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# Epigallocatechin-3-Gallate Attenuates Oxidative Stress and Inflammation in Obstructive Nephropathy via NF-κB and Nrf2/HO-1 Signalling Pathway Regulation

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Running title: Effect of EGCG on obstructive nephropathy

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*Abstract:* Oxidative stress and inflammation contribute importantly to the pathogenesis of chronic kidney disease (CKD). Epigallocatechin-3-gallate (EGCG), which is the most abundant and most active catechin polyphenol extracted from green tea, has been proved to have many bioactivities. In this study, the renoprotective effect of EGCG was evaluated in a widely used kidney disease model, the unilateral ureteral obstruction (UUO) mice model. After 14 days of EGCG administration, mean arterial blood pressure, body weight and obstructed kidney weight were measured. Levels of blood urea nitrogen (BUN) and creatinine (CR) and activities of glutamic-pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) in serum were estimated as indicators of renal function. Periodic acid-Schiff (PAS) staining was performed to observe the pathological changes of the obstructed kidney. Antioxidant enzymes and pro-inflammatory cytokine production were estimated to reflect the oxidative stress and inflammatory state in the obstructed kidney. Finally, the main proteins in the NF-κB and Nrf2 signalling pathway and DNA binding activity of NF-κB and Nrf2 were measured to investigate the effect of EGCG on these two pathways. The results demonstrated that EGCG could restore UUO-induced kidney weight

loss and renal dysfunction. In addition, UUO-induced oxidative stress and inflammatory responses in the obstructed kidney were also prevented by EGCG. Furthermore, EGCG could induce both NF-κB and Nrf2 nuclear translocation in the UUO kidney and promote heme oxygenase-1 (HO-1) production. These results indicated that the renoprotective effect of EGCG might be through its NF-κB and Nrf2 signalling pathway regulations.

**Keywords:** Epigallocatechin-3-gallate, unilateral ureteral obstruction, oxidative stress, inflammation, NF-κB, Nrf2

Chronic kidney disease (CKD) is characterized by a progressive loss of renal function, oxidative stress, chronic inflammation and glomerular and tubulointerstitial injury. Although clinical treatments prevent these pathological progressions, the therapeutic strategies remain limited. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, which both act on the renin-angiotensin system, show efficacy in reducing proteinuria, improving the kidney function and slowing progression to end-stage renal disease [1], but still are often inactive. Thus, it is urgent to develop new therapeutic strategies to manage the patients with CKD.

Unilateral ureteral obstruction (UUO) is one of the most widely used animal models of renal injury, which can mimic the pathological progression of chronic obstructive nephropathy and can reflect inflammation and oxidative stress in human CKD. Previous studies suggested that inflammatory response was observed as early as four hours after UUO

in rats [2, 3]. In addition, inflammatory cells recruitment after UUO stimulates production of numerous cytokines and vasoactive agents that sustain and enhance inflammation. Oxidative stress also contributes to the pathogenesis of UUO [4]. Increased concentrations of reactive oxygen species (ROS) have been observed in obstructed kidneys [5], as well as decreased activities of the antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [6, 7]. Actually, oxidative stress and inflammation are linked in the pathogenesis of kidney disease. Oxidative stress promotes pathological progression of kidney disease by up-regulating production of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-6 by activation of the nuclear factor kappa B (NF-κB). In turn, activated leukocytes in the inflammation also provoke oxidative stress through production of ROS and reactive nitrogen species (NOS). Thus, oxidative stress and inflammation form a vicious circle and promote renal damage together.

The Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2 related factor 2 (Nrf2)-antioxidant response elements (ARE) pathway is a major pathway which is involved in the regulation of the natural antioxidant defence system. By activating this pathway, numerous genes that encode antioxidative proteins are transcribed and the antioxidative mechanisms are initiated. NF-κB is an inducible transcription factor that regulates many genes involved in immune and inflammatory responses [8]. It was reported that NF-κB could also regulate renal Nrf2 expression in CKD rats [9]. Many studies have proved that targeting either NF-κB or Nrf2 signalling pathway could contribute to the recovery of animal models of kidney disease [10-13].

