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*Original Article*

# Arctic fox bile can alleviate carbon tetrachloride-induced acute liver injury

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**Abstract:** Objective: To investigate whether there is a protective role of Arctic fox bile against acute liver injury. Methods: Five groups were designed, namely CK, model, positive control, low-dose and high-dose groups. Mice in the latter three groups were administrated with Kuihua liver-protection tablet, low-dose and high-dose Arctic fox bile powder, respectively. At the 16 h before the last intragastric administration, mice in the model, positive control, low-dose and high-dose bile powder groups were intraperitoneally injected with $CCl_4$. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using AST Assay Kit and ALT Assay Kit, respectively; the blood total cholesterol (T-CHO) content was determined using the T-CHO Assay Kit; the liver glycogen content was measured using the Liver Glycogen Assay Kit; the malondialdehyde (MDA) content of the liver tissues was determined using MDA Assay Kit. Histopathological observation was also performed. Results: A classic $CCl_4$-induced acute liver injury model was successfully established using Kunming mice. Either low- or high-dose Arctic fox bile powder significantly reduced the serum AST and ALT levels, as well as liver MDA content in mice with $CCl_4$-induced acute liver injury; Arctic fox bile powder at either dose also decreased the T-CHO content and raised the liver glycogen content. Histopathological observation demonstrated that mice administrated with either low- or high-dose Arctic fox bile powder showed alleviated pathological symptoms, namely with less abnormalities in hepatocyte morphology, and mild inflammatory cells infiltration and hepatocyte necrosis. Conclusion: Arctic fox bile can be considered as a substitute to bear bile.

**Keywords:** Acute liver injury, Arctic fox bile, aspartate aminotransferase (AST) and alanine aminotransferase, total cholesterol, malondialdehyde content

## Introduction

A liver injury may occur, when liver cells or tissues are exposed to physical forces, biological factors (i.e. virus), or chemical factors like drugs or toxins, which will result in activation of hepatic stellate cells. This further leads to fibrosis and focal nodular hyperplasia of hepatocytes, followed by destruction of hepaticlobuli and formation of pseudolobuli, finally resulting in liver cirrhosis, when both morphological and functional changes occur to the liver. According to a statistical report, 10% of people have a liver injury in China. Currently, patients diagnosed with acute or chronic chemical liver injury are increasing due to the disordered modern life styles including abuse of alcohol, drugs.

Tauroursodeoxycholic acid (TUDCA), an ambiphilic bile acid, is the taurine conjugate form of ursodeoxycholic acid (UDCA) [1]. It has been applied clinically as the first-choice medicine for treatment of primary biliary cirrhosis for more than 20 years abroad. UDCA can preserve mitochondrial structure in chronic alcohol intoxication in mice with alloxan-induced diabetes [2] and improve liver functions in primary biliary cirrhosis, primary sclerosing cholangitis, pediatric cholestatic disorders, and cystic fibrosis [3]. Previous studies have reported that it can prevent amoxicillin-clavulanic acid-induced hepatotoxicity in mice because of its antioxidant properties [4]. In addition, another study has showed that UDCA has a protective effect against isoniazid plus rifampicin induced liver injury in mice [5]. And the mechanism underlying the protective role of UDCA is supposed to be mediated by displacement of toxic bile acids from the bile acid pool as well as choleretic,

immunomodulatory and cytoprotective properties [6]. TUDCA is found in huge amounts in the bile of bear, which thus has become one of the major causes for the hunting and death of bears, leading to a sharp drop in bear number. Additionally, obtaining bile from living bears is also strongly opposed by animal protectors, as this can cause great physical pain to bears. Synthesis of TUDCA by chemical or microbial methods has also been proven feasible, which however, are associated with various shortcomings.

In this context, we attempts to investigate whether the bile of Arctic foxes has a similar effect to bear bile on experimental liver injury. Arctic foxes, scientifically termed Alopex lagopus also belong to Carnivora like bears, which are now fed to provide fur. In China, a total of 130 million Arctic foxes are killed in the winter every year. Afterwards, their gallbladders are discarded. If Arctic fox bile also has protective role in liver injury, their bile can be reused for as a substitute to bear gallbladders, and this will have significant meanings to TUDCA production. Here, a classic $CCl_4$ (carbon tetrachloride)-induced acute liver injury model was established and used to investigate the role of Arctic fox bile in acute liver injury.

