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Original Article

Herb induced liver injury by Xianling Gubao Tablets: A case assessed for causality using updated RUCAM and integrated evidence chain

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ABSTRACT

Objective: A typical case of Xianling Gubao (XLGB) Tablets-induced liver injury was systematically studied in the clinic and the laboratory.
Methods: A patient with herb-induced liver injury (HILI) and a history of taking XLGB Tablets before disease onset was engaged as the study subject, and the case was diagnosed according to the updated Roussel Uclaf Causality Assessment Method (RUCAM) and the integrated evidence chain (iEC) method recommended by the *Guidelines for Diagnosis and Treatment of Herb-induced Liver Injury* (HILI Guidelines).
Results: Clinical history, biochemical indexes and imaging tests were used to exclude the influence of fundamental diseases and confusing liver diseases such as viral, alcoholic and autoimmune liver diseases on the diagnosis. Based on an investigation of the patient's medication history, she was suspected to have HILI caused by XLGB Tablets, as the patient was only taking an oral preparation of XLGB Tablets, and the influence of other drugs on the diagnosis was excluded. This patient with alanine aminotransferase (ALT) $\geq 3 \times$ upper limit of normal (ULN) and a calculated R of 6 was diagnosed with possible acute drug-induced hepatocellular injury. The relationship was considered "highly probable" (score of 9) using the updated RUCAM of 2016. Moreover, the fingerprint similarity between the preparation taken by the patient and a commercially available preparation was 0.99, suggesting that the patient was consuming XLGB Tablets rather than another drug. LC-MS technology and the Agilent Fake TCM-Drugs database were used to investigate the drug, and no chemical additions were found. Examination of the drug for pesticide residues, heavy metals, aflatoxins and other exogenous substances indicated compliance with the content limits of the *Chinese Pharmacopoeia*.
Conclusion: In summary, the final diagnosis of XLGB-induced liver injury reached the clinical diagnosis of HILI and was acute severe hepatocellular injury type by the updated RUCAM and iEC. Therefore, this study provides scientific evidence regarding the causality evaluation of compound preparations of traditional Chinese medicines-induced liver injury.

1. Introduction

With the recent popularization of herbal medicines and related products worldwide, reports of herb-induced liver injury (HILI) have risen on a yearly basis, attracting widespread attention both inside and outside of the industry. Clinical diagnosis of HILI faces significant challenges due to the complexity of herbal medicines in clinical use, as well as its own nature. *The Guidelines for Diagnosis and Treatment of Herb-induced Liver Injury* (HILI Guidelines) published and implemented by the China Association of Chinese Medicine (CACM) are a set of diagnostic guidelines and standards for HILI that reflect the complexity of herbal medicines, and have become one of the first CACM industry group standards to be promoted nationwide (Wang et al., 2018). The core of these guidelines is the recommended use of the integrated evidence chain (iEC)-based causality evaluation method, which improves the credibility of HILI diagnosis and causality evaluation. However, there are limited examples of the causal evaluation of liver injury using iEC as recommended by the HILI Guidelines; In particular, a causal evaluation of liver injury induced by compound preparations of traditional Chinese medicines (TCMs) has been rarely reported.

Recent years have been marked by increasing reports of liver injuries induced by compound preparations of TCMs, including oral preparations of Xianling Gubao (XLGB) Tablets and Zhuanggu

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Guanjie Pills in China (Zhang et al., 2023). There is no shortage of cases of severe and acute liver injury reported following adverse reactions to XLGB Tablets (Du et al., 2017; Li et al., 2019). However, most case reports lacked causality assessment using the updated Roussel Uclaf Causality Assessment Method (RUCAM) and iEC. This is because most reports are based on a retrospective rather than a prospective study design, and the information reported in the literature tends to be incomplete. Moreover, the quality of the herbs in XLGB, such as authentication and impurities, is not taken into account in the XLGB. XLGB is often used in combination with other chemical drugs in clinic, such as diclofenac sodium, omeprazole, cisapride, etc. Therefore, it is difficult to accurately judge causality between the drug and liver injury. To improve interobserver variability, the updated RUCAM of 2016 provided further specifications of criteria and instructions, such as liver tests with a high threshold: ALT of at least five times the upper limit of normal (ULN) and/or of alkaline phosphatase (ALP) of at least 2 × ULN, avoiding nonspecific liver injuries and providing a highly probable causality grading (Danan & Teschke, 2019; Teschke et al., 2020). Many publications on the updated RUCAM-based herb-induced liver injury cases have emerged (Teschke et al., 2020). Nevertheless, the updated RUCAM-based causality assessment method is not yet used to evaluate XLGB-induced liver injury frequently.

