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ORIGINAL

EFFECTS OF NEPHRITIS HEMOSTASIS PILLS ON IGA NEPHROPATHY RATS BASED ON XBP1/COSMC PATHWAY

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ABSTRACT

Objective: To observe the therapeutic effect of Shenyanzhixue/ nephritis hemostasis Pill on IgA nephropathy model rats, and to explore its mechanism based on X-box binding protein 1 (XBP1)/Cosmc pathway. **Methods:** The IgAN rat model was set by the combination of "bovine serum albumin (BSA) + subcutaneous injection of carbon tetrachloride (CCl4) + intravenous injection of lipopolysaccharide (LPS)". The rat model was randomly separated to Control-, Model-, Shenyanzhixue pill-, losartan potassium tablet-, or XBP1 inhibitor-group (n=8). The XBP1 inhibitor group was treated continuously for 4 weeks, and the other groups were treated continuously for 6 weeks. the levels of XBP1, Cosmc and Gd-IgA1 were detected. **Results:** Shenyanzhixue Pills can significantly reduce urinary protein and red blood cells (P<0.01), which is significantly better than that of Kesu subgroup. XBP1 inhibitor enhanced levels of Gd-IgA1 in blood and decreased Cosmc level in blood. The expression of blood Gd-IgA1 was drastically inhibited in the group, while XBP1 and Cosmc were increased. XBP1 inhibitor increased the deposition of IgA and C3; the Shenyanzhixuewan group significantly decreased their deposition, and decreased the deposition. Conclusion: XBP1 inhibitor significantly increased expression levels of Gd-IgA1 and decreased the expression levels of XBP1 and Cosmc in blood, intestinal tissue and kidney tissue, and increased urinary protein and red blood cells.

KEYWORDS: Hemostasis; Shenyanzhixuewan; XBP1; Cosmc; IgA

nephropathy.

1. INTRODUCTION

IgA nephropathy (IgAN) is caused by IgA-based immune-complexes deposition in the mesangial area of the glomerulus and impairing renal function. It is a common glomerulonephritis, accounting for about 39.6% to 44.6% of Chinese patients with primary glomerular disease (Vecchio et al., 2015). Approximately 40% of patients encounter end-stage renal disease (ESRD) (Rajasekaran et al., 2021). IgAN's pathogenesis is still not clear. However, increasing evidence suggests that multiple immune strikes are involved, namely galactose-deficient IgA1 (Gd-IgA1) formation (first strike); anti-Gd-Autoantibody synthesis of IgA1 (second hit); and Gd-IgA1 binds to autoantibodies to form circulating immune complexes (third hit). These circulating immune complexes containing Gd-IgA1 are accumulated in glomerular mesangium, triggering damage, and leading to IgAN (the fourth hit) (Suzuki, 2019). Thus, Gd-IgA1 is a key factor in the initiation and driving of IgAN. The normal IgA1 hinge region aggregates O-glycans formed by O-glycosylation, consisting of N-acetylgalactosamine (GalNAc), β-1,3-galactose, and sialic acid. GalNAc can bind to serine/threonine residues under the action of β-1,3-galactosyltransferase (C1GALT1) and its specific molecular chaperone, Cosmc, to normally glycosylate O-glycans. C1GALT1 plays a crucial role in IgA1O-glycosylation. When the expression and/or activity of C1GALT1 is down-regulated, it will inhibit the normal O-glycosylation of IgA1 and form Gd-IgA1 (Wu et al., 2018). Under normal physiological conditions, the molecular chaperone Cosmc can make C1GALT1 fold correctly in the endoplasmic reticulum, form an active dimer and move out to the Golgi apparatus to conduct a physiological function. When Cosmc is deficient or dysfunctional, C1GALT1 accumulates in the endoplasmic reticulum and cannot fold correctly, causing misfolded galactosyltransferase to agglutinate and form oligomeric complexes, which are transported out of the endoplasmic reticulum and stored in the cytoplasm. The result is that protease degrades and loses its physiological function. Therefore, the formation of active C1GalT1 requires the regulation of the molecular chaperone Cosmc in the endoplasmic reticulum (Zeng et al., 2020). There is increasing evidence that the downregulation of Cosmc expression is associated with abnormal glycosylation of IgA1 (Xing et al., 2020). Endoplasmic reticulum stress (ERS) is the dysfunction of endoplasmic reticulum caused by internal and external stimuli, which accumulates unfolded/misfolded proteins, and finally initiates unfolded protein response (UPR) (Smith & Wilkinson, 2017). As a key regulator in endoplasmic reticulum stress protection, X-box binding protein 1 (XBP1)(Li et al., 2017) can directly bind to the promoter region of inflammatory genes, regulate gene transcription, and a variety of inflammatory signals Or pathogenic infection can directly induce ERS (Li et al., 2021). The mechanism of ERS involved in XBP1 in IgAN is less studied by domestic and foreign scholars, and further research is needed on

the mechanism of Gd-IgA1 involved in the abnormal glycosylation of ERS in IgAN. Based on the above studies, we infer that XBP1 is involved in the regulation of Cosmc in the endoplasmic reticulum and then acts on Gd-IgA1. Shenyan Zhixue Pills is made based on the theory that the overall pathogenesis of IgAN belongs to the deficiency and the real, and the disease location is mainly in the lung, spleen and kidney(Chen et al., 2022) . The abnormal mucosal immunity is the core of the immune pathogenesis of IgAN caused by the excessive production of Gd-IgA1. The theory of "three viscera and three viscera-mucosal axis" aims to nourish the lung, spleen and kidney, clear heat and stop bleeding, and increase circulation and decrease stasis (Chen et al., 2022). Previous studies have confirmed that Shenyanzhixue Pills can significantly improve the clinical symptoms of IgAN patients, reduce urine red blood cells and urine protein, and decrease blood IgA content. It can reduce IgA in glomerulus mesangium in IgAN rats and reduce glomerular damage (Li, 2010; Wang, 2005). This study is to explore whether Shenyanzhixue Pill can reduce the generation of Gd-IgA1 by activating the XBP1/Cosmc pathway, reducing the deposition in renal tissue and reducing the IgAN. Our data provides a strong basis for Shenyanzhixue Pills in IgAN treatment.

2. Materials and methods

2.1 Experimental materials

2.1.1 Experimental animals:

40 8-week-old male SD rats (200±10g) were purchased from Department of Laboratory Zoology, Harbin Medical University. The animal study was approved by Animal Ethics Committee of the Heilongjiang Academy of Chinese Medicine.

