Drug Development and Industrial Pharmacy, 34:663–667, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701836537

informa
healthcare

Protection by Chinese Herbs Against Doxorubicin-Induced Focal and Segmental Glomerulosclerosis in Rats

YanQing Tong
Department of Nephrology, Jilin Chinese Medical Hospital, Changchun City, Jilin Province, China

Qian Wang
Department of Nephrology, Shaoguan Railway Medical Hospital, Shaoguan City, Guangdong Province, China

HuoMing Hou
Department of Nephrology, Jilin Chinese Medical Hospital, Changchun City, Jilin Province, China

The objective of this study was to evaluate the efficacy of Chinese herbs on Doxorubicin-induced focal and segmental glomerulosclerosis (FSGS) in rats. Twenty age-matched male Wistar rats were divided into two groups: group A (n = 10) given only water ad libitum served as the control group and group B (n = 10) was given Chinese herbs (40 ml/kg with drug concentration 1.75 g/ml) beginning at day zero. All rats were administered doxorubicin (7 mg/kg) intravenously. All the rats were placed in metabolic cages at day 0, 7, 14, 21, and 28, and daily proteinuria was measured. At day 28, the animals were killed by cervical dislocation, followed by immediate organ collection for histologic analysis of kidneys; blood was collected by tail vein and cardiac puncture (at day 28) for the measurement of serum albumin. Body weight (BW) and food intake were recorded. The rats in groups A and B demonstrated severe susceptibility to doxorubicin injection with the onset of proteinuria (80–100 mg/24h) at day 7. The rats in group B were partly resistant to doxorubicin nephropathy with decreasing proteinuria and increasing serum albumin compared with group A ($p < 0.05$). All 10 rats in group A developed at least 5% glomerulosclerosis with tubular casts at day 28. In contrast, the rats in group B developed less severe histologic renal disease. The difference in histologic scores between the two groups were significant at day 28 (12 in group B vs. 20 in group A, $p = 0.002$). Food intake of Group B animals progressively increased to reach 67–73% of those observed before the doxorubicin administration with 28–43% in Group A. After the 4-wk experimental period, BW in Group A decreased more significantly than that in Group B (-20 ± 3 and -16 ± 1%, respectively, $p = 0.035$, paired T test). Chinese herbs seem to reduce proteinuria and attenuate renal histologic severity in rats with doxorubicin-induced FSGS and may offer an alternative to the treatment of FSGS.

Keywords Chinese herbs; focal and segmental glomerulosclerosis (FSGS); histologic analysis

Address correspondence to YanQing Tong, Jilin Chinese Medical Hospital, Department of Nephrology, Gongnong Road, No. 1478, Changchun City, Jilin Province, P. R. China, 130021. E-mail: tyq1229@yahoo.com.cn

INTRODUCTION

Focal and segmental glomerulosclerosis (FSGS) is a glomerular disease characterized by marked proteinuria, steroid resistance, hypertension, and a high incidence of progression to renal failure. It is the most common progressive glomerular disease in children and is the second leading cause of end-stage renal disease (ESRD) in this age group; FSGS accounts for 20 to 25% of idiopathic nephrotic syndrome in adults (Korbet, Schwartz, & Edmund, 1994; McEnery, Alexander, Sullivan, & Tejani, 1993). It is a worrisome complication for nephrologists because of its high rate of incidence, the subsequent graft loss, and the inability to predict its occurrence.

Rats that are given doxorubicin develop a self-perpetuating glomerular nephropathy, which has an early onset. The animal model of nephropathy induced by doxorubicin has been the prototypical experimental model of primary FSGS, which is characterized by morphological changes resembling human FSGS (Okasora et al., 1992). In rats, a single i.v. injection of doxorubicin produces proteinuria and progressive renal disease within 5–7 days of exposure; histopathologically, animals exhibit early podocyte foot process fusion, followed by the development of typical lesions of FSGS, and progressive global sclerosis and interstitial fibrosis (Bertani et al., 1986; Yang Wang et al., 2000). Even in the absence of continued doxorubicin exposure, the glomerular damage progresses (Scholey, Miller, Rennke, & Meyer, 1989). As the animal model of doxorubicin-mediated nephropathy is reproducible and enables precise timing of the onset of renal injury, this model has been widely used to dissect the mechanisms that promote initiation and progression of nephropathy and test the efficacy of various therapies in preventing the deterioration of renal function (Fogo, 2003).

