



## Short Communication

# Intervention Effect of Total Glucoside of Paeony on Mesangial Proliferative Glomerulonephritis in Rats

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## ABSTRACT

Ten out of 30 rats were randomly selected as normal group. Remaining 20 rats were used to establish rat model of mesangial proliferative glomerulonephritis (MsPGN), and were equally divided into model group and total glucoside of paeony (TGP) group. TGP group was given 150 mg/kg TGP by gavage, normal group and model group were given the same amount of saline by gavage. After 12 weeks of intervention, 24-h urine protein quantity, serum levels of IL-6, IFN- $\gamma$ , and TNF- $\alpha$ , kidney ROS and MDA levels, relative content of TGF- $\beta$ 1 and CTGF in TGP group were lower than that in model group ( $P < 0.05$ ); serum levels of IL-2 and CTLA-4, SOD levels in TGP group were higher than that in model group ( $P < 0.05$ ). These results indicated that TGP could reduce urinary protein quantity, inhibit inflammatory response, and reduce expression of TGF- $\beta$ 1 and CTGF in kidney tissue of MsPGN rats.

### Article Information

Received 27 March 2020

Revised 15 May 2020

Accepted 12 June 2020

Available online 10 December 2020

### Authors' Contribution

XY conceived and designed study, data collection and analysis and wrote the manuscript. HW and LZ were responsible for data analysis. JW edited the manuscript. LW reviewed and edited the manuscript.

### Key words

Total glucosides of paeony (TGP), Mesangial proliferative glomerulonephritis (MsPGN), Rats, TGF- $\beta$ 1, CTGF

Mesangial proliferative glomerulonephritis (MsPGN) is a general term for a group of glomerular diseases with the proliferation of mesangial cells and the accumulation of extracellular matrix (ECM) as the main pathological changes (Jin *et al.*, 2019). The clinical manifestations of MsPGN are edema, low back pain, and fatigue. Laboratory tests can reveal proteinuria, microscopic hematuria or gross hematuria, hypoproteinemia, and hyperlipidemia (Cagdas *et al.*, 2005). The current treatment of MsPGN mainly includes glucocorticoids and cytotoxic drugs that regulate the immune response, but the effects are different and there are many adverse reactions. Total glycoside of paeony (TGP) is an effective ingredient extracted from the root of traditional Chinese medicine *Paeonia lactiflora* Pall. It mainly contains paeoniflorin, hydroxy-paeoniflorin, albiflorin, and benzoylpaeoniflorin (Naveed *et al.*, 2018). Studies have confirmed that TGP can prevent and treat some autoimmune diseases such as rheumatoid arthritis and autoimmune liver injury (Lin *et al.*, 2012; Liu *et al.*, 2006). However, experimental studies

of TGP in kidney diseases closely related to inflammation and immunity have rarely been reported.

Cytotoxic T lymphocyte-associated antigen (CTLA-4) is an important inhibitory molecule for T cell activation (Darlington *et al.*, 2002; Düzgün *et al.*, 2009). IL-2, secreted by T helper cells, can promote the production of antibodies and assist the immune response. IL-6, IFN- $\gamma$ , and TNF- $\alpha$  are cytokines related to the inflammatory response. They possess various physiological activities and can aggravate the inflammatory response (Schuerwegh *et al.*, 2003; Tufano *et al.*, 1991). When chronic nephritis occurs, there are a large number of immune complexes and cellular immune defects in patients, stimulating a variety of cells to produce IL-6, IFN- $\gamma$ , and TNF- $\alpha$ , and accelerating disease progression. Some studies have shown that TGF- $\beta$ 1 and CTGF are highly expressed in the renal tissue of MsPGN rats, and reducing the expression level of TGF- $\beta$ 1 and CTGF has a protective effect on the renal tissue (Iwano *et al.*, 1994; Liu *et al.*, 2004).

