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Aqueous extract from Aconitum carmichaelii Debeaux reduces liver injury in rats via regulation of HMGB1/TLR4/NF-KB/caspase-3 and PCNA signaling pathways

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ABSTRACT

Ethnopharmacological relevance: Aconitum carmichaelii Debeaux is a well-known Chinese herb that has been used to treat liver diseases for many years in China. We investigated the effects of aqueous extract from Aconitum carmichaelii Debeaux (AEACD) on acute liver failure and identified the possible mechanisms of these effects.

Material and methods: Specific pathogen-free (SPF) male Wistar rats were used to establish acute liver failure model by intraperitoneal injection of D-galactosamine (D-GalN) and treated with Stronger Neo-Minophagen C (SNMC) and AEACD by gavage. Then, the serum biochemical parameters, the pathological scores in the liver tissue, the mRNA expressions of toll- like receptor 4 (TLR4), nuclear factor kappa B (NF-κB), high mobility group box 1 (HMGB1) and caspase-3, the proliferating cell nuclear antigen (PCNA) positive rates were analyzed.

Results: The liver function was improved, the pathological scores were decreased, the expressions the TLR4, NF-κB, HMGB1, and caspase-3 were inhibited, and the PCNA positive rates were increased by both SNMC and AEACD, but AEACD induced greater effects.

Conclusions: AEACD protected liver function by inhibiting inflammatory reaction, apoptosis and promoting liver tissue regeneration in the acute liver failure rats induced by D-galactosamine.

1. Introduction

Acute liver failure (ALF) is defined by the presence of coagulopathy (International Normalized Ratio ≥ 1.5) and hepatic encephalopathy due to severe liver damage in patients without pre-existing liver disease (Lee, 2012; Lee et al., 2012). The clinical symptoms are complicated, including cerebral edema, coagulopathy, renal failure, metabolic disturbance, hemodynamic instability, and susceptibility to infection. Until recently, ALF has had a high mortality rate (widely reported to be $>80\%$) without liver transplantation (Nobuhisa et al., 2013). In Europe and the United States, transplantation is considered as the first-line therapy for acute liver failure (Polson and Lee, 2005). And the survival rates of patients have considerably improved with the advent of liver transplantation (Kayoko et al., 2012). However, due to the limited availability of donor organs and vicious progress of the disease, the mortality of ALF remains high (Zhu et al., 2013; Wang et al., 2013a, 2013b). Therefore, it is time to identify a novel strategy to cure ALF.

Traditional Chinese medicine (TCM) has been practiced

Abbreviations: AEACD, aqueous extract from Aconitum carmichaelii Debeaux; ALF, acute liver failure; D-GalN, D-galactosamine; TRL4, toll-like receptor 4; HMGB1, high-mobility group box 1 protein; H&E, hematoxylin and eosin; NF-κB, nuclear factor kappa B; PCNA, proliferating cell nuclear antigen; SNMC, Stronger Neo-Minophagen C; RT-PCRs, real-time reverse transcription polymerase chain reactions

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successfully on human bodies directly for centuries in China (Li et al., 2012a, 2012b; Xiong et al., 2013). Among the Chinese medicine herbs, Aconitum carmichaelii Debeaux is a well-known traditional Chinese herbal that was recorded in Ben Cao Gang Mu (a book of traditional Chinese medical cases from the Ming Dynasty, AD 1518–1593). Aconitum carmichaelii Debeaux (Zhou et al., 2015) with its active compounds are possessed of wide-reaching biological activities, including the effects on cardiovascular system, anti-inflammation and analgesic action, anti-tumor activity, the effect on the immune system, hypoglycemic and hypolipidemic effects, anti-aging effect, the effect of protecting kidney and the effect on energy metabolism, so it has been widely used to treat shock resulting from acute myocardial infarction, low blood pressure, coronary heart disease, chronic heart failure and liver diseases, such as liver fibrosis, cirrhosis, etc (Zhou et al., 2015; Wang et al., 2004', 2005). On the other hand, it had toxicity, such as cardio-toxicity, neurotoxicity, embryotoxicity and renal toxicity. Unprocessed Aconitum carmichaelii Debeaux contains high amounts of diterpenoid alkaloids (DAs) (Chen et al., 2013). However, if diterpenoid alkaloids (DAs) are exposed to hydrolysis, they can transform into monoester-diterpenoid alkaloids (MAs) or unesterified compounds, then toxicity is markedly decreased, but in the meantime, their pharmacological activities do not alter to weaken (Zhou et al., 2015; Kuang, 2002). Paozhi as a traditional Chinese processing approach, is a kind of detoxifying measure in traditional Chinese medicine and can decompose poisonous aconitum alkaloids into less or nontoxic derivatives and plays an important role in detoxification (Zhou et al., 2015; Chen et al., 2013). In traditional usage, decoction is in a position to transform the DAs into MAs then UAs, thus Aconitum carmichaelii Debeaux's toxicity shrink but its activities are considered not to be changed (Chen et al., 2013). The optimum decoction time is one hour (Gong et al., 2011). Thousands of years ago, ancient Chinese people had already used method of detoxifying Aconitum carmichaelii Debeaux by compatibility with other herbs. It could be found in Bencaojing Jizhu and Shanghan Lun, ect. In conclusion, the methods of processing, decoction and compatibility are found to make substantial contributions to the detoxification of Aconitum carmichaelii Debeaux (Zhou et al., 2015).