Green tea is believed to be one of the healthiest beverages because it is enriched with a variety of catechin polyphenols which exhibit anti-oxidative, anti-inflammatory, anti-cancerous and anti-infective effects [14-16]. Epigallocatechin-3-gallate (EGCG) is the most abundant and most active catechin polyphenol extracted from green tea [17]. Previous studies have evaluated the renal protective effect of EGCG in different animal models including the UUO model [18]. In Zhou's study, only part of the oxidative stress indicators and expression of Nrf2 were measured [18]. Here, we thoroughly investigated the renal protective effect of EGCG on UUO mice from renal functional restoring, anti-oxidative effect and anti-inflammatory effect. The changes of NF-κB and Nrf2 signalling pathway were in focus in this study as well.

## Materials and Methods

### *Animals*

Male C57BL/6 mice (n = 24, 6-8 weeks old), weighing 20-22 g were obtained from the Laboratory Animal Center of China Medical University (Shenyang, China). The mice were kept in an environmentally controlled room with a constant temperature (21~22°C) and humidity (75~80 %). The animals were exposed to a 12-hr light/dark cycle. The animals had free access to water and standard rodent diet *ad libitum*. All the experimental protocols were approved by the ethics committee of China Medical University. After one-week acclimatization, the mice were subjected to either sham operation or unilateral ureteral obstruction (UUO). In the UUO group, all the 12 mice were anaesthetized by 10% chloral

hydrate (3 ml/kg, i.p.), the left ureter was exposed and ligated at two points. The 12 mice in the sham group had their ureters separated but not ligated. Radom 6 mice in the UUO group and 6 mice in the sham group received 50 mg/kg EGCG (i.p. Biopurify phytochemicats Ltd, Chengdu, China) and the remaining 12 mice received the same amount of saline once a day for 14 days. At day 14, after the body weight was measured, all the mice were euthanized, and obstructed kidney tissue and blood samples were harvested. Obstructed kidneys of mice were weighed.

*Assessment of biochemical indices in serum*

Levels of blood urea nitrogen (BUN), creatinine (CR), glutamic-pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) activities in the serum were estimated using commercially available kits (JianCheng Biological Engineering Institute, Nanjing, China).

*Histopathologic examination*

Obstructed kidney samples were excised, fixed in 4% paraformaldehyde in 0.01 M PBS buffer for 12 hr, hydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Five-μm sections were prepared and stained with Periodic acid-Schiff (PAS) for histological examination.

*Detection of antioxidant enzymes and total bilirubin (TB) in kidney homogenate*

Each kidney tissue sample was weighed using an analytical balance, and 100 mg tissue of each sample was homogenized at 0.01 M PBS buffer (pH 7.2). After the homogenate was centrifuged, the debris was discarded and the supernatant was used for biochemical analyses.

The total protein content was measured using the BCA kit (Beyotime Institute of Biotechnology, Haimen, China). The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and TB in kidney tissue were estimated using commercially available kits (JianCheng) according to the manufacturer's protocols..

Measurements of myeloperoxidase (MPO) and inflammatory cytokines in kidney homogenate

MPO activity in kidney tissue was assayed using a MPO Assay Kit (Jiancheng) according to the manufacturer's instructions. TNF-α, IL-6 and IL-1β concentrations in the obstructed kidney tissue were measured using a commercial enzyme-linked immunosorbent assay kit (Boster, Wuhan, China) according to the manufacturer's protocol.

*Western blot analysis*

Total protein lysates were extracted from obstructed kidney tissues in NP-40 lysis buffer (Beyotime) containing 1% Triton X-100 with 1 mM PMSF. Protein extracts were centrifuged at 12,000 x *g* for 10 min. and the supernatants were collected. Nuclear and cytosolic proteins

were extracted using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime) following the manufacturer's instruction. Total protein content was determined using BCA protein assay kit (Beyotime). Equal amounts of protein (40 μg) were electrophoresed and subsequently transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked by 5% skimmed milk for 1 hr at room temperature. Primary antibody was diluted into proper concentration with blocking solution. The membranes were incubated overnight at 4°C with corresponding protein antibody (listed in table 1). The blots were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5000, Beyotime) for 1 hr at room temperature. The enhanced electrochemiluminescence (ECL) reagent (7 Sea Pharmtech, Shanghai, China) was added before the X-ray film was exposed. β-actin and lamin A were used as cytosolic and nuclear markers, respectively.

