Prophylaxis and Therapy of *Isaria felina* on Acute Renal Failure Induced by Glycerin in Rats

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Abstract: **Objective** To evaluate the prophylaxis and therapy of *Isaria felina* (IF) on glycerin-induced acute renal failure (ARF) in rats. **Methods** Forty male Wistar rats were divided into control, model, Uremic Clearance Granule (UCG, positive control), high- and low-dose IF groups. Rats in the high- and low-dose IF groups were ig administered with 200 and 100 mg/kg IF, respectively, while rats were ig administered with 3.6 g/kg UCG successively for 7 d to establish UCG group. The rats in model, control, and drug-treated groups were im injected with 8 mL/kg 50% glycerin after drinking was quitted for 24 h to induce ARF in rats. The drugs were continued to give thereafter. The level of blood urea nitrogen (BUN) and serum creatinine (SCr) were determined 24 and 72 h after the injection of glycerin, also the kidney was dissected for pathology examination. **Results** Im injection with 8 mL/kg 50% glycerin could successfully induce ARF in rats. The dose of 200 mg/kg IF could reduce the high levels of BUN and SCr, and ameliorate the pathological damage of the kidney. **Conclusion** IF has good protective and therapeutic effects on ARF and it is a potential and valuable Chinese herb for ARF.

Key words: acute renal failure; blood urea nitrogen; glycerin; *Isaria felina*; serum creatinine
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Introduction

Acute renal failure (ARF) is characterized by a rapid decline in glomerular filtration rate (GFR) over hours to days and the retention of nitrogenous waste products (Lee *et al*, 2009). ARF is a common critical disease in clinic, and the mortality of patients with ARF has remained 25%—70% despite the use of various pharmacologic agents (Thadhani, Pascual, and Bonventre, 1996; Liao *et al*, 2003). Therefore, a new therapeutic agent is required to promote the renal function recovery.

Natural *Cordyceps sinensis* (Berk.) Sacc. is one of the rare medicinal herbs in China that has the function of protecting the lung and the kidney (Wang *et al*, 2010; Xu *et al*, 2011). *C. sinensis* has protective and reparative function on kidney and is used to treat chronic nephropathy (Li *et al*, 2009). Because it is difficult to obtain in large quantity, the strains separated from *C. sinensis* were cultured to substitute for natural *C. sinensis*, such as Bailing Capsule, Jinshuibao Capsule (Chen, Liang, and Liu, 2006).

Isaria felina (IF) is a fungus isolated from the fructification of natural *C. sinensis*. It was identified as *Isaria feline* (DC.:Fr.) Fr. by GUO Ying-lan in Institute of Microbiology, Chinese Academy of Sciences. It is a new strain of *C. sinensis* and preserved by China General Microbiological Culture Collection Center, China Committee for Culture Collection of Microorganisms (CGMCC NO. 0706). For the purpose of providing possibly clinical application and improving nephro-protective effects, we conducted the experiment to evaluate the effectiveness of IF in the protection of renal function in glycerin-induced ARF rats.

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Materials and methods

Animals

The study was approved by the local ethical committee and referred to the National Research Council Guide for the Care and Use of Laboratory Animals.

Experiments were performed using 40 SPF age-matched male Wistar rats purchased from Medical Experimental Animal Center, Academy of Military Medical Sciences (Beijing, China) aged seven weeks with mean body weight (BW) of (225.96 ± 12.33) g. The rats were maintained in individual cages in a temperature-controlled [(23 ± 2) ℃] room on a 12h-12h light-dark cycle (light at 07:00 AM), and the humidity was kept between 40%—60%. They were fed a standard laboratory chow (0.6%—1.2% phosphate, 1.0%—1.8% calcium, 0.2% magnesium, and 20% crude protein) and allowed tap water *ad libitum*.

