Berberine protects diabetic nephropathy by suppressing epithelial-to-mesenchymal transition involving the inactivation of the NLRP3 inflammasome

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ABSTRACT

Accumulating evidence has implicated that berberine (BBR) has a beneficial effect on diabetic kidney disease (DKD), but its mechanism is not clear. The aim of this study was to assess whether berberine could alleviate tubulointerstitial fibrosis and attenuate epithelial-to-mesenchymal transition (EMT) and its possible molecular mechanism. 

High-fat diet (HFD) followed by injection of STZ was used to induce diabetic rats \( ^{\text{in vivo}} \). After the onset of diabetes, rats were treated with either BBR or saline for 12 weeks. 

In vitro, the human renal proximal tubular epithelial cell line (HK-2) was exposed to high glucose, with or without BBR. The influence of berberine on renal tubulointerstitial histological changes, markers of epithelial-to-mesenchymal transition (EMT) and (NOD-like receptor pyrin domain-containing protein 3) NLRP3 inflammasome expression were examined. Results showed that \( ^{\text{in vivo}} \) BBR could significantly ameliorate microalbumin and renal pathologic changes in diabetic rats. Immunofluorescence showed that BBR could inhibit EMT. Furthermore, BBR could down-regulate the level of the NLRP3 inflammasome in diabetic rats. Consistently, \( ^{\text{in vitro}} \) BBR suppressed high glucose-induced EMT and activation of NLRP3 inflammasome in HK-2. Our study demonstrated that BBR could inhibit high glucose-induced EMT and renal interstitial fibrosis by suppressing the NLRP3 inflammasome. BBR might be used as a novel drug to ameliorate tubulointerstitial fibrosis in DKD.

1. Introduction

Diabetic kidney disease (DKD) is a common and serious microvascular complication of diabetes mellitus. DKD has become the main cause of the end-stage renal disease (ESRD) \([1,2]\). Although DKD patients are clinically received lowering blood glucose or blood pressure treatment \( s \), the progression of DKD is not completely prevented and finally develops to ESRD \([3–5]\).

DKD is characterized by persistent albuminuria caused by renal interstitial fibrosis, which is triggered by the accumulation of extracellular matrix (ECM) in glomerular mesangium and tubulointerstitium and renal tubular epithelial-mesenchymal transition (EMT) \([6,7]\). Loss of E-cadherin and increased expression of \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA) are the characteristics of EMT \([8–10]\). Control of EMT has been identified and may be used in the intervention of tubulointerstitial fibrosis in future \([11]\). Growing studies indicate that inflammation may be involved in the progress of tubulointerstitial fibrosis \([12–14]\). In addition, our previous study has shown that activation of NF-\( \kappa \)B plays a role in renal inflammation and tubulointerstitial fibrosis in the progression of DKD \([15]\). Recent studies have demonstrated that the inflammasome of nucleotide binding oligomeric domain like receptor protein 3 (NLRP3) participated in the process of renal inflammation, leading to the occurrence and development of DKD \([16–19]\).

Therefore, targeting NLRP3 inflammasome may be a positive and potential therapy for DKD.

Berberine (BBR: \([\text{C20H18NO4}]^{+}\)) is an isquinoline alkaloid extracted from Chinese herbal medicine, is one of the main components of Coptis chinensis. Studies have shown that BBR has various metabolic benefits including antioxidant, anti-inflammatory and anti-

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fibrosis effects. In addition, increasing evidence show that BBR can effectively improve renal injury, inhibit mesangial cell proliferation and ameliorate tubulointerstitial fibrosis, suggesting that BBR might be used as a potential treatment for DKD [20,21]. However, the renal protective mechanism of BBR on DKD remains to be further explored. This study aims to assess whether berberine alleviates renal tubulointerstitial fibrosis and alleviates EMT and its potential molecular mechanism.

2. Materials and methods

2.1. Cell

HK-2 cells were cultured in Dulbecco’s modified Eagle’s medium/F12 (DMEM/F12) medium containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and maintained at 37 °C in a humidified atmosphere of 5% CO2. Cultured cells were divided into six groups: normal control group (NG group): 5.6 mmol/L glucose; osmotic pressure control group (OP group): 24.4 mmol/L mannitol; high glucose group (HG group): 30 mmol/L glucose; different concentrations of BBR (1, 10 or 100 μM) + high glucose group (BBR + HG group). After 48 h of BBR and HG treatment, the cells were collected for subsequent experiments.