In our preliminary study, favourable results (partial to complete remission) were obtained in three patients administered

Chinese herbs for 35–50 days, in whom a significant reduction of proteinuria was seen. We hypothesized that these Chinese herbs would have the same effect in the animal model of FSGS. In an attempt to evaluate the Chinese herbs' efficacy, we studied the herbs' action in FSGS rats.

SUBJECTS AND METHODS

Animals

Experiments were performed using 20 age-matched male Wistar rats (obtained from Jilin University, China) of mean body weight 235 g (range 182–286 g). The rats were maintained in individual cages at a temperature-controlled room (22°C) on a 12:12 h light-dark cycle (light at 0700). They were fed a 'standard laboratory chow (0.35 g NaCI, 20 g protein) and allowed tap water ad libitum. Body weight (BW) and food intake were recorded daily. All animal protocols were approved and monitored by our institutional animal care and use committee.

Induction of FSGS

FSGS was induced by the administration of doxorubicin (7 mg/kg) intravenously via a superficial femoral vein under light ether anaesthesia at day 0.

Drug Therapy

The rats were divided into two groups: group A (n = 10) given only water ad libitum served as the control group and group B (n = 10) was given Chinese herbs at an average dose of 40 ml/kg beginning at day 0.

The Chinese herbs administered was as follow: Tu Fuling (Smilax Glabra, Geographical origin: Sichuan) 50 g, Jue Chuang (Sanchezia nobilis, Geographical origin: Jiangsu) 30 g, Huang Qi (Astragalus membranaeus, Geographical origin: Gansu) 30 g, Di Long (Lumbricus, Geographical origin: Guangdong) 20 g, Dang Gui (Radix Angelica, Geographical origin: Gansu) 15 g, Chuan Xiong (Rhizoma Chuanxiong, Geographical origin: Sichuan) 15 g, Chan Tui (Periostracum Cicadae, Geographical origin: Shandong) 15 g. The seven crude drugs were mixed in 800 ml water, getting 100 ml liquor after the drugs decocted in 800 ml water (100ºC for 30 minutes twice). The liquor was filtered with 1.75 g/ml as the drug concentration.

Urinary Albumin Excretion

All the rats were weighed and placed in metabolic cages at day 0, 7, 14, 21, and 28, to determine albumin excretion in 24 h urine. Urine albumin was determined with a nephelometric method (Boehring Nephelometer Analyzer, Behringwerken AG, Marburg, Germany).

Serum Albumin Analysis

The blood was collected for the measurement of Serum albumin. At day 0, 7, 14, and 21, the blood was collected via the tail vein, and was collected by cardiac puncture at day 28.

Serum albumin was measured by single radial diffusion using anti-rat albumin antibodies (ICN, Cappel, Belgium) as previously described (Breuille et al., 1998).

Kidney Histologic Analysis

At day 28, the animals were killed by cervical dislocation, followed by immediate organ collection for histologic analysis of kidneys.

Kidney sections were stained with hematoxylin and periodic acid/Schiff reagent. Renal histology was analyzed with Transmission Electron Microscopy (792, Gatan, USA). Histology scored independently by two investigators (Yanming Chen and Hewei Wei) blinded to groups; discrepancies between scores were resolved by a third expert pathologist (Pingfu Liu). On each slide, we examined at least 100 glomeruli and scored four traits: glomerular injury, tubular cyst/cast formation, podocyte hyperplasia, and interstitial inflammation. We used the following semiquantitative scale: 0, no disease; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100% of tissue affected. In our first genome screen, we applied dichotomous criteria to define affection status: mice with ≥ 50 mg/24 h proteinuria and histologic evidence of ≥ 5% glomerulosclerosis at the time of death were classified as affected. Mice with < 50 mg/24 h proteinuria and normal histology were classified as unaffected (Zongyu et al., 2005).

Statistical Analyses

Mean values ± standard error of mean (SEM) or range with median values are given. The paired t-test was used.