*Electrophoretic mobility shift assay (EMSA)*

EMSA was performed using EMSA kit (Viagene Biotech Inc., Changzhou, China) according to the manufacturer's instruction. Briefly, the nuclear proteins were incubated with 1× binding buffer for 30 min. at room temperature and then incubated at room temperature for an additional 20 min. with 0.5 μl of biotin-labelled NF-κB or Nrf2 probe. The mixture was then subjected to a 6.5% acrylamide gel, transferred to a nylon hybridization membrane (Amersham Biosciences, Piscataway, NJ, USA) and DNA cross-linked under UV light for 30 min., probed with streptavidin-HRP buffer (1: 750) for 20 min., and then visualized with enhanced ECL reagent (7 Sea Pharmtech).

*Statistical analysis*

Data are given as mean ± SD. The data were analysed using the SPSS 19.0 statistical package. The treatments were compared using two-way analysis of variance (ANOVA) followed by LSD test. $P < 0.05$ was considered statistically significant.

## Results

*Effects of EGCG treatment on body and obstructed kidney weight changes*

Table 2 shows the effects of EGCG treatment on the body weight and obstructed kidney weight. At day 14 after UUO, we found no difference in body weight between the groups. However, the weight of obstructed kidney was markedly decreased in UUO mice ($P < 0.01$ *versus* sham-operated mice). EGCG treatment for 14 days partly, but significantly increased the obstructed kidney weight ($P < 0.01$),

*EGCG treatment improves renal function of UUO-induced mice*

The effect of EGCG treatment on renal function of UUO mice are shown in table 3. Levels of BUN and Cr were increased in the kidney of UUO mice ($P < 0.01$ *versus* sham-operated mice) and were partly restored by EGCE treatment ($P < 0.01$ *versus* UUO mice, $P < 0.05$ *versus* EGCG-treated sham mice). Activities of GPT and LDH were measured as non-specific markers of extensive cellular deterioration or systemic tissue injury as well. Both of the activities of the two enzymes were enhanced by UUO operation ($P < 0.01$ *versus*

sham-operated mice) and EGCG partly restored them ($P < 0.01$ *versus* UUO mice, $P < 0.01$ *versus* EGCG-treated sham mice).

*EGCG treatment ameliorates UUO-induced renal injury*

Renal histological findings with PAS staining are shown in fig. 1. The sham group showed normal glomeruli, tubule and tubulointerstitium (fig. 1A). After 14 days of UUO, the mice exhibited atrophied tubule, increased ECM deposition and sclerosis (fig. 1C). However, treatment with EGCG significantly attenuated the injuries in the UUO mice (fig. 1D). EGCG showed no obvious effect on glomeruli or tubule in sham-operated mice (fig. 1B).

*EGCG treatment ameliorates UUO-induced oxidative stress*

As shown in fig. 2, SOD, CAT and GSH-Px activities in the obstructed kidney decreased at day 14 after UUO operation as compared to the sham-operated group ($P < 0.01$). EGCG treatment significantly ameliorated the reduction of activities of these antioxidant enzymes as compared to the UUO group ($P < 0.01$). EGCG showed no significant effect on sham-operated mice.

*EGCG treatment ameliorates UUO-induced inflammatory response*

Inflammatory response was quantified through measuring MPO activity and levels of inflammatory cytokines in kidney tissue homogenates. UUO mice showed significantly greater kidney MPO activity than mice in the sham group (fig. 3A, $P < 0.01$). EGCG treatment produced a significant reduction in kidney MPO activity as compared to that in UUO mice ($P < 0.05$). Compared with mice in the sham group, mice that underwent UUO treatment demonstrated increased TNF-α, IL-6 and IL-1β levels. Treatment with EGCG markedly decreased levels of these cytokines in the obstructed kidney. EGCG did not change inflammatory status of the sham-operated mice.

*EGCG treatment inhibits UUO-induced NF-κB activation*

NF-κB signalling pathway is believed to be another important pro-inflammatory signalling pathway in human and experimental kidney disease, and NF-κB showed protective effect in animal models of kidney disease [19, 20]. In this study, expression of inhibitors of NF-κBα (IκBα) and phosphorylated IκBα (p-IκBα) in the kidney tissue and expression of NF-κB in the nucleus of kidney tissue were detected by western blot. As shown in fig. 4, the phosphorylated level of IκBα was significantly higher in the kidneys of UUO mice (fig. 4B, $P < 0.01$ *versus* the sham-operated mice). Accordingly, total IκBα expression in the UUO kidney was significantly lower (fig. 4A, $P < 0.01$ *versus* the sham-operated mice). Thereby, the nuclear accumulation of NF-κB in the kidneys of UUO mice markedly increased compared with the sham-operated mice (fig. 4C, $P < 0.01$). However, the expression of

