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## Abstract

**Background:** *Radix Astragali* was one of the main compositions of 'Modified Danggui Buxue Decoction' used for treatment of various kidney diseases. Astragaloside IV was the active composition of *Radix Astragali*. Astragaloside IV liposomes were used for the treatment of adriamycin-induced nephropathy (AN) rats. The aim of the study was to study the effect of astragaloside IV liposomes on AN rats, and to test through regulating the expression of nephrin, integrins and integrin-linked kinase (ILK) in renal tissues.

**Methods:** The rats were given a single tail intravenous injection of adriamycin (6 mg/kg) within 1 week, and then divided into four groups including normal, model, benazepril and astragaloside IV liposomes group. They were all orally administered dosage of benazepril and astragaloside IV liposomes once daily for 8 weeks.

**Results:** Astragaloside IV liposomes significantly reduced the proteinuria of AN rats at 28, 42 and 56 days. astragaloside IV liposomes could increase the mRNA and protein expression of nephrin, integrin  $\alpha_3$ , down-regulate the expression of ILK to alleviate the podocyte damage and restore glomerular selective filtration function.

**Conclusions:** Astragaloside IV liposomes could enhance renal function and protect podocyte to ameliorate the adriamycin-induced nephritic syndrome.

**Key words:** Astragaloside IV, Liposomes, Adriamycin-induced nephropathy, Podocyte

## Introduction

Glomerular filtration barrier (GFB) is a 3 layer structure - the endothelial cell, the basement membrane and the epithelial cell. Epithelial cell localizes at the outside of glomerular, which is named as podocyte. Glomerular basement membrane (GBM) localizes at the middle of GFB of glomerular, which are made up of collagen, fibronectin and laminin, and so on. Collagen and fibronectin are the main composition of extracellular matrix (ECM) and set up the structure of GBM. Podocyte stick to GBM through the adhesion function of integrin. Many membrane proteins located on the surface of the foot process of podocyte. These proteins located at the vicinity area and form the special structure through the intermolecular action. The special structure is named as slit membrane (SD), which includes some special proteins, such as nephrin, podocin and CD2AP, and so on. The structure of SD is the zipper sample which constitutes the molecular channel/barrier to prevent plasma protein pass through the renal corpuscle (Gavin and moin, 2010; Jaakko and Karl, 2007). Recently, some researches have confirmed that the expression of nephrin, which is closely related to the fusion of the podocyte's foot process, prevented proteinuria and renal fibrosis (Hiroshi, et al.2009).

As we know, cell functions include proliferation, differentiation, migration, morphogenesis and apoptosis. All of these functions had intimate relationship with the metabolic action of ECM. The family of integrin is the prototypic heterodimeric trans-membrane matrix receptors, which has a special affinity to the ligands of ECM (Andrew and Shoukat,2009).The intracellular domain of integrin plays a role on the bidirectional signaling transduction (including outside-in and inside-out) through the sticky spot proteins and  $\alpha$ -auxiliary muscle protein. ILK also had affinity action with ECM via integrin. Some of the integrin play important roles in adhering foot processes with GBM. Podocytes are modulated by integrin and GBM, which probably play a crucial role in foot process maturation (Korhonen, et al., 1990). ILK acts with the cytoplasmic domains of  $\beta$ -integrin and accepts the integrin signaling transduction. ILK also interacts with nephrin and builds a molecular bridge to cell-matrix signaling transduction and cell-cell signaling transduction (Dai, et al., 2006). Conditional knockout of ILK would lead to massive proteinuria, glomerulosclerosis and premature death in mice (Dai, et al.2006; El-Aouni, et al.2006). ILK expression is up-regulated in a wide variety of chronic glomerulonephritis (CGN) in both experimental and clinical settings (Kretzler, et al.2001; Teixeira, et al.2005).

Furthermore, ILK is a key mediator of podocyte dysfunction and proteinuria in many forms of CGN (Kretzler, et al.2001). In this context, it is plausible that ILK acts as a downstream signaling of TGF- $\beta$ ; it plays a crucial role in mediating podocyte (Simone, et al.2005). ILK could exert an influence to cytoskeleton and lead to the dysfunction of adhesion action, which result in the loss of podocytes in the renal disease. Some studies have confirmed that ILK and some kinds of integrin can affect the filter function of GN (Jill and Leon, 1999; Jordan A and Jordan M, 2000) and the expression of SD (Shoji and Shuji, 2004; Simone and Matthias, 2005).

