



Original Article

# Bile Drainage Improves Survival in Amatoxin-induced Severe Liver Injury: A Prospective Pilot Cohort Study



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## Abstract

**Background and aims:** Amatoxin-containing mushroom poisoning causes fatal acute liver failure with >50% mortality despite maximal medical therapy. Interrupting the enterohepatic recirculation of amatoxins is a mechanistically rational but unproven therapeutic strategy. This study aimed to evaluate the efficacy and safety of biliary drainage (BD) in patients with pre-acute liver failure caused by amatoxin-containing mushroom poisoning. **Methods:** In this prospective cohort study (ChiCTR2300073442), consecutive adults with amatoxin-induced pre-acute liver failure received standardized care (silibinin, N-acetylcysteine, dehydration). Patients undergoing percutaneous or endoscopic BD were compared to non-BD controls. The primary outcome was survival to hospital discharge. **Results:** Nine patients were enrolled (mean age: 63.3 ± 15.6 years; 44.4% female). All five patients who underwent BD (performed at a median of three days after ingestion) survived (100%), whereas only one of the four non-BD patients survived (25%;  $P = 0.048$ ). BD initiated a rapid biochemical recovery: within 48 h, mean alanine and aspartate transaminase levels decreased by 67.6% and 91.6%, respectively, from their peak levels ( $P < 0.001$ ), and the international normalized ratio decreased from 1.99 to 1.27 ( $P = 0.008$ ). Non-survivors in the non-BD group progressed to multiorgan failure. Procedure-related complications (transient pancreatitis/amylasemia) occurred in three of the five BD patients but resolved with conservative management. **Conclusions:** Timely BD was associated with 100% survival after amatoxin-induced pre-acute liver failure, contrasting sharply with 75% mortality in non-BD controls. The dramatic biochemical improvement after BD supports enterohepatic recirculation interruption as a mechanistic intervention. BD represents a potentially definitive, life-saving intervention for this lethal poisoning as a preliminary finding; larger, multicenter studies are required to confirm the observed association between BD and survival.

**Keywords:** Amatoxin; Mushroom poisoning; Hepatotoxicity; Acute liver failure; Bile drainage; Enterohepatic recirculation.

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## Introduction

Amatoxin-containing mushroom poisoning is a life-threatening medical emergency with a persistently high mortality rate. Although cases are relatively uncommon, they occur worldwide—particularly in regions where foraging is prevalent—and can progress from initial gastrointestinal symptoms to fulminant hepatic failure within days after ingestion.<sup>1–5</sup> It typically produces gastrointestinal symptoms within 6–24 h and acute liver failure within three to five days.<sup>4,5</sup> Despite advances in critical care and the availability of putative antidotes (silibinin, penicillin G, N-acetylcysteine), mortality remains unacceptably high (10%–30% overall and >50% among those who develop acute liver failure), and definitive rescue by liver transplantation is constrained by organ availability, contraindications, and rapid clinical deterioration.<sup>6–9</sup> This combination of rarity, rapid clinical decline, and limited therapeutic options creates an urgent need for mechanistically informed interventions to prevent irreversible liver injury. Given the practical and ethical constraints of accruing large samples in a rare, rapidly fatal condition, pre-specified interim or pilot clinical data—clearly labeled as such—can be important to inform frontline care and guide timely, larger confirmatory studies.

The lethality of amatoxins stems fundamentally from their enterohepatic recirculation (EHR),<sup>10,11</sup> a pharmacokinetic process that prolongs hepatic exposure to the toxins by promoting their reabsorption from the intestine. After intestinal absorption, α-amanitin is actively transported into hepatocytes via organic anion-transporting polypeptides. Within hepatocytes, the toxin inhibits RNA polymerase II, triggering apoptotic and necrotic cell death. Crucially, unmetabolized toxin is excreted into the bile via multidrug resistance-associated protein 2 (MRP2/ABCC2), reabsorbed in the intestine, and returned to the liver via the portal vein—creating a vicious cycle of sustained hepatocellular injury.<sup>12</sup> This process explains why plasma toxin levels often decline while hepatic damage progresses relentlessly days after ingestion.<sup>13</sup> Con-

sequently, interrupting the EHR of amatoxins has long been proposed as a rational therapeutic strategy.<sup>8</sup>

Bile drainage (BD), via percutaneous transhepatic gallbladder drainage (PTGBD) or endoscopic nasobiliary drainage (ENBD), offers a direct means to physically remove amatoxin-laden bile, thereby disrupting its EHR.<sup>14</sup> Experimental models demonstrate that BD significantly reduces hepatic amatoxin uptake and accelerates its systemic clearance.<sup>15,16</sup> Thus far, sporadic case reports and small series have suggested that BD may improve survival in severe poisoning<sup>15,17</sup>; however, these reports lack robust comparative data, standardized protocols, and prospective validation. Crucially, no prospective study has yet evaluated the efficacy of BD within a controlled framework of standardized medical care.<sup>8,18</sup>