p-IκBα in the kidney was significantly decreased, and the expression of IκBα was significantly increased by EGCG treatment in the UUO mice ($P < 0.01$ *versus* UUO mice). The NF-κB accumulation in the nuclear fraction of kidney was markedly decreased by treatment with EGCG in the UUO mice as well ($P < 0.01$ *versus* UUO mice). NF-κB activation is also associated with increased DNA binding activity. As shown in fig. 4D, the formation of the DNA-NF-κB complex was markedly induced by UUO, and the high UUO complex was markedly reduced after EGCG treatment. These data strongly suggest that EGCG diminished UUO-induced NF-κB nuclear translocation and blunted NF-κB DNA binding activity.

EGCG treatment promotes nuclear accumulation of Nrf2 and heme oxygenase-1 (HO-1) expression

As shown in fig. 5, EGCG treatment significantly increased the nuclear accumulation of Nrf2 and induced the Nrf2 binding to an ARE consensus element in the kidney of UUO mice ($P < 0.01$ *versus* UUO mice). EGCG also markedly increased TB production, which is catalyzed by HO-1, and HO-1 protein expression in the kidney of UUO mice ($P < 0.01$ *versus* UUO mice). Notably, UUO itself could induce Nrf2 nuclear translocation, as well as induce Nrf2 DNA binding and HO-1 expression, which is accordance with previous studies [21, 22].

## Discussion

In this study, the effect of EGCG on kidney of UUO mice was evaluated. We found that EGCG administration significantly improved the renal function and increased the weight of obstructed kidney in UUO mice. In addition, increased activities of antioxidant enzymes and levels of pro-inflammatory cytokines induced by UUO were normalized by EGCG treatment. Furthermore, the nuclear accumulation of NF-κB was inhibited while the nuclear accumulation of Nrf2 was promoted by EGCG. The effect of EGCG on these two signalling pathway might contribute to the anti-oxidative and anti-inflammatory effects of EGCG in the kidney.

UUO mouse was chosen as the experimental model in this study. In this animal model, consistent with a previous study [23], we found a considerable atrophy in the tubule, which might thereby cause the large weight reduction in the obstructed kidney. In addition, renal function was found impaired in UUO mice, which represented as increased levels of serum BUN and Cr and activities of GPT and LDH. For renal function of UUO models, different studies showed discrepant results [24-26] and there is no reliable explanation for the discrepancy between these observations, and the clear reason still needs to be revealed. In our study, EGCG treatment prevented the tubular injury and restored the weight of obstructed kidney. The sharp increase of the indicators of renal function was decreased by EGCG as well, indicating the renoprotective effect of EGCG in UUO mice.

In the kidney of UUO models, cytokines and growth factors produced by damaged tubular cells, macrophages and myofibroblasts promote an inflammatory state. TNF-α and

IL-1, the major pro-inflammatory cytokines, play a principal role in the inflammatory cell recruitment in the obstructed kidney [27, 28]. Both TNF-α and IL-1 production have been found to be augmented in the obstructed kidney [29, 30]. Oxidative stress has also been implicated in the pathogenesis of obstructive nephropathy [31]. Levels of oxidative stress markers are observed to have increased while levels of anti-oxidative enzymes are found to be decreased in the obstructed kidney. In this study, both inflammation and oxidative stress were observed in the obstructed kidney, which indicated a vicious circle between inflammation and oxidative stress. However, EGCG administration inhibited inflammatory response by decreasing the production of cytokines and diminished oxidative stress through enhancing the activities of antioxidative enzymes. These results suggest that the renoprotective effect of EGCG may source from its anti-inflammatory and anti-oxidative effects.

