Int J Clin Exp Med 2016;9(7):12811-12817
www.ijcem.com /ISSN:1940-5901/IJCEM0018697

Original Article

Protective effects of panaxadiolsaponins on liver and kidney injury in rats with severe acute pancreatitis

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Received October 28, 2015; Accepted March 25, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Severe acute pancreatitis (SAP) is a serious necro-inflammatory disease with a high mortality rate due to lack of specific treatments. Previous studies have shown that panaxadiolsaponins (PDS) involved in the regulation of immune function and protection against cell injury. The aim of our study was to investigate the protective effects of PDS on liver and kidney injury in rats with SAP. Sixty-four rats were divided into four groups (n=16): Sham Operation (SO) group, SAP group, Dexamethasone (DEX) group and PDS group. The results showed that PDS significantly decreased the level of serum AMS and GLU of SAP rats ($P<0.05$), while DEX did not affect the level of serum AMS and GLU. DEX retarded the increasing of serum Hb and HCT of SAP rats ($P<0.05$), and PDS retarded the increasing of Hb, RBC and HCT ($P<0.05$). DEX and PDS significantly decreased the level of serum BUN, TNF-α and IL-6 ($P<0.01$). Results of pathological analysis showed that PDS could protect against liver and kidney injury in SAP rats. To the best of our knowledge, this is the first report that PDS could protect against liver and kidney injury in SAP rats and had better therapeutic effects than DEX, indicating a potential drug to be used in treating SAP.

Keywords: Severe acute pancreatitis, panaxadiolsaponins, liver, kidney

Introduction

SAP is one of the most common necro-inflammatory diseases associated with high mortality rates. It has been reported that mortality rate of SAP ranges from 15% to 30% [1]. SAP is characterized by pancreatic necrosis, systemic inflammatory response syndrome and multiple organ dysfunctions [2]. Multiple organ dysfunctions are the most important determinant of the high mortality of SAP, among which liver and kidney injury are frequently happened. Therefore, developing new medicine or treatment approaches for reducing liver and kidney injury is important for decreasing the mortality rate of SAP. Previous studies have shown that panaxadiolsaponins (PDS), a Chinese ginseng herb extract, can modulate immune function and prevent tissues and cells from injury [3, 4]. The present study was conducted to investigate the protective effects of PDS on liver and kidney injury in rats with SAP.

Material and methods

Material

Clean-grade healthy Wistar rats weighing 250-300 g were purchased from the Laboratory Animal Research Center of Jilin University (China). Sodium taurocholate was purchased from Sigma-Aldrich Co. LLC (USA). PDS were provided by Professor Chunyan Zhao from Natural Medicine Research laboratory of Jilin University. Radioimmunoassay kit (RIA kit) for detecting TNF-α and IL-6 were provided by Radioimmunoassay institute of Technology Development Center of PLA General Hospital. The study was approved by the ethics committee of Jilin University.

Experiment design

All the rats were allowed water ad libitum and without food for 12 hours before the experimental procedure. Sixty-four rats were randomly divided into the following four groups (n=16):

Table 1. Effects of different treatments on level of AMS, GLU and Ca^{2+} in serum

Groups	AMS (U/L)	GLU (mmol/L)	Ca^{2+}
SO	1406.33±193.57	6.21±0.61	2.28±0.07
SAP	7814.80±3712.3**	8.52±1.35**	2.04±0.16*
DEX	5891±1914.01	10.28±1.42	2.07±0.15
PDS	2621.4±1059.1#	6.07±1.44#	2.14±0. 19

*P<0.05, compared with the SO group; **P<0.01, compared with the SO group; #P<0.05, compared with the SAP group. AMS: amylase; GLU: glucose; SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group.