2.2. Animal model and experimental protocol

T2DM was induced in rats with a high-fat diet (HFD) followed by a single tail intravenous injection of STZ as previously reported [22]. Thirty healthy 6-week-old male Sprague-Dawley (SD) rats were purchased from Beijing Huafukang Bioscience Co., Inc and housed under a 12:12 h light-dark cycle with free access to water and food. The rats were randomly divided into the normal control group (NC group, n = 10) and the diabetes group (n = 20). The normal control group was fed a standard diet continuously. The diabetes group was fed with a high-fat diet freely to induce dyslipidemia for 6 weeks, then 30 mg/kg STZ which was dissolved in 0.1 M citrate-phosphate buffer (pH 4.5) was injected intravenously. Fasting blood glucose (FBG) ≥ 16.7 mmol/L was considered a successful diabetic model. Then the diabetic rats were divided into two subgroups: the BBR group (n = 10) and the DM group (n = 10). BBR group were orally treated with BBR (150 mg/kg-d) dissolved in 0.5% carboxymethyl cellulose every day for 12 weeks. The DM group and NC group were orally given with the same volume of 0.5% carboxymethyl cellulose. Body weight and FBG were measured once per two weeks. After 12 weeks of treatment, 24-h urine, blood, and kidney samples were collected for subsequent experiments. All experimental procedures and protocols were approved by the Animal Research Committee of Tianjin Medical College.

2.3. Metabolic analyses

The kidney weight was measured and the ratio of kidney weight to body weight (KW/BW) was calculated. Urinary albumin to creatinine ratio (ACR). Urea nitrogen (BUN) was measured using Urea Nitrogen Colorimetric Detection Kit (Arbor Assays, Ann Arbor, MI, USA). Serum and urinary creatinine were determined by Creatinine Colorimetric/Fluorometric Assay Kit (Biovision, Milpitas, CA, USA). Serum HbA1c was determined by Glycated Hemoglobin (HbA1C) ELISA Kit (Wuxi DonglinSci&Tech Development Co., Ltd., Wuxi, China). Total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and triglyceride (TG) were determined by gel filtration high-performance liquid chromatography.

2.4. Histological examination

Paraffin-embedded kidney sections were cut into 4 μm thick and deparaffinized with xylene. Hematoxylin and eosin (H&E) staining were used to evaluate the renal injury. Tissue damage was examined in a blinded manner and scored according to the percentage of damaged tubules: 0, no damage; 1, less than 25% damage; 2, 25%–50% damage; 3, 50%–75% damage; and 4, more than 75% damage as reported. Paraffin-embedded kidney tissue sections were deparaffinized and stained with Masson trichrome. The extent of renal fibrosis was assessed based on the amount of collagen deposition using an inverted microscope (DMI8, Leica, Germany), and collagen quantification was analyzed using ImageJ software. The area of fibrotic lesions was expressed as a percentage of fibrotic area relative to the whole area.

2.5. Real-time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was purified from the kidney or the cultured HK-2 cell with TRizol reagent (Invitrogen, Carlsbad, CA) and reverse transcribed into cDNA using Primerscript RT master mix (TaKaRa). The relative mRNA levels were detected with quantitative real-time PCR using the SYBR Green PCR Kit (TaKaRa) on ABI PRISM 7300Sequence Detector according to the manufacturer’s instructions. The primers were as follows: GAPDH, forward: 5′-CAGTGCCAGCCTGCTCTA T-3′, reverse: 5′-AGGGGCCATCCACAGTCTTC-3′; KIM-1,
2.6. Immunohistochemistry

The 4-μm thick paraffin sections of kidneys were deparaffinized in xylene and hydrated in graded ethanol and treated with 3% H2O2 for 10 min to inhibit endogenous peroxidase activity. Then the sections were blocked with 10% normal goat serum for 40 min. The slides were incubated with primary anti-NLRP3 (1:100), anti-ASC (1:100), anti-Caspase-1 (1:100) and IL-1β (1:100) at 4°C overnight. On the second day, horseradish peroxidase (HRP)-labeled goat anti-rabbit/mouse secondary antibody were added to the sections at room temperature for 1 h, and then the protein bands were observed by enhanced chemiluminescence (ECL) (Thermo, Rockford, USA). Densitometric analysis was performed using the Bio-Rad Quantity One Software. The relative expression of each target protein was normalized to the GAPDH band.