Although NF-κB signalling pathway can be activated by both oxidative stress and inflammatory factors, it considered a prototypical pro-inflammatory signalling pathway for its role in the expression of other pro-inflammatory genes including cytokines, chemokines and adhesion molecules [32]. Activated NF-κB signalling has been found in a variety of human kidney disease [20, 33-35] and experimental renal disease [36-39]. There are at least two separate pathways for NF-κB activation: the canonical pathway and the alternative pathway. NF-κB activated by the canonical pathway regulates expression of pro-inflammatory and cell survival genes. When activated by pro-inflammatory cytokines such as TNFa and IL-1, IκB kinase (IKK) complex promotes the phosphorylation inhibitor of κB (IκB) [40]. Phosphorylated IκB becomes ubiquitylated and subsequently degraded by the

proteasome. Loss of inhibition, NF-κB proteins translocate to the nucleus and bind to their cognate DNA binding sites to regulate the transcription of associated genes. In contrast to NF-κB that contributes to immune and inflammatory response in the kidney disease, the nuclear factor erythroid 2-related factor 2 - Kelch-like ECH-associated protein 1 (Nrf2-Keap1) pathway is one of the most critical endogenous defence systems which regulate antioxidant and anti-inflammatory cellular responses in the body. Under normal state, Nrf2 is retained by Keap1 in the cytoplasm. In Keap1, a cysteine-rich IVR domain is responsible for repression of Nrf2. When oxidative stress or other covalent modification of thiols occur in some of these cysteine residues, Nrf2 dissociates from Keap1 and translocates to the nucleus where it binds to the antioxidant response elements. Nrf2 is also believed to be an anti-inflammatory modulator for its regulation of NF-κB. The Nrf2-Keap1 pathway is involved in the control of NF-κB through decreasing IκBα phosphorylation, thereby reducing NF-κB nuclear accumulation [41]. Nrf2 knockout mice showed elevated NF-κB activity and TNF-α expression [42]. Similarly, over-expression of HO-1, whose gene transcription is regulated by Nrf2, resulted in stabilization of IκB and inhibition of NF-κB [43]. On the other hand, the inhibition of HO-1 activity suppressed NF-κB p65 activity [44]. In this study, we found a down-regulation of total IκB protein but an increased phosphorylated IκB protein in the obstructed kidney of UUO mice. Subsequently, nuclear accumulation of NF-κB was increased. In the determination of Nrf2-Keap1 pathway, we found a slight but significant increase of Nrf2 nuclear accumulation and HO-1 production in the obstructed kidney of UUO mice. These results indicate that Nrf2-Keap1 pathway may imitate a compensatory mechanism against inflammatory response mediated by NF-κB. As expected, after treatment

with EGCG for 14 days, the Nrf2 signalling pathway was more activated. Total and phosphorylated IκB proteins were restored to normal levels and the activity of NF-κB signalling was inhibited by EGCG treatment. These results indicate that the NF-κB signalling pathway inhibition and Nrf2-Keap1 pathway activation may contribute to the renoprotective effect of EGCG.

In this study, we found that EGCG could improve the renal dysfunction induced by UUO operation. This renoprotection may be due to its anti-inflammatory and anti-oxidative effects. The underlying mechanisms may include its NF-κB signalling pathway inhibition and Nrf2-Keap1 pathway activation effects. However, clear mechanisms, such as whether EGCG directly or indirectly promote Nrf2 nuclear translocation, whether the IκB phosphorylation inhibited effect of EGCG is Nrf2 dependent or independent, still need to be further studied. Nonetheless, the powerful anti-inflammatory and anti-oxidative effects make EGCG a novel and potentially advantageous therapeutic agent for treatment of obstructive nephropathy.

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**Table 1. Conditions of the primary antibody incubation**

| Antibody | Dilution | Company |
|---|---|---|
| IκBα antibody | 1:1000 | Wanleibio, Shenyang, China |
| p-IκBα antibody | 1:500 | Bioss Beijing, China |
| NF-κB antibody | 1:1000 | Wanleibio |
| HO-1 antibody | 1:200 | Santa Cruz Biotechnology, Santa Cruz, CA, USA |
| Nrf2 antibody | 1:1000 | wanleibio |
| Lamin A | 1:1000 | Wanleibio |
| β-actin | 1:1000 | Wanleibio |

**Table 2 Physiological index of mice (mean ± SD, n = 6)**

| Groups | sham | Sham + EGCG | UUO | UUO + EGCG |
|---|---|---|---|---|
| **BW (g)** | 23.4±1.4 | 23.4±1.3 | 21.3±1.8 | 22.4±1.6 |
| **LKW/BW (g/100 g)** | 1.32±0.14 | 1.30±0.11 | 0.90±0.07$^{\#\#}$ | 1.08±0.07$^{\S\S,**}$ |

$^{\#\#}P < 0.01$ *versus* sham group, $^{\S\S}P < 0.01$ *versus* sham + EGCG group, ** $P < 0.01$ *versus* UUO group. BW: body weight, LKW: left kidney weight.

