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Fucoidan alleviates the hepatorenal syndrome through inhibition organic solute transporter α/β to reduce bile acids reabsorption

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ABSTRACT

The high levels of bile acids are a critical factor in hepatorenal syndrome. Organic solute transporter α/β (Ostα/β) participate in bile acids reabsorption in the kidney. Fucoidan has the great potential in protecting against liver and kidney injury. However, whether Ostα/β increase bile acids reabsorption in bile duct ligature (BDL)-induced hepatorenal syndrome and the blockade of fucoidan are still not clear. Male mice that received BDL were given to fucoidan (at 12.5, 25 and 50 mg/kg) through intraperitoneal injection once daily for three weeks. The serum, liver and kidney samples of these experimental mice were collected to carry out biochemical, pathological and Western blot analysis. In this study, fucoidan significantly lowered serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), decreased serum levels of uric acid, creatinine and uric nitrogen, restored the deregulation of the renal urate transporter 1 (URAT1), organic anion transporter 1 (OAT1), and organic cation/carnitine transporter 1/2 (OCTN1/2), consistence with alleviation BDL-induced liver and kidney dysfunction, inflammation and fibrosis in mice. Furthermore, fucoidan significantly hampered Ostα/β and reduced bile acids reabsorption in BDL-induced mice, protected against AML12 and HK-2 cells injury in vitro. These results demonstrate that fucoidan alleviates BDL-induced hepatorenal syndrome through inhibition Ostα/β to reduce bile acids reabsorption in mice. Therefore, suppression of Ostα/β by fucoidan may be a novel strategy for attenuating hepatorenal syndrome.

1. Introduction

Hepatorenal syndrome often occurs in the later stages of severe liver disease [Gupta et al., 2021]. It is characterized by high mortality and poor prognosis, with more than half of the patients with concomitant cirrhosis and renal failure dying within one month of diagnosis [Davenport et al., 2017; Fukazawa and Lee, 2013; Fede et al., 2012]. Up to now, liver transplantation is the only curative treatment of hepatorenal syndrome. The specific drug for hepatorenal syndrome is still very deficient. Therefore, it is necessary to search novel drug for the prevention and treatment of hepatorenal syndrome.

Bile acids are synthesized by hepatocytes, stored in the gallbladder and eventually excreted by the kidney. They play an important role in fat metabolism. Despite not being harmful at physiologic levels, bile acids are cytotoxic at high concentrations. High concentrations of bile acids increase oxidative stress and induce cell death pathways, resulting in tissue dysfunction [Perez and Briz, 2009; Herman-Edelstein et al., 2018]. Clinical studies have found that hypercholeacidemia plays a causative role in liver cirrhosis associated acute kidney injury [Lopez-Ruiz and Juncos, 2021]. Increased delivery and filtration of bile acids during cirrhosis result in high enough intrarenal levels to be cytotoxic [Lopez-Ruiz and Juncos, 2021]. Therefore, lowering the level of bile acids may be an effective way to prevent and treat hepatorenal syndrome.

Many transporters are closely involved in controlling bile acids homeostasis. The organic solute transporters α/β (Ostα/β) form a functional basolateral transporter in the ileum and renal proximal tubules. The previous study showed that Ostα-deficient mice display less liver injury during cholestasis, with lower levels of circulating bile acids than wild-type (WT) mice [Soroka et al., 2010]. Moreover, decreasing bile acids

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; OAT1, organic anion transporter 1; OCTN1/2, organic cation/carnitine transporter 1/2; Ostα/β, the organic solute transporter α/β; URAT1, urate transporter 1.

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pool after BDL by adaptation of renal transporters Ostα and Ostβ could protect mouse liver from cholestatic injury [Jang et al., 2012]. Of note, a recent study showed that adaptive changes of tubular bile acids transporters responses to rat liver cirrhosis, which causes acute kidney injury [Donadei et al., 2022]. In this regard, the regulation of Ostα and Ostβ may reduce hypercholeacidemia to alleviate hepatorenal syndrome.

Fucoidan, which can be found in brown algae, is comprised of a wide spectrum of fucans, ranging from typical fucoidans (major components) containing mainly fucose, sulfate and no uronic acid, to algin and laminaran (minor components) [Li et al., 2020]. Fucoidan derived from almost all species appear to lack toxicity in vitro and in vivo [Chung et al., 2010; Song et al., 2012; Kin et al., 2010]. Up to now, numerous biological activities of fucoidan, including antiviral [Kuznetsova et al., 2017; Li et al., 2017], antioxidant [Abdel-Daim et al., 2020], antitumor [Cho et al., 2016; Teng et al., 2015], anti-inflammatory [Choi et al., 2010; Raghavendran et al., 2011], anticoagulant [Mansour et al., 2019], have been extensively studied. These known biological activities of fucoidan have been investigated for therapeutic uses against injury, infection, chronic inflammation, fibrosis and neuronal damage [Lim et al., 2015], which indicated that fucoidan has the important medicinal value.

Many studies have found that fucoidan can effectively ameliorate mouse/rat liver injury induced by carbon tetrachloride or diethylnitrosamine or BDL [Hayashi et al., 2008; Nakazato et al., 2010; Hong et al., 2011; Li et al., 2016; Chale-Dzul et al., 2020]. Fucoidan not only has the protective effect to the liver injury, but also has the potential in alleviation renal injury in mice [Yu et al., 2020; Wang et al., 2022]. Notably, fucoidan can ameliorate adenine-induced hypercholeacidemia and down-regulated expression of renal URAT1 in mice [Zhang et al., 2018]. However, blockade of fucoidan on BDL-induced hyperbilirubinemia in hepatorenal syndrome and the possible mechanism in mice is still unclear. Therefore, the effect of fucoidan on BDL-induced hepatorenal syndrome and the possible molecular mechanism was investigated.