Although interrupting EHR is theoretically rational, the Chinese Expert Consensus<sup>19</sup> does not recommend invasive BD, citing the belief that biliary excretion contributes little to overall toxin clearance and a lack of clinical proof; conversely, recent mechanistic and toxicokinetic data document<sup>11,20</sup> high amatoxin concentrations in bile and provide a plausible biological basis for EHR interruption. This discordance presents a practical therapeutic dilemma, and to address it, we designed a prospective clinical study (Chinese Clinical Trial Registry: ChiCTR2300073442) to evaluate the real-world effectiveness of BD in patients with amatoxin-induced acute liver failure who were receiving maximal medical therapy. We hypothesized that timely BD would interrupt EHR, accelerate toxin clearance, mitigate ongoing liver injury, and improve survival compared with standard medical therapy alone; here we report the results of a pre-specified interim analysis of the ongoing trial.

## Methods

### Study design and ethical oversight

We designed a prospective, non-randomized interventional cohort study that was scheduled to be conducted between July 1, 2023, and December 31, 2028, at the First People's Hospital of Yunnan Province. This report presents the results of an interim analysis of the above study, from July 1, 2023, to September 2025. The study protocol was registered with the Chinese Clinical Trial Registry (ChiCTR2300073442) and approved by the institutional review board of the First People's Hospital of Yunnan Province (KHLL2023-KY106-GZ2024-7-8). Written informed consent was obtained from all patients included in this interim analysis or from their legally authorized representatives.

### Patient selection

The inclusion criteria were as follows: (1) age  $\geq$  18 years; (2) a documented history of ingesting poisonous mushrooms, confirmed either morphologically (via provided mushroom specimens or photographs) as containing amatoxin-producing species or through laboratory detection of toxic amanitin components in biological specimens such as blood or urine; (3) onset of gastrointestinal symptoms (e.g., anorexia, nausea, vomiting, abdominal distension)  $>$  6 h after mushroom ingestion, accompanied by progressive elevations in liver biochemical indices and coagulation parameters, with serial liver-function and coagulation tests performed at 8-h intervals demonstrating findings consistent with the early (prodromal) stage of acute liver failure; (4) duration of illness  $\leq$  7 days; and (5) provision of written informed consent.

Patients were excluded if they met any of the following criteria: (1) hemodynamic instability or critical illness with an anticipated survival  $<$  24 h; (2) pregnancy or breastfeeding;

and (3) any other condition that, in the investigator's judgement, would render the patient unsuitable for participation.

### Diagnosis of amatoxin poisoning

The diagnosis of amatoxin poisoning was consistent with the criteria outlined in the Chinese Expert Consensus on Amatoxin-Containing Mushroom Poisoning.<sup>19</sup> For toxin detection, bile samples, along with contemporaneous blood and urine samples from the BD group, as well as blood and urine samples from the non-drainage group, were sent to the Ningxia Center for Disease Control and Prevention for analysis. The detection method was ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UP-LC-ESI-MS/MS).<sup>21</sup> While all five bile samples tested positive for amatoxin, all blood and urine samples from both groups returned negative results. It should be noted that the reliability of toxin detection in bile is currently unstable. The diagnosis of amatoxin poisoning was confirmed after considering the toxin-detection results, the examination of mushroom specimens/photographs, and the clinical and laboratory data of the patients.

### Intervention protocol

Eligible patients were assigned to one of two groups based on their (or their legal representative's) consent for BD: those who provided consent underwent the BD procedure, while those who declined constituted the non-drainage (control) group. All patients in both cohorts received standard medical therapy. Patients in the intervention group ( $n = 5$ ) received standard medical therapy and underwent ultrasound-guided PTGBD or ENBD within 8 h of diagnosis. Continuous external BD was maintained, with daily measurement and recording of the bile output. Patients in the control group ( $n = 4$ ) received maximal standard medical therapy, consisting of fluid management, silibinin, charcoal, N-acetylcysteine infusion, blood purification therapy, and supportive measures as required. Details are provided below. Silibinin (oral capsules): 50–100 mg/kg (up to 2 g per dose), continued as clinically indicated. N-acetylcysteine (IV): loading dose 150 mg/kg over 60 min (not to exceed 10 g), then 12.5 mg/kg/h for 4 h, followed by 6.25 mg/kg/h for 16 h; the 16 h infusion can be repeated if severe hepatic dysfunction persists. Dose reduction for body weight  $<$  40 kg was applied. Penicillin G (IV): 300,000 U/kg/d divided into three doses; maximum total daily dose 25,000,000 U. Activated charcoal: initial single dose 50 g, then 0.25 g/kg every 4 h as continued therapy when indicated. Blood purification was protocol-guided and initiated on admission when prespecified clinical criteria were met; modality selection followed defined timing windows to maximize comparability between groups. Within 48 h post-ingestion, the primary objective was removal of circulating free amatoxins, typically using adsorption/hemoperfusion combined with continuous renal replacement therapy (CRRT; e.g., continuous veno-venous hemofiltration (CVVH) or continuous veno-venous hemodiafiltration (CVVHDF)). At 2–3 days (organ-damage phase), the focus shifted to removal of redistributed toxin and organ support, primarily with plasma exchange (PE)  $\pm$  double plasma molecular adsorption system (DPMAS) and/or PE combined with hemofiltration. In advanced stages (acute liver failure, hepatic encephalopathy, or renal dysfunction), PE  $\pm$  CRRT (CVVH/CVVHDF), DPMAS+PE, or other CRRT modalities were used to clear toxins, correct metabolic disturbances, and support failing organs.