Table 2. Effects of different treatments on routine blood indexes

Groups	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	Hb (g/L)	HCT
SO	10.80±2.86	7.12±0.53	145.20±9.63	0.46±0.03
SAP	11.52±5.04	8.94±1.55*	175.17±26.02*	0.58±0.07*
DEX	10.52±1.42	8.50±0.82	137±7.30#	0.47±0.03#
PDS	8.46±1.59	7.11±0.89#	143.4±3.44#	0.46±0.04#

*P<0.05, compared with the SO group; #P<0.05, compared with the SAP group. WBC: White blood cell; RBC: Red blood cell; Hb: hemoglobin; HCT: hematocrit; SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group.

Table 3. Effects of different treatments on liver function

Groups	ALT (U/L)	ALP (U/L)	ALB (g/L)
SO	42.50±8.38	175.4±27.46	27.37±0.80
SAP	105.80±17.01**	178.4±17.91	24.38±0.54**
DEX	93.2±36.80	174.8±34.42	25.24±2.62
PDS	92.75±26.30	218.5±14.03#	24.36±0.73

**P<0.01, compared with the SO group; #P<0.05, compared with the SAP group. ALT: Alanine aminotransferase; ALP: alkaline phosphatase; ALB: albumin; SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group.

Sham Operation (SO) group, SAP group, Dexamethasone (DEX) group and PDS group.

Preparation of SAP models

First, the rats were anesthetized by an intraperitoneal injection of 2.5% sodium pentobarbital (0.2 mL/100 g) and junction of pancreaticobiliary ductal and duodenal was found after opening the abdominal cavity. Then 5% sodium taurocholate (0.01 ml/kg) was used to prepare the SAP model by retrograde injection into the pancreatic duct through an epidural catheter in the duodenal papilla. Rats in the SO group were subjected to the same surgical procedure but without infusion of 5% sodium taurocholate. Two minutes after finishing drug injection, edema and bleeding in pancreas was detected indicating that SAP models were successfully made. At last, the abdominal cavity was closed. All the rats were treated with 2 ml of 0.9% NaCl solution after the operation.

Therapeutic regimen

Ten minutes after successful operation, the corresponding drug was given. In DEX group, SAP rats were administered with DEX (0.5 mg/100 g) by intraperitoneal injection. In PDS group, SAP rats were administered with PDS (2.5 mg/100 g) by intraperitoneal injection. Rats in SAP group and SO group were administered with same amount of 0.9% NaCl solution by intraperitoneal injection.

Collection of samples

All the rats were sacrificed at 6 h after preparation of SAP models. Blood samples, liver, pancreas and kidneys were then collected. Blood samples were centrifuged (3000 rpm/min) for 3 min. Supernatants were aliquoted into sterilized 1.5-ml microcentrifuge tubes and stored at -20°C for later use. Parts of Livers, pancreas and kidneys from each rat were fixed by immersion in 4% paraformaldehyde, the other parts of livers and kidneys wereplaced in liquid nitrogen.

Evaluation of serum biochemical parameters

Level of amylase (AMS), Alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP) , blood urea Nitrogen (BUN), creatinine (CREA), Calcium (Ca^{2+}) and glucose (GLU) in serum was determined by using the Hitachi 7600 automatic biochemical analyzer. White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and hematocrit (HCT) were determined by using automatic hematology analyzer XE-2100. Serum TNF-α and IL-6 were determined using a RIA kit.

Histopathological evaluation of livers and kidneys

Livers, pancreas and kidneys samples fixed by 4% paraformaldehyde were embedded in par-

Table 4. Effects of different treatments on kidney function

Groups	BUN (mmol/L)	CREA (mmol/L)
SO	5.37±1.15	25.66±2.65
SAP	12.98±1.73**	33±6.44*
DEX	8.4±2.10##	32.2±4.86
PDS	9.66±1.02##	28.6±1.67

*P<0.05, compared with the SO group; **P<0.01, compared with the SO group; ##P<0.01, compared with the SAP group. BUN: blood urea Nitrogen; CREA: creatinine; SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group.

Table 5. Effects of different treatments on level of TNF-α and IL-6 in serum

Groups	TNF-α (pg/ml)	IL-6 (pg/ml)
SO	0.48±0.06	24.25±12.39
SAP	0.91±0.19**	76.06±27.01**
DEX	0.56±0.09##	31.14±10.33#
PDS	0.606±0.098#	30.99±12.46##

**P<0.01, compared with the SO group; #P<0.05, compared with the SAP group; ##P<0.01, compared with the SAP group. SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group.