In our preliminary experiments, the prescription of Chinese herbs 'Modified Danggui Buxue Decoction' was used for the treatment of the nephritic syndrome and so on (Wei, et al, 2012; Wei et al, 2012). *Radix Astragali* is one of the main composition of 'Modified Danggui Buxue Decoction' and Astragaloside IV is the active composition of *Radix Astragali*. In this text, we try to verify whether astragaloside IV can protect the pathological changes of podocyte through regulating the expression of integrin and ILK.

## Methods and Materials

### Animals and Treatment

Thirty-two male albino rats of the Sprague Dawley strain (140 $\pm$ 10 g) were bought from Shanghai laboratory animal center. We randomly divided them into four groups by the way of random number table method. Four groups were named as the control, model, benazepril and astragaloside IV liposomes groups according to the different treatment, with 8 in each group. Except the rats in the control group (sham

treatment), those in the other groups were administered adriamycin (7.5 mg/kg) in a single injection intravenously through the tail vein. All procedures of experiment were approved by the Animal Ethics Committee of the First Affiliated Hospital of Soochow University.

We, respectively, gave the rats water or benazepril through intragastric administration and astragaloside IV liposomes through the injection to vena caudalis according to the model, benazepril and astragaloside IV groups, once daily for 8 weeks. Benazepril and astragaloside IV liposomes were given at the doses of 10 mg/kg/d and 1.2mg/kg/d, respectively. The dose of astragaloside IV liposomes also corresponded to the minimum effective dose in inhibition of the TGF- $\beta$ 1 mRNA expression in AN rats in our preliminary experiment. In the experimental period, the body weight of the rats was recorded every week. At the end of the 8-week experimental period, the animals were sacrificed and the kidneys were excised.

### Drugs, Reagents and Instruments

Adriamycin (Shenzhen, China) was purchased from Wanle Pharmaceuticals. Astragaloside IV (Beijing, china) was purchased from National Institutes for Food and Drug Control. Benazepril (Beijing, China) was purchased from Novartis.

The preparation of astragaloside IV liposomes included astragaloside IV 10mg, Bean phospholipids 90mg, cholesterol 30mg, anhydrous ethanol 2-3ml and some of phosphate buffer. Astragaloside IV liposomes would be made by the following steps: (1) Configuration of the phosphate buffer solution (PBS): disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) 0.37g and sodium di-hydrogen phosphate 2.0g ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ), then add distilled water to dissolve them and dilute to 1000 ml. (2) Preparing the liquor of Astragaloside IV: Astragaloside IV 10mg, phospholipid 90mg and cholesterol 30mg into a 50ml beaker, add anhydrous ethanol and put the breaker in thermostat-controlled water bath at 65-70°C and stir them to dissolve. (3) Preparing the initial product of Astragaloside IV liposomes: 10 ml PBS in the other small beaker and stir in magnetic at the 65-70°C thermostat-controlled water bath, then slowly inhale the liquor of Astragaloside IV with a syringe injection at 65-70 °C, then stir around 30 min without the taste of alcohol, next to complement liquor to 10 ml with water. (4) Then product of liposomes would be made by ultrasound, every 5 seconds, and 2 seconds, repeated 60 times. Next to get the liposomes of astragaloside IV by the 0.45 $\mu\text{m}$  microporous membrane filter. (5) Determination particle size of liposomes: 1ml of astragaloside IV liposomes and determine the particle size on the instrument of Zetasizer Nano ZS90. The average particle size of astragaloside IV liposomes was about 72.61nm.

The kit of urine-trace albumin was purchased from Taiyang biotechnology Co. Inc. (Shanghai, China). The enhanced chemiluminescence kit was purchased from Amersham (Arlington Heights, Italy). Nephryn, integrin  $\alpha_3$  and ILK was provided by Abcam Co. Inc(Cambridge, UK). The second antibody immunoglobulin (IgG) was provided by Abcam Co. Inc(Cambridge, UK). The Trizol reagent was purchased from LIFE (Grand Island, New York, USA). The horseradish peroxidase (HRP)-coupled secondary antibodies, anti- $\beta$ -tubulin monoclonal antibody and secondary antibody were provided by Santa Cruz Biotechnology (Dallas, Texas, USA.). The automatic biochemistry analyzer was OLYMPUS AU-2700(Tokyo, Japan). The computer assisted image analysis was designed by Leica Co. Ltd (Wetzlar, Germany). Enzyme-Linked Immunosorbent Assay was JETLIA-962 System (Beijing,China). The particle size was determined by the instrument of Zetasizer Nano ZS90 (Malvern, Worcestershire, UK.).