Oral preparations of XLGB are new national medicines that combine Miao folk prescriptions with modern pharmaceutical technology. Preparations such as XLGB Capsules and XLGB Tablets have been shown to nourish the liver and kidneys, activate blood circulation, and strengthen tendons and bones, and other benefits. The formulation of XLGB consists of six herbs: *Epimedii Folium* (Yinyanghuo in Chinese), *Psoraleae Fructus* (Buguzhi in Chinese), *Dipsaci Radix* (Xuduan in Chinese), *Salviae Miltiorrhizae Radix* et *Rhizoma* (Danshen in Chinese), *Anemarrhenae Rhizoma* (Zhimu in Chinese), and *Rehmanniae Radix Praeparata* (Shudihuang in Chinese). No toxic herbs were found in typical recipes. As a national essential drug (Zhang, 2009), XLGB has been extensively applied to treat osteoporosis, osteoarthritis, menopausal syndrome, aseptic bone necrosis and bone fractures for roughly twenty years, with definite therapeutic effects. Long-term toxicological experiments have confirmed the safety and efficacy of XLGB for the treatment of osteoporosis (Cheng, Liu, et al., 2013; Wu et al., 2017). A randomized, multicenter, double-blind, placebo controlled clinical trial showed that XLGB could reduce incidence of corticosteroid-induced osteonecrosis of femoral head, with 14.4% (21 of 146 cases) and 6.98% (9 of 129 cases) in the placebo group and XLGB group (Li et al., 2018).

Therefore, we employed XLGB Tablets as an example and used a causality evaluation method based on the updated RUCAM of 2016 and iEC recommended by the HILI Guidelines to systematically study a typical case of HILI caused by XLGB Tablets alone. Based on both clinical and laboratory analysis, we analyzed the objective reality and risk factors of XLGB-induced liver injury in order to provide scientific evidence for the causality evaluation of liver injury associated with compound preparations of TCMs.

2. Materials and methods

2.1. Case presentation

In March 2017, a 75-year-old female patient was admitted to the outpatient clinic of 302 Military Hospital due to acute hepatitis. The patient complained that after taking the self-purchased drug "XLGB Tablets (batch No. 161108)" half a month ago, she developed cold-like symptoms accompanied by yellow urine and fatigue. Later, her family found that her complexion was yellow, and the degree was gradually aggravated, accompanied by yellow urine, fatigue, abdominal distension and poor appetite. Local hospitals found that serological markers of hepatitis A, B, C and E were negative, and her liver biochemistry was analyzed to determine serum levels of ALT (665 U/L), aspartate aminotransferase (AST, 494 U/L), total bilirubin (TBIL, 496.35 μmol/L), albumin (27 g/L), creatinine (118.84 μmol/L) and coagulation activity (34%). Beyond this, there were no significant abnormalities in routine bloodwork, and an abdominal B-ultrasound did not indicate cirrhosis or ascites. The patient did not mention any history of close contact with hepatitis patients; history of infectious and genetic diseases; history of blood transfusion; history of unhealthy diet or food allergy; history of cataracts; infectious history of typhoid fever tuberculosis or scarlet fever; chronic organ disease history of the heart, brain, lung or kidney; history of surgical trauma; history of allergy to cephalosporin, penicillin or metronidazole. The patient's family history was negative for inherited metabolic diseases. In addition, she denied any history of long-term non-local residence, history of contact with epidemic water or epidemic foci, history of contact with radioactive substances or poisons, history of inhalation of harmful dust, or history of drinking, smoking or tourism.

The patient was admitted into our hospital for further diagnosis and treatment. Her temperature was 36.5 °C with a pulse of 84 beat/min, respiration of 18 breath/min and arterial blood pressure of 115/70 mmHg. Her nutrition was moderate, a wheelchair was used to transfer her into the ward in an automatic position, and body check was cooperative. She was alert, oriented and cooperative. Her face was yellowish. She exhibited severe icterus of the sclerae and skin. Petechiae were seen at the veni-puncture site of both hands, liver palm was negative, and spider nevi were absent. Enlargement of systemic and superficial lymph nodes was not observed, and the patient's heart and lung examinations were normal. The abdomen was flat and soft, and no abdominal wall varices were observed. No pressure pain or rebound pain was reported. The liver was not reached under the right rib nor subxiphoid. Murphy's sign was negative. The spleen was not reached under the left rib. The upper border of the liver was located in the right midclavicular line between the fifth ribs, with no percussion. The shifting dullness test was negative, and bowel sounds were observed five times per minute without hyperfunction. Mild oedema of both lower limbs was observed. Physiological reflex was intact, and pathological signs were not drawn out. Flapping wing tremor was negative.