RESULTS

Animal Characteristics

The administration of doxorubicin decreased food intake, especially during the first week where rats ate only 15–25% of the pre-administration intake (20–25 g). Thereafter, food intake of Group B animals progressively increased to reach 67–73% of those observed before the doxorubicin administration with 28–43% in Group A. In this study, BW was similar in both groups at the beginning of the study. After the 4 wk experimental period, BW in Group A decreased more significantly than that in Group B (–20 ± 3 and –16 ± 1%, respectively, p = 0.035, paired t-test).

Albumin Changes

The rats in groups A and B demonstrate severe susceptibility to doxorubicin injection with the onset of proteinuria

(80–100 mg/24 h) at day 7. The rats in group B are partly resistant to doxorubicin nephropathy with decreasing proteinuria and increasing serum albumin after therapy compared with group A ($p < 0.05$, paired *t*-test, Figures 1 and 2).

Renal Pathology

Renal pathology changes in doxorubicin-mediated nephropathy are shown in Figure 3. We found that all 10 rats were affected in group A, developing at least 5% glomerulosclerosis with tubular casts at day 28. In contrast, the rats in group B developed less severe histologic renal disease. The difference in histologic scores between the two groups were significant at day 28 (12 in group B vs. 20 in group A, $p = 0.002$, paired *t*-test).

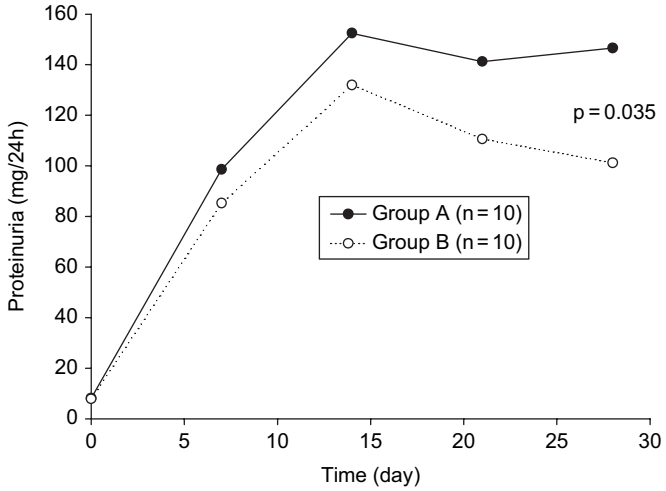

FIGURE 1. Changes of proteinuria during the process.

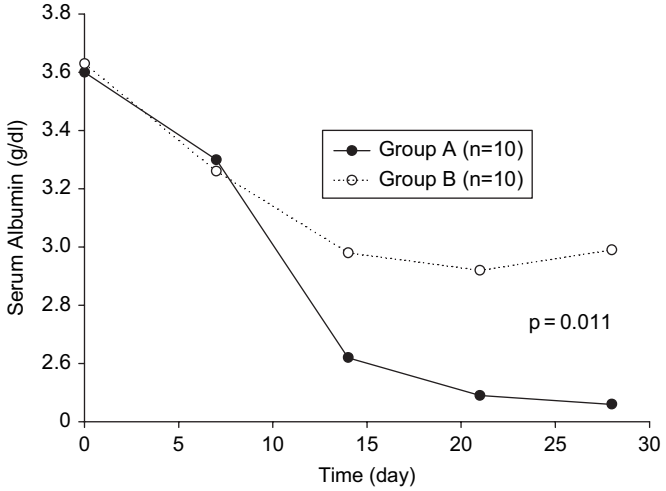

FIGURE 2. Changes of serum albumin during the process.

DISCUSSION

FSGS is becoming an increasingly important cause of the nephritic syndrome in both adults and children. It is currently the most common primary glomerular disease to progress to ESRD (Braden et al., 2000). Massive proteinuria and altered glomerular histology are characteristic features of FSGS. These changes indicate a damaged glomerular filtration barrier.

All the Chinese herbs in the experiment proved valuable for proteinuria reduction (Jun-long, 2005; Lei, & Xiaomei, 2006; Li-qiang, Qiong, Tiegang, & Xiaojie, 2005; Shiping, Rong, & Bingqian, 1989; Yonghua, et al., 2005). These properties have prompted their compound prescription use in the management of humans and experimental animals with massive proteinuria. With that in mind, we treated two groups of rats with single intravenous dose of doxorubicin in order to analyze kidney histology and to investigate the mechanism of Chinese herbs on doxorubicin-induced renal injury.