2. Materials and methods

2.1. Reagents and antibodies

Fucoidan (purity $\geq$ 98%) was got from Lmai Biotechnology Co., Ltd (Shanghai, China). Its average molecular weight of the commercial fucoidan is 189 kDa. The assay kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), hydroxyproline, uric acid, creatinine, uric nitrogen, and total bile acids were bought from Jiancheng Biotechnology Co., Ltd (Nanjing, China). Pierce™ BCA protein assay kit was obtained from Thermo Scientific (Schwerte, Germany). Rabbit anti-Ostα (A10127) was got from BOSTER Biological Technology Co., Itd (Wuhan, China). Rabbit anti-Ostβ (Bs-2128R) was obtained from BIOSS Biological Technology Co., Itd (Beijing, China). Rabbit anti-OAT1 (Abs-138102), anti-OCTN1 (Abs-147470) and anti-OCTN1 (Abs-148655) were purchased from ABSIN Biological Technology Co., Itd (Shanghai, China). The rabbit anti-GAPDH (60004-1-Ig), anti-URAT1 (14937-1-AP) and HRP-conjugated rabbit anti-IgG (SA00001-2) were got from Proteintech Group, Inc. (Chicago, USA).

2.2. Animal experiments

2.2.1. Experimental design

BDL is shorter time for achieving liver fibrosis. In the short term, elevated levels of bile acids increase oxidative stress in tissues, which is conducive to the success of the model. However, estrogen has an antioxidant effect that may extend modeling time. It is known that females secrete much estrogen than males. Thus, this study chose male mice for the experiments. One hundred male ICR mice (20–22 g) were purchased from Nantong University (Nantong, China) (Production license: SCXK (Su) 2019-0001). The mice were maintained under a controlled temperature (22 $\pm$ 2 °C), relative humidity (55 $\pm$ 5%), and 12-h light/12-h dark cycle with the lights from 9:00 a.m. to 9:00 p.m. The mice were fed a standard chow and water ad libitum. Before the experiment, the mice adapted to the environment for one week. The mice were excluded if the echoes in the liver were uneven, enhanced, or coarse with ultrasonic detector. The mice were divided into five groups (n = 15/group): (a) sham control mice; (b) BDL-control mice which received saline; (c-e) fucoidan-treated mice which received 12.5, 25 and 50 mg/kg fucoidan and BDL. Doses of fucoidan used in this study were selected based on the previous study [Hayashi et al., 2008].

2.2.2. Development of BDL and treatment

Mice model with hepatorenal syndrome were established by BDL. The mice were anesthetized with 10% chloral hydrate. The abdomen was sterilized with 75% alcohol. Then, the abdomen was opened along the midline of the abdomen to expose the lower edge of the liver and the duodenum. The liver lobe was turned up to the diaphragm and the duodenum was pushed to the lower abdomen, which exposed the common bile duct. It was doubly ligated with 5-0 silk and transected between the ligated sites. Followed disinfection with 3% iodine and 75% alcohol, the mice were placed in a thermal blanket to keep them warm until they woke up. According to the literature, liver fibrosis is achieved between one week to four weeks [Marques et al., 2012]. After the BDL one week, fucoidan was diluted in saline and administered once a day for the last 2 weeks. All drugs were administered between 2:30 p.m. and 3:30 p.m. Animal welfare and experimental procedures were carried out in accordance with the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' enacted by National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) and the related ethnical regulations of Yancheng Teachers University. All efforts were made to minimize animal suffering.

2.3. Blood and tissue collection

At the end of the animal experiment, these animals were anesthetized using 60 mg/kg sodium pentobarbital (Sigma-Aldrich Inc.) after a 12 h fast. Blood samples were collected from the mice hearts. The blood samples were centrifuged (5 000×*g*, 10 min) to get the serum. Then, these serum samples were stored at −80 °C for biochemical assays. Liver and kidney samples were rapidly dissected on ice followed by a wash with 0.01M phosphate buffer solution. Parts of them were immediately fixed with paraformaldehyde for histological study, while others were stored at −80 °C for biochemical and Western blot analysis, respectively.

2.4. Histopathological analysis

Formalin-fixed liver specimens were embedded in paraffin and cut into 4 μm-thick slices. The tissue sections were placed in xylene for 10 min, followed by rehydration in 100%, 90% and 70% ethanol, respectively. Then these slides were washed three times in tap water.

For the general tissue morphology, hematoxylin and eosin (H&E) staining were employed. Slides were stained with hematoxylin for 40 s. After three times wash, sectioned slides were then dipped four times in ammonia water and rinsed four times with tap water followed by 20 dips in 95% ethanol. Then the sections were stained in eosin for 10 s. Slides were dehydrated twice in 95% ethanol, three times in 100% ethanol, treated twice with xylene and mounted using mounting media. Each section was assessed under light microscopic fields.

To evaluate liver fibrosis, Masson and Sirius red staining was carried out. Nuclei were stained with hematoxylin for 30 s, and then washed in running water for 10 min. Followed stained in Masson ponceau red acid solution for 10 min, slides were washed with 2% glacial acetic acid solution. These samples were stained with aniline blue solution for 5 min after differentiation with 1% phosphomolybdic acid for 3–5 min. These slides were soaked for 1 min in 0.2% glacial acetic acid solution. Then, they were dehydrated twice in 95% ethanol, three times in 100% ethanol, treated twice with xylene and mounted using mounting media.