### Urine and Blood Chemistry

The rats were placed in metabolic cages and collected 24-h urine on days 7, 28, 42 and 56. Rats were bred by an identical diet and unrestricted water intake. We would test every rat's 24-h urine protein excretion on time. All of the samples of urine (5 ml) would be centrifuged at 5 000 $\times$ g for 5 min. The supernatant of the samples were collected and stored at -80°C until they were tested.

### Preparation of Kidney Samples

Immediately after death, the rats' bilateral kidneys were removed and weighted after removal of the capsule of kidneys. The kidneys were washed by pre-cooled saline solution and wiped dry by filter paper. The renal cortical tissue was carefully dissected from the medulla. Then, we would perfuse by cold saline solution at 4 °C via the vessel of the kidney at least three times to remove any remaining blood. Next, the right renal cortical tissue (50 mg) of every rat was snap-frozen immediately and stored at -80 °C for subsequent RNA extraction.

### Immunohistochemistry Analysis of Nephryn, Integrin $\alpha_3$ and ILK Expression in Renal Tissue

The procedure was performed by the SP staining system. Renal tissue samples were fixed in 10% neutral formaldehyde, dehydrated with ethanol, and embedded in paraffin. Serial sections (4  $\mu\text{m}$ ) were collected sequentially on glass slides. The paraffin was removed from the sections with xylene and rehydrated in graded ethanol. In order to retrieve antigenicity from formalin fixation, we incubated the sections for 10 min in 10 mM sodium citrate buffer using a microwave oven. Endogenous peroxidase activity was blocked by further pretreatment with 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and methanol. Finally, the sections were incubated with primary monoclonal antibodies against nephryn (1/100) or integrin  $\alpha_3$  (1/100) or ILK (1/100) overnight at 4°C. The sections were washed thoroughly in phosphate-buffered saline (PBS) solution and incubated with rabbit anti-rat IgG biotinylated secondary antibody for 30 min, followed by the avidin-conjugated peroxidase complex.

Finally, the sections were stained with diaminobenzidine (DAB). Negative controls were obtained by replacing specific antisera with PBS solution. Brownish yellow granular or linear deposits in the cells or matrix were interpreted as positive areas. Semi-quantitative evaluation was performed by computer-assisted image analysis. The mean optical density of nephryn, integrin  $\alpha_3$  and ILK staining was measured at 400-times original magnification in 30glomerulus of coded sections for each rat.

### RT-PCR and Western Blot Analysis

Total RNA was extracted from the kidney cortex with the use of a one-step phenol-guanidinium isothiocyanate procedure using Trizol reagent. The total RNA concentration was determined by absorbance at 260 nm with the use of a spectrophotometer. First-strand complementary DNA (cDNA) synthesis was performed in a 20- $\mu\text{L}$  reaction containing 1  $\mu\text{g}$  of total RNA, 50 U murine leukemia virus reverse transcriptase, 20U RNase inhibitor, 2.5  $\mu\text{mol}$  oligo (dT), 2 $\mu\text{L}$  of polymerase chain reaction (PCR) buffer, 1 mmol deoxynucleotide triphosphate (dNTP), and diethyl-pyrocabonate-treated water. The reaction was performed at 25 °C for 10 min, 42 °C for 90 min, and 99 °C for 5 min with the use of a

thermocycler. The resultant cDNA was stored at  $-20^{\circ}\text{C}$ . The primers for nephrin were P1 5'-GCCCAATACACACAGAACGTC-3' and P2 5'-CATTCACTCTGCGTCATCA-3'. The primers for integrin  $\alpha_3$  were P1 5'-CCCTCGCTTTGTAGGTTA-3' and P2 5'-GTCCTGTGTCAGCTCCACT-3'. The primers for ILK were P1 5'-GAAGCTGCTGCAGTACAAAGCTGAC-3' and P2 5'-CCGGAGTGTGTTTCAGAGTCCCAT-3'. The primers for  $\beta$ -actin were P1 5'-GTTGTCCTGTATGCCTCTG-3' and P2 5'-GGAGCCAGGGCAGTAATCT-3'. The PCR reactions were performed in a final volume of 25  $\mu\text{L}$  containing cDNA, 0.1 mmol dNTP, 2 mmol  $\text{MgCl}_2$ , 0.2  $\mu\text{mol}$  each of upstream and downstream primers, 2 U thermo stable DNA polymerase. A two-step cycling program was used and consisted of the following: initial template melting step at  $94^{\circ}\text{C}$  for 3 min, denaturing at  $94^{\circ}\text{C}$  for 30 s, annealing and extension at  $55^{\circ}\text{C}$  for 30 s, and final extension at  $72^{\circ}\text{C}$  for 7 min. The PCR products (nephrin 203 bp, integrin  $\alpha_3$  126 bp, ILK 310bp and  $\beta$ -actin 548 bp) were analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light. The gels were photographed and the volume density was calculated with the use of edge operator algorithm. To control for variation in RNA quality and reverse transcription (RT) efficiency between different samples, the expression of nephrin, integrin  $\alpha_3$  and ILK was corrected by the expression of  $\beta$ -actin and shown as the relative volume density per unit of the  $\beta$ -actin.