The purpose of this study was to investigate the effect of TGP on MsPGN rats and observe its influence on serum levels of IL-2, IL-6, IFN- $\gamma$ , CTLA-4, TNF- $\alpha$  and content of TGF- $\beta$ 1 and CTGF in MsPGN rats kidney tissue.

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0030-9923/2021/0001-0001 \$ 9.00/0  
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### Materials and methods

Thirty male SD rats weighing 180-210 g were selected as experimental animals. Ten out of 30 rats were randomly selected as the normal group. The remaining 20 rats were sterilized by intraperitoneal anesthesia before they were removed with the left kidney from the back side and rested for 1 week. After pre-immunization, the experimental rats were injected intraperitoneally with bovine serum albumin every day. The immunization dose started from 1 mg per animal, and increased by 1 mg daily until 5 mg. The dose was continued to increase by 1 mg weekly until 10 mg. Urine protein test was performed on the 5<sup>th</sup> weekend of modeling. Positive urine protein showed that the MsPGN rat model was successfully established. The remaining 20 rats in this experiment showed significant proteinuria. They were randomly divided into the model group and the TGP group each of 10 rats according to body weight.

The TGP group was given 150 mg/kg TGP by gavage once a day at the 6<sup>th</sup> week of modeling. The normal group and the model group were given the same amount of saline by gavage. All rats were treated for 12 weeks. The 24-h urine was collected in metabolic cages before and after 12<sup>th</sup> week of administration intervention. After 12 weeks, the animals were sacrificed. Blood was collected aseptically, serum was separated. Kidney tissue was taken.

Routine biochemical method was used to detect 24-h urine protein quantity in rats. The serum levels of IL-2, IL-6, IFN- $\gamma$ , CTLA-4, and TNF- $\alpha$  were detected by ELISA. The kidney was cut into two roughly equal halves along the coronal plane, and either half of the kidney tissue was taken to detect the content of ROS, MDA, and SOD in the kidney tissue by ELISA. The other half of the kidney tissue was fixed in 4% paraformaldehyde, dehydrated, transparentized, paraffin-embedded, and sectioned (thickness: 3  $\mu$ m). The expression of TGF- $\beta$ 1 and CTCF in the kidney tissue was detected by indirect immunofluorescence. A computer image analysis system (Image-Pro Plus) was used for semi-quantitative analysis. The 10 fields of view ( $\times$ 200) were randomly and non-repeatedly selected from each section. Semi-quantitative scoring was determined as the percentage of positive area of the selected glomerulus in view occupied in the whole glomerulus area, and the average was taken.

SPSS 25 statistical software (SPSS, Chicago, IL, USA) was used for statistical processing. The measurement data were expressed by Mean $\pm$ SD, and the comparison among multiple groups was performed by one-way analysis of variance, and between two groups was assessed by the LSD-t test. The  $P < 0.05$  was considered statistically significant.

### Results and discussion

Urine protein is a main clinical indicator of renal damage (Ležaić *et al.*, 2010). Before the intervention, the 24 h urine protein quantity in the TGP group and the model group was higher than that in the normal group ( $P < 0.05$ , Fig. 1). After 12 weeks of intervention, the 24 h urine protein quantity in the TGP group was significantly lower than that in the model group ( $P < 0.05$ , Fig. 1). This result indicated that TGP could reduce urinary protein quantity in MsPGN rats, which was basically consistent with the report of Zhang *et al.* (2009).

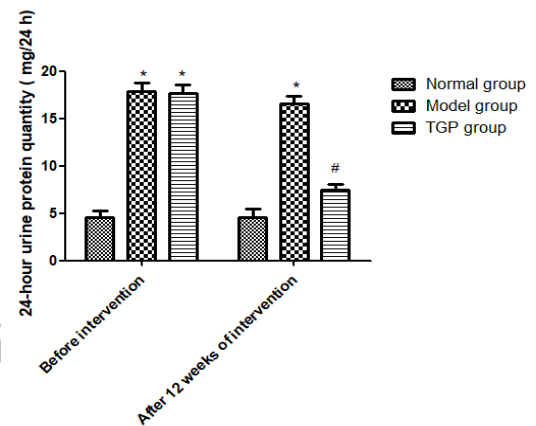


Fig. 1. Effect of total glucoside of paeony on 24 h urine protein quantity in different rat groups (n=10). \* $P < 0.05$ , compared with the normal group. # $P < 0.05$ , compared with the model group.