In our study, doxorubicin-induced nephritic syndrome in the rat (the animal model we used) resembles FSGS in the human, both biochemically and morphologically.

To further explore the effect of Chinese herbs, we carried out the study in an attempt to validate the antiproteinuric action of the drug. In the study, the administration of doxorubicin is associated with malnutrition, essentially due to a reduction of food intake. We are able to show a significant recovery of food intake and progressive decrease of proteinuria and increase of serum albumin after Chinese herbs administration in FSGS rats. During the 4 weeks of its administration, we also observed that these Chinese herbs attenuate the histologic severity of FSGS.

The basic mechanism for FSGS is still obscure. Indirect evidence in humans and animal models suggest that FSGS is associated with oxidants, interstitial fibrosis, proliferation, and cytokine production (de Oliveira et al., 1999; Kriz et al., 1998; Muda, Feriozzi, Rahimi, & Faraggiana, 1998; Musante et al., 2007).

Various Chinese herbs have been used alone, or in combination with others, in the treatment of various renal diseases (Fu & Kong, 2007; Qang, Du, Lu, & Wang, 1989; Su, He, & Chen, 1993; Zhao, 1988). For instance, the Chinese name of “Dang Gui,” literally means “state of return,” and refers to its ability to regulate “qi” and “blood” to maintain a normal state of well-being. Its medical use was first recorded in the “Shen Nong Ben Cao Jing” during the 1st century; it was subsequently listed in the 22nd edition of the United States Dispensatory (Wood & LaWell, 1937). We can only speculate on the mechanism underlying the protective effect of these Chinese herbs on experimental FSGS. The components of the drug could prove beneficial through a wide range of actions. For instance, the herbs Huang Qi and Dang Gui show antioxidant (Chengwen et al., 1996; Dragland et al., 2003; Li, Yu, & Li, 2007; Liu et al., 2003) and antifibrosis effects (Li-qiang et al., 2006), have strong protective effects on renal tubular damage, regulate tumor necrosis factor (TNF)-α, basic fibroblast growth factor (bFGF) and many other cytokines (Sheng et al., 2005;