Each section was assessed under light microscopic fields. Slides were stained in Sirius red stain for 60 min and washed two times in 0.5% acidified deionized water. Slides were then dehydrated in 70%, 90% and 100% ethanol and dipped in xylene twice and mounted with mounting media. The collagen deposition in selected samples was quantified using ImageJ 1.46 software.

2.5. Determination of biochemical parameters

Serum activities of AST and ALT were detected with standard diagnostic kits according to the manufactures' instructions, respectively. Briefly, 20 μL of buffer solution I and 5 μL of serum samples were added to assay wells. At the same time, 20 μL of buffer solution I was added to the control wells. Then, these 96-well plates were incubated at 37 °C for 30 min. 5 μL of serum samples were added to control wells, and a serious of standards with different concentrations were injected into standard wells. After 20 μL of buffer solution II addition, the plate was incubated at 37 °C for 30 min. The reaction in each well was stopped with 200 μL of stop solution. Lastly, the absorbance of each well at 510 nm was tested with the microplate reader. After the standard curve was made, serum activities of AST and ALT were calculated, respectively.

Serum level of uric acid was detected with standard diagnostic kit according to the manufacturer's instruction. 4 μL of serum samples, standard and distilled water were added to assay wells, standard wells and the control well, respectively. Followed 180 μL solution I addition, the 96-well plate was incubated at 37 °C for 5 min. The absorbance of each well at 600 nm was tested with the microplate reader. After 60 μL of solution II addition, the 96-well plate was incubated at 37 °C for 5 min. The absorbance of each well at 600 nm was measured again. Lastly, serum level of uric acid was calculated according to the formula provided in the instruction.

The determination of serum creatinine level was similar to that of uric acid. The only difference was that the absorbance was measured at 546 nm.

Serum level of uric nitrogen was tested with the kit according to the manufacturer's instruction. 10 μL of serum samples, standard and distilled water were added to the assay tubes, the standard tube and the control tube, respectively. After 125 μL of enzyme buffer solution was added into these tubes, they were incubated at 37 °C for 10 min. Then, 500 μL of phenol developer solution and 500 μL of alkaline sodium hypochlorite solution were added. The absorbance of each tube at 630 nm was measured after incubation at 37 °C for 10 min. Lastly, serum level of uric nitrogen was calculated according to the formula provided in the instruction.

Serum level of total bile acids was tested with the kit according to the manufacturer's instruction. 3 μL of serum samples were added into assay wells and 3 μL of standard was added into the control well. Followed 200 μL of buffer solution I and 50 μL of buffer solution II addition, the plate was incubated at 37 °C for 2 min. The absorbance of each well at 405 nm was read. Lastly, serum level of total bile salt was calculated according to the formula provided in the instruction.

The liver concentrations of hydroxyproline were detected with standard diagnostic kit according to the manufactures' instructions. In brief, 100 mg of liver tissues were thoroughly hydrolyzed at 95 °C for 20 min. 3 mL of hydrolysates and a serious of standards with different concentrations were injected into 10 mL tubes, respectively. After 0.5 mL of solution I, solution II and solution III were sequentially added, these tubes were incubated at 65 °C for 5 min. Finally, the absorbance of each tube at 550 mm was measured with the microplate reader. After the standard curve was made, liver concentrations of hydroxyproline were calculated, respectively. The wet weight of liver samples was used to normalize to the data of hydroxyproline levels.

Table 1
Effects of fucoidan on serum parameters in BDL-induced hepatorenal syndrome model.

Group	Sham +	BDL +	BDL + Fucoidan			
	Vehicle	Vehicle	12.5	25	50	mg/kg
AST (U/L)	2.39 ± 0.456	20.81 ± 6.420$^{\#\#}$	16.23 ± 4.265	9.14 ± 1.032	7.13 ± 0.496*	
ALT (U/L)	4.57 ± 0.830	10.97 ± 1.692$^{\#\#}$	8.88 ± 1.316	4.196 ± 0.921**	2.88 ± 0.506***	
Uric acid (μmol/L)	296.8 ± 18.14	479.4 ± 29.58$^{\#\#}$	419.0 ± 79.48	388.1 ± 24.40	320.0 ± 34.42*	
Creatinine (μmol/L)	182.5 ± 57.10	442.0 ± 100.8$^{\#}$	146.1 ± 56.55*	135.5 ± 66.50*	185.4 ± 20.16	
Uric nitrogen (μmol/L)	19.27 ± 0.589	25.01 ± 1.669$^{\#\#}$	18.57 ± 1.608**	19.22 ± 0.811*	19.71 ± 0.818*	

Data are expressed as the mean ± SEM (n = 4–6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus sham-vehicle. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$ versus BDL-vehicle. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

2.6. Cell culture

These previous researches have reported that high levels of bile acids are cytotoxic and can cause tissue dysfunction [Perez and Briz, 2009; Herman-Edelstein et al., 2018]. To verify that high levels of bile acids can cause liver and kidney damage and evaluate the blockade of fucoidan, we chose to culture the mouse normal hepatocytes AML12 cells and renal epithelial cell lines HK-2 cells. AML12 and HK-2 cells were supported by Beijing zhongkezhijian Biotechnology Co. Ltd (Beijing, China). AML12 cells were grown in DMEM/F12 supplemented with 10% fetal bovine serum and 40 ng dexamethasone. HK-2 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum. These cells were cultured in a humidified 5% CO_2 atmosphere at 37 °C, respectively. During experiments, these cells were plated in 96 well plates (1.0×10^5 cells/mL). After these cells were attached to the bottom of the well, they were made quiescent by incubation in serum-free DMEM/F12 or RPMI 1640 for 12 h. Then, serum samples (dilution 1:50) from these five groups in the animal experiment were respectively added into AML12/HK-2 cells. These cells were cultured for 48 h. The cell activity was tested by MTT method.