To maximize comparability, all enrolled patients were managed by the same multidisciplinary team (emergency, ICU, infectious diseases, and hepatology physicians) after

**Table 1. Bile drainage management in five patients**

Patient number	Time from ingestion to bile drainage (d)	Approach	Complication	Drainage duration	drainage volume (mL/d)
1	3	PTGBD	None	2 mo	80–400
3	3	PTGBD	None	2 mo	100–500
5	4	ENBD	Transient elevation of amylase/lipase	3 d	100–200
7	3	ENBD	Transient elevation of amylase/lipase	4 d	100–300
8	3	ENBD	Pancreatitis	5 d	100–260

PTGBD, percutaneous transhepatic gallbladder drainage; ENBD, endoscopic nasobiliary drainage.

uniform protocol training.

BD was performed by interventional radiology (PTGBD) or by ENBD according to local expertise and anatomical considerations. Key procedural variables are summarized here and in Table 1: timing of drainage (median 3 days post-ingestion, range 3–4 days), procedural approach (PTGBD in two patients; ENBD in three patients), documented drainage duration (PTGBD: 2 months; ENBD: 3–5 days), and daily drainage volumes (range 80–500 mL/d). Observed procedure-related events are reported in Table 2 and included transient amylase/lipase elevations in two ENBD cases and one episode of pancreatitis; no complications were recorded for PTGBD cases. Standard post-procedural management (antibiotic prophylaxis, daily drainage monitoring, and criteria for catheter removal) was applied.

**Data collection and outcomes**

The primary endpoint was the 28-day survival rate. The secondary endpoints were the in-hospital mortality rate and biochemical recovery rate. We also recorded any complications, such as gastrointestinal bleeding, infections, renal failure, and so on.

**Measurements**

At baseline, we recorded the following for all patients: demographic data, latency period (h) defined as the interval between mushroom ingestion and symptom onset, time to admission (d) defined as the interval between mushroom ingestion and hospital admission, and laboratory parameters.

Serial monitoring of liver-function and coagulation markers, including alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), international normalized ratio (INR), and activated partial thromboplastin time (APTT), was performed at admission; before the BD procedure; at 24 h, 48 h, 72 h, and 96 h after admission; and at discharge.

Safety assessment was conducted by analyzing all pro-

cedure-related adverse events (e.g., bleeding, pancreatitis).

**Sample-size estimation**

A power calculation was performed to detect an anticipated 50% absolute difference in survival rates between the two groups. Based on a two-sided significance level of 0.05 ( $\alpha = 0.05$ ) and 80% power to compare two independent proportions, a target sample size of approximately 20 patients (10 patients per group) was indicated.<sup>22</sup> An interim analysis was performed after the enrollment of nine participants.<sup>21</sup>

**Statistical analysis**

Statistical analyses were performed using Free Statistics version 2.3 beta. Continuous variables were presented as median (IQR) and compared between groups using the Mann–Whitney *U* test. Categorical variables were analyzed using the Fisher exact test. For longitudinal data analysis, the Friedman test was employed, with the Dunn post-hoc test applied for pairwise comparisons where appropriate. Effect sizes were reported as odds ratios (ORs), each accompanied by its respective 95% confidence intervals (CIs). A two-sided *P*-value of less than 0.05 was considered statistically significant for all tests.

**Results**

**Study population and feasibility of BD**

A total of nine patients with amatoxin-induced pre-acute liver failure were enrolled in this prospective cohort. All patients had confirmed mushroom poisoning based on their clinical history and the examination of mushroom specimens or the detection of amatoxin. The BD procedure was technically successful in all five patients assigned to the intervention group. BD was performed at three to four days after mushroom ingestion and was carried out via PTGBD in two patients and ENBD in three patients. No procedure-related complications

**Table 2. Longitudinal changes in liver-function and coagulation parameters following bile drainage in patients with mushroom poisoning-induced liver failure (n = 5)**