**Table 3 Parameters for kidney function (mean ± SD, n = 6)**

| Groups | Sham | Sham + EGCG | UUO | UUO + EGCG |
|---|---|---|---|---|
| **BUN (mg/dL)** | 15.62±3.72 | 15.53±3.98 | 53.64±12.75$^{\#\#}$ | 26.86±6.30$^{\S,**}$ |
| **Creatinine (μmol/L)** | 61.53±11.46 | 64.81±13.51 | 217.00±43.87$^{\#\#}$ | 99.36±25.22$^{\S,**}$ |
| **GPT (U/L)** | 34.95±8.98 | 38.77±9.92 | 178.38±43.97$^{\#\#}$ | 88.21±21.07$^{\S\S,**}$ |
| **LDH (U/L)** | 1211.89±271.79 | 1084.93±312.04 | 3992.78±911.92$^{\#\#}$ | 2126.54±466.47$^{\S\S,**}$ |

$^{\#\#}P < 0.01$ *versus* sham group, $^{\S}P < 0.05$ *versus* sham + EGCG group, $^{\S\S}P < 0.01$ *versus* sham + EGCG group, ** $P < 0.01$ *versus* UUO group. BUN: blood urea nitrogen, GPT: glutamic-pyruvic transaminase, LDH: lactate dehydrogenase.

**Figure legends**

**Figure 1. Histological findings of kidney sections after 14 days of UUO stained by periodic-acid Schiff.** Compared with the sham group (A), the UUO group showed cell proliferation and sclerosis (C). Treatment with EGCG showed no effect on sham mice (B) but markedly attenuated UUO-induced injury (D). Typical injury and recovery are shown by arrows. Scale bar: 100μm.

**Figure 2. Effect of EGCG on UUO-induced oxidative stress in the kidney of mice.** Superoxide dismutase (SOD) (A), catalase (CAT) (B) and glutathione peroxidase (GSH-Px) (C) activity in obstructed kidneys decreased at day 14 after UUO operation, and EGCG treatment ameliorated the reduction. Values were represented as means ± standard deviation (SD) ($n = 6$). $^{\#\#}P < 0.01$ *versus* sham group, $^{**}P < 0.01$ *versus* UUO group.

**Figure 3. Effect of EGCG on UUO-induced inflammation in the kidney of mice.** Myeloperoxidase (MPO) activity (A), Tumour Necrosis Factor-α (TNF-α) (B), interleukin (IL)-6 (C) and IL-1β (D) levels in obstructed kidneys increased at day 14 after UUO operation and EGCG prevented UUO-induced inflammatory response. Values arerepresented as means ± standard deviation (SD) ($n = 6$). $^{\#\#}P < 0.01$ *versus* sham group, $^{**}P < 0.01$ *versus* UUO group.

**Figure 4. Effect of EGCG on NF-κB signalling pathway in the kidney of UUO mice.** Protein expression of IκBα (A), phosphorylated IκBα (B) and nuclear protein expression of NF-κB (C) in the obstructed kidney of mice were detected by Western blot. NF-κB DNA binding activity was measured by electrophoretic mobility shift assay (EMSA) (D). UUO induced increased IκBα phosphoryation and proteolysis of total IκBα, and thereby increased NF-κB nuclear accumulation and its binding to DNA in the kidney. EGCG treatment reversed these effects. Values are represented as means ± standard deviation (SD) ($n = 6$). $^{\#\#}P < 0.01$ *versus* sham group, $^{**}P < 0.01$ *versus* UUO group.

**Figure 5. Effect of EGCG on Nrf2 signalling pathway in the kidney of UUO mice.** EGCG contributes to Nrf2 nuclear accumulation (A), induces Nrf2 DNA binding (B) and promotes HO-1 expression (C) and thereby increased the total bilirubinin (TB) production (D) in the UUO kidney. Values are represented as means ± standard deviation (SD) ($n = 6$). $^{\#\#}P < 0.01$ *versus* sham group, $^{\S\S}P < 0.01$ *versus* sham + EGCG group, $^{**}P < 0.01$ *versus* UUO group.