2.1.2 Main reagents:

Major reagents and venders are TRIZOL (China Tiangen Biotechnology), Urine Protein Kit (Shanghai Zhicheng Biotechnology Co., Ltd., China), ELisa Kit (China Jiangsu Enzyme Immunology Industry Co., Ltd.), Hematoxylin-Eosin Staining Solution (Sinopharm), neutral gum (Sinopharm), hematoxylin staining solution (Jiangsu Addison, China), DAB color development kit (DAKO, Germany), PVDF membrane (Millipore, USA), Kodak Medical X-ray film (Kodak, USA), STF083010 (MCE, USA), Anti-XBP1 antibody (lot number ab37152, UK abcam), Anti-COSMC antibody (lot number ab229831, UK abcam), goat anti-rat IgA alpha chain (FITC) (lot number ab97184, British abcam), FITC fluorescent Anti-C3c antibody (batch number ab182890, British abcam).

2.1.3 Main instruments:

Fluorescence quantitative PCR instrument (China Shanghai Hongshi

Medical Equipment Co., Ltd.), ultra-trace spectrophotometer (Thermo, USA), ultra-clean workbench (China Sujing Antai), electrophoresis instrument (China CAVOY), enzyme Standard instrument (Diatek, China), scanner (EPSON, Japan), incubator (Shanghai Jinghong, China), automatic biochemical analyzer (Beckman, USA), microscope (Olympus, Japan), automatic biochemical analyzer (China) Mindray), upright optical microscope (Nikon, Japan), imaging system (Nikon, Japan), etc.

2.2 Methods

2.2.1 Rat model preparation

The model was set using the method of bovine serum albumin (BSA) + subcutaneous injection of carbon tetrachloride (CCl4) + intravenous injection of lipopolysaccharide (LPS) for 6 weeks. Subcutaneous injection of castor oil 0.5ml + CCl40.1ml was conducted once a week for 9 weeks, the 6th and 8th week of tail vein injection of LPS 0.05mg, modeling time for 10 weeks. For control group: 0.1% dilute hydrochloric acid was administered every other day with acidified water, 2 ml each time, for 6 weeks. 0.6 ml of normal saline was injected subcutaneously, once a week, for 9 weeks; saline (0.2ml) was provided via tail vein on 6th and 8th week brine. At the end of the ninth week, one rat in each group was randomly sacrificed, and the kidney tissue was taken for immunofluorescence staining to confirm that the IgAN model was successful.

2.2.2 Grouping and administration method:

Animals were randomly separated to Control-, model-, Shenyanzhixue-, losartan potassium-, and XBP1 inhibitor (STF083010)-group (n=8). The Shenyan Zhixue Pill group was administered from the 10th weekend to the 16th weekend of the experiment. The dose was determined by converting the equivalent dose between the body surface area between humans and rats. Losartan potassium (20 mg/kg.d) was provided from the end of 10th to 16th week of the experiment. Control- and model-group were administered with distilled water from 10th week to 16th week; XBP1 inhibitor Drugs were administered from the 10th weekend to the 14th weekend of the experiment. After the STF083010 reagent was completely dissolved in dimethyl sulfoxide (DMSO), it was diluted in saline to 3 mg/ml, and the dosage was 5 mg/Kg, once a week for 4 consecutive weeks, intraperitoneal injection.

2.2.3 Experimental drugs:

Shenyan Zhixue Pill: 0.04g/capsule, provided by the Preparation Office of Heilongjiang Academy of Traditional Chinese Medicine, #170903. Heilongjiang Provincial Academy of Chinese Medical Sciences (Harbin, HeilongJiang, PR China). Losartan potassium tablets: 50mg/tablet, Hangzhou Merck & Co., Ltd., batch number: H20000371.

2.2.4 Specimen collection:

Ten weeks after modeling, 2nd, 4th, and 6th weekends after administration of the experimental rats, the rats were put into the rat metabolism cage, and 24h urine was collected. (During this period, fasting and water), and centrifuged, supernatants were kept at −80 °C; at experiment end, rats were anesthetized, and blood was collected, centrifuged, and serum was kept at −80°C. Colon tissue at 8 cm from the anus was taken out, the contents were washed, and the intestinal specimens were stored in liquid nitrogen for future use. Right kidneys were collected to cryopreservation tube and kept at a −80 °C. Left kidneys were fixed with 4% PFA to prepare tissue sections.

2.2.5 Detection indicators

1) Urinary erythrocyte count and 24-hour urine protein quantitative detection in each phase: Urine sediment counting method was carried out to measure urine red blood cell number, and biuret colorimetry was used to detect the 24-hour urine protein quantification.

2) Determination of the levels of XBP1, Cosmc and Gd-IgA1 in serum: according to the instructions of the kit, the contents of XBP1, Cosmc and Gd-IgA1 in serum were detected by ELISA.

3) Detection of XBP1 and Cosmc in intestine and kidney by RT PCR method: Middle RNA was isolated from intestine and kidney using Trizol, and reverse transcribed into cDNA. Design specific primers, prepare a reaction system, and perform PCR amplification. The reaction conditions are 95°C,10m, 40x (95°C,30s, 55°C,30s, 60°C,30s). Fold changes were calculated by 2-ΔΔCt. All primers are shown in Table 1.

Table 1: Primer sequences.

GENE	FORWARD PRIMER （5'->3'）	REVERSE PRIMER （5'->3'）
COSMC	CAGAGCGTAACCGAGTGGG	TGAAAGCATGTTTCCGCATCT
XBP1	CACAGACTGCGCGAGATAGA	AGCTGGAGTTTCTGGTTCTCT
GAPDH	TACTGTTGTCCAGCTACGGC	CGTCCAAATCCATTGATGCCC

4）Western blot was used to measure XBP1 and Cosmc in intestine and kidney: total protein of intestine and kidney of each group was extracted by protein extraction kit, and the protein concentration of XBP1 and Cosmc was detected by BCA protein concentration assay kit; Quantification was carried out according to different concentrations. The protein samples were subjected to SDS-PAGE, PVDF membrane transferred, blocked, added with I antibody (anti-XBP1, Cosmc and GAPDH, 1:1 000), incubated at 4°C for 12h, washed with TBST, Ⅱ anti-IgG (1:5000) was incubated at RT for 1h; the membrane was

washed according to the above method, the exposure conditions were adjusted according to different light intensities, and ECL luminescent solution was added to develop and fix. Observe the results and take pictures, and use Image J for analysis of optical density.

5) Determination of XBP1 and Cosmic protein levels in intestine and kidney by immunohistochemistry: intestine and kidney were fixed, dehydrated, and embedded in paraffin. Serial sectioning, routine dehydration, immunohistochemical staining, blocking, dropwise addition of primary antibody at 4 °C overnight in the dark, secondary antibody incubation for 30 min, DAB staining, routine dehydration, clear, neutral gum sealing. Observation and shooting under the field of view of light microscope, brownish yellow color was considered positive, and Image-Pro Plus 6.0 (Media Cybernetics) was used to analyze cumulative positive value of each photo Optical density value (IOD).