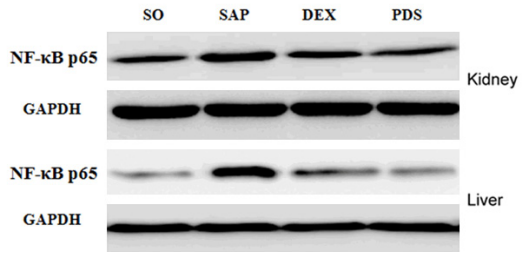

Figure 1. Effects of different treatments on expression of NF-κB pathway detected by western blotting. SO: Sham Operation group; SAP: Severe acute pancreatitis group; DEX: Dexamethasone group; PDS: Panaxadiolsaponins group; GAPDH: a reference Protein.

affin and then tissue sections (4-μm thickness) were prepared. The sections were deparaffinized and stained with hematoxylin and eosin (HE) for histopathological examination under microscope.

Western blot

Approximately 0.1 g of liver and kidney tissue was washed three times with PBS, and then homogenized and resuspendeda lysis buffer (Tris-HCl 50 mmol/L, EDTA 5 mmol/L, NaCl 150 mmol/L, sodium deoxycholate 1%, Na_3VO_4 500 μmol/L, Triton X-100 0.5%, AEBSF 10 μmol/L, NaF 10 mmol/L) on ice for 30 min. Proteins were extracted from tissues using ultrasonication and their concentration were determined using the Bradford reagent (Sigma, German). Proteins were separated on an 8%-13% SDS-polyacrylamide gel (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membrane was incubated for 2 h in PBS plus 0.1% Tween-20 and 5% nonfat skim milk to block nonspecific binding. Then the membranes were incubated overnight at 4°C with antibodies against NF-κB p65 (Santa Cruz Biotechnology, USA, 1:1000 dilution). After washing, proteins were visualized using an ECL detection kit with the secondary antibody (Santa Cruz Biotechnology, USA, 1:3000 dilution) for 2 h.

Statistical analysis

Values are presented as the mean ± standard deviation (SD). SPSS 18.0 software was used for statistical analysis. Student's test was used to compare the differences between DEX or PDS groups and SO or SAP groups, respectively. Significant difference was considered at P<0.05.

Results

Levels of serum AMS, GLU and Ca^{2+} in different groups were shown in **Table 1**. Compared to SO rats, serum AMS and GLU in SAP group significantly increased (P<0.01), while serum Ca^{2+} in SAP group significantly decreased (P<0.05). Treating with PDS significantly decreased level of serum AMS and GLU (P<0.05), while treating with DEX could not affect the level of serum AMS and GLU (P>0.05). Treating with DEX or PDS did not change the level of serum Ca^{2+}. As shown in **Table 2**, level of RBC, Hb and HCT in SAP rats increased significantly (P<0.05), while treating with DEX retarded the increasing of Hb and HCT (P<0.05), and PDS retarded the increasing of Hb, RBC and HCT (P<0.05).

Effects of different treatments on liver function were shown in **Table 3**. Liver function of SAP rats was destroyed indicated by the increasing of serum AST and decreasing of serum ALB (P<0.01). Treating with PDS or DEX did not affect the level of AST and ALB. As shown in