Drugs

The strain of IF was separated from the fructification of the natural *C. sinensis*. The culture medium composed of 0.6% peptone, 1.2% yeast extract, 2.4% sugar, 0.05% $MgSO_4$, and 0.1% KH_2PO_4. They were dissolved in distilled water and pH value was adjusted to 6.5—7.0 with saturated aqueous solution of $NaHCO_3$. A volume of 500 mL culture medium in a triangular flask (3000 mL) was sterilized for 40 min under 0.15 MPa. Then the strain of IF was inoculated and cultured with shaking (180 r/min) for 72 h at 23—26 ℃. The fermentation liquid was collected and centrifuged at 2000 r/min for 5 min. The mycelium was baked at 80 ℃, powdered, sifted through a 0.15 mm sieve and used in this study.

Glycerin (for injection) was produced by Jiangxi Ipsen Pharmaceutical Co., Ltd. (China) and adjusted to 50% by sterile water for injection. Uremic Clearance Granule (UCG) was produced by Consun Pharmaceutical Group (Inner Mongolia, China). Blood urea nitrogen (BUN) kit and serum creatinine (SCr) kit were provided by Nanjing Jiancheng Bioengineering Institute (China).

Treatment schedule

Forty Wistar rats were kept for a week to adapt to the experimental environment. The rats were randomly divided into control, model, UCG (positive control), high- and low-dose IF groups (n = 8).

From the first day on, the rats were ig administered with 200 and 100 mg/kg IF to establish the high- and low-dose IF groups, respectively, while the rats were ig administered with 3.6 g/kg UCG in water suspension successively for 11 d to establish the UCG group. The dosage used was approximately counted according to the ratio of body surface area and body weight. The rats in the control and model groups were ig administered with the same amount of distilled water.

The blood was sampled from caudal vein on day 7, levels of BUN and SCr were determined.

The rats in the model group and the drug-treated groups were im injected with 8 mL/kg 50% glycerin after drinking was stopped for 24 h to induce ARF in rats on day 8. The drugs were continued to give thereafter. The levels of blood BUN and SCr were determined 24 and 72 h after the injection of glycerin. Kidney, liver, spleen, and thymus were dissected and weighed, and viscera indexes were calculated.

Renal histology

The left kidneys were fixed in the 10% neutral formalin, dehydrated in increasing concentration of ethanol, cleared with xylene, and embedded in paraffin. Five micrometer sections were prepared from kidney paraffin blocks and stained with HE staining using standard procedures. The microscopic scoring of the kidney sections was carried out in a blind fashion by a pathologist who was unaware of the treatment groups for histological examination.

Statistical analysis

Statistical calculations were carried out with SPSS 17.0 for Windows software package. Results were expressed as $\bar{x} \pm s$ and were analyzed by ANOVA and LSD. $P < 0.05$ was considered statistically significant.

Results

Effects of IF on serum biochemical parameters

Levels of BUN and SCr on day 0 showed no significant difference among each group ($P > 0.05$). After being treated for 7 d, the levels of BUN and SCr had little change, and there showed no significant difference among each group ($P > 0.05$) (Table 1).

Changes of serum biochemical parameters after injection of glycerin

Im injection with 8 mL/kg 50% glycerin could

Table 1 Levels of BUN and SCr before and after treatment for 7 d (n = 8)

Groups	Dosages	BUN / (mmol·L^{-1})		SCr / (μmol·L^{-1})	
		day 0	day 7	day 0	day 7
control		9.05 ± 0.87	9.06 ± 0.27	68.75 ± 10.39	68.20 ± 9.16
model		8.81 ± 0.85	9.02 ± 0.86	71.84 ± 7.76	70.40 ± 6.72
UCG	3.6 g·kg^{-1}	8.84 ± 0.67	9.03 ± 0.73	69.85 ± 8.32	71.13 ± 8.07
IF	200 mg·kg^{-1}	8.94 ± 0.77	8.98 ± 0.65	70.51 ± 8.26	70.23 ± 8.25
	100 mg·kg^{-1}	8.82 ± 0.75	9.03 ± 0.56	68.61 ± 7.39	69.24 ± 7.12

successfully induce ARF in rats. Cruenturesis was observed after the injection of glycerin for 3 h. BUN and SCr increased significantly 24 h after the injection of glycerin (P < 0.05) and the dose of 200 mg/kg IF reduced the high levels of BUN and SCr significantly (P < 0.05). IF (100 mg/kg) and UCG groups also showed the effects of decreasing the high levels of BUN and SCr, but there showed no significant difference compared with the model group (P > 0.05) (Table 2).