6) Influence of renal histopathology: Direct immunofluorescence method: Paraffin sections of rat kidney tissue were added dropwise with FITC-labeled IgA and C3 fluorescent antibodies, and the cells were protected from light at 4°C overnight. Anti-fluorescence quencher was added dropwise, and the slides were sealed with neutral gum. The precipitation of IgA and C3 in kidney tissue was observed and photographed under the field of fluorescence microscope. Image-pro plus 6.0 was used to convert green/red into black/white (black was considered positive), and analyze each photo to get IOD. Light microscopy staining: The left kidney tissue was taken, fixed in 4% paraformaldehyde, dehydrated in ethanol, transparentized, immersed in wax, embedded in paraffin and sectioned to 3-μm slices, stained with HE, Masson, and PAS, respectively, and observed under a microscope. The shape and size of the glomerulus and renal tubule, the number of cells in the glomerulus, the presence or absence of immune complex, the degree of tubulointerstitial damage, the degree of fibrosis of renal tissue, and the blue collagen fibers were photographed.

2.2.6 Statistical methods:

SPSS 25.0 and GraphPad Prism 8 software were used for statistics and graphing. The data were normally distributed and were represented as mean ± SD (x ± s). For comparison among multiple groups, one-way ANOVA test was applied. $P<0.05$ was defined significant.

3. Results

3.1 Effects of Shenyanzhixue Pills on hematuria and proteinuria in IgAN rats

Compared to controls at 10w, urine red blood cells and urine protein of rats in each group increased drastically; except for controls, no significant difference was found between groups, indicating that modeling was successful.

Compared to models group, inhibitor sharply increased from 12 weeks (ie, 2 weeks of administration), and the urine red blood cells increased significantly at 14w, but there was no statistical difference. Compared to model, Shenyanzhixuewan group and the Kesu subgroup both decreased significantly from 12 weeks, and the decrease was more significant at 16 weeks (P<0.01). The Shenyanzhixuewan group was much better than Kesu subgroup (P<0.01) (Figure 1). Results showed that XBP1 inhibitor further aggravated the urinary protein and red blood cells of IgAN rats, which was abolished by Shenyanzhixue Pills.

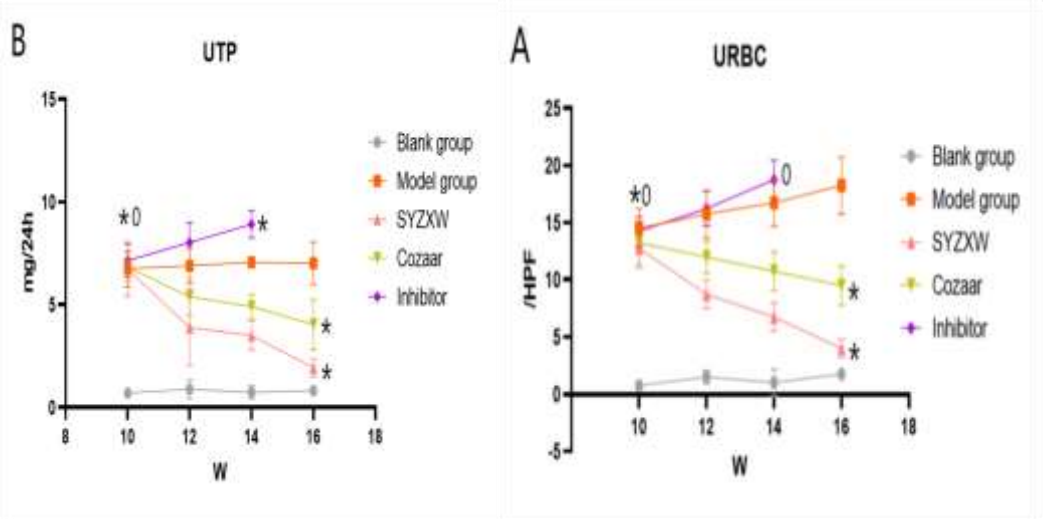


Figure 1: The effect of Shenyanzhixue Pills on urine protein quantification and urine red blood cell count in IgAN rats. A) The influence of urinary protein quantification. B) Effect of urine red blood cell count.

3.2 The effect of Shenyanzhixue Pills on serum Gd-IgA1, XBP1 and Cosmc in IgAN rats by ELISA

Gd-IgA1 was higher in model rats than that blank; Gd-IgA1 in inhibitor group was significantly higher than model rats; Shenyanzhixuewan and Kesu subgroups had much lower levels than model rats; Shenyanzhixuewan showed a better effect than Kesu (Figure 2A).

Compared to blanks, XBP1 in model rats was significantly decreased; inhibitor further inhibited XBP1 without statistically difference; Shenyanzhixuewan and Kesu subgroups were significantly higher than models; Kesu subgroup was better than Shenyanzhixuewan group (Figure 2B). Compared to blanks, Cosmc of models was significantly decreased, and Cosmc of inhibitor group was decreased compared to models. Shenyanzhixuewan group was better than Kesu subgroup (Figure 2C). Results indicated that XBP1 inhibitor further increased the expression of Gd-IgA1 by reducing the expression of XBP1 and Cosmc; Shenyanzhixue Pill enhanced XBP1 and Cosmc but suppressed Gd-IgA1.

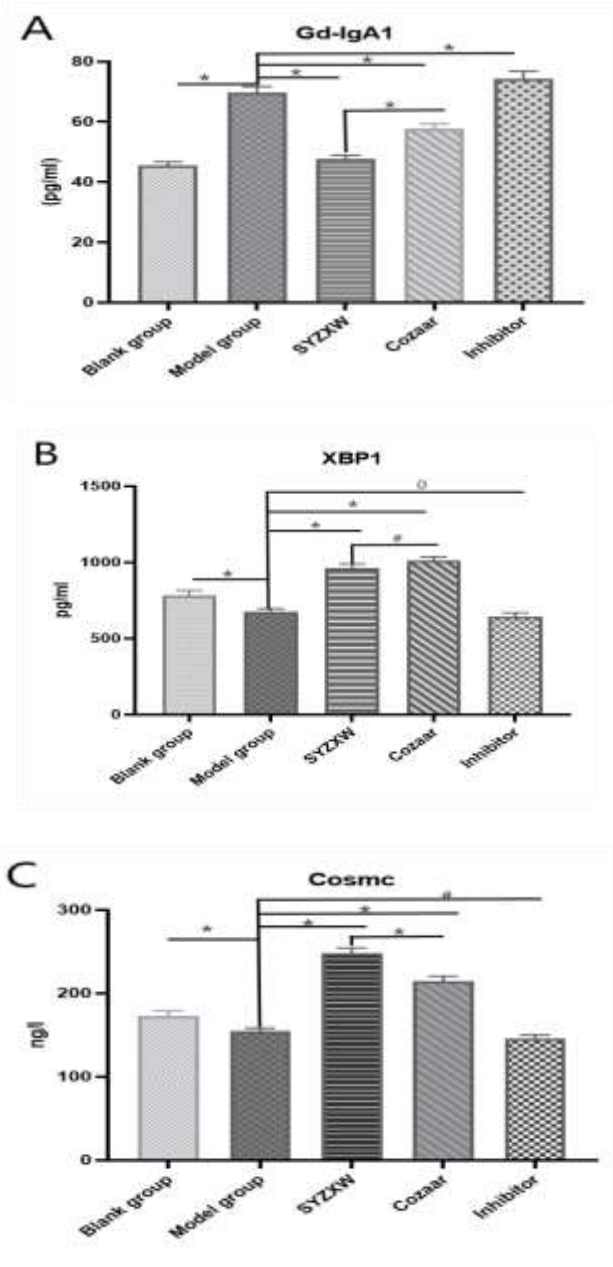


Figure 2: The effect of each group on serum Gd-IgA1, XBP1 and Cosmc of IgAN rats. (*P<0.01; #P<0.05; OP>0.05) A) The effect of Gd-IgA1. B) Effects of XBP1. C) Influence of Cosmc.