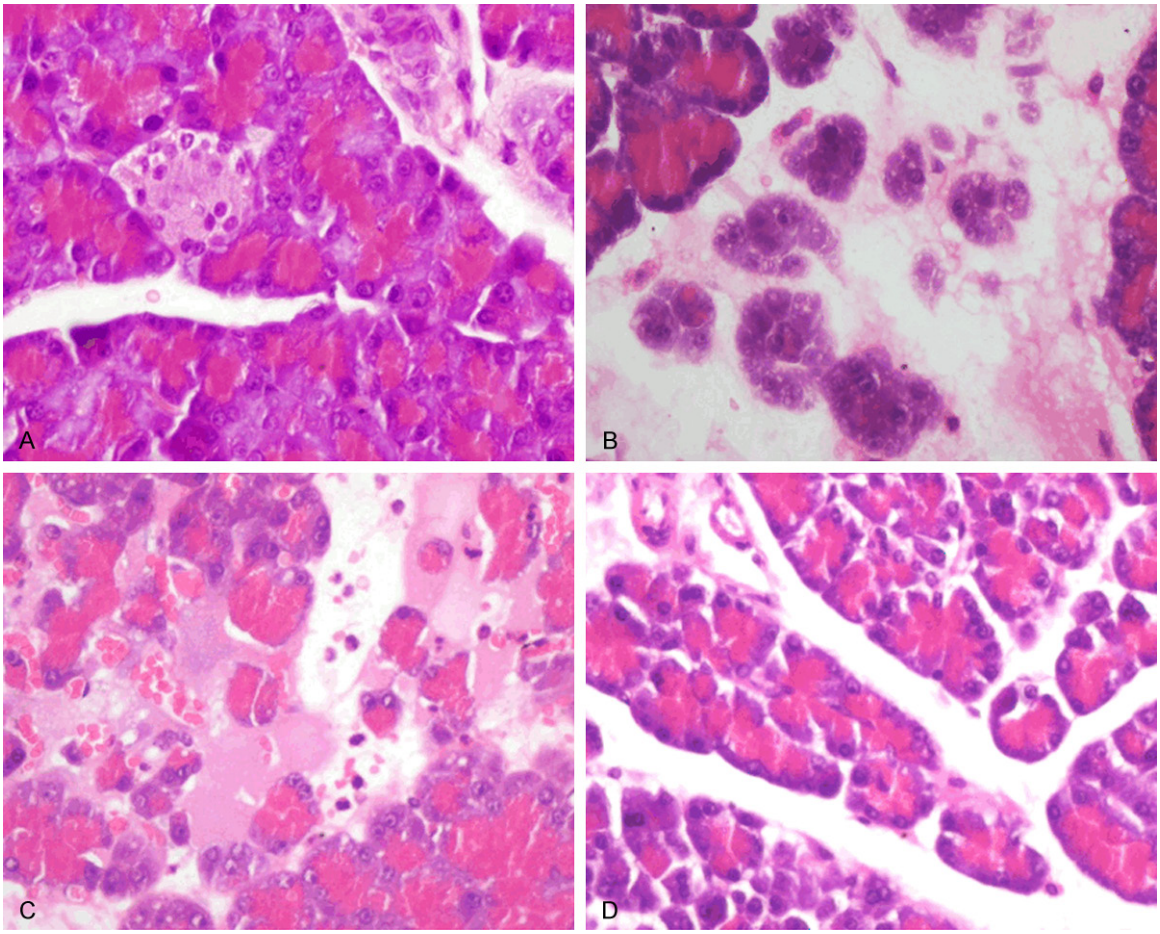

Figure 2. HE staining of pancreas from different groups (×400). A. SO group; B. SAP group: Necrosis of pancreas and inflammatory cell infiltration; C. DEX group: Degree of necrosis of pancreas and inflammatory cell infiltration were lower than that in SAP group; D. PDS group: Degree of necrosis of pancreas and inflammatory cell infiltration were lower than that in DEX group.

Table 4, kidney function of SAP rats was destroyed indicated by the increasing of serum BUN ($P<0.01$) and CREA ($P<0.05$), and treating with DEX or PDS significantly decreased the level of serum BUN ($P<0.01$). Effects of different treatments on level of serum TNF-α and IL-6 were shown in **Table 5**. The results showed that level of serum TNF-α and IL-6 of SAP rats increased significantly ($P<0.01$), and treating with DEX or PDS significantly inhibited the increasing of serum TNF-α and IL-6. Similar trend was observed for the expression of NF-κB p5 in livers and kidneys (**Figure 1**).