	Pre-drainage	48-h post-drainage	Pre-discharge	<i>P</i> -value*
ALT (U/L)	3,452 (2,495–4,876)	1,118 (278–1,584)	143 (87–160)	<0.001
AST (U/L)	2,521 (1,569–3,607)	213 (61–715)	29 (18–33)	<0.001
TBIL (µmol/L)	46.1 (43.1–83.5)	40.3 (29.9–85.4)	20.4 (19.3–28.5)	0.003
INR	1.99 (1.66–2.11)	1.27 (1.24–1.39)	1.03 (1.00–1.09)	0.008
APTT (s)	43.5 (32.6–51.8)	39.4 (35.5–45.1)	31.2 (28.0–37.5)	0.014

Normal ranges: ALT/AST < 40 U/L, TBIL < 21 µmol/L, INR = 0.8–1.2, APTT = 25–35 s. Values are expressed as median and interquartile range. ALT, alanine transaminase; AST, aspartate transaminase; TBIL, total bilirubin; INR, international normalized ratio; APTT, activated partial thromboplastin time. \*All *P*-values are <0.05, indicating statistically significant differences.

occurred in the PTGBD subgroup, and the drainage tubes were removed after two months. In the ENBD subgroup, two patients developed elevated pancreatic enzymes, and one patient experienced acute pancreatitis, which resolved after five days of medical management. The nasobiliary tubes were removed within three to five days after placement.

### Baseline laboratory parameters

Baseline laboratory values were measured at three to four days after the ingestion of foraged mushrooms and included the serum levels of ALT, AST, lactate dehydrogenase, TBIL, creatinine, D-dimer, hemoglobin, and lactate; APTT, prothrombin time, and INR; and the red blood cell and platelet counts. None of the laboratory parameters, except for a lower blood urea nitrogen (BUN) in the BD group, significantly differed between the two groups (all  $P > 0.05$ ). This isolated reduction of BUN, likely related to prerenal factors in acute severe liver injury, did not reflect a difference in intrinsic organ function, as markers such as creatinine and bilirubin were comparable (Table 3).

Complications present at admission included gastrointestinal bleeding and infections. The incidence of gastrointestinal bleeding was 0% in the BD group versus 25% in the control group ( $P = 0.04$ ), whereas that of infections was 20% in the BD group versus 25% in the control group ( $P = 1$ ). These complications reflected the severity of poisoning at baseline rather than procedure-related events.

### Primary outcome: survival

BD was associated with a significantly improved survival rate. All five patients in the BD group survived (100%), whereas three of the four patients in the control group died (75% mortality). This difference was statistically significant ( $P = 0.048$ , Fisher exact test; OR = 21.0, 95% CI: 0.8–563). In the control group, three patients who met the liver transplantation criteria after maximal therapy did not receive a graft due to financial constraints and subsequently opted for withdrawal of care, resulting in death.

### Time to intervention and its impact on survival

The mean duration from mushroom ingestion to hospital admission was significantly shorter in the BD group ( $2.2 \pm 0.8$  days) than in the control group ( $3.8 \pm 1.0$  days). A delay of more than three days in hospital presentation was strongly predictive of mortality: only 25% of patients who presented to the hospital at three days after mushroom ingestion survived, whereas 100% of patients who were admitted within three days survived ( $P = 0.005$ ). The mean latency period (from ingestion to symptom onset) did not differ between the groups ( $8.8 \pm 2.2$  h vs.  $9.0 \pm 3.4$  h,  $P = 0.903$ ).

### Biochemical trajectory and hepatic recovery

Serial monitoring revealed a robust and rapid improvement in the markers of hepatocellular injury and hepatic synthetic function following BD (Table 2, Fig. 1). Within 48 h after BD, the mean ALT and AST levels decreased significantly by 67.6% and 91.6% from their pre-drainage peaks, respectively ( $P < 0.001$  for both). This improvement continued progressively, with mean ALT and AST levels normalizing or nearing normalization by the time of discharge (143 U/L and 29 U/L, respectively;  $P < 0.001$  vs. pre-drainage levels). Coagulopathy, as measured using the INR, showed significant and rapid correction (pre-drainage mean: 1.99; 48 h post-treatment: 1.27; pre-discharge: 1.03;  $P = 0.008$ ). Similarly, APTT significantly improved after BD ( $P = 0.014$ ). TBIL levels also demonstrated a clear downward trend after drainage,

reaching near-normal levels by the time of discharge ( $P = 0.003$ ). In contrast, the surviving patient in the control group showed a much slower biochemical recovery, and the non-survivors exhibited progressive deterioration in liver-function and coagulation parameters.

### BD procedure and safety

BD was performed at a median of three days (interquartile range [IQR]: 3–5 days) after mushroom ingestion (Table 1), via PTGBD ( $n = 2$ ) or ENBD ( $n = 3$ ). Three patients (60%, all ENBD) experienced procedure-related complications: transient elevation of serum amylase/lipase ( $n = 2$ ) and mild pancreatitis ( $n = 1$ ). All complications were self-limiting or resolved with conservative management and did not impact overall survival or the trajectory of liver recovery. In the ENBD subgroup, all three patients experienced transient pancreatic enzyme elevations, and one developed pancreatitis; there was no clear evidence that these events prolonged hospitalization or adversely affected the trajectory of liver recovery in this cohort.