After 12 weeks of intervention, the serum levels of IL-6, IFN- $\gamma$ , IL-2, CTLA-4, and TNF- $\alpha$  were significantly different in the three groups ( $P < 0.05$ , Table I). Among them, the serum levels of IL-6, IFN- $\gamma$ , and TNF- $\alpha$  in the model group were higher than those in the normal group ( $P < 0.05$ , Table I), and the levels of IL-2 and CTLA-4 were lower ( $P < 0.05$ , Table I). The levels of IL-6, IFN- $\gamma$ , and TNF- $\alpha$  in the TGP group were lower than those in the model group ( $P < 0.05$ , Table I), and the levels of IL-2 and CTLA-4 were higher ( $P < 0.05$ , Table I). This result indicated that TGP could increase the content of CTLA-4 and IL-2, inhibit the release of inflammatory factors and control the progression of MsPGN.

After 12 weeks of intervention, the differences in kidney ROS, MDA, and SOD levels were statistically significant among the three groups ( $P < 0.05$ , Fig. 2). Among them, the kidney ROS and MDA levels in the model group were higher than those in the normal group ( $P < 0.05$ , Fig. 2), and the SOD levels were lower ( $P < 0.05$ , Fig. 2). The kidney ROS and MDA levels in the TGP group were lower than those in the model group ( $P < 0.05$ , Fig. 2),

**Table I.** Effect of total glucoside of paeony on serum biochemical indicators in each rat group after 12 weeks of intervention.

Index	Normal group (n=10)	Model group (n=10)	TGP group (n=10)	P value
IL-6(pg/mL)	31.47±2.54	66.85±4.33*	38.02±3.94 <sup>#</sup>	<0.05
IFN-γ(pg/mL)	43.28±6.79	113.46±9.21*	57.35±4.77 <sup>#</sup>	<0.05
IL-2 (pg/mL)	352.84±12.95	88.03±7.52*	201.24±8.61 <sup>#</sup>	<0.05
CTLA-4 (pg/mL)	273.77±15.05	152.36±8.94*	213.87±12.72 <sup>#</sup>	<0.05
TNF-α(ng/mL)	0.325±0.048	0.517±0.031*	0.396±0.055 <sup>#</sup>	<0.05

\*P<0.05, compared with the normal group. <sup>#</sup>P<0.05, compared with the model group.