2.8. Immunofluorescence

Frozen kidney sections (6 μm) were fixed in 100% acetone for 5 min and blocked with 5% normal bovine serum at room temperature for 60 min. Then the sections were incubated overnight with 15 antibodies (rabbit anti-E-cadherin 1:200, mouse anti-α-SMA 1:200) at 4°C. After being washed with PBS three times, the sections were incubated with 2nd antibodies (Alexa Fluor 488 goat anti-rabbit 1:200; Alexa Fluor 549 anti-mouse 1:200) and counterstained with DAPI. Fluorescence microscopy was used to analyze 10 fields of vision in each kidney.

2.9. Statistical analysis

The results of all continuous variables were expressed as mean ± standard deviation (SD). Statistical analysis was performed using ANOVA to compare differences among three and more groups and unpaired Student’s t-tests to compare differences between two groups using GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). p < 0.05 was defined as statistically significant.

3. Results

3.1. Effects of berberine on metabolic parameters

As shown in Table 1, the levels of blood glucose, Hba1C, TC and TG in the DM group were significantly higher than NC group, (p < 0.05), but there was no significant difference between the DM group and BBR
The ratio of KW/BW and ACR in DM rats was significantly higher than those in the NC group but markedly decreased after BBR treatment ($p < 0.01$). Serum creatinine and BUN were no significant differences among these three groups ($p > 0.05$).

### 3.2. Berberine attenuated renal tubulointerstitial fibrosis in diabetic nephropathy

HE staining showed that BBR treatment significantly reduced tubular atrophy and inflammatory cell infiltration compared with the DM rats (Figure 1A). Masson’s trichrome staining revealed that compared with the NC group, collagen deposition in tubulointerstitium of diabetic rats increased ($p < 0.05$), while BBR treatment significantly reduced the collagen deposition (Figure 1B, $p < 0.05$). Consistent with this result, western blot analysis and RT-PCR showed that the protein and mRNA levels of KIM-1, collagen I, collagen IV and FN in the kidney were significantly increased in the DM group, but partially decreased after BBR treatment (Figure 1C,D, $p < 0.05$).

### 3.3. Effect of berberine on EMT in diabetic nephropathy

In the present study, the expression levels of E-cadherin and α-SMA in the kidney were examined by immunofluorescence and western blot (WB). Immunofluorescence results demonstrated that the expression of E-cadherin and α-SMA in the DM group were significantly decreased and increased, respectively, however, BBR treatment partially reversed these changes (Figure 2A, $p < 0.05$). The protein level of E-
Cadherin was decreased in the kidney of the DM group compared with the NC group, and it was partially recovered by BBR treatment, in addition, the expression of α-SMA was significantly increased in the DM group compared with the NC group, however, BBR treatment significantly decreased the expression of α-SMA (Figure 2B, \( p < 0.05 \)).

### 3.4. Effects of berberine on NLRP3 inflammasome activation in the kidney of diabetic rats

Western blot analysis showed that compared with the NC group, the protein levels of NLRP3, ASC, Cleaved caspase-1 and Cleaved IL-1β were significantly increased in the DM group, while BBR treatment reduced the expression of NLRP3, ASC, Cleaved caspase-1 and Cleaved IL-1β (Figure 3B, \( p < 0.05 \)). Furthermore, we examined the mRNA levels of NLRP3, ASC, pro-caspase-1 and pro-IL-1β in the kidney by real-time PCR. The mRNA levels of NLRP3, ASC, pro-caspase-1 and pro-IL-1β were all significantly upregulated in the DM group compared with the NC group, while those in the BBR treatment group were down-regulated (Figure 3C, \( p < 0.05 \)). The above results indicated that BBR treatment could suppress the activation of NLRP3 inflammasome in diabetic rats.