2.7. Western blot analysis

Total proteins of liver and kidney tissue were extracted by Radio immunoprecipitation assay (RIPA) buffer. The protein concentration of liver and kidney samples was quantified by the BCA method. Then, the concentration of all protein samples was unified to 1 mg/mL. Twenty microgram protein were resolved by SDS-PAGE, transferred to polyvinylidene fluoridemembranes, and then immunoblotted using specific primary antibodies including anti-Ostα (dilution 1:1000), anti-Ostβ (dilution 1:1000), anti-URAT1 (dilution 1:1000), anti-UAT1 (dilution 1:1000), anti-OCTN1 (dilution 1:1000), anti-OCTN2 (dilution 1:1000) and anti-GAPDH (dilution 1:2000) at 4 °C overnight, followed by HRP-conjugated anti-rabbit IgG antibody (dilution 1:5000). Immunoreactive bands were visualized via enhanced chemiluminescence and quantified via densitometry using ImageJ (version 1.42q, National Institutes of Health).

2.8. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was performed using a one-way analysis of variance (ANOVA), followed by the Dunnett test. In all statistical comparisons, $P < 0.05$ was considered to be

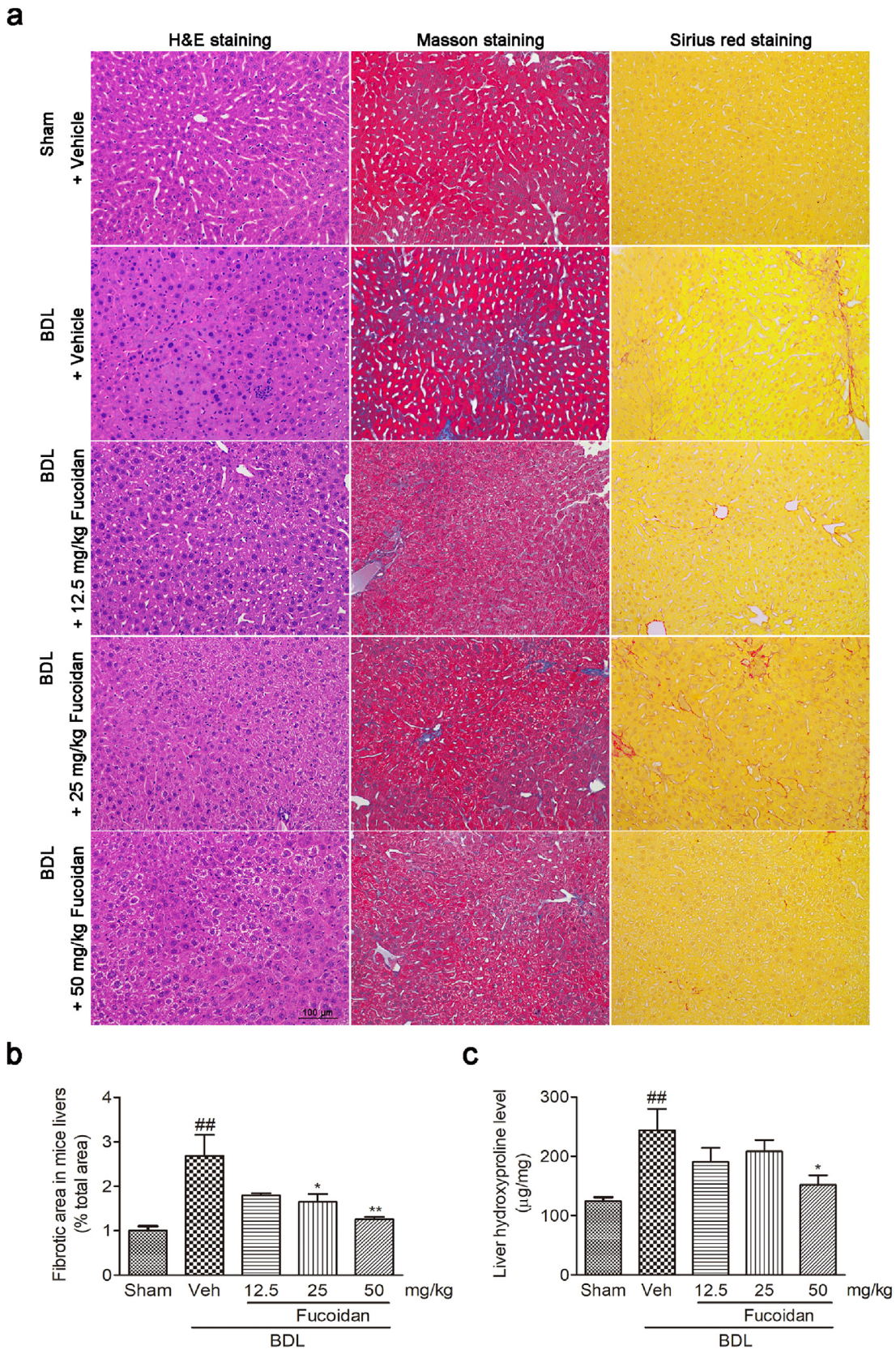

Fig. 1. Fucoidan alleviated BDL-induced liver histological changes in mice. (**a**) Histology of liver sections were examined by H&E, Masson and Sirius red staining, respectively (magnification × 200; bar: 100 μm). (**b**) Fibrotic area of the Sirius red-stained liver section was quantitatively compared (n = 3). **(c)** The hepatic level of hydroxyproline was detected with standard diagnostic kit (n = 5–6). Data are expressed as the mean ± SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus sham-vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$ versus BDL-vehicle.