3.3 The effects of Shenyanzhixue Pills on XBP1 and Cosmc in intestinal tissue and kidney tissue of IgAN rats by qt-PCR

Compared to blanks, XBP1 and Cosmc in intestine of models decreased; the expression of the inhibitor group decreased compared to models, but there was no statistical significance; the expression of Shenyanzhixuewan group enhanced compared to models, or cosine subgroup (XBP1 P>0.05, Cosmc P<0.01) (Figure 3A, B). Compared to blanks, XBP1 and Cosmc in kidney of models were suppressed; the expressions of inhibitor group were suppressed

compared to models; the Shenyanzhixuewan group Compared to models, the expression was enhanced, but showed no statistical difference compared to cosine subgroup (Figure 3 C, D). These results showed that XBP1 inhibitor suppressed the expression of XBP1 and Cosmc in intestinal tissue and kidney tissue; Shenyanzhixue Pill increased the expression of XBP1 and Cosmc in intestinal tissue and kidney tissue.

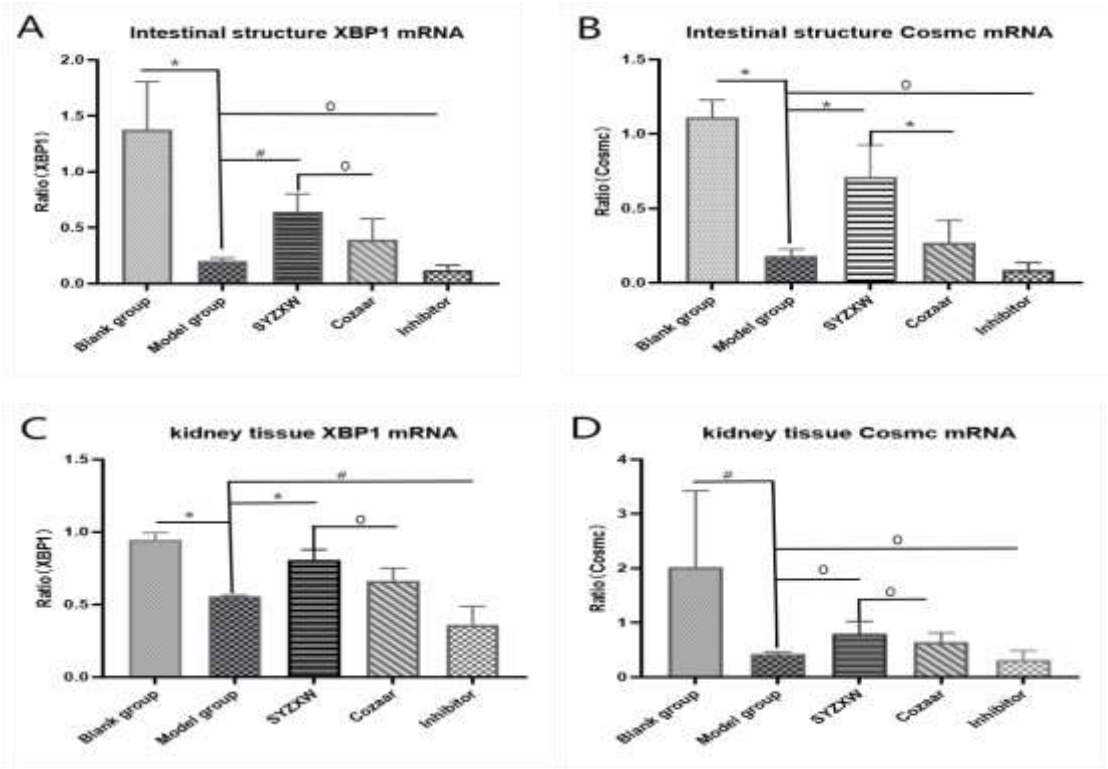


Figure 3. The effects of each group on XBP1 and Cosmc in intestinal tissue and kidney tissue of IgAN rats. (*P<0.01; #P<0.05; 0P>0.05) A) Influence of intestinal XBP1. B) Effects of intestinal Cosmc. C) Effects of XBP1 in kidney tissue. D) Effects of renal tissue Cosmc.

3.4 Western blot method to investigate effects of Shenyanzhixue Pills on XBP1 and Cosmc in intestin and kidney of IgAN rats

Compared to blanks, XBP1 and Cosmc in the intestine of models was decreased; the expression of inhibitor group was decreased compared to models; the expression of Shenyanzhixuewan group was enhanced compared to models. The expression of the subgroups of cosine increased (P<0.05) (Fig. 4A, B, C). Compared to blanks, XBP1 and Cosmc in kidney of models was decreased; the expression of inhibitor group was decreased compared to models; the expression of Shenyanzhixuewan group was enhanced compared to models. The expression of cosine subgroups increased, but there was no statistical abnormality (P>0.05) (Fig. 4D, E, F). These data suggested that XBP1 inhibitor suppressed XBP1 and Cosmc in intestine and kidney; Shenyanzhixue Pill increased the expression of XBP1 and Cosmc in intestinal

tissue and kidney tissue.