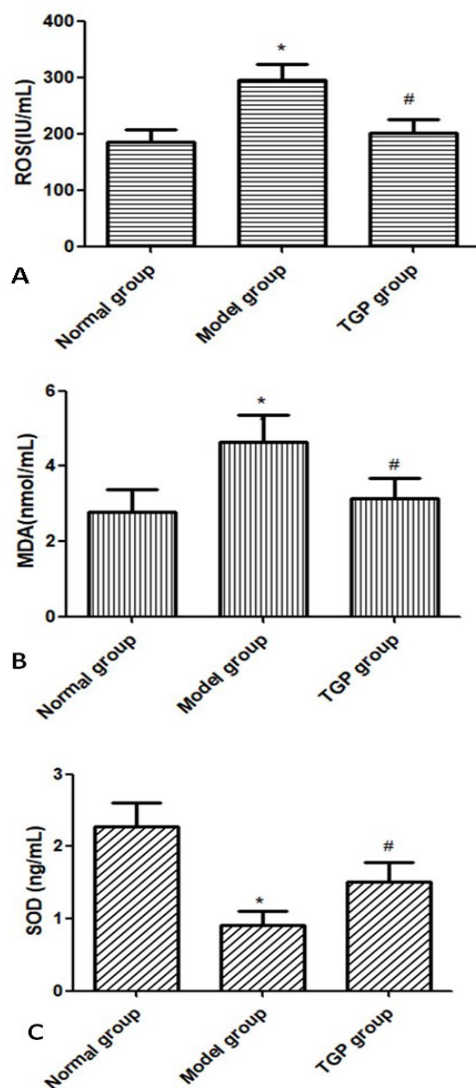


Fig. 2. Effect of total glucoside of paeony on kidney ROS (A), MDA (B), and SOD (C) content in the three rat groups after 12 weeks of intervention (n=10). \*P<0.05, compared with the normal group. <sup>#</sup>P<0.05, compared with the model group.

and the SOD levels were higher (P<0.05, Fig. 2). This study revealed that TGP could reduce ROS and MDA content and increase SOD content in MsPGN rats, indicating that TGP could improve the antioxidant capacity of MsPGN rats in vivo, adjust the disorder of the body's oxidant-antioxidant system, and inhibit lipid peroxidation.

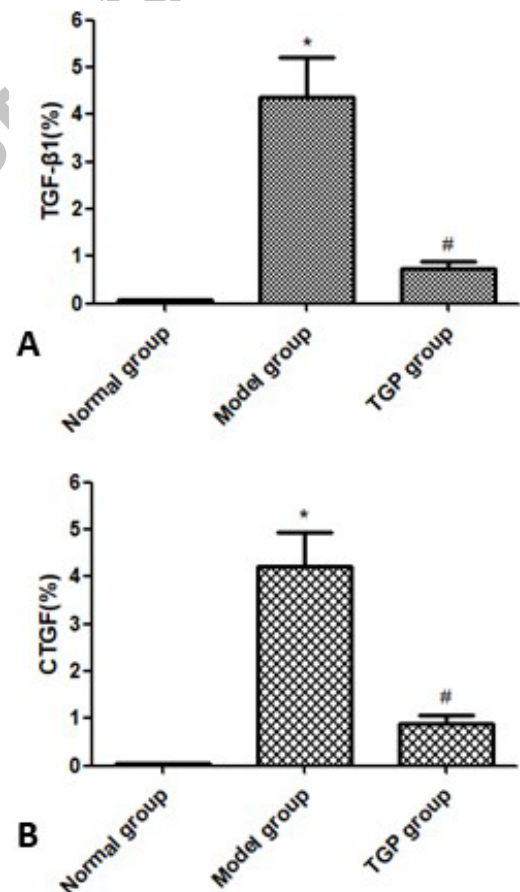


Fig. 3. Effect of total glucoside of paeony on kidney TGF-β1 (A) and CTGF (B) in the three groups after 12 weeks of intervention (n=10). \*P<0.05, compared with the normal group. <sup>#</sup>P<0.05, compared with the model group.

The CTGF is an important downstream effector molecule that promotes fibrosis mediated by TGF- $\beta$ 1, playing part of the biological role of TGF- $\beta$ 1 (Ito et al., 2010). After 12 weeks of intervention, the relative content of TGF- $\beta$ 1 and CTGF in the kidney tissue of the model group was higher than that in the normal group ( $P < 0.05$ , Fig. 3); and compared with the model group, the relative content of TGF- $\beta$ 1 and CTGF was reduced in the TGP group ( $P < 0.05$ , Fig. 3). It revealed that TGP could significantly reduce the expression of TGF- $\beta$ 1 and CTGF in renal tissue of MsPGN rats, suggesting that TGP could delay the occurrence and development of glomerular fibrosis.

In conclusion, TGP can reduce urinary protein quantity, inhibit inflammatory response, and reduce the expression of TGF- $\beta$ 1 and CTGF in kidneys of MsPGN rats.

#### Acknowledgement

This study was supported by Shandong province key research and development plan(2018GSF118163), Science Technology Development Plan of Shandong Province Medical and Health (2017WS009).

#### Statement of conflict of interest

The authors have declared no conflict of interest.

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