Pathological changes of Liver, pancreas and kidneys in different groups were shown in **Figures 2-4**. As shown in **Figure 2**, Necrosis of pancreas and inflammatory cell infiltration were observed in SAP group. PDS or DEX significantly decreased the degree of necrosis of pancreas and inflammatory cell infiltration of SAP rats, where the decreased degree of PDS group was lower than DEX group. As shown in **Figure 3**, inflammatory cell infiltration in liver was observed in SAP group, and vacuolar degeneration of liver cells and disordered structure of hepatic lobules were observed in DEX group, while the above symptoms were not observed in PDS group. As shown in **Figure 4**, obvious degeneration and necrosis of renal tubule were observed in SAP and DEX group, while there is only minor lesion of renal tubule in PDS group.

Discussion

SAP is a serious necro-inflammatory disease with a high mortality rate due to lack of specific treatments. DEX is frequently used for the treatment of SAP patients, which has anti-inflammatory, anti-toxic, and anti-shock effects

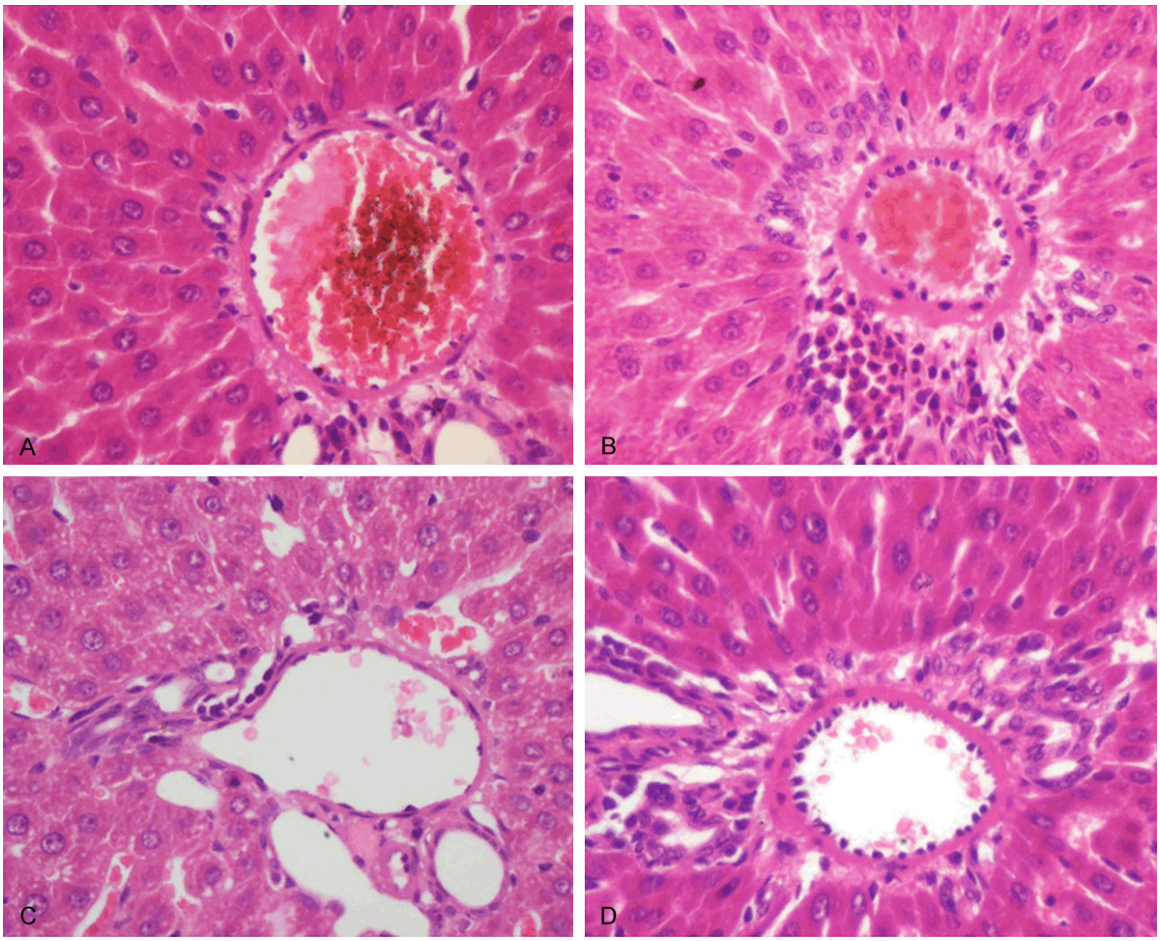

Figure 3. HE staining of livers from different groups (×400). A. SO group; B. SAP group: inflammatory cell infiltration; C. DEX group: vacuolar degeneration of liver cells and disordered structure of hepatic lobules; D. PDS group: cells were in good condition.