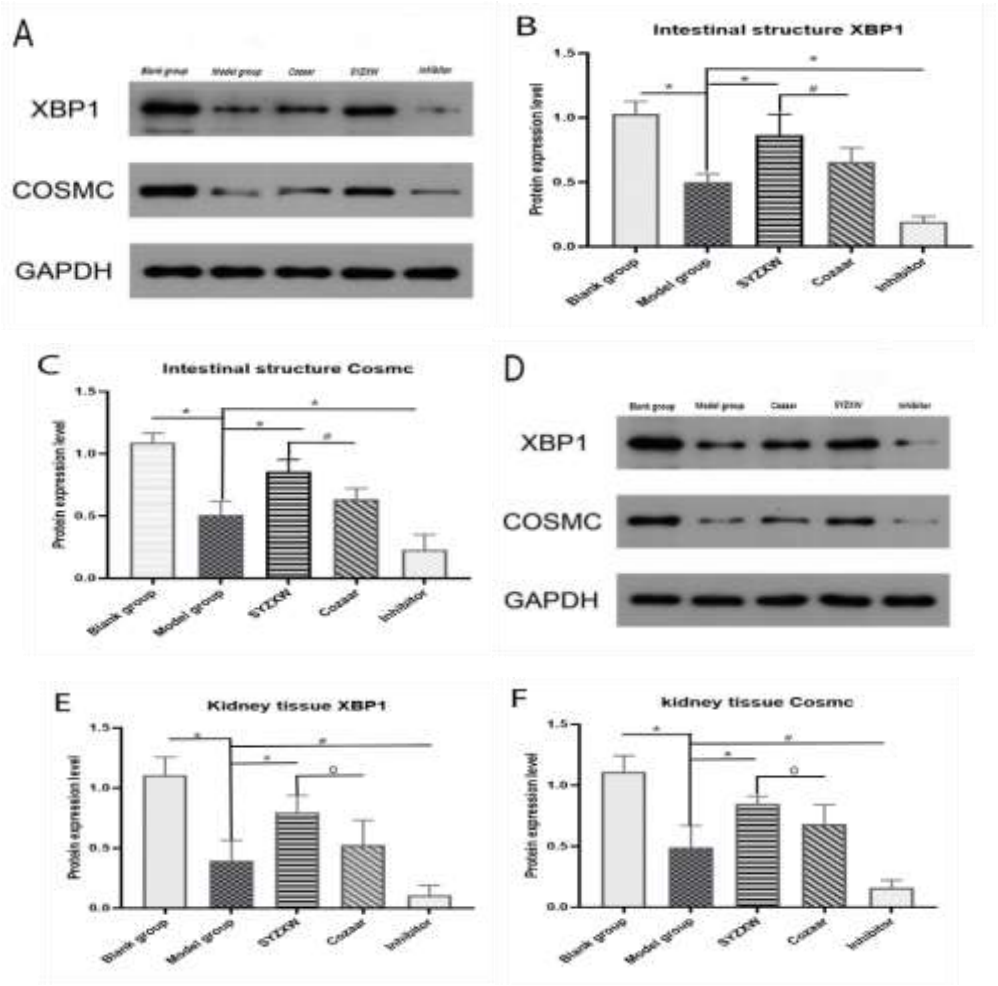


Figure 4: The effects of each group of WB on XBP1 and Cosmc in the intestinal tissue and kidney tissue of IgAN rats. (*P<0.01; #P<0.05; OP>0.05) A) XBP1 and Cosmc WB bands in intestinal tissue. B) Effects of XBP1 in intestinal tissue. C) Influence of intestinal tissue Cosmc. D) XBP1 and Cosmc WB bands in kidney tissue. E) Effects of XBP1 in kidney tissue. F) Effects of renal tissue Cosmc.

3.5 Immunohistochemical method to investigate effects of Shenyanzhixue Pills on XBP1 and Cosmc in intestine and kidney of IgAN rats

Compared to blanks, XBP1 and Cosmc in intestine of model rats were decreased; the expression of inhibitor group was suppressed compared to models without statistical difference; the expression of the Shenyanzhixuewan group was increased compared to models, the expression was enhanced in all subgroups compared to Cosine subgroups (Figure 5 A, B, E, F). Compared to blanks, XBP1 and Cosmc in kidneys of models were decreased, the expression of inhibitor group was suppressed compared to models without statistical difference, and the expression of Shenyanzhixuewan group was up-regulated compared to model or Cosu subgroup (Figure 5C, D, G, H). These data

suggested that XBP1 inhibitor suppressed XBP1 and Cosmc in intestine and kidney; Shenyanzhixue Pill increased the expression of XBP1 and Cosmc in intestinal tissue and kidney tissue.

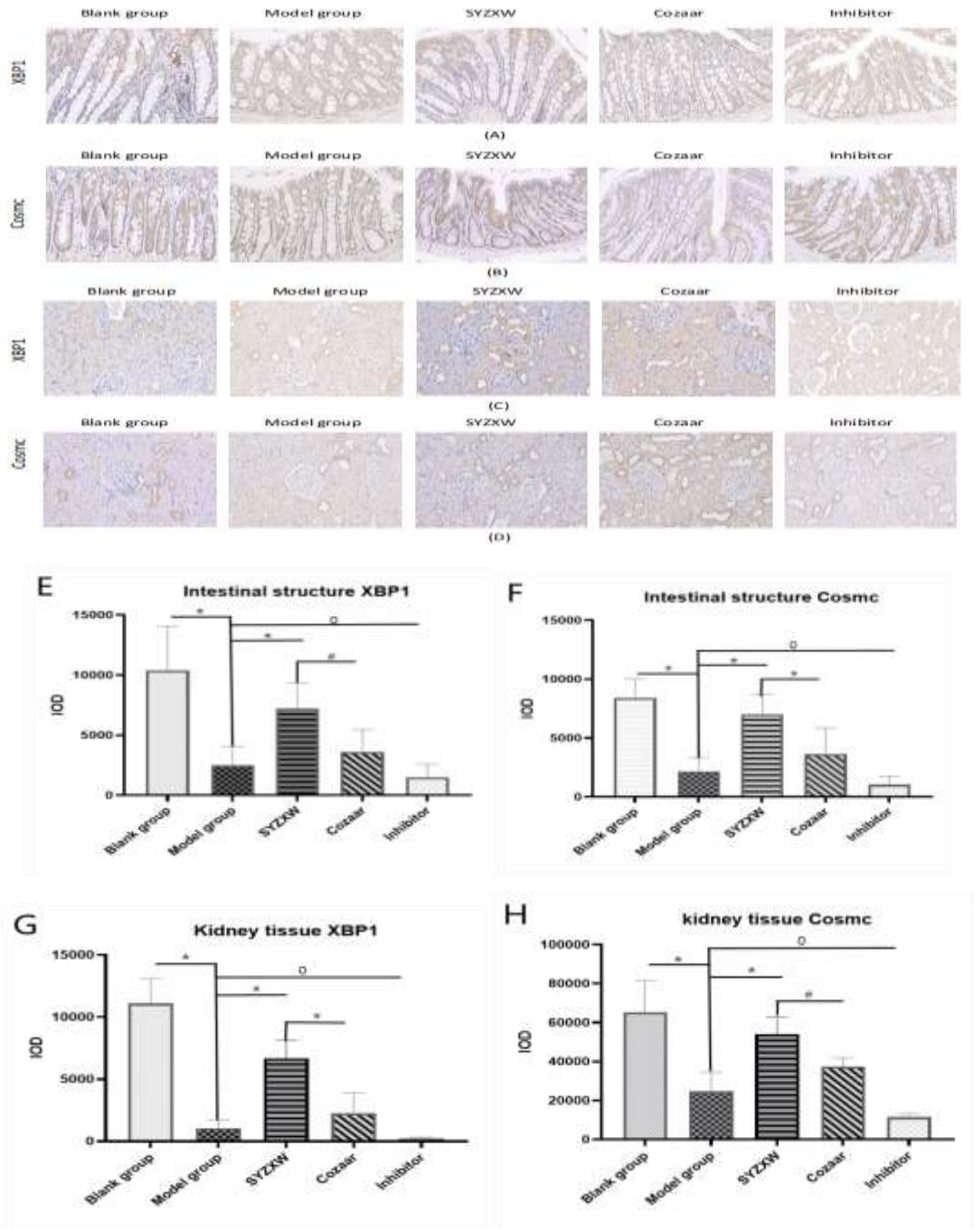
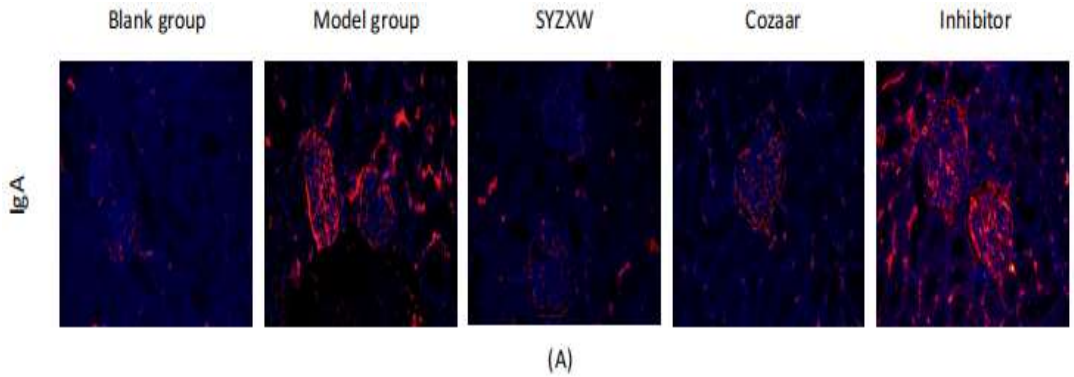