Western blot analysis was performed as previously described. In brief, the kidneys were homogenized in 3 ml of 10 mM KCl buffer (pH 7.7). The homogenates were centrifuged at  $14\,000\times g$  for 15 min. The supernatants were collected and the protein extracts were separated by electrophoresis on a 12% SDS polyacrylamide gel for 2 h at 150 V and transferred electrophoretically to a nitrocellulose membrane for 1 h at 100 V at  $4^{\circ}\text{C}$ . The membranes were blocked 1h at room temperature by immersion into a Tris-borate-saline Tween-20 (TBST-20) buffer conating 5% milk. The membrane was then incubated for overnight at  $4^{\circ}\text{C}$  with primary antibody. Then, the membrane was washed several times with TBST-20 buffer and incubated with horseradish peroxidase (HRP)-coupled secondary antibodies for 2 h. The membrane then was developed using an enhanced chemiluminescence kit. Expression of  $\beta$ -actin was studied as an internal control using anti- $\beta$ -actin monoclonal antibody (1:1 000) and its secondary antibody (goat anti-rabbit IgG-HRP, 1:5 000). For quantitation of protein expression, the relative intensity of them was determined using NIH Image software. The specificity of the antiserum against was confirmed by the absorption test.

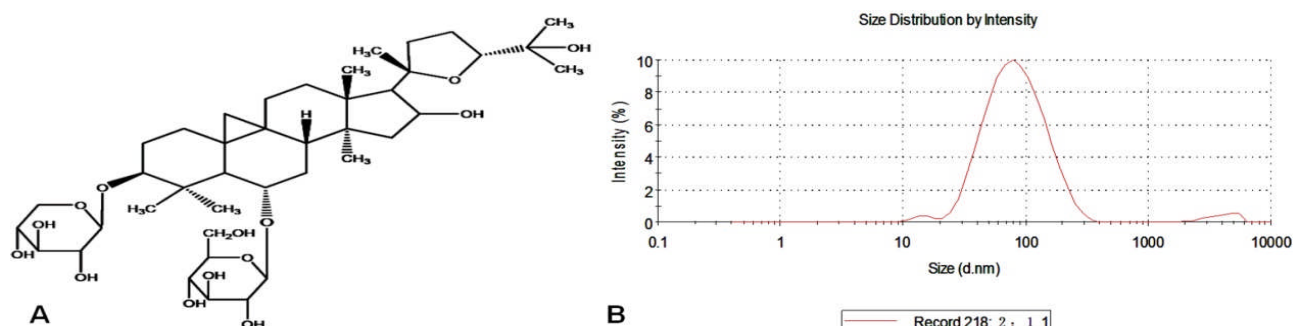
### Statistical Analysis

Reporter assays were performed in quadruplicate. Western blot analyses were repeated 2–3 times independently and individual data were subjected to densitometric analysis. Data were expressed as means $\pm$ s.e. Statistical analysis was performed using the non-parametric Mann–Whitney U-test in different groups. A P-value  $< 0.05$  was considered to indicate a statistically significant difference.

## Results

### Preparation of Astragaloside IV Liposomes

The chemical structure is shown in Figure 1A, and the side of astragaloside IV liposomes by the analyzer of Zetasizer Nano ZS90 (Fig.1B). **Figure 1: The chemical structure (A) of and the side of astragaloside IV liposomes by the analyzer of Zetasizer Nano ZS90 (B)**