### 3.5. Berberine reversed high glucose-induced EMT in HK-2 cell

HK-2 cells were incubated with high glucose for 48 h, and were harvested for protein and mRNA analysis. As shown in Figure 4A,B, high glucose could decrease the protein and mRNA levels of E-cadherin and increase the expression of α-SMA (\( p < 0.05 \)), while BBR could reverse these changes in a dose-dependent manner (\( p < 0.05 \)). These data suggested that BBR could inhibit HG-induced EMT in HK-2 cells.

### 3.6. Effects of berberine on the expression of NLRP3 inflammasome in HK-2 cells

To determine the effects of berberine on the expression of NLRP3 inflammasome in HG-induced HK-2 cells, the expression of protein and mRNA of NLRP3 inflammasome was measured using western blotting and RT-PCR. Both protein and mRNA levels of NLRP3, ASC, Caspase-1 and IL-1β were all markedly higher in the
Figure 3. Effect of berberine on NLRP3 inflammasome in the kidney. (A) Immunostaining for the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), Caspase-1 and interleukin-1beta (IL-1β) protein. (B) Western blot of NLRP3, ASC, Caspase-1 and IL-1β proteins in different groups. (C) The mRNA expression levels of NLRP3, ASC, Caspase-1 and IL-1β. NC, normal control group; DM, diabetic group; DM + BBR, berberine-treated diabetic group. *P < 0.05 between the NC and the DM groups; #P < 0.05 between the DM and the DM + BBR groups.
HG-induced group compared with the NG group; however, treatment with BBR reduced the expression of NLRP3, ASC, Caspase-1 and IL-1β in a dose-dependent manner (Figure 5A,B, \( p < 0.05 \)). These results of the cell experiment were consistent with the results of the animal experiment, which indicated that BBR could inhibit HG-induced EMT involving the inactivation of the NLRP3 inflammasome (Figure 6).

4. Discussion

DKD is believed to be one of the most serious microvascular complications of diabetes, which is highly prevalent in ESRD. At present, western medicine mainly focuses on controlling blood glucose, hypertension and inhibiting the renin-angiotensin system, which can only partially ameliorate but not prevent the development of renal failure [23]. BBR is extracted from Chinese herbs, which has a potential clinical application as a drug for the treatment of diabetes and its complications. In the present study, we demonstrated that BBR could ameliorate renal tubulointerstitial fibrosis by inhibiting hyperglycemia-induced EMT via inactivation of the NLRP3 inflammasome both in vivo and in vitro.

Although the glomerulus, especially mesangium, has always been the focus of DKD, tubulointerstitial injury is also a major feature of DKD and an important predictor of renal dysfunction [24–26]. This pathological phenomenon occurs due to the accumulation of interstitial ECM such as fibronectin, type I collagen and type IV collagen in the kidney. Although the origin of myofibroblasts is still controversial, EMT is considered as the main source of myofibroblasts. In DKD, EMT of renal tubular epithelial cells is believed to participate in the progress of tubulointerstitial fibrosis through the accumulation of renal matrix protein. EMT induced by hyperglycemia is generally considered to be the initial factor leading to matrix accumulation and deposition, so EMT is also considered to be an important mechanism of renal fibrosis and renal dysfunction in DKD [27]. Therefore, targeting EMT is considered a potential therapy to delay the progression of tubulointerstitial fibrosis in DKD [28].

The current study demonstrated that the expression of α-SMA was significantly decreased and the expression of E-cadherin was increased in BBR-treated diabetic rats, suggesting that the inhibition of EMT might be an important mechanism for BBR to exert a renoprotective effect and inhibit the progression of tubulointerstitial injury.

The activation of NLRP3 inflammasome plays an important role in initiating and aggravating diabetic tubulointerstitial injury [29]. The NLRP3 inflammasome complex activates caspase-1 and then increases the production of some inflammatory cytokines, such as IL-
Furthermore, the inflammatory cytokines IL-1β, MCP-1 and VCAM-1 play an important role in mediating the tubulointerstitial injury [30]. It is becoming more evident that targeting the NLRP3 inflammasome is considered a potential therapeutic approach for DKD [31,32].