## Materials and methods

### *Arctic fox bile source and processing*

The 2.5 mL of fresh Fox bile was provided by Suihua Fox Breeding Farm (Heilongjiang Province, China), which was identified to be collected from Arctic foxes. The fresh bile was dried into powder by drying at 37°C for 48 h.

### *Establishment of $CCl_4$-induced liver acute injury model and drug treatment*

The Kunming mice of clean grade were purchased from the Experimental Animal Center of Zhejiang University of Traditional Chinese Medicine. All animal studies have been approved by the local Ethics Committee and performed in accordance with the ethical standards.

A total of 60 Kunming mice (18±2.0 g of weight), were divided into five groups, namely the blank control group (CK), the $CCl_4$-induced acute liver injury group (model), the Kuihua liver-protection tablet group (positive control group), low-dose bile powder group (low-dose group) and high-dose bile powder group (high-dose group), male and female in half in each group. Mice in the Kuihua liver-protection tablet group were administrated with Kuihua liver-protection tablet solution (Chinese Medicine Chemistry Teaching and Research Room, College of Pharmacy, Zhejiang Chinese Medical University) at 4.2 g/kg; mice in the low-dose and high-dose bile powder solution groups were administrated with low-dose Arctic fox bile powder (5 g/kg) and high-dose Arctic fox bile powder (10 g/kg) respectively. All the drug solutions were administrated by gavage successively for 10 days, once every day. Meanwhile, distilled water of the same volume was administrated to mice in the CK and model groups in the same manner. At the 16 h before the last intragastric administration, mice in the $CCl_4$-induced liver acute injury group, Kuihua liver-protection tablet group, low-dose bile powder group and high-dose bile powder group were intraperitoneally injected with 0.2% $CCl_4$ solution in soy oil (Luhua Group. Co., Laiyang, China) at 10 mL/kg, and soy oil of the same volume was given to mice in the CK, both followed by starvation until execution. All the mice were killed by decollation 1 h after the last intragastric administration, and the blood was collected. Later, the collected blood was centrifuged to obtain serum.

### *Determination of serum and liver indicators in mice*

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using AST Assay Kit (Fosun Long March Medical Science Co., Ltd, Shanghai, China) and ALT Assay Kit (Fosun Long March Medical Science Co., Ltd, Shanghai, China), respectively; the total cholesterol (T-CHO) level in the blood was determined using the T-CHO Assay Kit (Huili Biotech. Co., Ltd., Changchun, China); the liver glycogen content was measured using the Liver Glycogen Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by the thiobarbituric acid (TBA) method following the manufacturer's instruction; the malondialdehyde (MDA) content of the liver tissues was determined using MDA Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by the thiobarbituric acid (TBA) method following the manufacturer's instruction.

**Table 1.** Comparison of different indicators in mice with $CCl_4$ (carbon tetrachloride)-induced acute liver injury between different groups

| Group | Alanine amino-transferase (U/L) | Aspartate amino-transferase AST(U/L) | Malondialdehyde | Total cholesterol | Liver glycogen |
|---|---|---|---|---|---|
| CK | 38.84±8.14 | 92.78±38.90 | 7.80±1.43B | 5.75±1.50 | 4.04±1.75 |
| Model group | 86.92±9.12▲ | 141.23±10.64▲ | 14.86±3.16A | 17.08±1.03▲ | 1.39±0.19▲ |
| Kuihua liver-protection tablet group | 58.04±4.00*,▲ | 63.82±2.86*,▲ | 4.85±1.63C | 16.43±1.39*,▲ | 38.45±2.94*,▲ |
| Low-dose bile powder group | 72.28±5.27*,▲ | 89.29±4.43* | 5.09±0.95C | 6.20±1.07*,▲ | 26.10±2.61*,▲ |
| High-dose bile powder group | 69.05±6.20*,▲ | 105.99±9.31* | 5.28±1.13C | 6.23±1.39*,▲ | 51.67±3.96*,▲ |

*Indicates a significant difference with the model group (P<0.05); ▲Indicates a significant difference with the CK (P<0.05).

*Histopathological observation*

Liver tissues were collected from mice in each group, which were first fixed in 4% formalin for 24 h, followed by washing under the tap water for 6 h and immersion in 70% alcohol solution successively. Subsequently, the liver tissues were dehydrated using a dryer, followed by conventional paraffin embedding, sectioning and HE staining, successively. Finally, the sections were observed under alight microscope (Nikon Co., Tokyo, Japan).