**Figure 1**

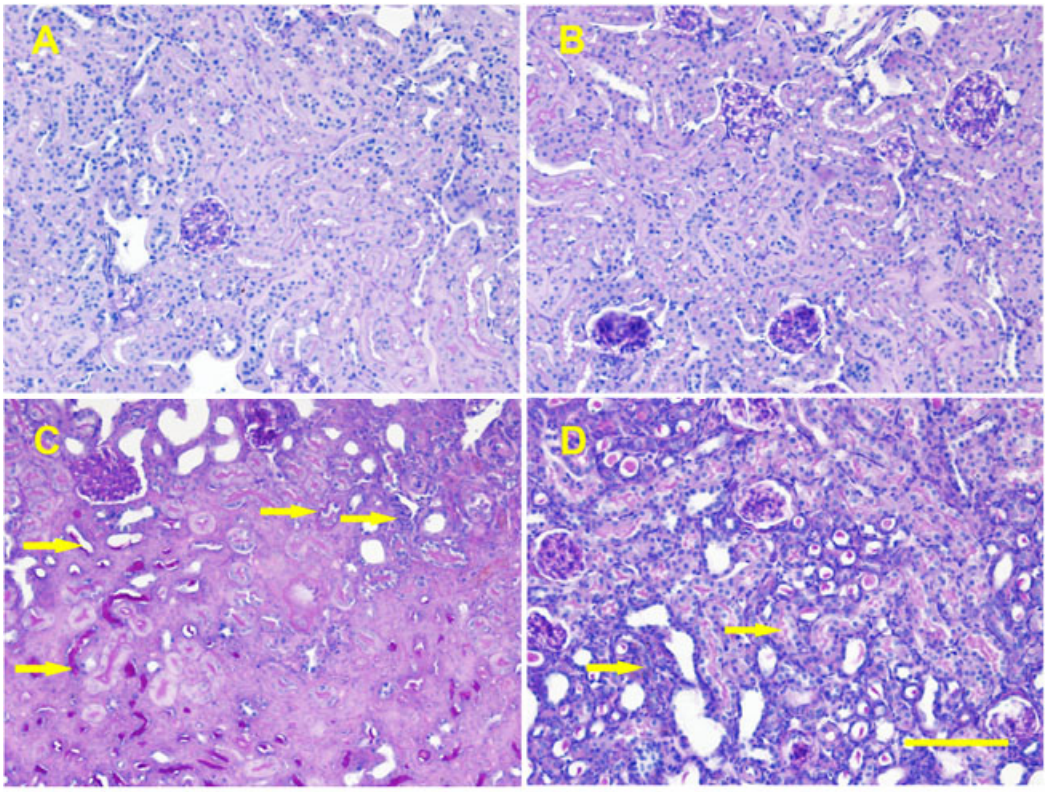


**Figure 2**

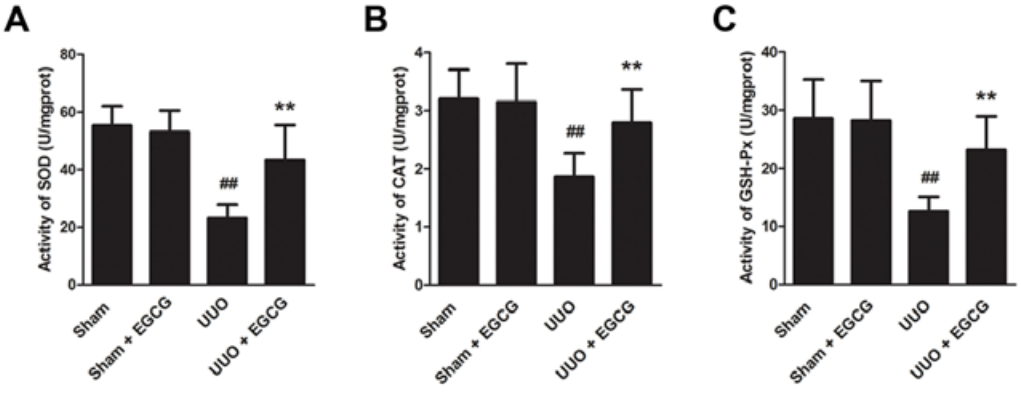