Levels of BUN and SCr in rats increased further higher 72 h after the injection of glycerin. It showed significant difference when compared with the control group (P < 0.05). The levels of BUN and SCr decreased significantly in IF treatment groups. It showed the significant difference when compared with the model group (P < 0.05) (Table 2).

Body weight and viscera index changes

There was no significant difference in body weight showed before the experiment (P > 0.05). The BW of rats decreased significantly after being injected with glycerin. But the BW of rats in the model group decreased most, which showed significant difference in the final stage of the experiments compared with the control group (P < 0.05) (Table 3).

Renal index was significantly higher at 72 h after the injection of glycerin when compared with the control group (P < 0.05) while renal index in 200 mg/kg IF group was significantly lower compared with the model group (P < 0.05). Liver index increased, spleen and thymus indexes decreased in the model group, while liver index decreased, spleen and thymus indexes increased in IF and UCG groups, but there was no significant difference showed (P > 0.05) (Table 4).

Renal pathology

Kidneys in the control group shaped like horsebean and were colored henna with lustre. Kidneys were swelled to varied degrees and colored pale without lustre in all other groups. Renal pathology in

Table 2 Levels of BUN and SCr after injection of glycerin for 24 and 72 h

Groups	Dosages	BUN / (mmol·L^{-1})		SCr / (μmol·L^{-1})	
		24 h	72 h	24 h	72 h
control		8.92 ± 0.31	9.54 ± 0.19	68.20 ± 2.54	72.60 ± 7.62
model		42.49 ± 5.03*	51.28 ± 16.61*	337.33 ± 96.63*	503.49 ± 232.17$_{*}$
UCG	3.6 g·kg^{-1}	37.45 ± 4.52*	35.65 ± 9.22*	272.35 ± 43.55*	256.67 ± 33.54$^{*\triangle}$
IF	200 mg·kg^{-1}	33.50 ± 4.29$^{*\triangle}$	23.62 ± 8.12$^{*\triangle}$	200.20 ± 41.35$^{*\triangle}$	146.67 ± 36.78$^{*\triangle}$
	100 mg·kg^{-1}	35.78 ± 4.36*	28.84 ± 8.25$^{*\triangle}$	228.24 ± 40.13*	182.67 ± 38.44$^{*\triangle}$

$^{*}P$ < 0.05 *vs* control group $^{\triangle}P$ < 0.05 *vs* model group; same as below

Table 3 Body weight changes of rats in each group after treatment (n = 8)

Groups	Dosages	BW_I / g	BW_F / g	$BW_F - BW_I$ / g
control		226.02 ± 10.93	227.08 ± 11.25	1.18 ± 0.63
model		225.68 ± 13.82	201.88 ± 11.31*	−23.54 ± 4.28
UCG	3.6 g·kg^{-1}	226.54 ± 12.21	204.55 ± 12.86	−21.03 ± 6.25
IF	200 mg·kg^{-1}	227.68 ± 10.72	211.63 ± 11.96	−16.75 ± 5.42
	100 mg·kg^{-1}	225.51 ± 10.11	208.63 ± 12.22	−17.47 ± 4.83

Notes: BW_I was average body weight at initial; BW_F was average body weight in final stage of experiments