### Discussion

This prospective study demonstrated that BD significantly improved survival after mushroom poisoning-induced pre-acute liver failure, achieving 100% survival (5/5) compared to 25% survival (1/4) in the control group ( $P = 0.048$ ). This study represents the first clinical validation of mechanistic insights from preclinical models, which posit biliary toxin clearance as pivotal for interrupting the EHR of  $\alpha$ -amanitin.<sup>11</sup> Our findings directly address the critical evidence gap highlighted in the 2024 comprehensive review by Kayes *et al.*, which concluded that, owing to limited data, the efficacy of BD needs to be verified to determine its usefulness and role in treating amatoxin poisoning.<sup>8</sup> While this assessment accurately reflected the pre-existing literature (which comprised only case reports),<sup>17</sup> our prospective cohort provides the first rigorous clinical validation of the life-saving potential of BD.

Four key advances emerge from the findings of our study:

1. *Empirical evidence supporting amatoxin EHR*: This study detected amatoxins in all five bile samples, while contemporaneous blood and urine samples were negative, providing preliminary evidence supporting the hypothesis that toxins are concentrated in bile via EHR. These findings validate the mechanistic rationale for EHR interruption via BD. However, limitations related to assay sensitivity, sample matrix effects, and timing constraints mean this evidence remains preliminary. Future studies require standardized bile toxin assays and prospective plasma-bile paired sampling to quantitatively define amatoxin kinetics and strengthen diagnostic foundations.
2. *Redefining the therapeutic window*: The 2018 Chinese guidelines identify pre-acute liver failure as a critical intervention phase.<sup>23</sup> Our data reveal that hospital admission  $>3$  days after mushroom ingestion predicted mortality (OR = 15.2, 95% CI: 1.8–128.3), extending beyond the conventional 6–24 h toxin-absorption window.<sup>24</sup> Crucially, BD remained effective even at a median of three days after mushroom ingestion, suggesting that BD mitigates ongoing EHR of the toxin—a phenomenon recently quantified in porcine models.<sup>25</sup> These findings challenge the dogma that therapies are futile beyond 48 h.<sup>26</sup>
3. *Multiorgan protection mechanism*: Beyond hepatic recovery (ALT  $\downarrow$ 68% at 48 h,  $P < 0.001$ ), BD attenuated extra-hepatic injury. Reversal of coagulopathy, as indicated by the normalization of APTT ( $\leq 40$  s), occurred by one day after BD ( $P = 0.014$ ), which contrasted with the progres-