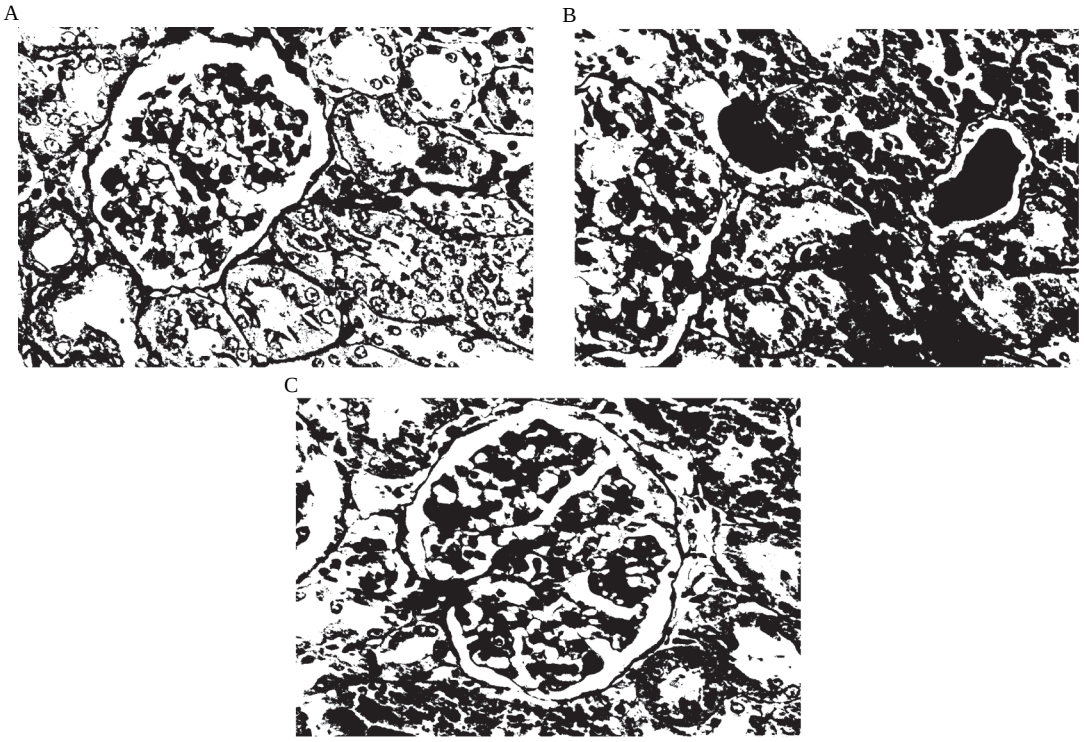

FIGURE 3. (A) (× 400) is from a rat with no detectable disease before doxorubicin injection; (B) (× 400) is from a rat with severe histologic renal disease in group A at day 28. (C) (× 400) is from a rat with less severe histologic renal disease in group B at day 28. Histology scores: B = 2; C = 1. The affected kidney (B) show microcystic tubular dilatation, tubular casts, and FSGS. Glomerular damage is less severe in C.

Xu et al., 2002). Chan Tui inhibit the reactive oxygen species (ROS) generation, nitric oxide (NO) production, and nuclear factor-κB (NF-κB) activity as well as the expression of pro-inflammatory molecules such as inducible nitric oxide synthase (iNOS), interleukin (IL)-6, TNF-α, and cyclooxygenase (COX)-2 (Xu et al., 2006). Di Long could reduce the platelet aggregation at the glomeruli of kidney and regulate the serum levels of IL-2, IL-2R and IL-6 (Yousheng, Xiaoli, & Qing, 2005). Chuan Xiong has antiproliferative effect (Jianwu, Zhuwen, & Xiaochuan, 2004). The extract of Tu Fuling could significantly recover the decrease of IL-1, TNF and NO induced by lipopolysaccharide, down-regulate over-activated macrophages, and up-regulate the dysfunctional T lymphocytes (Jiang & Xu, 2003). However, this multi-herb formula is prepared in decocted extracts. One has to expect the possibility that some chemical interactions take place among natural constituents existing in the component herbs of the formula during decoction. Decoction may change the extraction rates of the active ingredients or produce new artificial substances, which may then exhibit new pharmacological activities. The details of these changes will require future clarification.

In China, traditional medicinal herbs are often used in conjunction with Western pharmacological agents. Such an approach has been advocated for the management of experimental and clinical FSGS. The combination of cyclophosphamide and a Chinese herbal drug was found to be superior to either in slowing the progression of FSGS in patients (Hongjun & Honyu, 2002). With the continuous increase in the consumption of botanical supplements in the world, it might be beneficial in future studies to explore the therapeutic potential of these Chinese herbs in the clinical management of human FSGS.

In summary, we studied the action of some Chinese herbs on doxorubicin-induced FSGS in rats. We showed that the antiproteinuric effect of these Chinese herbs was of a significant nature in our animal model; the renal histologic severity also was attenuated, which may possibly ameliorate the natural progressive course of the disease. Our results in this particular experimental model support the notion that these Chinese herbs are useful in preventing FSGS. Although it is difficult to extrapolate animal data to humans, we still recommend Chinese herbs as a beneficial treatment of the nephrotic syndrome in FSGS. In conclusion, Chinese herbs seem to reduce proteinuria and attenuate renal histologic severity in rats with doxorubicin-induced FSGS and may offer an alternative to the treatment of FSGS.

ACKNOWLEDGMENTS

This research was funded by the Administration of Traditional Chinese Medicine. We thank Hewei Wei, MD, Yanming Chen, MD, and Pingfu Liu, MD, for helpful assistance in preparing the manuscript.

REFERENCES

Bertani, T., Rocchi, G., Sacchi, G., Mecca, G., & Remuzzi, G. (1986). Adriamycin-induced glomerulosclerosis in the rat. *Am. J. Kidney Dis., 7*, 12–19.

Braden, G. L., Mulhern, J. G., O'Shea, M. H., Nash, S. V., Ucci, A. A, & Germain, M. J. (2000). Changing incidence of glomerular disease in adults. *Am. J. Kidney Dis., 35*, 878–883.