[5, 6]. Previous studies have shown that PDS involved in regulation of immune function and protection against cell injury [3, 4, 7]. As liver and kidney injury is one of the reasons for high mortality rate of SAP, in the present study, we compared the protective effects of PDS and DEX on liver and kidney injury in SAP rats. The results showed that PDS had better protective effects on liver and kidney injury in SAP rats, compared with DEX, indicating a potential drug to be used in treating SAP.

SAP is associated with high level of serum GLU and AMS, while results of the present study showed that PDS could decrease the level of serum AMS and GLU, compared to DEX and SAP group. These results indicated that PDS could protect pancreas of SAP. Our pathological analysis for pancreas of SAP rats also showed that PDS had a better protective ability for pancreas of SAP rats than DEX. Previous study has also shown that PDS has antidiabetic activity by regulating glucose metabolism [8, 9]. Our results also showed that PDS has better ability to retarded the increasing of Hb, RBC and HCT, compared with DEX. Although the mechanism of PDS regulating blood parameters is still unknown, there is evidence indicating that PDS could promote proliferation and differentiation of hematopoietic progenitor cells [7].

Although results of the present study showed that treating with PDS or DEX did not affect the level of serum AST and ALB, pathological analysis for livers of SAP rats showed that DEX and PDS could protect against liver injury in which effects of PDS were better than DEX. SAP rats were associated with the increasing level of serum BUN and CREA, however, treating with