**Table 3. Demographic and clinical parameters of patients with amatoxin-containing mushroom poisoning**

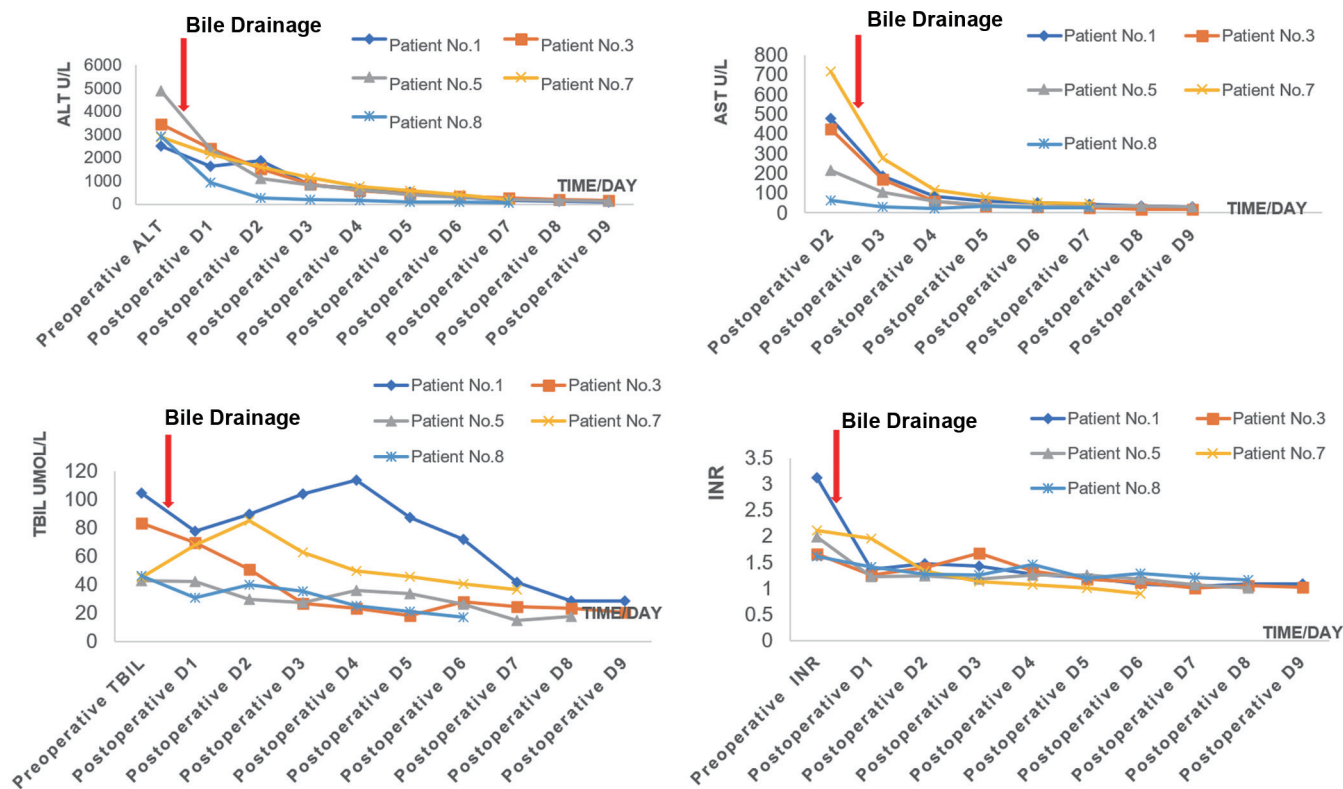
Variable	Total (n = 9)	No bile drainage (n = 4)	Bile drainage (n = 5)	P-value
Sex, n (%), female	4 (44.4)	2 (50)	2 (40)	1
Age, mean ± SD	63.3 ± 15.6	60.5 ± 15.7	65.6 ± 17.0	0.658
Survived, n (%)	6/9 (66.7%)	1 (25%)	5 (100%)	0.048*
Latency period (h), mean ± SD	8.9 ± 2.8	8.8 ± 2.2	9.0 ± 3.4	0.903
Time to admission (d), mean ± SD	2.9 ± 1.2	3.8 ± 1.0	2.2 ± 0.8	0.036*
Tracheal infection, n (%)	2 (22.2)	1 (25)	1 (20)	1
GI bleeding, n (%)	2 (22.2)	1 (25)	1 (20)	0 (0)
Arrhythmia, n (%)	1 (11.1)	1 (25)	0 (0)	1 (20)
Penicillin, n (%)	5/9 (55.56)	2 (50)	3 (60)	1
Charcoal, n (%)	7 (77.8)	3 (75)	4 (80)	1
Silibinin, n (%)	9 (100)	4 (100)	5 (100)	1
AST (U/L), mean ± SD	3,235.8 ± 2,122.2	3,224.8 ± 1,728.5	3,244.6 ± 2,601.3	0.99
ALT (U/L), mean ± SD	2,824.4 ± 1,229.2	2,378.5 ± 772.8	3,181.2 ± 1,488.6	0.364
TBIL (µmol/L), mean ± SD	67.8 ± 41.3	74.4 ± 57.1	62.5 ± 29.8	0.695
DBIL (µmol/L), median (IQR)	22.7 (17.0, 28.0)	20.5 (15.3, 47.1)	24.0 (17.0, 28.0)	0.806
Ammonia (µmol/L), median (IQR)	82.0 (31.5, 114.0)	141.0 (108.5, 175.5)	34.0 (24.0, 88.0)	0.101
Cr (mmol/L), mean ± SD	78.7 ± 29.2	82.9 ± 43.1	75.4 ± 16.9	0.728
BUN (mmol/L), mean ± SD	12.2 ± 2.8	14.4 ± 1.8	10.5 ± 2.2	0.026*
LAC (mmol/L), median (IQR)	2.6 (2.2, 3.2)	2.8 (2.5, 4.9)	2.3 (1.8, 3.2)	0.462
Troponin (ng/mL), median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.805
Myoglobin (ng/mL), median (IQR)	69.9 (56.2, 230.8)	167.7 (87.1, 270.5)	62.0 (56.2, 69.9)	0.624
Potassium (mmol/L), mean ± SD	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	0.949
Calcium (mmol/L), mean ± SD	2.0 ± 0.2	2.1 ± 0.2	2.0 ± 0.1	0.624
HCO <sub>3</sub> (mmol/L), mean ± SD	16.6 ± 2.4	15.5 ± 2.4	17.5 ± 2.1	0.223
APTT (s), mean ± SD	48.6 ± 13.3	51.4 ± 18.0	46.3 ± 9.9	0.604
PT (s), median (IQR)	23.5 (19.4, 25.3)	25.0 (22.9, 49.0)	22.2 (19.4, 23.5)	0.389
INR, mean ± SD	2.5 ± 1.4	3.0 ± 2.0	2.1 ± 0.6	0.363
TT (s), mean ± SD	23.0 ± 7.0	23.3 ± 8.3	22.8 ± 6.8	0.924
D2 (µg/mL), median (IQR)	1.5 (1.1, 2.7)	1.7 (1.4, 3.7)	1.3 (1.0, 2.7)	0.462
PTA (%), median (IQR)	0.4 (0.3, 0.5)	0.3 (0.3, 14.0)	0.4 (0.4, 0.5)	0.624
WBC count (×10 <sup>9</sup> /L), mean ± SD	8.6 ± 3.4	8.6 ± 4.0	8.5 ± 3.5	0.968
RBC count (×10 <sup>12</sup> /L), mean ± SD	5.2 ± 0.9	4.7 ± 0.6	5.5 ± 1.0	0.2
Hb (g/L), mean ± SD	146.7 ± 29.8	138.0 ± 40.5	153.6 ± 20.3	0.472
PLT count (×10 <sup>9</sup> /L), median (IQR)	53.0 (31.0, 153.0)	50.5 (39.8, 78.0)	148.0 (31.0, 169.0)	0.624