Table 4 Viscera index changes of rats in each group after treatment

Groups	Dosages	Renal indexes / ($\times 10^{-2}$)	Liver indexes ($\times 10^{-2}$)	Spleen indexes ($\times 10^{-2}$)	Thymus indexes ($\times 10^{-2}$)
control		0.646 ± 0.039	3.14 ± 0.14	0.197 ± 0.014	0.092 ± 0.010
model		1.027 ± 0.081*	3.44 ± 0.27	0.176 ± 0.030	0.073 ± 0.022
UCG	3.6 g·kg^{-1}	0.954 ± 0.035*	3.23 ± 0.26	0.188 ± 0.035	0.074 ± 0.018
IF	200 mg·kg^{-1}	0.934 ± 0.024*△	3.19 ± 0.28	0.196 ± 0.039	0.077 ± 0.019
	100 mg·kg^{-1}	0.955 ± 0.027*	3.22 ± 0.25	0.189 ± 0.029	0.075 ± 0.016

the control group was shown in Fig. 1A. All rats developed marked structural damage 72 h after being injected with glycerin. The damages included tubular dilatation and protein cast in renal tubules. In the model group, renal tubules distended seriously and were filled with protein casts (Fig. 1B). All the values were much less in UCG group (Fig. 1C) and IF groups (Figs. 1D and 1E) than those in the model group. The pathological changes in 200 mg/kg IF group were less obvious than those in other glycerin-treated groups. The results indicated that IF had protective roles and tissue-repairing effects on glycerin-induced ARF.

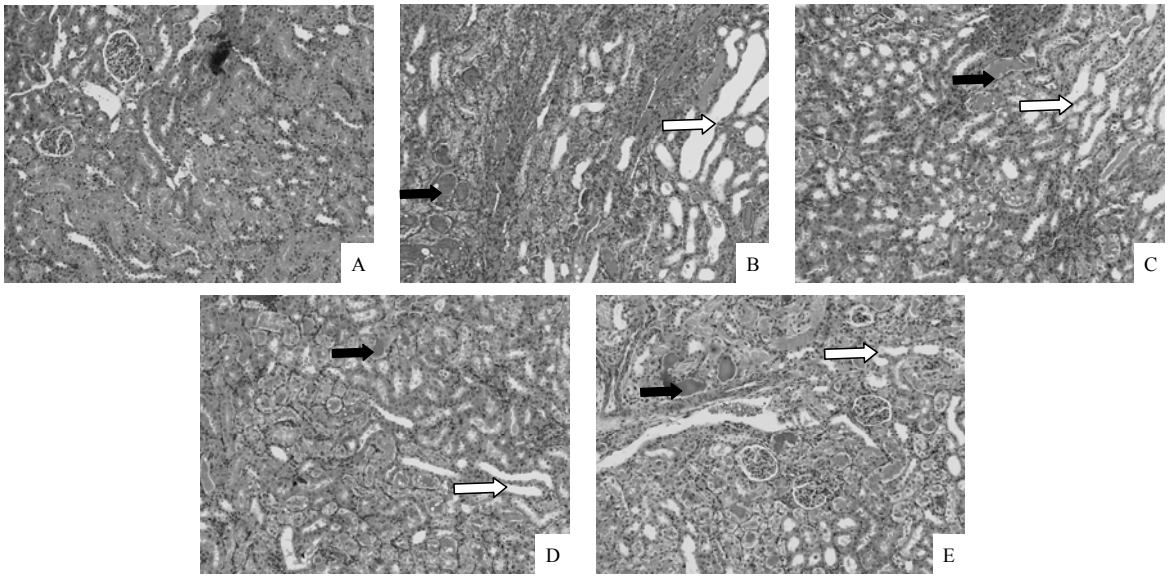

Fig. 1 Representative photographs of renal tissue under light microscope

A: control group B: model group C: 3.6 g/kg UCG-treated group D: 200 mg/kg IF-treated group E: 100 mg/kg IF-treated group

⇨ showing tubular dilatation ➡ showing protein cast in some renal tubules

Discussion

At present, studies on IF are quite limited. Guo *et al* (2005) first isolated a kind of peptide isarfelin with antifungal and insecticidal activities from IF and identified its structure and physicochemical properties. Insecticidal cyclodepsipeptides isariins B—D were isolated from IF and their structures were identified (Baute *et al*, 1981; Deffieux *et al*, 1981). Ikumoto *et al* (1991) have studied physiologically active compounds in the extracts of cultured mycelia of IF. There was no research report on the preventive and therapeutic effects of IF on ARF.