**Figure 3**

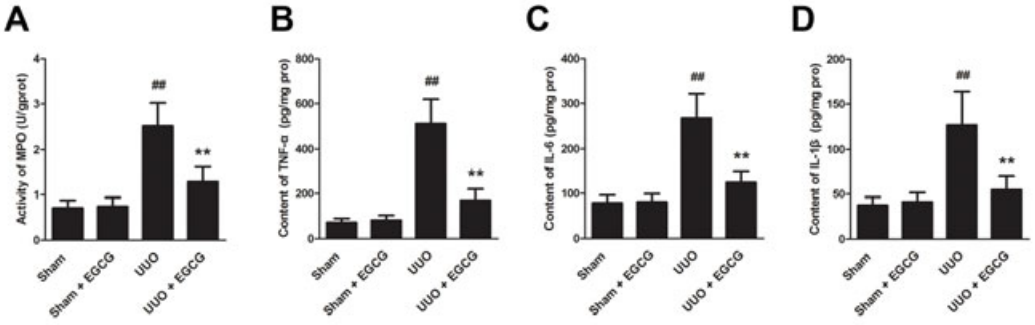


**Figure 4**

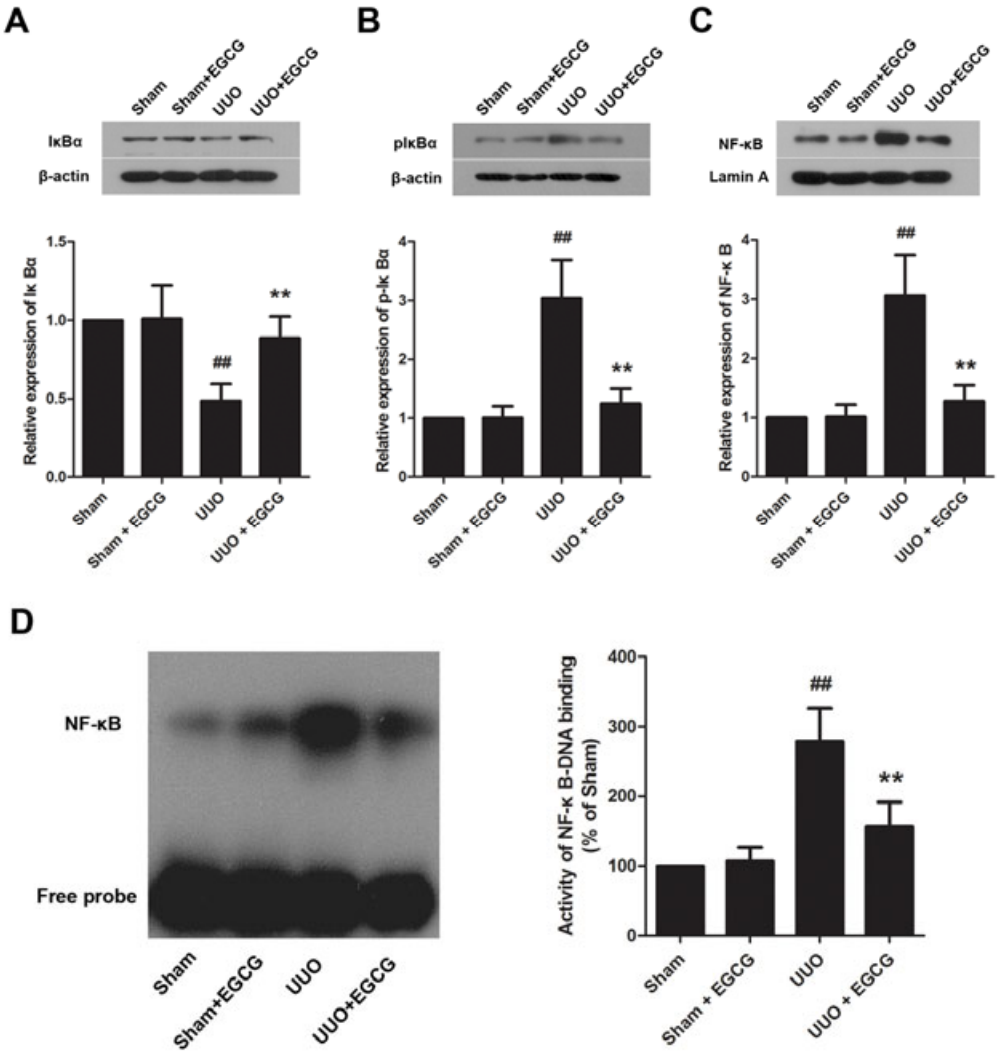

**Figure 5**