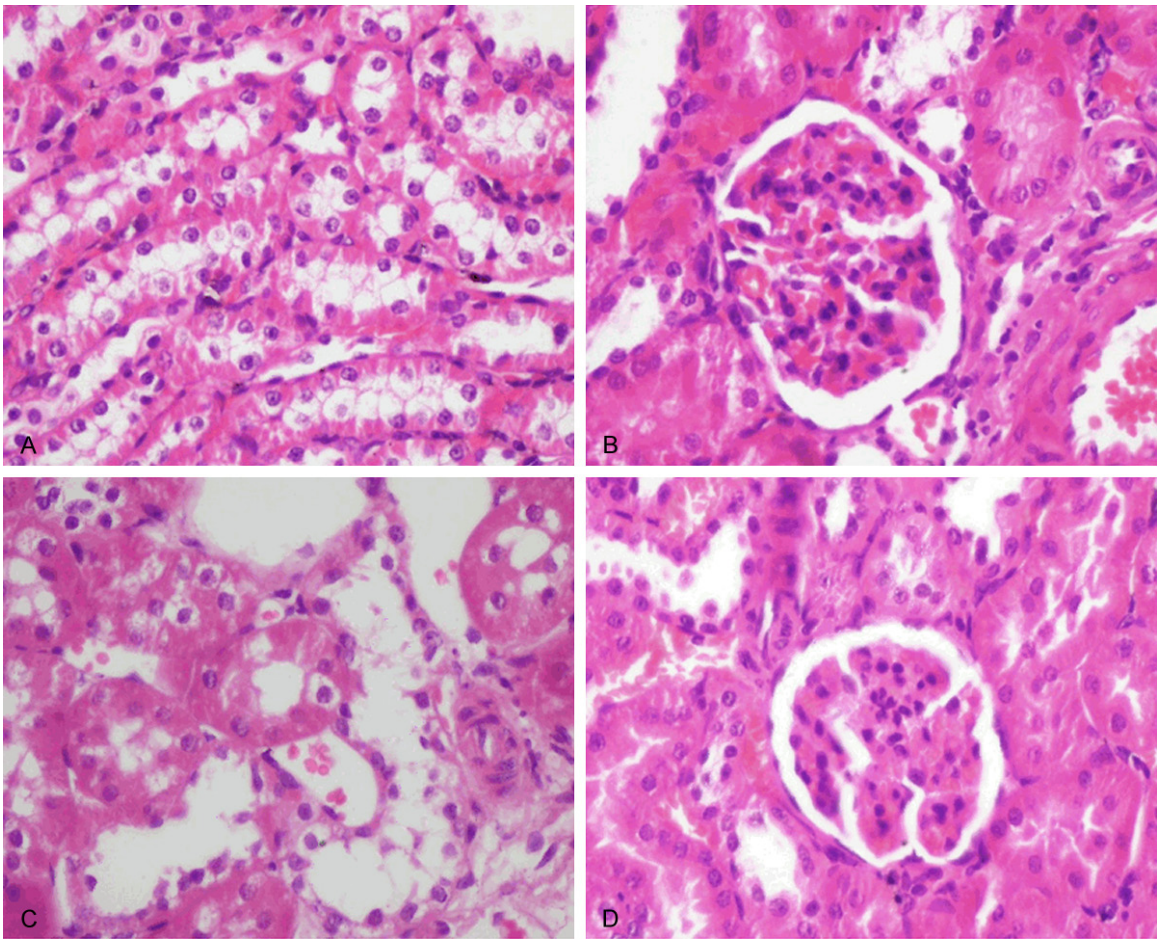

Figure 4. HE staining of kidneys from different groups (×400). A. SO group: structure of renal tubule; B. SAP group: degeneration and necrosis of renal tubule; C. DEX group: degeneration and necrosis of renal tubule; D. PDS group: minor lesion of renal tubule.

DEX or PDS significantly decreased the level of serum BUN. Pathological analysis showed that obvious degeneration and necrosis of renal tubule were observed in SAP and DEX group, conversely, minor lesion of renal tubule was observed in PDS group. These results indicated that PDS could prevent liver and kidney from injury in SAP rats. This role may be reasons for the antioxidant and anti-inflammatory action of PDS. Oxidative stress is a main factor in the pathogenesis of SAP [10]. It has been shown that PDS could induce the antioxidant enzymes which are important for maintaining cell viability by lowering the level of oxygen radical [11]. PDS has protective effects on the cardiac functions after burn injury possibly through its enhancement of superoxide dismutase activity and the reduction of both the levels of free radicals [12]. Recently, a study has shown that ginseng extract protects the kidney from gentamicin-induced acute kidney injury via the mechanism of modulation of oxidative stress [13]. As inflammatory mediators, TNF-α and IL-6 are up-regulated in serum of SAP patients or rats [14, 15]. Results of this study showed that treating with PDS significantly inhibited the increasing of serum TNF-α and IL-6 in SAP rats. This result was according with previous study. PDS could enhance immunity by improving animal immune organ weight and plasma TNF-α and IL-6 [7, 16]. Furthermore, our results also showed that expression of NF-κB p65 in livers and kidneys of rats in all the groups has the same trend as serumTNF-α and IL-6. Previous study has demonstrated that NF-κB signaling pathways areresponsible for release of IL-6 under inflammatory responses [17].

Although results of the present study indicated that PDS may be a potential drug to be used in

treating SAP, their protective roles in other organ injuries of SAP such as lung and intestinal injury and clinical outcome of SAP patients are still unknown. Therefore, more extensive studies are needed before PDS is used to treat SAP patients.

In conclusion, the present study showed that PDS had better protective effects on liver and kidney injury in SAP rats, compared with DEX.

Disclosure of conflict of interest

None.

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