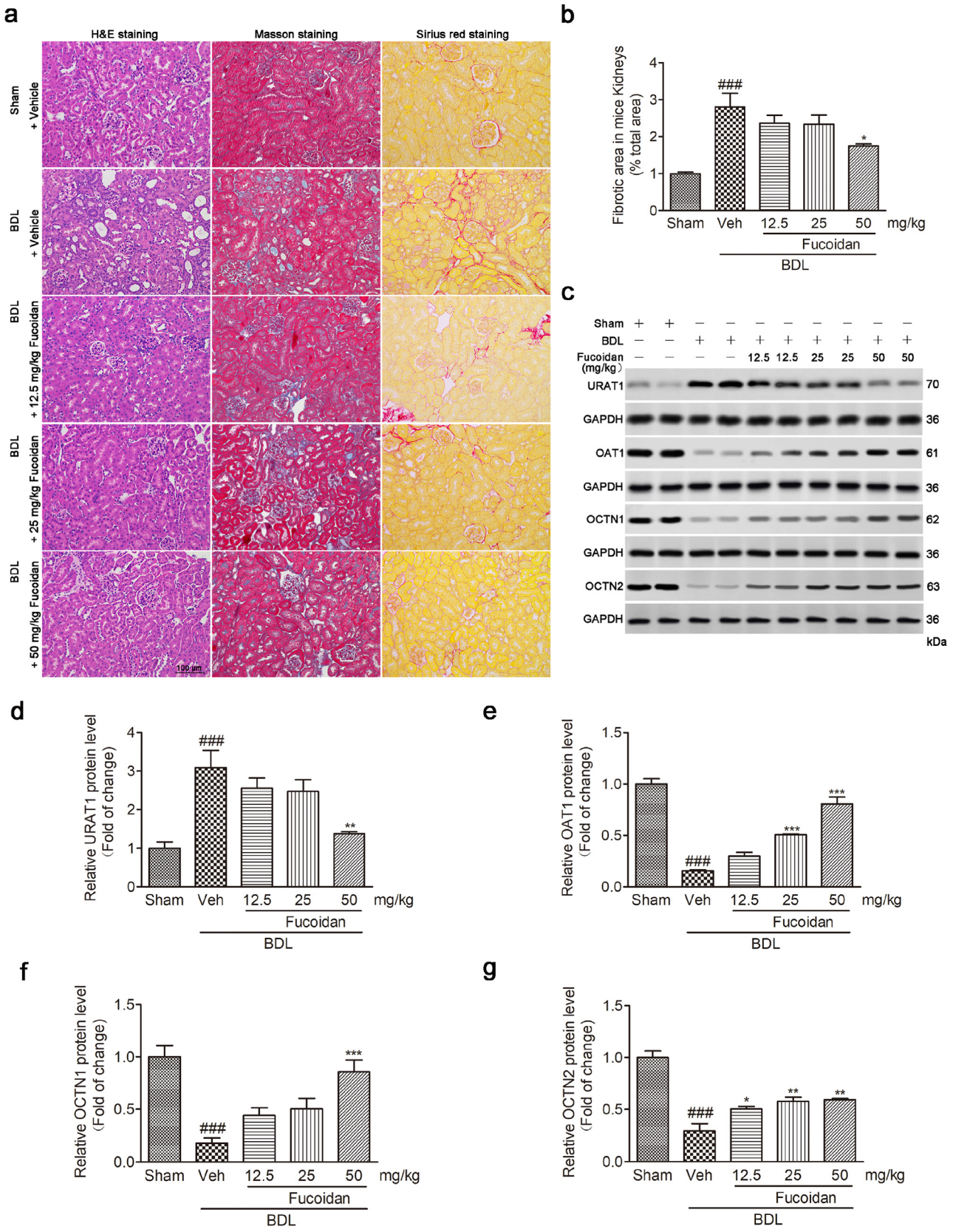

Fig. 2. Fucoidan ameliorated BDL-induced renal injury in mice. (**a**) Histology of kidney sections were examined by H&E, Masson and Sirius red staining, respectively (magnification × 200; bar: 100 μm). (**b**) Fibrotic area of the Sirius red-stained kidney section was quantitatively compared (n = 3). **(c)** The representative blots of URAT1, OAT1, OCTN1 and OCTN2. Relative protein levels of URAT1, OAT1, OCTN1 and OCTN2 were normalized to GAPDH (n = 4). (**d**) Renal protein level of URAT1. (**e**) Renal protein level of OAT1. (**f**) Renal protein level of OCTN1. (**g**) Renal protein level of OCTN2. Data are expressed as the mean ± SEM. $^{###}P < 0.001$ versus sham-vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus BDL-vehicle.

significant. Figures were obtained by the Statistical Analysis System (GraphPad Prism 5, GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Fucoidan ameliorated BDL-induced liver and kidney dysfunction in mice