Previous studies have shown that BBR has a wide range of pharmacological activities and has been demonstrated to exert protective effects against DN progression by ameliorating a variety of pathological changes. Such as, BBR could reduce renal injury and inflammatory response, and podocyte apoptosis by inhibiting TLR4/NF-κB pathway [33]. Additionally, BBR could protect glomerular podocytes via positively regulating Drp1-mediated mitochondrial dynamics [20]. In this study, we observed that diabetic rats displayed significant renal tubulointerstitial injury and fibrosis, meanwhile, the NLRP3 inflammasome was activated as indicated by the increased expression of NLRP3, ASC, Caspase-1 and IL-1β. While berberine obviously suppressed the activation of the NLRP3 inflammasome by decreasing the expression of NLRP3, ASC, caspase-1 and IL-1β in diabetic rats. Our vitro experiments also indicated that berberine could suppress the activation of NLRP3. Additionally, BBR could decrease the immunoreactivity of TLR4/NF-κB and Drp1 in the renal tissue of diabetic rats. These findings suggest that berberine may have a potential therapeutic role in the treatment of diabetes-induced renal injury.
of NLRP3 inflammasome. These findings support that administration of berberine may be a potential therapeutic for DKD by inhibiting NLRP3 inflammasome. Our study further revealed that BBR could inhibit high glucose-induced tubular epithelial-to-mesenchymal transition and renal interstitial fibrosis by suppressing NLRP3 inflammasome.

In conclusion, our study demonstrates that berberine can effectively inhibit EMT and renal interstitial fibrosis induced by high glucose by blocking NLRP3 inflammation. These findings suggest that berberine can be used as a new therapeutic drug to ameliorate tubulointerstitial fibrosis in DKD.

Disclosure statement
The authors declare no conflict of interest.

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References

[1] Afkarian M, Zelnick LR, Hall YN, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. Jama. 2016;316(6):923–910.

[2] Keating ST, van Diepen JA, Riksen NP, et al. Epigenetics in diabetic nephropathy, immunity and metabolism. Diabetologia. 2018;61(1):6–20.

[3] Kim Y, Park CW. New therapeutic agents in diabetic nephropathy. Korean J Intern Med. 2017;32(1):11–25.

[4] Perco P, Mayer G. Molecular, histological, and clinical phenotyping of diabetic nephropathy: valuable complementary information? Kidney Int. 2018;93(2):308–310.

[5] A/L B Vasanth Rao VR, Tan SH, Candasamy M, et al. Diabetic nephropathy: an update on pathogenesis and drug development. Diabetes Metab Syndr. 2019;13(1):754–762.

[6] Zeng LF, Xiao Y, Sun L. A glimpse of the mechanisms related to renal fibrosis in diabetic nephropathy. Adv Exp Med Biol. 2019;1165:49–79.

[7] Pourghasem M, Shafi H, Babazadeh Z. Histological changes of kidney in diabetic nephropathy. Caspian J Intern Med. 2015;6(3):120–127.

[8] Sheng L, Zhuang S. New insights into the role and mechanism of partial epithelial-mesenchymal transition in kidney fibrosis. Front Physiol. 2020;11:569322.

[9] Cruz-Solbes AS, Youker K. Epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (EndMT): role and implications in kidney fibrosis. Results Probl Cell Differ. 2017;60:345–372.

[10] Loeffler I, Wolf G. Epithelial-to-mesenchymal transition in diabetic nephropathy: fact or fiction? Cells. 2015;4(4):631–652.

[11] Wang Z, Chen Z, Li B, et al. Curcumin attenuates renal interstitial fibrosis of obstructive nephropathy by suppressing epithelial-mesenchymal transition through inhibition of the TLR4/NF-κB and P3K/AKT signalling pathways. Pharm Biol. 2020;58(1):828–837.

[12] Sabapathy V, Stremksa ME, Mohammad S, et al. Novel immunomodulatory cytokine regulates inflammation, diabetes, and obesity to protect from diabetic nephropathy. Front Pharmacol. 2019;10:572.