Figure 5: The effects of each group on XBP1 and Cosmc in intestinal tissue and kidney tissue of IgAN rats were detected by immunohistochemistry. (*P<0.01; #P<0.05; OP>0.05) A) Expression of intestinal XBP1. B) Intestinal Cosmc expression. C) Shadow expression of XBP1 in kidney tissue. D) Expression of Cosmc in kidney tissue. E) Expression analysis of intestinal XBP1. F) Expression analysis of intestinal Cosmc. G) Expression analysis of XBP1 in kidney tissue. H) Expression analysis of Cosmc in kidney tissue.

3.6 Effects of Shenyanzhixue Pills on renal pathology in IgAN rats

In model or inhibitor group, IgA was diffusely deposited in mesangial region of the glomerulus in a mass or granular form, combined with C3 deposition, which was basically consistent with the distribution of IgA. In the Shenyanzhixuewan group and the Kesu subgroup, a small amount of IgA and C3 were deposited along the mesangial region of the glomerulus as agglomerates and granular deposits; IOD of IgA and C3 in models was different from that of blanks; deposition in inhibitor group was further increased than models; deposition in Shenyanzhixuewan group was significantly weakened compared to models, which was sharply lower in Shenyanzhixuewan group than in models. The deposition in both groups was weakened. It can be seen that Shenyanzhixue Pills can reduce the deposition of IgA and C3 in rat kidney tissue (see Figure 6). The results of HE, Masson, and PAS staining (see Figure 7) showed that: the normal blank group rat renal tissue structure was basically normal, the glomerular structure was complete, the stroma and cells in the mesangium did not increase, the vascular loop structure was clear, and the glomerular capsule was clear. The size of the lumen was normal, the renal tubular structure was intact, and there was no obvious edema. Proximal convoluted tubule epithelial cells were arranged in an orderly manner, brush border structure was normal, and distal convoluted tubule epithelial cells were regularly arranged. There were few blue collagen fibers in the kidney tissue. In model or inhibitor group, mesangial cells were moderately and severely proliferated and the mesangial matrix increased, inflammatory cells infiltrated glomerular visual field, and individual neutrophils were also seen, and the proximal convoluted tubule epithelial cells were irregularly arranged; some The glomerular basement membrane and mesangial area showed blue line-like areas, plaque-like blue areas were scattered in the interstitial tubules, and the vascular collagen fibers were also colored blue; the mesangium of the rats in the Shenyanzhixuewan group Mild-moderate cell increase and matrix hyperplasia, and individual inflammatory cell infiltration can be seen in the visual field. The glomerular basement membrane and the mesangium are rarely seen in the blue line-like area. It can be seen that Shenyanzhixue Pills can significantly reduce kidney injury.



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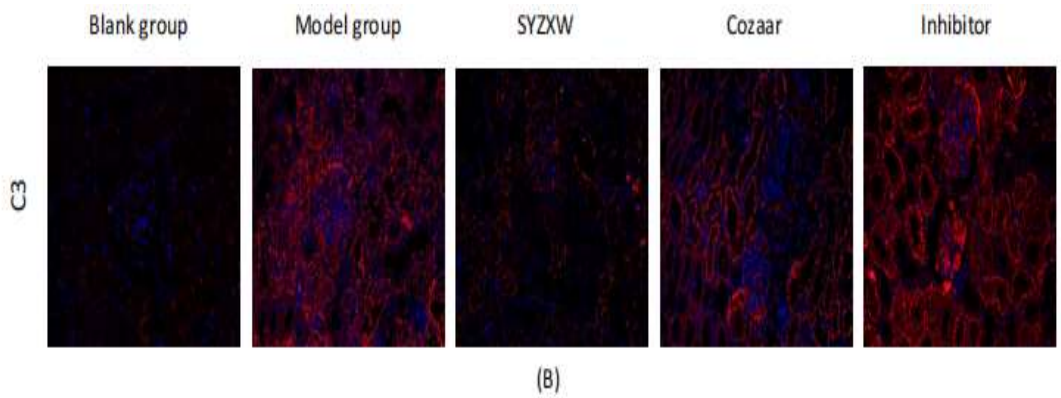