*Statistical methods*

All the data were expressed in the form of mean ± standard deviation. SPSS software (version 13.0, Chicago, USA) was used to perform ANOVA and multiple-comparison to determine whether there is significant difference between different groups. P<0.05 was considered to be statically significant.

## Results

*Effect of Arctic fox bile powder on serum AST and ALT levels*

There were significant differences in serum ALT and AST levels between the model group and the control group (P<0.05, **Table 1**), indicating that the $CCl_4$-induced acute liver injury model has been successfully established. Compared with mice in the model group, mice administrated with Kuihua liver-protection tablet, low-dose bile powder and high-dose powder had lower serum ALT levels (P<0.05), suggesting that Arctic fox bile can reduce serum ALT level. Meanwhile, the AST level in both the low-dose group and the high-dose group was also significantly lower than that of the model group (P<0.05), indicating that either the low- or high-dose Arctic fox bile powder can decrease the serum AST level. However, either the ALT or AST levels in both low- and high-dose groups was higher than that of the positive control group, indicating that Kuihua liver-protection tablet has a better effect on declining ALT and AST levels than Arctic fox bile powder at either dose.

*Effect of Arctic fox bile powder on blood T-CHO level*

There was significant difference in serum T-CHO level between the model group and the control group (P<0.05), indicating that the $CCl_4$-induced acute injury model has been successfully established. Compared with mice in the model group, mice administrated with Kuihua liver-protection tablet, low-dose bile powder and high-dose powder had a lower blood T-CHO level (P<0.05, **Table 1**), suggesting that like Kuihua liver-protection tablet, Arctic fox bile at either dose can also reduce the blood T-CHO level.

*Effect of Arctic fox bile powder on liver glycogen content*

Mice in the model group had significantly lower liver glycogen content compared to the CK; further, mice in the Kuihua liver-protection tablet group, low-dose group and the high-dose group had significantly higher liver glycogen content compared to the model group (P<0.05, **Table 1**), suggesting that like Kuihua liver-protection tablet, both the low- and high-dose Arctic fox bile powder can raise the liver glycogen content.

*Effect of Arctic fox bile powder on liver MDA content*

The MDA content in the model group was significantly higher than that of the CK group (P<0.05), indicating that the $CCl_4$-induced acute liver injury model has been successfully established. Furthermore, mice in either the positive

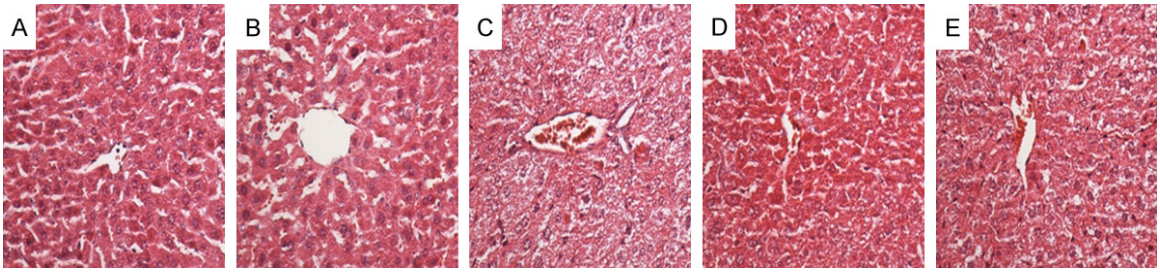


**Figure 1.** Histopathological observation of liver tissues (40×). A. CK; B. Model group; C. Kuihua liver-protection tablet group; D. Low-dose Arctic fox bile powder group; E. High-dose Arctic fox bile powder group.

group, the low-dose group or the high-dose group had significantly lower MDA content compared to the model group ($P<0.05$, **Table 1**), indicating both the low-dose and high-dose Arctic fox bile powder can decrease the MDA content in the liver as Kuihua liver-protection tablet.

*Histopathological observation*

According to the histopathological observation, portal veins and lobular central veins were clearly visible in the CK group; no abnormalities were observed in hepatocyte morphology; hepatocytes were observed to contain abundant cytoplasm that was dyed into red; no binucleated cells were observed; hepatic sinusoids were clearly visible; no infiltration by some inflammatory cells was observed (**Figure 1A**). In contrast, in the model group, portal veins and lobular central veins were hard to be distinguished; a large number of hepatocytes were swollen; spotty necrosis of some hepatocytes was observed; a lot of binucleated cells were also seen; liver sinusoids become obvious (**Figure 1B**). In the Kuihua liver-protection tablet group, portal veins and lobular central veins were clearly visible; however, swelling and spotty necrosis of some hepatocytes were also observed (**Figure 1C**), indicating that Kuihua liver-protection tablet can alleviate the in. In the low-dose group, portal veins and lobular central veins were clearly visible; swelling of many hepatocytes was also observed in some areas; spotty necrosis of hepatocytes was also observed occasionally (**Figure 1D**). In the high-dose group, swelling of some hepatocytes was observed; no notable infiltration by inflammatory cells was observed (**Figure 1E**).

