X.H. Sun, L. Gan, X.L. Yang, H.B. Xu. Puerarin protects rat pancreatic islets from damage by hydrogen peroxide. Eur J Pharmacol 2006, 529, 1-7.
19 L. Bebrevska, K. Foubert, N. Hermans, S. Chatterjee, E. Van Marck. In vivo antioxidative activity of a quantified Pueraria lobata root extract. J Ethnopharmacol 2010, 127, 112-117.
20 R.M. Han, Y.X. Tian, E.M. Becker, M.L. Andersen, J.P. Zhang, L.H. Skibsted. Puerarin and conjugate bases as radical scavengers and antioxidants: molecular mechanism and synergism with betacarotene. J Agric Food Chem 2007, 55, 384-391.
21 J.G. Shen, M.F. Yao, X.C. Chen, Y.F. Feng, Y.H. Ye, Z.H. Tong, Effects of puerarin on receptor for advanced glycation end products in nephridial tissue of streptozotocin-induced diabetic rats. Mol. Biol. Rep. 2009, 36, 2229–2233.
22 H.M. Chiang, S.H. Fang, K.C. Wen, S.L. Hsiu, S.Y. Tsai, Y.C. Hou, Y.C. Chi, P.D. Chao. Life-threatening interaction between the root extract of Pueraria lobata and methotrexate in rats. Toxicol. Appl. Pharmacol. 2005, 209, 263–268.
23 H.J. Chung, M.J. Chung, S.J. Houng, J. Jeun, D.K. Kweon, C.H. Choi, J.T. Park, K.H. Park, S.J. Lee, Toxicological evaluation of the isoflavone puerarin and its glycosides. Eur. Food Res. Technol. 2009, 230, 145–153.
24 Y. P. Hwang, H. G. Kim, T. T. Hien, M. H. Jeong, T. C. Jeong, H. G. Jeong. Puerarin activates endothelial nitric oxide synthase through estrogen receptor-dependent PI3-kinase and calcium-dependent AMP-activated protein kinase. Toxicol Applied Pharmacol, 2011, 257, 48-58.

25 C.-M. Liu, J.-Q. Ma, Y.-Z. Sun. Puerarin protects rat kidney from lead-induced apoptosis by modulating the PI3K/Akt/eNOS pathway. Toxicol Applied Pharmacol, 2012, 258, 330-342.
26 S. Sarkar, M. Pranava, R.A. Marita. Demonstration of the hypoglycemic action ofMomordica charantia in a validated animalmodel of diabetes. Pharmacol Res1996;33:1–4.
27 D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 1967, 70, 158-169.
28 H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 1979, 95, 351-358.
Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
29 S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 1974, 47, 469–474.
30 H.J. Kim, I.G. Chae, S.G. Lee, H.J. Jeong, Effects of fermented red ginseng extracts on hyperglycemia in streptozotocin-induced diabetic rats. In: Eun-Ju Lee, E.J., Lee, I.S. (Eds.), J. Ginseng Res. 2010, 34, 104-112.
31 V. Chen, C.D. Ianuzzo, Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. Can. J. Physiol. Pharmacol. 1982, 60, 1251-1256.
32 S.B. Sharma, A. Nasir, K.M. Prabhu, P.S. Murthy, G. Gev. Hypoglycaemic andhypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana inalloxan-induced diabetic rabbits. J Ethnopharmacol 2003, 85, 201–206.
33 N. Haugaard. Cellular mechanisms of oxygen toxicity. Physiol Rev 1968, 48, 311–373.
34 R.F. Del Maestro. An approach to free radicals in medicine and biology. Acta Physiol Scand 1980, 492, 153–168, Suppl.
35 K. Welt, J. Weiss, R. Martin, T. Hermsdorf, S. Drews, G. Fitzl, Ginkgo biloba extract protects rat kidney from diabetic and hypoxic damage. Phytomedicine 2007, 14,196–203.
36 M.M. Anwar, A.R. Meki, Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 2003, 135, 539–547.
37 S. Bhatia, R. Shukla, S.Venkata Madhu, J. Kaur Gambhir, K. Madhava Prabhu, Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin. Biochem. 2003, 36, 557–562.
38 K. Miyauchi, Y. Takiyama, J. Honjyo, M. Tateno, M. Haneda. Upregulated IL-18 expression in type 2 diabetic subjects with nephropathy: TGF-β1 enhanced IL-18 expression in human renal proximal tubular epithelial cells. Diabetes Res Clin Pract, 2009, 83, 190-199.
39 H. Y. Lan, A. C.-K. Chung. TGF-β/Smad Signaling in Kidney Disease. Seminars in Nephrology, 2012, 32, 236-243.