It was reported that acute tubular necrosis (ATN) induced by im injection of glycerin was similar to ATN in clinic especially seriously wounded-induced ATN (Wilkes and Mailloux, 1986). At the beginning of the experiment, we used the dosage of 10 mL/kg glycerin to induce ARF and found that the mortality of the animal was very high. So in this study, we selected the dosage of 8 mL/kg 50% glycerin to induce ARF. The results showed that im injection with 8 mL/kg 50% glycerin could increase BUN and SCr levels in rats significantly. Renal pathology showed serious tubular dilatation and vast protein cast in renal tubules. So it showed that im injection with 8 mL/kg 50% glycerin could successfully induce ARF.

It was reported that the pathological changes were most serious at 72 h after the injection of glycerin and then begin to recover (Liao and Cheng, 1994). So we selected 72 h as the detection time to evaluate the protective effects of IF on ARF of rats. The result showed that 200 mg/kg IF could decrease the high levels of BUN and SCr induced by glycerin and ameliorate the pathological damage of the kidney.

In conclusion, IF has good protective and therapeutic effects on ARF induced by glycerin in rats, and it is a potential and valuable Chinese herb to promote renal function recovery.

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References

Baute R, Deffieux G, Merlet D, Baute MA, Neveu A, 1981. New insecticidal cyclodepsipeptides from the fungus *Isaria felina*. I. Production, isolation and insecticidal properties of isariins B, C and D. *J Antibiot* 34(10): 1261-1265.

Chen XW, Liang D, Liu HF, 2006. *Chronic Renal Failure*. China Medical Science Press: Beijing.

Deffieux G, Merlet D, Baute R, Bourgeois G, Baute MA, Neveu A, 1981. New insecticidal cyclodepsipeptides from the fungus *Isaria felina*. II. Structure elucidation of isariins B, C and D. *J Antibiot* 34(10): 1266-1270.

Guo YX, Liu QH, Ng TB, Wang HX, 2005. Isarfelin, a peptide with antifungal and insecticidal activities from *Isaria felina*. *Peptides* 26(12): 2384-2391.

Ikumoto T, Sasaki S, Namba H, Toyama R, Moritoki H, Mouri T, 1991. Physiologically active compounds in the extracts from Tochukaso and cultured Mycelia of *Cordyceps* and *Isaria*. *Yakugaku Zasshi* 111(9): 504-509.

Lee DW, Kwak IS, Lee SB, Song SH, Seong EY, Yang BY, Lee MY, Sol MY, 2009. Post-treatment effects of erythropoietin and nordihydroguaiaretic acid on recovery from Cisplatin-induced acute renal failure in the rat. *J Korean Med Sci* 24(Suppl1): S170-S175.

Li Y, Xue WJ, Tian PX, Ding XM, Yan H, Pan XM, Feng XS, 2009. Clinical application of *Cordyceps sinensis* on immunosuppressive therapy in renal transplantation. *Transplant Proc* 41(5): 1565-1569.

Liao HJ, Cheng XM, 1994. The preparation of acute renal failure models. *J Nephrol Transplant* 3(2): 155-157.

Liao ZX, Wang H, Peng RX, Kong R, 2003. Antagonistic effect of sodium ferulate on glycerol-induced renal oxidative injury in mice. *Acta Pharm Sin* 38(12): 900-903.

Thadhani R, Pascual M, Bonventre JV, 1996. Acute renal failure. *N Engl J Med* 334: 1448-1460.

Wang Y, Yin H, Lv X, Wang Y, Gao H, Wang M, 2010. Protection of chronic renal failure by a polysaccharide from *Cordyceps sinensis*. *Fitoterapia* 81(5): 397-402.

Wilkes BM, Mailloux LU, 1986. Acute renal failure. Pathogenesis and prevention. *Am J Med* 80(6): 1129-1136.

Xu H, Li S, Lin Y, Liu R, Gu Y, Liao D, 2011. Effectiveness of cultured *Cordyceps sinensis* combined with glucocorticosteroid on pulmonary fibrosis induced by bleomycin in rats. *China J Chin Mater Med* 36(16): 2265-2270.