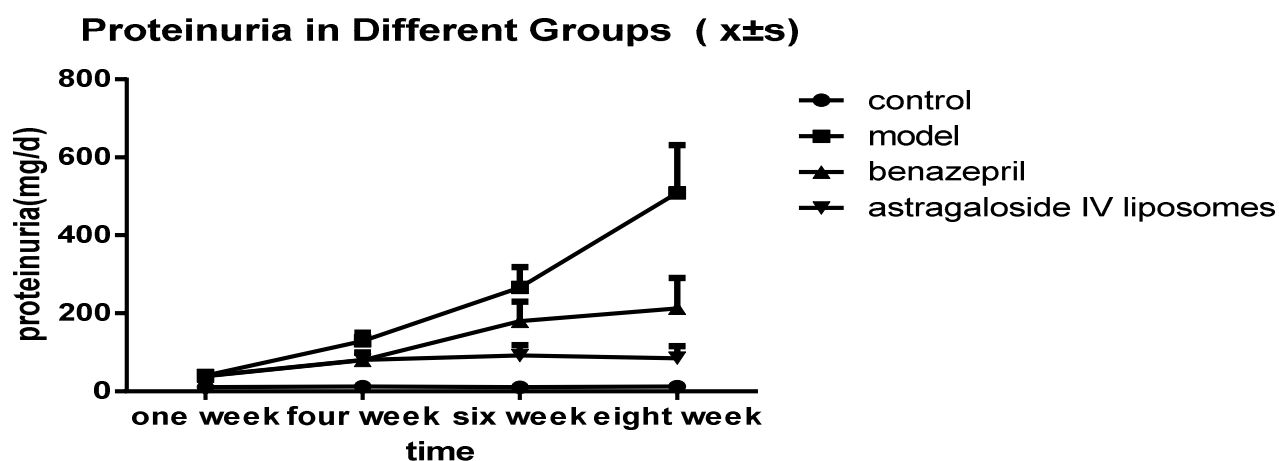
### Effects of Astragaloside IV liposomes on the 24-H Urine Protein

Urine protein is the hallmark of chronic glomerular nephritis. We first used astragaloside IV liposomes to examine its effect on the leakage of protein in AN rats. When AN rats were treated with astragaloside IV liposomes or benazepril, both of them decreased the leakage of urine protein. Astragaloside IV liposomes had an obvious reduction in urine protein than the treatment of benazepril ( $P < 0.05$ , Table 1, Fig.2). These results indicated that astragaloside IV liposomes decreased proteinuria via protecting podocyte.

**Table 1:** Proteinuria in Different Groups after Injection of Adriamycin ( $\bar{x} \pm s$ )

Group	n	proteinuria (mg/d)			
		One week	Four weeks	Six weeks	Eight weeks
Control	8	11.19 $\pm$ 3.16	12.03 $\pm$ 4.11	10.59 $\pm$ 3.87	12.16 $\pm$ 4.42
Model	8	40.29 $\pm$ 7.03*	129.12 $\pm$ 21.54*	266.54 $\pm$ 51.72*	509.14 $\pm$ 122.35*
Benazepril	8	38.77 $\pm$ 8.56	79.76 $\pm$ 20.23	179.62 $\pm$ 50.04 <sup>Δ</sup>	213.11 $\pm$ 77.64 <sup>Δ</sup>
astragaloside IV liposomes	8	39.65 $\pm$ 7.72	80.05 $\pm$ 18.46	91.37 $\pm$ 27.16 <sup>Δ</sup> ▲	85.26 $\pm$ 30.49 <sup>Δ</sup> ▲