In this study, BDL significantly enhanced serum activities of ALT ($P = 0.0028$) and AST ($P = 0.0018$), increased serum levels of uric acid ($P = 0.0046$), creatinine ($P = 0.0402$) and uric nitrogen ($P = 0.0060$) in mice (Table 1). These results indicate that BDL causes liver and kidney dysfunction in mice. Fucoidan was found to remarkably reduce serum activities of ALT (25 mg/kg, $P = 0.0029$; 50 mg/kg, $P = 0.0006$) and AST (50 mg/kg, $P = 0.0266$), decreased serum levels of uric acid (50 mg/kg, $P = 0.0186$), creatinine (12.5 mg/kg, $P = 0.0263$; 25 mg/kg, $P = 0.0210$) and uric nitrogen (12.5 mg/kg, $P = 0.0058$; 25 mg/kg, $P = 0.0137$; 50 mg/kg, $P = 0.0237$) (Table 1). These results suggest that fucoidan is able to effectively recover BDL-induced liver and kidney dysfunction in mice.

3.2. Fucoidan relieved BDL-induced liver inflammation and fibrosis in mice

H&E, Sirius red and Masson staining showed the liver histological changes, including inflammatory cell infiltration, hepatocyte necrosis, perisinusoidal or portal/peripotal fibrosis in BDL-induced mice livers (Fig. 1a). Consistently, the liver fibrotic area ($P = 0.0014$) (Fig. 1b) and the hepatic level of hydroxyproline ($P = 0.0046$) (Fig. 1c) were significantly increased in BDL-induced model. These results indicate that BDL induces liver inflammation and fibrosis in mice.

As we expected, fucoidan effectively alleviated the liver inflammation, protected hepatocyte against necrosis, reduce collagen deposition in BDL-induced model (Fig. 1a). Fucoidan also significantly decreased the liver fibrotic area (25 mg/kg, $P = 0.0304$; 50 mg/kg, $P = 0.0045$) and the hepatic level of hydroxyproline (50 mg/kg, $P = 0.0417$) in BDL-induced model (Fig. 1b and c). These results suggest that fucoidan can alleviate BDL-induced liver inflammation and fibrosis in mice.