[13] Rayego-Mateos S, Morgado-Pascual JL, Opazo-Rios L, et al. Pathogenic pathways and therapeutic approaches targeting inflammation in diabetic nephropathy. JUMS. 2020;21(11):3798.

[14] Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. Nat Rev Nephrol. 2020;16(4):206–222.

[15] Ma ZJ, Zhang XN, Li L, et al. Tripterygium glycosides tablet ameliorates renal tubulointerstitial fibrosis via the Toll-like receptor 4/nuclear factor kappa B signaling pathway in high-fat diet fed and streptozotocin-induced diabetic rats. J Diabetes Res. 2015;2015(390428):390428.

[16] Kim YG, Kim SM, Kim KP, et al. The role of inflammasome-dependent and inflammasome-independent NLRP3 in the kidney. Cells. 2019;8(11):1389.

[17] Chen K, Feng L, Hu W, et al. Optineurin inhibits NLRP3 inflammasome activation by enhancing mitophagy of renal tubular cells in diabetic nephropathy. Faseb J. 2019;33(3):4571–4585.

[18] Qiu YY, Tang LQ. Roles of the NLRP3 inflammasome in the pathogenesis of diabetic nephropathy. Pharmacol Res. 2016;114:251–264.

[19] Wen L, Yang H, Ma L, et al. The roles of NLRP3 inflammasome-mediated signaling pathways in hyperuricemic nephropathy. Mol Cell Biochem. 2021;476(3):1377–1386.

[20] Ni WJ, Zhou H, Ding HH, et al. Berberine ameliorates renal impairment and inhibits podocyte dysfunction by targeting the phosphatidylinositol 3-kinase-protein kinase B pathway in diabetic rats. J Diabetes Investig. 2020;11(2):297–306.

[21] Wang S, He B, Hang W, et al. Berberine alleviates tau hyperphosphorylation and axonopathy-associated with diabetic encephalopathy via restoring PI3K/akt/GSK3β pathway. J Alzheimers Dis. 2018;65(4):1385–1400.

[22] Danda RS, Habiba NM, Rincon-Choles H, et al. Kidney involvement in a nongenetic rat model of type 2 diabetes. Kidney Int. 2005;68(6):2562–2571.

[23] Shikata K. Recent evidence in the etiology and treatment for diabetic kidney disease. J Diabetes Investig. 2021;12(5):694–696.

[24] Najafian B, Alpers CE, Fogo AB. Pathology of human diabetic nephropathy. Contrib Nephrol. 2011;170:36–47.

[25] Powell DW, Bertram CC, Cummins TD, et al. Renal tubulointerstitial fibrosis in OVE26 type 1 diabetic mice. Nephron Exp Nephrol. 2009;111(1):e11–e19.
[26] Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. Nat Rev Nephrol. 2020;16(6):317–336.

[27] Yang Y, Wang Y, He Z, et al. Trimetazidine inhibits renal tubular epithelial cells to mesenchymal transition in diabetic rats via upregulation of Sirt1. Front Pharmacol. 2020;11:1136.

[28] Satirapoj B. Tubulointerstitial biomarkers for diabetic nephropathy. J Diabetes Res. 2018;2018:2852398.

[29] Wu R, Liu X, Yin J, et al. IL-6 receptor blockade ameliorates diabetic nephropathy via inhibiting inflammasome in mice. Metabolism. 2018;83:18–24.

[30] Zhang Y, Li Z, Wu H, et al. Esculetin alleviates murine lupus nephritis by inhibiting complement activation and enhancing Nrf2 signaling pathway. J Ethnopharmacol. 2022;288:115004.

[31] Ram C, Jha AK, Ghosh A, et al. Targeting NLRP3 inflammasome as a promising approach for treatment of diabetic nephropathy: preclinical evidences with therapeutic approaches. Eur J Pharmacol. 2020;885:173503.

[32] Alyaseer AAA, de Lima MHS, Braga TT. The role of NLRP3 inflammasome activation in the epithelial to mesenchymal transition process during the fibrosis. Front Immunol. 2020;11:883.

[33] Zhu L, Han J, Yuan R, et al. Berberine ameliorates diabetic nephropathy by inhibiting TLR4/NF-κB pathway. Biol Res. 2018;51(1):9.