## Discussions

$CCl_4$ can induce hepatotoxicity after being metabolized to trichloromethyl free radical ((.) $CCl_3$), which is thus extensively used to establish an induced acute liver injury model for investigating the pathological mechanism underlying this disease [7]. In the present study, mice in the model group revealed significantly high ALT and AST levels, blood T-CHO content, as well as MDA content, while significantly low liver glycogen content, as compared to the CK group, indicating a $CCl_4$-induced acute liver injury model has been established successfully.

AST and ALT were both routine biomarkers of liver function. AST is a pyridoxal phosphate (PLP)-dependent transaminase enzyme, which catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, and thus is an important enzyme in amino acid metabolism [8], primarily existing in mitochondrion. ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, which is mainly present in cytoplasm. Under normal physiological conditions, ALT and AST are mainly distributed within the liver epithelialcells, which are seldom detected in serum. However, when a liver injury occurs, ALT and AST will be released into blood due to necrosis of epithelial cells, and the content detected in serum is thus positively correlated with the severity of liver injury. In our study, the ALT and AST levels were significantly increased in mice with the $CCl_4$-induced liver injury compared to the control mice, while Kuihua liver-protection tablet, low-dose bile powder and high-dose powder significantly reduced the ALT and AST levels, indicating that Arctic foxes bile may have a similar role in alleviating the $CCl_4$-induced liver injury like Kuihua liver-protection tablet.

Liver synthesizes high-density lipoproteins, which removes fats and cholesterol from cells, including within artery wall atheroma, and

transports it, mainly to the liver for excretion or re-utilization [9]. When a liver injury occurs, the cholesterol delivery is obstructed and the blood cholesterol content will rise. In humans, liver is also a primary organ to synthesize glycogen aside from muscle, and liver glycogen is storage pool to maintain a stable blood glucose level [10]. When liver injury occurs, glycogen synthesis is hampered, and the glycogen content will decline. In the present study, both the low- and high-dose Arctic fox bile powder can reduce the T-CHO content and raise the liver glycogen content in mice with $CCl_4$-induced acute liver injury remarkably, similar to Kuihua liver-protection tablet. It thus can be concluded that Arctic fox bile powder can alleviate $CCl_4$-induced injury to liver and has a beneficial role in maintaining the normal biological functions of liver.

Lipid peroxidation has been widely accepted to contribute to pathological changes at both the subcellular and tissue levels following liver injury [11]. Many aldehydes are produced during the peroxidative decomposition of unsaturated fatty acids. MDA, together with HNE are two most important lipid peroxidation end-products, which can modify DNA bases, yielding promutagenic lesions, and cause protein damage by addition reactions with lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups [12]. In the present study, the MDA content was significantly higher in the $CCl_4$-induced acute liver injury group, and Kuihua liver-protection tablet, low-dose bile powder and high-dose powder were found capable to reduce the MDA content markedly. Thus, it can be inferred that Arctic fox bile has a positive role in alleviating $CCl_4$-induced oxidative stress.

Additionally, histopathological observation also confirmed that mice with $CCl_4$-induced acute liver injury exhibited alleviated pathological symptoms after administration with Arctic fox bile powder, namely with less abnormalities in hepatocytes morphology, and relieved inflammatory cells infiltration and hepatocyte necrosis.

Taken together, Arctic fox bile powder at either the low- or high-dose can reduce the serum ALT and AST levels, blood T-CHO content, liver MDA content and increase the liver glycogen content, suggesting a similar role of Arctic fox bile to Kuihua liver-protection tablet in alleviating the $CCl_4$-induced liver injury, and the latter is a frequently used medicine in China for liver injury therapy. Although the liver-protective effect of Arctic fox bile has not been compared to that of bear bile, it has a comparable, or even better efficacy than Kuihua liver-protection tablet, and thus can be considered as a substitute to bear bile.

## Disclosure of conflict of interest

None.

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