And our previous studies had shown that the formula mainly consisted of Aconitum carmichaelii Debeaux was found to be very effective in treating chronic hepatitis B and liver failure, and no drug-related side effects were observed in the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (HU et al., 2012). But we still do not know how dose Aconitum carmichaelii work. Therefore, the present study aimed at confirming the effects of AEACD on acute liver failure and exploring the underlying mechanism using a combination of pharmacologic and genetic approaches. Our findings indicate that AEACD is an useful therapeutic agent for ALF through inhibiting the activation of HMGB1/TLR4/NF-κB/ caspase-3 inflammatory and necrotic pathway and promoting the PCNA expressions.

2. Materials and methods

2.1. Animals

Specific pathogen-free (SPF) male Wistar rats weighing 150 ± 20 g were provided by Shanghai Experimental Animal Co., Ltd. (Shanghai, China). The rats were housed in the animal center of Chengdu University of Traditional Chinese Medicine with a temperature of 20–25 °C and relative humidity of 50–70% on a 12-h dark/light cycl and provided a standard pelleted diet and water ad libitum. This study was approved by the Animal Ethics Committee of Chengdu University of Traditional Chinese Medicine and conducted in accordance the internationally accepted principles for laboratory animal use and care according to the US guidelines (NIH Publication no. 85–23, revised in 1985).

2.2. Chemicals and reagents and kits

The PCNA immunohistochemistry kit was purchased from Boster Bioengineering Co., Ltd. (Wuhan, China). The TLR4, NF-κB, HMGB1, and caspase-3 monoclonal antibodies were from Sigma. The CHE detection kit was purchased from Meikang Biotechnology Co., Ltd. (Ningbo, China). The TBIL and ALB detection kits were supplied by Mike Biotechnology Co., Ltd. (Sichuan, China). The PT detection kit was purchased from American STAGO Inc. The ALT and AST detection kits were obtained from Biosino Bio-technology & Science Inc. (Beijing, China). D-galactosamine (D-GalN) was purchased from Hongbang Medical Technology Co., Ltd. (Shanghai, China). Stronger Neo-Minophagen C (SNMC) was used as a positive control in this study and was purchased from Minophagen Pharmaceutical Co., Ltd. (Tokyo, Japan) and mixed with distilled water to a final concentration of 1.56 mg/ml.

2.3. Preparation of aqueous extract from Aconitum carmichaelii Debeaux

Aconitum carmichaelii Debeaux (Medicinal part: Radix; Origin: Sichuan Province) was purchased from Sichuan Chinese Herbs Co., Ltd. (Sichuan, China) and validated by a pharmacologist according to the Pharmacopoeia of the People's Republic of China (2010). Its' voucher specimen was deposited at the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, Sichuan, China). The optimal extraction process was as following: 40 g Aconitum carmichaelii Debeaux (lot#: 2012010426) was added in 400 ml water, soaked for 30 min, decocted 3 times by the traditional instruments tisanes casserole, 1 h each time (at least 30 min after boiling), and the decoctions were combined, filtered and concentrated to 95 ml by the distilling apparatus. Each milliliter contained raw herbs 0.42 g (40 g/95 ml = 0.42 g/ml).

2.4. Experimental design

The 70 SPF male Wistar rats were randomly divided into the following four groups: a control group, a model group, a Stronger Neo-Minophagen C (SNMC) group, and an AEACD group (experimental group). The rats in the control group (10 rats) were given distilled water by gavage; The rats in the model group (20 rats) were given distilled water by gavage and injected with D-GalN 1.4 g/kg intraperitoneally three days after the gavage; The rats in the SNMC group (20 rats) were given SNMC 15.6 mg/kg/d by gavage and injected with D-GalN 1.4 g/kg intraperitoneally three days after the gavage; The rats in the experiment group (20 rats) were given AEACD 4.2 g/kg/d by gavage and injected with D-GalN 1.4 g/kg intraperitoneally three days after the gavage. The gavages were administered for 5 days. The dosages of SNMC and MSND that are used on humans in the clinic are 150 mg/60 kg/d (2.5 mg/kg/d) and 40 g/60 kg/d (0.7 g/kg/d), respectively. And according to the following formula $Dose_{rat} = Dose_{human} \times (habeas\ index_{rat}/habeas\ index_{human}) \times (body\ weight_{human}/body\ weight_{rat}) \times 2/3$ (Zhu et al., 2013; Li et al., 2012a, 2012b), the dosages of SNMC and AEACD used on rats in this study were calculated.

2.5. Sample collection

36 h after the D-GalN injection, Six-milliliter blood samples were obtained from the femoral artery of the living rats and centrifuged for 10 min at 3000 r/min to get the serum for the detection of serum alanine aminotransferase (ALT), aspartate