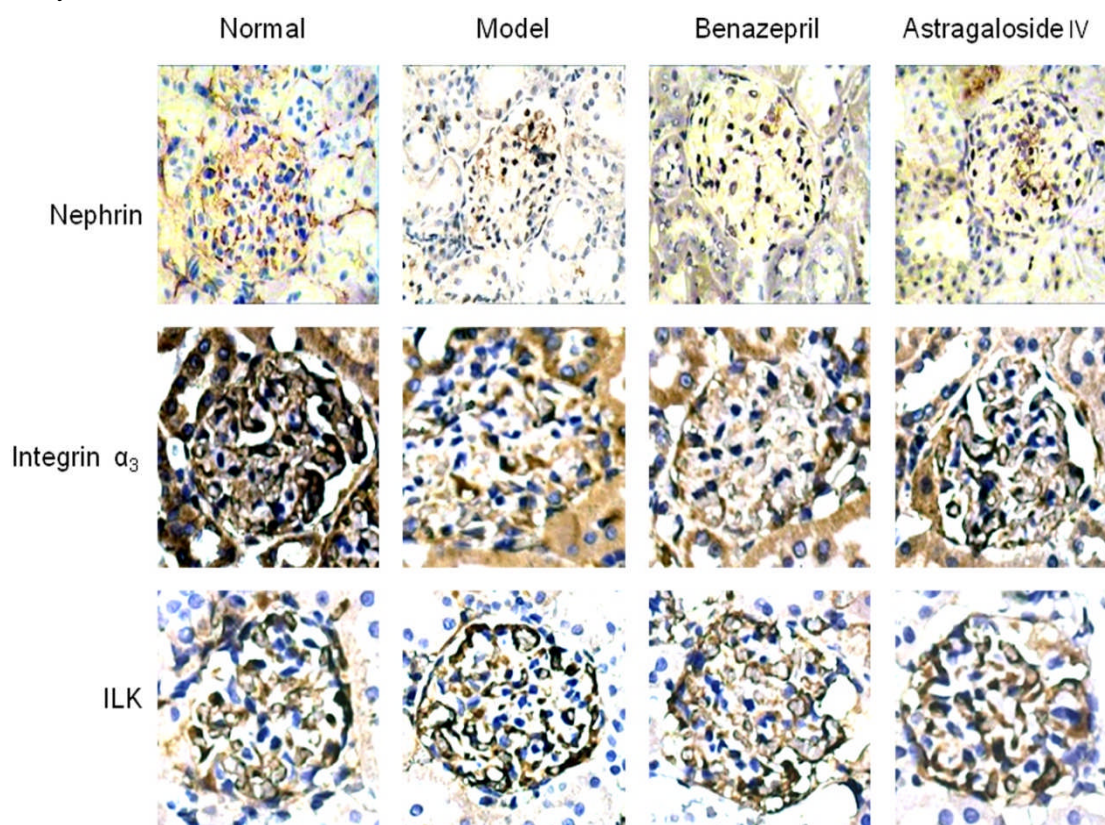
Notes: \*  $P < 0.05$ , compared with the control group; <sup>Δ</sup> $P < 0.05$ , compared with the model group. <sup>▲</sup> $P < 0.05$ , compared with the benazepril group



**Figure 2:** The proteinuria in the different groups at the different time

Effect of Astragaloside IV Liposomes on the Expressions nephrin, integrin  $\alpha_3$  and ILK in the kidney

We previously reported that astragaloside IV liposomes had the ability of protecting podocyte through regulating the expression of nephrin. To elucidate mechanisms underlying the effects of astragaloside IV liposomes through regulating the expression of integrin  $\alpha_3$  and ILK, immunohistochemistry was used to test the expression of these molecules. As shown in Fig.3, compared with sham/control rats, nephrin and integrin  $\alpha_3$  were decreased, ILK was up-regulated in model rats. All of these changes in models were largely attenuated by the treatment with either astragaloside IV liposomes or benazepril. The analysis data were shown in table 2.



**Figure 3:** The expression of nephrin, integrin  $\alpha_3$ , and ILK at the kidney tissue (DAB×400)

**Table 2:** The ratio of optical density

Group	n	nephrin	integrin $\alpha_3$	ILK
Control	6	0.436±0.052	0.401±0.063	0.214±0.019
Model	6	0.201±0.042*	0.165±0.034*	0.351±0.033*
Benazepril	6	0.263±0.029 <sup>Δ</sup>	0.201±0.029 <sup>Δ</sup>	0.294±0.042 <sup>Δ</sup>
astragaloside IV liposomes	6	0.319±0.031 <sup>Δ▲</sup>	0.258±0.031 <sup>Δ▲</sup>	0.267±0.031 <sup>Δ▲</sup>

Notes: \*  $P < 0.05$ , compared with the control group; <sup>Δ</sup> $P < 0.05$ , compared with the model group. <sup>▲</sup> $P < 0.05$ , compared with the benazepril group

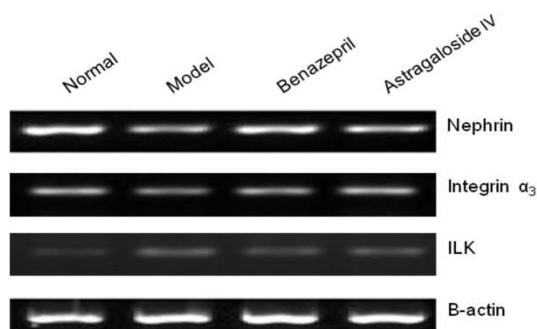
**Effects of Astragaloside IV Liposomes on the Expression of the mRNA and Protein of Nephrin, Integrin  $\alpha_3$  and ILK in the Kidney Cortex**