SD, standard deviation; CT, computed tomography; MRI, magnetic resonance imaging; GI, gastrointestinal; AST, aspartate transaminase; ALT, alanine transaminase; TBIL, total bilirubin; DBIL, direct bilirubin; IQR, interquartile range; Cr, creatinine; BUN, blood urea nitrogen; LAC, lactate; APTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; TT, thrombin time; D2, D-dimer; PTA, prothrombin activity; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelet; Latency period, time from mushroom ingestion to symptom onset; Time to admission, time from mushroom ingestion to hospital admission. \**P* < 0.05.

sive deterioration of APTT (peak > 180 s) observed in the control group. This aligns with the finding of Ward *et al.* that  $\alpha$ -amanitin directly activates thrombin via endothelial glyocalyx degradation.<sup>6</sup>

4. *Clinical translation and procedural safety*: While silibinin remains the first-line treatment for amatoxin-containing mushroom poisoning,<sup>27</sup> its substantial failure rate in

stage III toxicity<sup>24</sup> necessitates the development of alternative treatments to interrupt EHR. Current modalities intended to reduce toxin burden fall into three broad categories, each with important mechanistic limitations. First, extracorporeal blood-purification techniques (for example, plasmapheresis and hemodialysis) are effective at removing toxin from the circulating plasma com-



**Fig. 1. Dynamic changes in serum liver enzymes and coagulation parameters after bile drainage.** ALT, alanine transaminase; AST, aspartate transaminase; TBIL, total bilirubin; INR, international normalized ratio.

partment, but do not access or clear toxin that has been excreted into and retained within the biliary tree or gallbladder; as a consequence, plasma concentrations can rebound as biliary stores are re-released into the gut and reabsorbed. Second, gastrointestinal decontamination with activated charcoal can adsorb amatoxins present in the intestinal lumen, and thereby partially interrupt enterohepatic recycling; however, the effect is limited to luminal contents, and charcoal cannot directly remove toxin sequestered in the biliary tract or gallbladder. Third, combined approaches (extracorporeal clearance plus repeated charcoal administration) may attenuate circulating levels more effectively than either approach alone, yet they remain unable to eliminate the biliary reservoir of toxin. A recent review<sup>28</sup> similarly emphasizes these limitations of blood purification and charcoal-based strategies in the setting of biliary sequestration.

By contrast, targeted BD directly removes toxin from the biliary compartment, and therefore addresses a source that is inaccessible to standard extracorporeal or luminal adsorption methods. This mechanistic distinction may explain why interruption of EHR via BD could provide a therapeutic advantage in cases where significant biliary accumulation has occurred. Given these complementary but non-overlapping mechanisms, a rational therapeutic strategy might combine timely BD with extracorporeal clearance and repeated activated charcoal to reduce both biliary and circulating toxin loads; prospective evaluation of such multimodal approaches is warranted. The 100% technical success of BD in our study, along with the minor procedure-related complications (mostly mild pancreatitis/elevated transaminases), underscores its feasibility. Nonetheless, since the BD procedure is associated

with some complications, procedural expertise and careful monitoring of the patient's condition are required.

Patients in the BD group were admitted to the hospital significantly earlier than patients in the control group (mean: 2.2 vs. 3.8 days after mushroom ingestion,  $P = 0.049$ ), which warrants careful consideration as a potential confounder. Delayed presentation likely reflects more severe initial symptoms (owing to more cycles of EHR of the toxin, amplifying hepatocellular damage) or barriers to care, both of which can independently contribute to worse outcomes. However, several factors may mitigate the effects of this confounder in our study: (1) The baseline markers of liver injury (ALT, AST, and TBIL) and coagulopathy (INR) were comparable between the BD and control groups at three to four days after mushroom ingestion, indicating similar initial hepatotoxic burden. (2) The surviving patient in the control group presented the latest (on day 5) yet survived, while fatalities occurred in patients presenting earlier (days 3–4). (3) Crucially, BD was initiated at a median of three days after mushroom ingestion (range: 3–4 days), a timeframe historically associated with peak toxicity and high mortality, yet resulted in universal survival in our cohort.