Breuille, D., Arnal, M., Rambourdin, F., Bayle, G., Levieux, D., & Obled, C. (1998). Sustained modifications of protein metabolism in various tissues in a rat model of long-lasting sepsis. *Clin. Sci., 94*, 413–423.

Chengwen, S., Yan, J., Guogan, Z., Shu, Z., Yongdi, W., & Zhanyuan, A. (1996). Study on antioxidant effect of Astragalus polysaccharide. *Chinese Pharma. Bull., 12*, 161–163.

de Oliveira, J., Xavier, P., Carvalho, E., Ramos, J., Magalhaes, M., Mendes, A., Faria, V., & Guerra, L. (1999). T lymphocyte subsets and cytokine production by graft-infiltrating cells in FSGS recurrence post-transplantation. *Nephrol. Dial. Transplant., 14*, 713–716.

Dragland, S., Senoo, H., Wake, K., Holte, K., & Blomhoff, R. (2003). Several culinary and medicinal herbs are important sources of dietary antioxidants. *J. Nutr., 133*, 1286–1290.

Fogo, A. B. (2003). Animal models of FSGS. Lessons for pathogenesis and treatment. *Semin. Nephrol., 23*, 161–171.

Fu, N. L., & Kong, Z. X. (2007). Clinical observation on treatment of anaphylactic purpura nephritis by traditional Chinese herbs and Western medicine. *Zhongguo Zhong Xi Yi Jie He Za Zhi, 27*, 739–742.

Hongjun, W., & Honyu, C. (2002). Combine TCM and western medicine (WM) to treat focal sectional glomerular sclerosis (FSGS) by putting emphasis on tonifying kidney and removing masses. *J. Zhejiang Coll. Tcm., 26*, 19–21.

Jiang, J., & Xu, Q. (2003). Immunomodulatory activity of the aqueous extract from rhizome of smilax glabra in the later phase of adjuvant-induced arthritis in rats. *J. Ethnopharmacol., 85*, 53–59.

Jianwu, X., Zhuwen, Y., Xiaochuan, W. (2004). Effect of tetramethylpyrazine injection on proliferation of glomerulus mesangium cells in rats. *Herald. Med., 23*, 611–613.

Jun-long, R. (2005). Astragalus injection's therapeutic effects on urine protein of adriamycin-induced nephropathic rats. *Chinese Remedies & Clinics, 5*, 767–769.

Korbet, S. M., Schwartz, M. M., & Edmund, J. L. (1994). Primary focal segmental glomerulosclerosis: Clinical course and response to therapy. *Am. J. Kidney Dis., 23*, 773–783.

Kriz, W., Hosser, H., Hahnel, B., Gretz, N., & Provoost, A. (1998). From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: A histopathological study in rat models and human glomerulopathies. *Nephrol. Dial. Transpl., 13*, 2781–2798.

Li, S. Y., Yu, Y., & Li, S. P. (2007). Identification of antioxidants in essential oil of radix Angelicae sinensis using HPLC coupled with DAD-MS and ABTS-based assay. *J. Agric. Food Chem., 55*, 3358–3362.

Li-qiang, M., Lei, Q., & Xiaomei, L. (2006). Astragalus and angelica mixture ameliorates renal interstitial fibrosis by a multiple manner. *Chinese Pharma. Bull., 22*, 296–302.

Liu, J. H., Ho, S. C., Lai, T. H., Liu, T. H., Chi, P. Y., & Wu, R. Y. (2003). Protective effects of Chinese herbs on D-galactose-induced oxidative damage. *Methods. Find. Exp. Clin. Pharmacol., 25*, 447–452.

McEnery, P. T, Alexander, S. R., Sullivan, K., & Tejani, A. (1993). Renal transplantation in children and adolescents: The 1992 Annual Report of the North American Renal Transplant Cooperative Study. *Pediatr. Nephrol., 7*, 711–720.

Muda, A., Feriozzi, S., Rahimi, S., & Faraggiana, T. (1998). Expression of TGF-{beta} receptors type I and II in human glomerulonephritis. *Nephrol. Dial. Transplant., 13*, 277–282.