In order to further analyze the expression level of nephrin, integrin  $\alpha_3$  and ILK in different group, RT-PCR and western blot were also used. The mRNA expression of ILK in the renal cortex was significantly increased while nephrin and integrin  $\alpha_3$  expression was significantly decreased in the model group as compared with the control group ( $P < 0.05$ , Table 3, Figure 4). Compared with the model group, the expression of ILK was decreased, while nephrin and integrin  $\alpha_3$  were increased in the group of benazepril and astragaloside IV liposomes ( $P < 0.05$ , Table 3). Meanwhile, astragaloside IV liposomes had better effect than benazepril on attenuating the changes in AN rats. The protein's expression of nephrin, integrin  $\alpha_3$  and ILK in the kidney cortex had the same results as the mRNA expression of nephrin, integrin  $\alpha_3$  and ILK (Table 3, Figure 5). Compared with the model group, the expression of ILK was decreased, while nephrin and integrin  $\alpha_3$  were increased in the group of benazepril and astragaloside IV liposomes.

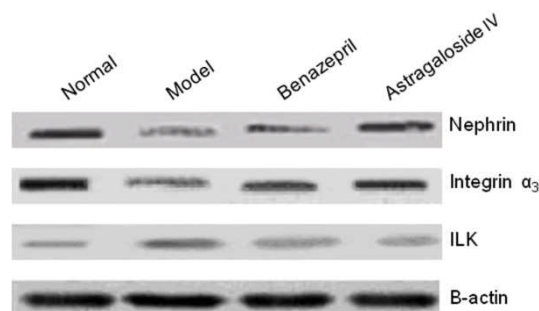
**Table 3:** The quantitative analysis of expression of nephrin、 integrin  $\alpha_3$  and ILK by the way of RT-PCR and Western blot (  $\bar{X} \pm S$  )

Notes: \*  $P < 0.05$ , compared with the control group; <sup>Δ</sup> $P < 0.05$ , compared with the model group. <sup>▲</sup> $P < 0.05$ , compared with the benazepril group

Group	n	mRNA			Protein		
		nephrin/ $\beta$ -actin	integrin $\alpha_3$ / $\beta$ -action	ILK/ $\beta$ -action	nephrin/ $\beta$ -actin	integrin $\alpha_3$ / $\beta$ -action	ILK / $\beta$ -action
Control	6	0.313±0.023	0.452±0.021	0.223±0.017	0.485±0.021	0.489±0.036	0.213±0.028
Model	6	0.206±0.027*	0.255±0.026*	0.347±0.021*	0.253±0.024*	0.251±0.039*	0.379±0.021*
Benazepril	6	0.250±0.029 <sup>Δ</sup>	0.306±0.019 <sup>Δ</sup>	0.314±0.019 <sup>Δ</sup>	0.316±0.035 <sup>Δ</sup>	0.321±0.033 <sup>Δ</sup>	0.333±0.027 <sup>Δ</sup>
astragaloside IV liposomes	6	0.281±0.023 <sup>Δ▲</sup>	0.352±0.022 <sup>Δ▲</sup>	0.287±0.017 <sup>Δ▲</sup>	0.411±0.016 <sup>Δ▲</sup>	0.413±0.041 <sup>Δ▲</sup>	0.287±0.032 <sup>Δ▲</sup>



**Figure 4:** RT-PCR analysis of nephrin、 integrin  $\alpha_3$  and ILK expression



**Figure 5:** Western blot analysis of nephrin, integrin  $\alpha_3$  and ILK expression

## Discussion

Glomerulosclerosis is the main pathology changes in most of CGN. Adriamycin induced nephropathy is considered to be a classic rat model of CGN, such as hyperproteinuria and the pathology change and so on, and these characteristics in pathology were similar to the pathological model induced by puromycin. All of that could lead to the damage of podocyte and tubulointstitial fibrosis, resulting proteinuria (Saad, et al, 2001; Aihua and Songming, 2012). Proteinuria acts as not only the hallmark of CGN, but also an independent risk factor to the renal function. Antiproteinuric treatment, especially with ACE inhibitors (ACEI) or angiotensin II (ARB) receptor blockers, had been recognized as the instrumental way in maximizing renoprotection (Abbate, et al., 2006; Pavenstadt, et al., 2003). In our previous report, we confirmed that astragaloside IV liposomes had better therapeutic efficacy than ACEI (Simone and Matthias, 2005). In the present study, we demonstrated that astragaloside IV liposomes decrease proteinuria.