Procedure-related pancreatic enzyme elevations (observed in all ENBD patients) and one case of pancreatitis were generally mild and reversible in our series; nevertheless, these safety signals should be prospectively monitored and explicitly integrated into the benefit–risk assessment of BD, particularly when ENBD is employed.

We note that three non-survivors in the control group did not undergo liver transplantation because of financial constraints, underscoring a critical ethical and practical dilemma in resource-limited settings. In addition to economic barriers,

limited availability of donor livers and regional transplant capacity further restrict access to definitive therapy. Although Yunnan Province has made active investments in prevention and clinical capacity, these systemic constraints may confound outcome comparisons and impede optimal care for some patients. To address this, we recommend policy measures to ensure equitable access to life-saving care, including medical financial assistance programs, expedited emergency referral or 'green channel' pathways for critically ill poisoning patients, strengthened regional referral and transplant networks, efforts to increase organ donation and optimize allocation, and consideration of living-donor transplantation or bridging therapies where appropriate. Financial and resource barriers should not determine access to potentially curative interventions, and we urge health authorities and professional societies to prioritize mechanisms that remove these obstacles.

Several limitations of the present study must be acknowledged. First, the small sample size inherent to this rare poisoning is a major limitation, precluding definitive conclusions or sophisticated multivariate adjustment; accordingly, the findings should be regarded as preliminary and hypothesis-generating. Second, the non-randomized allocation introduces selection bias. Although care was prospectively standardized and delivered by the same trained multidisciplinary team to mitigate heterogeneity, residual confounding remains possible and limits causal inference. Another potential limitation is that BD was occasionally withheld due to family refusals, often driven by perceived hopelessness in the most critically ill patients. Consequently, our study could not evaluate the true efficacy and safety of BD in this high-risk subgroup. Nevertheless, the magnitude and consistency of the survival difference, coupled with the dramatic and rapid biochemical response specifically linked to the intervention, strongly suggest a true therapeutic effect. These limitations mandate cautious interpretation of the study findings and highlight the urgent need for larger, ideally randomized, multicenter trials to confirm the efficacy of BD and refine patient selection and treatment timing.

Despite its limitations, this study provides highly promising evidence that percutaneous or endoscopic BD, when performed within the critical window of established pre-acute liver failure (typically three to five days after mushroom ingestion; median time to BD in our cohort was three days), may be a life-saving intervention for rigorously selected patients with severe amatoxin poisoning when performed in centers with appropriate technical expertise. Routine implementation requires confirmation in larger, preferably randomized, multicenter studies. On the basis of our findings, we propose that BD should be strongly considered in all patients with confirmed severe amatoxin poisoning who exhibit progressive liver injury and coagulopathy despite 24–48 h of maximal medical therapy, particularly before meeting definitive liver transplantation criteria. These conclusions are preliminary and hypothesis-generating; larger prospective studies are required to define optimal timing. The choice between PTGBD and ENBD should be individualized based on local expertise, coagulation status, and anatomy. Future research must focus on: (1) confirming these findings in larger, prospective cohorts or randomized trials; (2) defining the optimal timing of BD initiation relative to mushroom ingestion and biochemical trajectory; (3) refining patient-selection criteria to maximize benefit and minimize procedural risk; (4) quantifying amatoxin concentrations in drained bile versus serum to directly confirm toxin removal; and (5) exploring the potential synergy between BD and enhanced elimination techniques.

## Conclusions

In this prospective clinical study, BD was associated with a striking 100% survival rate in patients with severe amatoxin-induced pre-acute liver failure, compared to 25% survival in patients who did not undergo BD and were managed with maximal medical therapy alone. The rapid and significant improvement in liver injury and synthetic function markers following BD provides strong mechanistic support for interrupting EHR as a key therapeutic strategy. While limited by the small sample size and non-randomized allocation, these interim results suggest that timely BD may dramatically alter the historically grim prognosis of this devastating poisoning. Pending further validation, BD should be integrated early into the management algorithm for severe amatoxin intoxication as a potentially definitive, liver-saving procedure.

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## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Conceptualization (JG, BH, XM), data acquisition (XM, YS and WY), statistical analysis (XM), writing—original draft (XM), and writing—review and editing (JG, BH). All authors have read and agree to the published version of the manuscript.

## Ethical statement

The study protocol was registered with the Chinese Clinical Trial Registry (ChiCTR2300073442). The study was performed in accordance with the Declaration of Helsinki (as revised in 2024) and approved by the institutional review board of the First People's Hospital of Yunnan Province (KHLL2023-KY106-GZ2024-7-8). Written informed consent was obtained from all patients included in this interim analysis or from their legally authorized representatives.

## Data sharing statement

The individual deidentified participant data that underlie the results reported in this article (text, tables, figures, and appendices) will be made available upon reasonable request. Proposals should be directed to the corresponding author. Data requestors will need to sign a data access agreement. The study protocol and statistical analysis plan are available in the Chinese Clinical Trial Registry (ChiCTR2300073442).

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