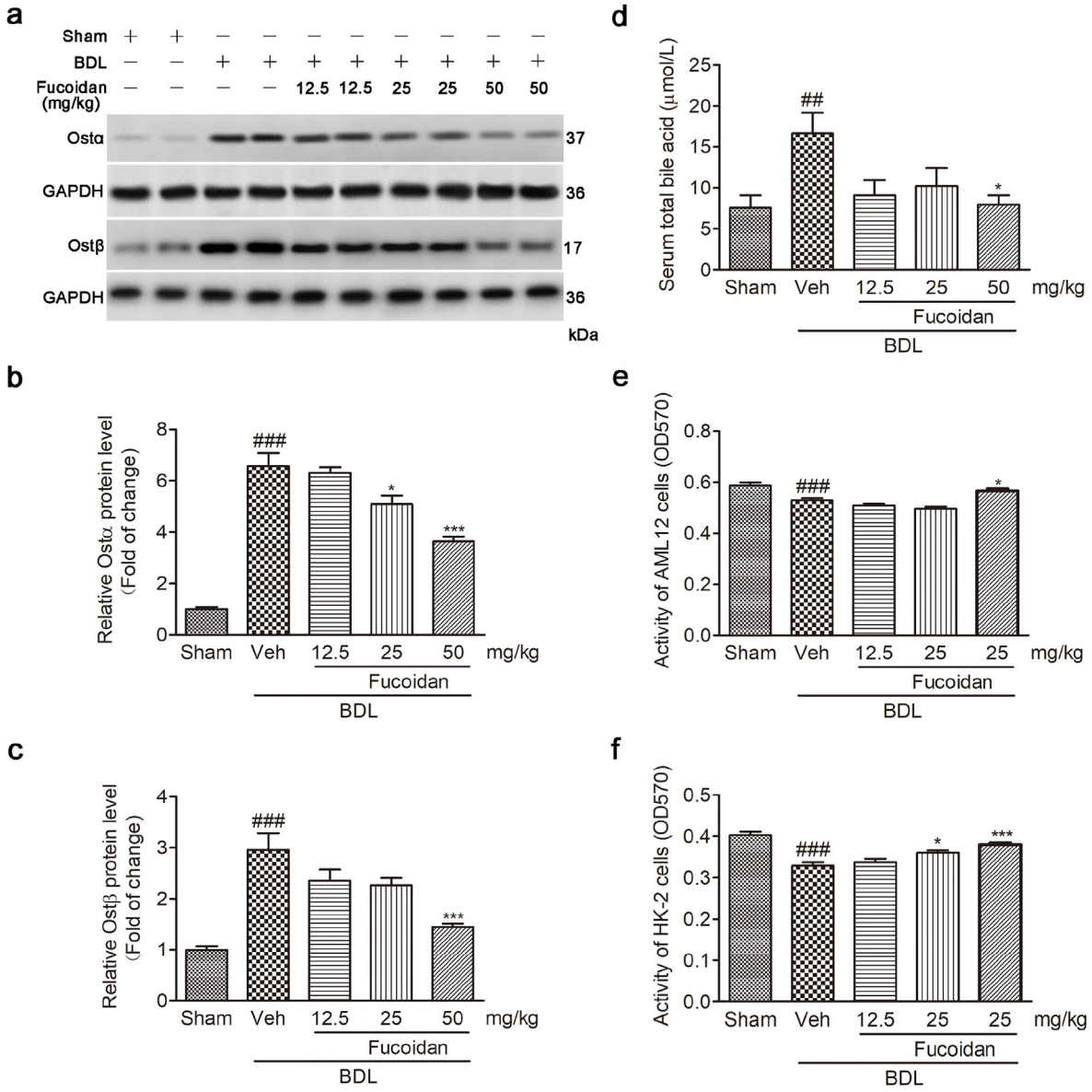

Fig. 3. Fucoidan reduced bile salt reabsorption by inhibition Ostα and Ostβ in BDL-induced mice. (a) The representative blots of Ostα and Ostβ. Relative protein levels of Ostα and Ostβ were normalized to GAPDH (n = 4). **(b)** Renal protein level of Ostα. **(c)** Renal protein level of Ostβ. **(d)** The serum level of total bile acid was detected with standard diagnostic kit (n = 5–6). **(e–f)** Activities of AML12 and HK-2 cells treated with serum samples from animal experiment were determined with MTT method ((n = 7–8). Data are expressed as the mean ± SEM. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus sham-vehicle; $^{*}P < 0.05$, $^{***}P < 0.001$ versus BDL-vehicle.

3.3. Fucoidan alleviated BDL-induced kidney injury

H&E, Sirius red and Masson staining results showed extensive tubular dilatation with signs of epithelial cell necrosis, leukocyte infiltration, tubulointerstitium and glomerular fibrosis (Fig. 2a). The kidney fibrotic area ($P = 0.0008$) was significantly increased in BDL-induced mice (Fig. 2b). In addition, the renal protein level of URAT1 ($P = 0.0003$) was significantly up-regulated, while the renal protein levels of OAT1 ($P < 0.0001$), OCTN1 ($P < 0.0001$) and OCTN2 ($P < 0.0001$) were down-regulated in BDL-induced mice (Fig. 2c–g). These results suggest that BDL causes renal injury in mice.

In fact, fucoidan was found to attenuate atrophy of the tubuli with better preservation of the cellular architecture in large parts of the cortical area, inflammation and fibrosis (Fig. 2a), lower the kidney fibrotic area (50 mg/kg, $P = 0.0251$) (Fig. 2b). Fucoidan also significantly down-regulated the protein level of URAT1 (50 mg/kg, $P = 0.0020$), up-regulated protein levels of OAT1 (25 mg/kg, $P = 0.0001$; 50 mg/kg, $P < 0.0001$), OCTN1 (50 mg/kg, $P = 0.0004$) and OCTN2 (12.5 mg/kg, $P = 0.0210$; 25 mg/kg, $P = 0.0023$; 50 mg/kg, $P = 0.0015$) in BDL-induced mice kidneys (Fig. 2c–g). These results indicate fucoidan relieves BDL-induced renal injury in mice.

3.4. Fucoidan reduced bile acids reabsorption by inhibition Ostα and Ostβ in BDL-induced mice

Next, we explored the possible mechanism of fucoidan on the alleviation BDL-induced hepatorenal syndrome in mice. Compared with the sham-control mice, renal protein levels of Ostα ($P < 0.0001$) and Ostβ ($P < 0.0001$) were significantly up-regulated in BDL-induced mice (Fig. 3a–c). Consistence with these changes, the serum level of total bile acids ($P = 0.0076$) was notably increased in model mice (Fig. 3d). When serum samples from BDL-induced mice were used to cultured AML12 ($P = 0.0003$) and HK-2 ($P < 0.0001$) cells, activities of these cells were significantly decreased (Fig. 3e and f). These data indicate that the up-regulation of bile salt transporters contributes to cause hypercholeacidemia, which results in liver and kidney injury.

Importantly, fucoidan was found to down-regulate renal protein levels of Ostα (25 mg/kg, $P = 0.0130$; 50 mg/kg, $P < 0.0001$) and Ostβ (50 mg/kg, $P = 0.0002$) (Fig. 3a–c), lower the serum level of total bile acids (50 mg/kg, $P = 0.0229$) in BDL-induced mice (Fig. 3d). Fucoidan also significantly increased activities of AML12 (50 mg/kg, $P = 0.0469$) and HK-2 (25 mg/kg, $P = 0.0157$; 50 mg/kg, $P < 0.0001$) cells exposed serum samples from this animal experiment (Fig. 3e and f). These results suggest that fucoidan can reduce hypercholeacidemia by inhibition Ostα and Ostβ, which may protect against liver and kidney injury induced by BDL in mice.

4. Discussion

The present study was the first to demonstrate that fucoidan prevented BDL-induced hepatorenal syndrome in mice. Furthermore, fucoidan down-regulated Ostα and Ostβ to reduce bile acids reabsorption, and alleviated hypercholeacidemia, which may contribute to protect against liver and kidney injury in BDL-induced hepatorenal syndrome in mice (Fig. 4).

In this study, we observed significant enhance in serum activities of AST and ALT, indicating BDL caused liver dysregulation in mice. The further results from histopathological evaluation and the hepatic level of hydroxyproline assay verified that BDL induced liver inflammation and fibrosis in mice. Consistence with the previous study [Li et al., 2016], fucoidan could effectively alleviate BDL caused liver inflammation and fibrosis, in parallel with the restoration of liver function in mice.