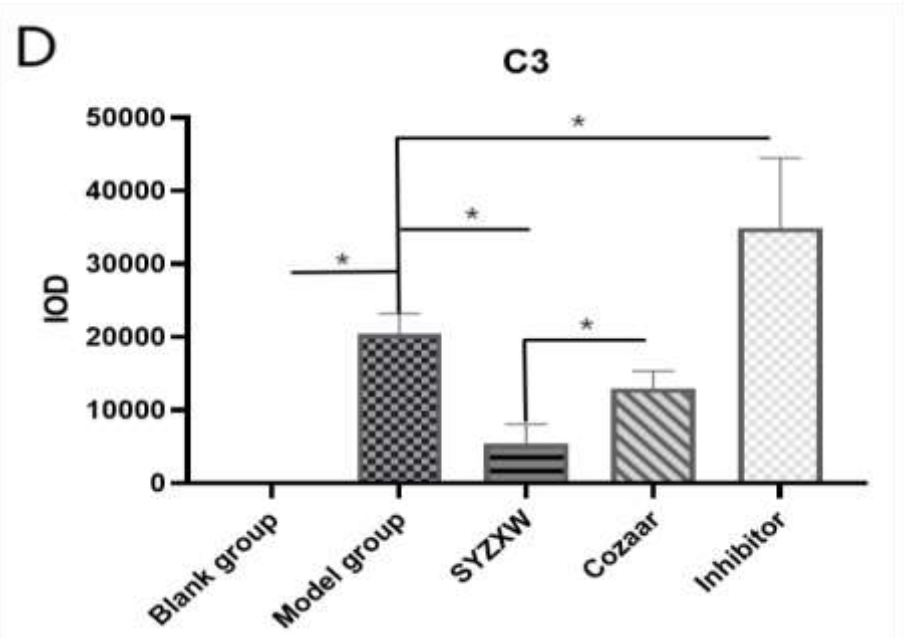

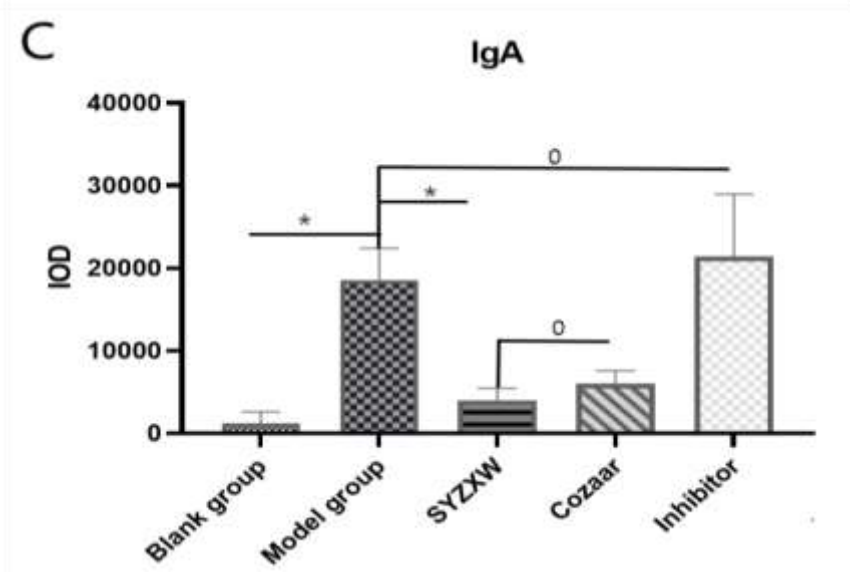

Figure 6: The deposition of IgA and C3 in kidney. (*P<0.01; OP>0.05) A) IgA fluorescence performance. B) C3 fluorescence performance. C) IOD comparison of IgA. D) IOD comparison of C3.

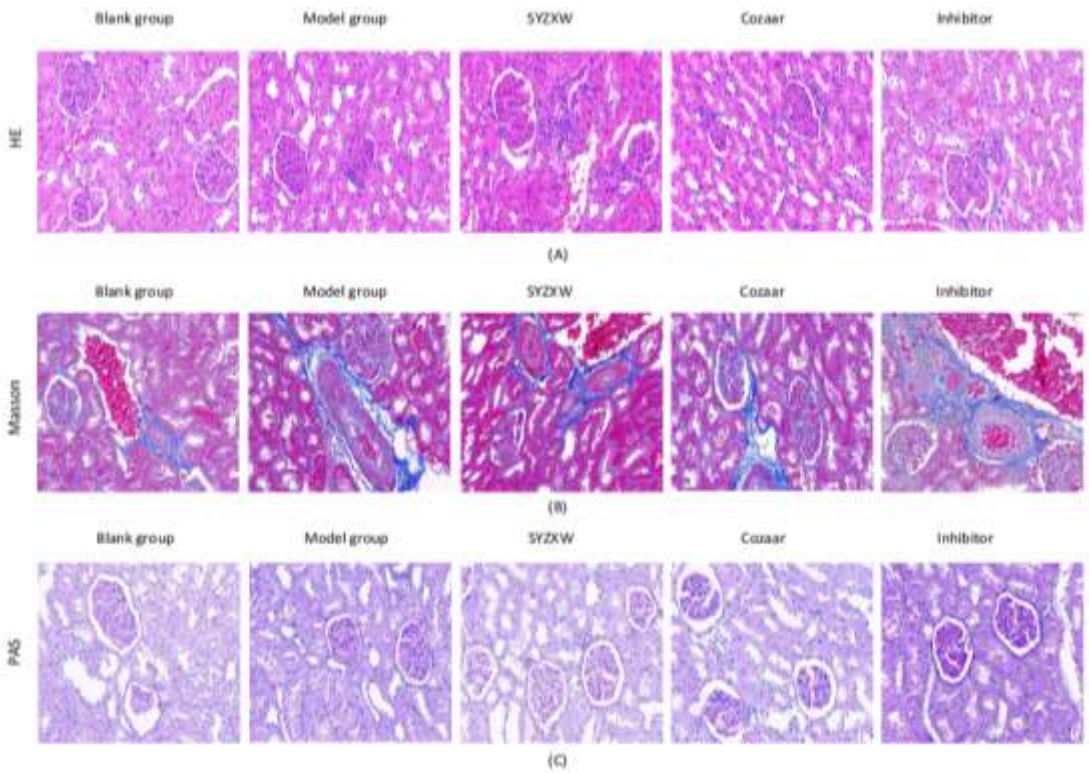

Figure 7: Kidney pathological changes. A) HE staining. B) Masson staining. C) PAS staining.