Musante, L., Candiano, G., Petretto, A., Bruschi, M., Dimasi, N., Caridi, G., Pavone, B., Del Boccio, P., Galliano, M., Urbani, A., Scolari, F., Vincenti, F., & Ghiggeri, G. M. (2007). Active focal segmental glomerulosclerosis is associated with massive oxidation of plasma albumin. *J. Am. Soc. Nephrol., 18*, 799–810.

Okasora, T., Takikawa, T., Utsunomiya, Y., Senoh, I., Hayashibara, H., Shiraki, K., Kasagi, T., & Shimizu, F. (1992). Suppressive effect of superoxide dismutase on adriamycin nephropathy. *Nephron., 60*, 199–203.

Qiong, Z., Tiegang, Y., & Xiaojie, G. (2005). Effects of flushing dose of Llgustrazine on haemorheology in nephrotic syndrome. *Chinese J. Integr. Nephrol., 6*, 393–394.

Scholey, J. W., Miller, P. L., Rennke, H. G., & Meyer, T. W. (1989). Effect of converting enzyme inhibition on the course of Adriamycin-induced nephropathy. *Kidney Int., 36*, 816–822.

Sheng, B. W., Chen, X. F., Zhao, J., He, D. L., & Nan, X. Y. (2005). Astragalus membranaceus reduces free radical-mediated injury to renal tubules in rabbits receiving high-energy shock waves. *Chin. Med. J. (Engl.), 118*, 43–49.

Shiping, M., Rong, Q., & Bingqian, H. (1989). Immunological inhibition and anti-allergic action of cryptotympana atrata fabricius. *China. J. Chinese Materia. Med., 14*, 42–45.

Su, Z. Z., He, Y. Y., & Chen, G. (1993). Clinical and experimental study on effects of man-shen-ling oral liquid in the treatment of 100 cases of chronic nephritis. *Zhongguo Zhong Xi Yi Jie He Za Zhi, 13*, 269–272, 259–260.

Wang, S. Q., Du, X. R., Lu, H. W., Wang, T. L., & Li, H. (1989). Experimental and clinical studies of "Shen Yan Ling" in treatment of chronic glomerulonephritis. *J. Tradit. Chin. Med., 9*, 132–134.

Wang, Y., Wang, Y. P., Tay, Y-C., & Harris, D. C. H.. (2000). Progressive adriamycin nephropathy in mice: Sequence of histologic and immunohistochemical events. *Kidney Int., 58*, 1797–1804.

Wood, H., & LaWell, C. H. (1937). *The dispensatory of the United States of America*. (22nd ed.). Philadelphia: J. B. Lippincott.

Xu, M. Z., Lee, W. S., Han, J. M., Oh, H. W., Park, D. S., Tian, G. R., Jeong, T. S., & Park, H. Y. (2006). Antioxidant and anti-inflammatory activities of N-acetyldopamine dimers from periostracum Cicadae. *Bioorg. Med. Chem., 14*, 7826–7834.

Xu, X. Y., Li, L. H., Wu, L. S., Zhao, C. L., & Lin, H. Y. (2002). Adjustment effect of Radix Astragalus and Radix Angelicae sinensis on TNF-alpha and bFGF on renal injury induced by ischemia reperfusion in rabbit. *Zhongguo Zhong Yao Za Zhi, 27*, 771–773.

Yonghua, X., Yunqi, L., Yuxia, L., & Jinxiang, G. (2005). Effect of shuizhidilong injection on concentrations of plasma endothelin and D-dimer in patients with primary nephrotic syndrome. *J. Binzhou. Med. Coll., 28*, 89–90.

Yousheng, Y., Xiaoli, L., Qing, X. (2005). Effect of earthworn and earthwormmud (EW&EWM) on nephritis induced by cationic bovine serum albumin in rats. *China J. Modern Med., 15*, 1974–1983.

Zhao, G. H. (1988). Modified five herb decoction in treating infantile acute nephritis. *J. Tradit. Chin. Med., 8*, 216.

Zheng, Z., Schmidt-Ott, K. M., Chua, S., Kirk, A. F., Rachelle, F. Z., Paul, P., Barasch, J., D'agati, V. D., & Gharavi, A. G. (2005). A Mendelian locus on chromosome 16 determines susceptibility to doxorubicin nephropathy in the mouse. *PNAS, 102*, 2502–2507.