The pseudopodia of podocyte connect with GBM through the special conjunction. There are some special proteins, such as nephrin and podocin, located at the pseudopodia through reciprocal chiasmata and form the hiatus membrane. The special structure is named as glomerular podocyte slit membrane (GPSD), which takes part in the molecular size selectivity and maintains the alien of glomerular filtration rate (GFR)(Pavenstadt, et al, 2003). The abnormal expression of GPSD has intimate relationship with proteinuria (Guan N, et al, 2003; Yuan H, et al, 2002). As to heritage nephropathy, the acquired nephropathy and animal experimental nephropathy are abnormal expression of nephrin may be related to proteinuria (Nakatsue, et al, 2005). Some studies find that the abnormal expression of nephrin causes the breakdown of GPSD's structure and high level of proteinuria (Hauser, et al, 2009; Tryggvason, et al, 2006). The low expression of nephrin has intimate connection with podocyte and the aggravation of proteinuria, indicating that the expression of nephrin may indirectly reflect the damage of the podocyte (Nakhoul F., et al., 2005). The protein of nephrin is one of the representative molecular structures of GPSD.

Integrin has the adhesive function and signaling transduction function (Malgorzata, et al., 2010). Some kinds of integrin, as to the adhesive molecular on the podocyte, take part in the action to GBM. Some studies have confirmed that the damage of podocyte has intimate relationship with the injury of integrin (Jordan, et al, 2000). We believed that some kinds of integrin were involved in the production of proteinuria (Shoji and Shuiji, 2004). ILK is a serine/threonine protein kinase and takes part in a variety of cell signaling pathways (Hannigan, et al., 1996). ILK plays an important role in many ways, such as regulating cell's adhesion, apoptosis, spreading and migration, and so on (Andrew and Shoukat, 2009). The adhesive actions maintain the integrity structure of tissue (Chuanyue and Shoukat, 2001). ILK and integrin take part in the cell signaling pathway through the integrin's intracellular segment and their action to cytoskeleton. According to the downstream of integrin, ILK mediated the morphogenesis of kidney through the conduction of integrin at the embryonic period (Burn, et al, 2007). ILK directly or indirectly mediated the interaction among the cells and ECM. The integrin, ILK and their downstream cytokines set up the cell signaling pathway to mediate the adhesion actions among the cells and ECM. They also have important actions on the growth of kidney and the development of kidney disease (Simone and Matthias, 2005; Ruiz-Torres, et al, 2005). Therefore, we deduced that integrin, ILK and its downstream cytokines played a key role in maintaining the integrity of GFB.

Radix Astragali has been used for the treatment of night sweats, deficiency of qi (e.g., fatigue, weakness, and loss of appetite) and diarrhea. It contains many types of active components - astragaloside IV is one of the most important active components. Experimental researches confirmed that astragaloside IV liposomes can delay the course of renal fibrosis through regulating the level of TGF- $\beta_1$  and TIMPs/MMPs to decrease ECM in the kidney. This result is consistent with the new point of view to the mechanism of fibrosis for CGN (Youhua, 2010). In this

study, AN rats experienced high levels of proteinuria, and Astragaloside IV liposomes reduced proteinuria was associated with a 75% reduction at the end of 8-week treatment. These results appeared similar to the study with retinoic acid in a rat model of glomerular damage (Wagner, et al, 2000)

Our research demonstrated that a lower expression of nephrin and integrin- $\alpha_3$  in the kidney cortex of the AN rats and a higher expression of ILK. It indicated that decreasing the ratio of integrin- $\alpha_3$ /ILK may lead to the impairment of podocyte and the damage of GFB. In the next, we can see the high level of proteinuria. Benazepril, an angiotensin-converting enzyme inhibitor, was shown to be able to reduce proteinuria and decrease the expression of ILK for AN rats (Yingjian, et al., 2009; DAI, et al., 2012). We hypothesized that astragaloside IV liposomes might employ a similar mechanism in mediating its' effect on reduction of proteinuria excretion. The study showed that astragaloside IV liposomes could regulate the expression of nephrin, integrin- $\alpha_3$  and ILK in the kidney cortex. These findings thus substantiated the hypothesis and provided the effect of astragaloside IV liposomes in the treatment of AN rats. We elucidated that the decrease in the level of nephrin was correlated with the suppression of integrin- $\alpha_3$ .

In conclusion, the study demonstrated that astragaloside IV liposomes had effective function on AN rats. The benefits of treatment were associated with the expression of nephrin, integrin- $\alpha_3$  and ILK. These results indicated that astragaloside IV liposomes may be a promising therapeutic candidate for the treatment of CGN.

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**Conflict of interest:** None

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