Liver fibrosis/cirrhosis caused by BDL can gradually induce renal injury and dysfunction [Varga et al., 2018; Trojnar et al., 2020]. In this study, we also found that BDL significantly increased serum levels of uric acid, creatinine and uric nitrogen in mice, which suggested BDL induced renal dysfunction following liver fibrosis. Renal transporters URAT1and OAT1 contribute to urate homeostasis in kidney [Hosoyamada 2021; Taniguchi et al., 2021]. Deregulation of renal organic cation/carnitine transporter OCTN1 and OCTN2 participate in the progress of kidney dysfunction in hyperuricemic rodents [Wang et al., 2015]. Based on these previous reports, we detected renal protein levels of URAT1, OAT1,

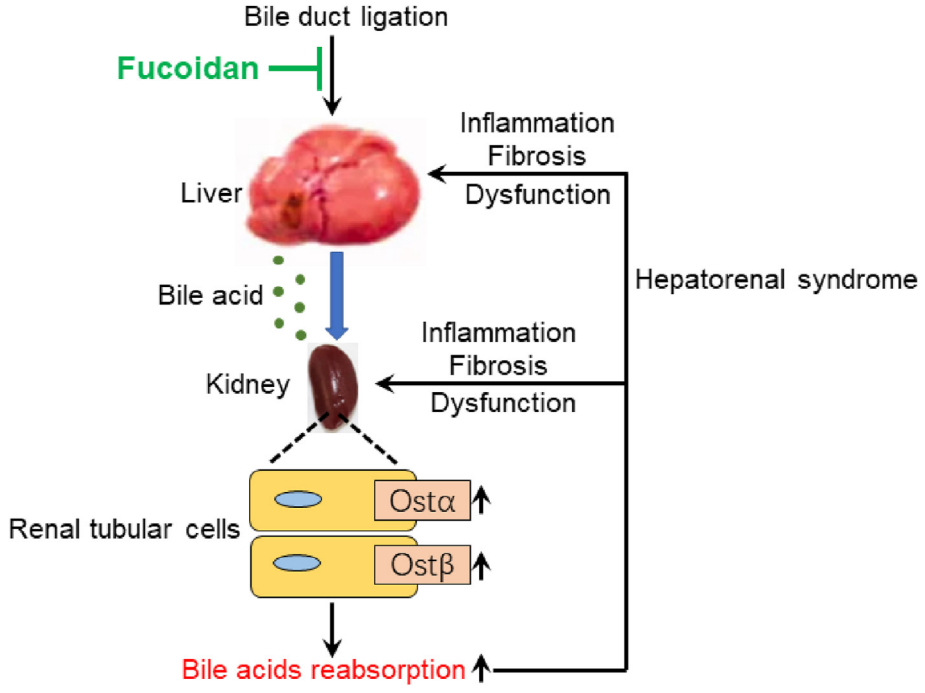

Fig. 4. The hypothetical mechanisms by which fucoidan prevents BDL-induced hepatorenal syndrome through down-regulating Ostα and Ostβ. Fucoidan inhibits Ostα and Ostβ, and reduces bile acids reabsorption in BDL-induced model mice. These effective actions may subsequently protect against liver and kidney injury, resulting in the attenuation of BDL-induced hepatorenal syndrome.

OCTN1 and OCTN2. Consistence with renal dysfunction in BDL-induced mice, URAT1, OAT1, OCTN1 and OCTN2 were notably deregulated. Previous researches have reported that fucoidan is able to ameliorate renal inflammation and fibrosis [Yu et al., 2020; Wang et al., 2022; AlKahtane et al., 2020]. In fact, we observed fucoidan effectively regulated URAT1, OAT1, OCTN1 and OCTN2, restored renal dysfunction and alleviated renal inflammation and fibrosis in BDL-induced mice.

Due to exhaustive blockade of bile flow flowing, the high level of circulatory bile acids cause a serious inflammatory response and oxidative stress, which leads to liver and kidney injury [Sánchez-Valle et al., 2012]. Therefore, it is necessary to focus our attention on bile acids metabolism to understand the pathological mechanism of hepatorenal syndrome. The up-regulated renal organic solute transporters Ostα and Ostβ have been proven to promote bile acids reabsorption to exacerbate liver injury [Soroka et al., 2010; Jang et al., 2012]. In this study, we observed increase in the serum level of total bile acids and up-regulation of renal protein levels of Ostα and Ostβ in BDL-induced mice. When these serum samples contained with the high level of total bile acids were used to culture AML12 and HK-2 cells, we found that activities of these cells were significantly reduced. These results indicate that de-regulation of Ostα and Ostβ increase bile acids reabsorption, which may cause liver and kidney injury in BDL-induced hepatorenal syndrome in mice. As we expected, fucoidan was able to down-regulate renal protein levels of Ostα and Ostβ and decrease the serum level of total bile acids in BDL-induced mice, and enhance activities of AML12 and HK-2 cells treated with serum samples contained with the high level of total bile acids in vitro. These data suggest that the protection of fucoidan on BDL-induced hepatorenal syndrome may attribute to hamper the renal Ostα and Ostβ.

In conclusion, fucoidan inhibited the renal Ostα and Ostβ, reduced bile acids reabsorption, alleviated BDL-induced hepatorenal syndrome in mice. Therefore, inhibition the renal Ostα and Ostβ by fucoidan may be a potential therapeutic strategy for the attenuation of hepatorenal syndrome in clinic.

CRediT authorship contribution statement

Xiaojuan Zhao: Funding acquisition, Formal analysis, Writing – original draft. **Ting Yang:** Investigation. **Jiayan Zhou:** Investigation. **Yanli Chen:** Investigation. **Qian Shen:** Investigation. **Jiankang Zhang:** Investigation. **Qianqian Qiu:** Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: None.

Data availability

The original data to this article have been deposited in link. The data can be found online at https://figshare.com/s/06990e0c0dc3a83590f6.

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