4. Discussion

The occurrence and development of IgAN is closely related to Gd-IgA1. It is also related to the differential expression of molecular chaperone Cosmc. Cosmc is a protein with type II membrane topology and is widely distributed in the body, especially in the kidney and liver. Cosmc proteins exert their physiological functions in the endoplasmic reticulum. In recent years, the evidence on molecular chaperones has become more and more clear. They can assist in the correct folding, assembly, transport, degradation and misfolding of proteins, and inhibit protein aggregation. They can further maintain normal protein homeostasis, participate in assisting in antigen presentation, and genetic material. It regulates cell division/apoptosis and mediates normal cellular physiological activities such as autophagy and transmembrane transport of plasma membrane proteins such as mitochondria (Cho & Shan, 2018; Sun et al., 2019). These studies suggest that in IgAN the molecular chaperone Cosmc can act as a rate-limiting "valve" for regulating the activity of C1GALT1 and plays an irreplaceable regulatory function in the endoplasmic reticulum. Therefore, the abnormal expression of Cosmc is the key to the production of Gd-IgA1. Glycosylation, a post-translational modification, is catalyzed by glycosyltransferases to combine sugars with various protein amino acid residues into glycosidic bonds to form glycoproteins, which are involved in cell adhesion and molecular recognition in the body. Processes such as signal transduction start from the endoplasmic reticulum

and end in the Golgi apparatus (Wang et al., 2020). The ER plays a key role in protein synthesis, processing, and transport (Wang & Kaufman, 2016). UPR accompanies autophagy, slows the influx of nascent proteins into the endoplasmic reticulum by regulating transcription, translation and post-translational processes. It also upregulates proteins involved in degradation of ER proteins, improves the folding and processing capacity of the endoplasmic reticulum for proteins and the ability to prevent misfolding, increase protein degradation ability to reverse ERS (M. Wang et al., 2018). The function of UPR is mediated by three endoplasmic reticulum transmembrane sensors: PERK, IRE1α, and ATF6, which activate their respective signal transduction pathways and induce autophagy in cells. IRE1α is an endoplasmic reticulum type I transmembrane protein that selectively cleaves XBP1 to complete the regulation of ERS. Studies have shown that XBP1 overexpression in intestinal inflammation can reduce of NF-kB and myeloperoxidase, TNF-a, IL-6 and IL-1b to inhibit inflammation (H. Zhang et al., 2017). XBP1 overexpression can also prevent the stimulation of oxidative stress (Y. Zhang et al., 2017). In recent years, more and more evidence has shown that mucosal immune dysregulation is the core of the pathogenesis of IgAN caused by excessive production of Gd-IgA1. Repeated infection of respiratory and gastrointestinal mucosa can promote the progression and deterioration of IgAN (Harabuchi & Takahara, 2019; Hu et al., 2020; Q. Wang et al., 2018; Yuzawa et al., 2016). When the human mucosal barrier is damaged by exogenous pathogenic microorganisms, the antigen triggers Toll-like receptors (TLRs), causing the body's antigen-presenting cells (such as dendritic cells, etc.) to activate and release a variety of inflammatory factors. Cells and Chemokine production can also recruit other immune cells to infection site. Studies have shown that interleukin-6 (IL-6), BAFF, and APRIL can stimulate Gd-IgA1 formation in B cells or plasma cells of the body (ang & Wu, 2020; Buren et al., 2007); It is the promoter region of IgA molecule (Suzuki et al., 2011); The low expression of XBP1 may cause abnormal mucosal immunity, participate in the regulation of molecular chaperone Cosmc, and lead abnormal glycosylation. XBP1 participates in the regulation of C1GalT1 by Cosmc in the endoplasmic reticulum and then acts on Gd-IgA1. Our results prove that Shenyanzhixuewan can reduce Gd-IgA1. The potential mechanism is that treatment of IgAN may regulate the Gd-IgA1 pathway by up-regulating XBP1 and Cosmc proteins. Our results indicated that levels of Gd-IgA1 in the blood of model rats were sharply increased, XBP1 and Cosmc in blood, intestine and kidney were significantly decreased, and the proteinuria and red blood cells in urine were significantly increased. These data further support the involvement of XBP1/Cosmc pathway in the regulation of Gd-IgA1 in the development of IgAN. Compared to models, XBP1 inhibitor drastically increased Gd-IgA1 in blood, decreased levels of XBP1 and Cosmc proteins in blood, intestinal tissue and kidney tissue, and increased urine protein and urine red blood cells. These data indicate that XBP1 regulated IgAN Process. The expressions of XBP1 and Cosmc in XBP1 inhibitor group were

decreased than models, but no significant difference in the distribution was found, which may be related to the dose and time of XBP1 inhibitor (STF083010) applied in this experiment. Both Shenyanzhixue Pills and Cosuya can effectively reduce urine protein and urine red blood cells, and significantly reduce the expression of Gd-IgA1 in blood compared to models. Data also demonstrate that Shenyan Zhixue Pill is better than Kesuya. Shenyanzhixue Pills sharply decreased the deposition of IgA and C3 in kidneys and alleviate kidney injury. Compared to models, XBP1 and Cosmc in the blood of Cosaiya were enhanced, but XBP1 and Cosmc in the intestinal tissue and kidney tissue was not significantly increased. This data suggests that Cosaiya might not regulate those two, although it can be very effective in controlling disease. Therefore, the Shenyanzhixue Pills combined with Kesuya can be used to treat IgAN, reflecting the combination of traditional Chinese and Western medicine and multi-target disease control. To sum up, this study revealed that Shenyanzhixuewan reduced the production of Gd-IgA1 by promoting the XBP1/Cosmc pathway, and improved proteinuria, erythrocyte urine and renal tissue pathological damage in IgAN rats; thereby preventing or delaying the occurrence and development of IgAN (Fig. 8). These data encouraged us to understand the mechanism of ERS involved in abnormal glycosylation and better reveal the scientific connotation of the "three viscera-mucosal axis" theory in the treatment of IgAN.

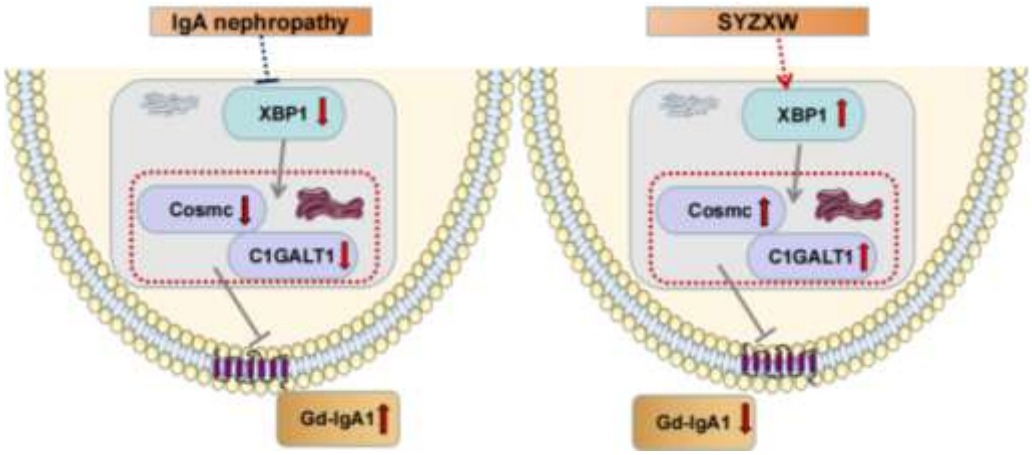

Figure 8: Schematic diagram of XBP1/Cosmc-mediated Gd-IgA1 pathway and the mechanism of action of SYZW on XBP1/Cosmc pathway to reduce Gd-IgA1 in IgAN rats.

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Data Availability Statement:

Data are collected and available by email requests

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Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The authors have declared that they were no competing interests.

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