Laboratory results from the patient's initial visit were shown in Table 1. An abdominal B-ultrasound revealed diffuse damage to the liver parenchyma, splenomegaly, effusion in the abdominal cavity, hepatic cysts, and inflammatory changes to the gallbladder. The prothrombin time (PT) was significantly prolonged, and the international normalized ratio (INR) was significantly increased on day 5; however, coagulation function gradually improved with plasma transfusion, protein supplementation and improvement of coagulation (Fig. 1A and B). After symptomatic treatments, such as anti-jaundice efforts, liver protection and enzyme reduction, levels of ALT, AST, direct bilirubin (DBIL) and TBIL gradually decreased (Fig. 1C and D). On May 15, the patient's liver tests indicated serum levels of AST (33 U/L), ALT (13 U/L) and albumin (ALB, 37 g/L), cholinesterase (1172 U/L), coagulation activity (49.3%), DBIL (64.5 μmol/L), TBIL (71.1 μmol/L) and INR (1.36) were significantly decreased. The patient's liver function and bilirubin continued to improve. The patient wanted to return home for further treatment, so she took medicine to leave the hospital.

2.2. HILI diagnosis process analysis

2.2.1. Diagnostic criteria of HILI

The liver biochemistry criteria for diagnosing HILI is based on the updated RUCAM (Danan & Teschke, 2019): (1)

Table 1
Initial laboratory tests.

Items	Units	Results
HGB	g/L	140
WBC	L	10.58×10^9
RBC	L	4.58×10^{12}
EO	%	0.1
PLT	L	139×10^9
PT	–	24.2
INR	–	2.15
TP	g/L	44
ALB	g/L	29
GLO	g/L	24
ALT	U/L	366
AST	U/L	192
ALP	U/L	173
GGT	U/L	134
CRE	μmol/L	240
HAV IgM	–	negative
HEV IgM, IgG	–	negative
HBsAg	–	negative
HBeAg	–	negative
HBeAb	–	negative
HBcAb	–	negative
HBsAb	–	negative
HBc IgM	–	negative
Anti-HCV	–	negative
HIV Ag/Ab	–	negative
CMV-IgM	–	negative
EBV-IgM	–	negative
ANA, Anti-AMA and Anti-SMA	–	negative
Serum Ceruloplasmin	g/L	0.3
HCG	mIU/mL	0.26

Note: HGB, hemoglobin; WBC, white blood cell count; EO, eosinophils; PLT, platelet count; PT, prothrombin time; INR, international normalized ratio for prothrombin time; DBIL, direct bilirubin; TBIL, total bilirubin; TP, total protein; ALB, albumin; GLO, globin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyltransferase; CRE, creatinine; HAV, hepatitis A virus; HEV, hepatitis E virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis Be antigen; HBeAb, hepatitis Be antibody; HBcAb, hepatitis B core antibody; HBsAb, hepatitis B surface antibody; Anti-HCV, antibodies to hepatitis C virus; CMV, cytomegalovirus; EBV, ebstein-barr virus; HCG, human chorionic gonadotropin; Anti-HIV, antibodies to human immunodeficiency virus; ANA, antinuclear antibody; Anti-AMA, anti-nitochondrial antibody; Anti-SMA, antismooth muscle antibody.

ALT $\geq$ 5 $\times$ ULN; or ALP $\geq$ 2 $\times$ ULN, particularly with a rise of 5′-nucleotidase or γ-glutamyl transpeptidase and the exclusion of bone disease-induced ALP elevation; or (2) ALT $\geq$ 3 $\times$ ULN and TBIL $\geq$ 2 $\times$ ULN.

2.2.2. Exclusion of non-drug etiologies of liver injury

In diagnosing HILI, non-drug etiologies of liver injury, including viral, autoimmune, alcoholic, inherited metabolic, biliary and vascular-related causes, as well as other systemic dysfunction, should be reasonably excluded using physical examinations, laboratory tests, imaging techniques, and other appropriate methods.

2.2.3. Determination of type of liver injury

According to the criteria of liver injury type described in the HILI Guidelines, ALT $\geq$ 3 $\times$ ULN and R $\geq$ 5 were defined as hepatocellular injury type. ALP $\geq$ 2 $\times$ ULN and R $\leq$ 2 were defined as cholestasis type. ALT $\geq$ 3 $\times$ ULN, ALP $\geq$ 2 $\times$ ULN and 2 < R < 5 were defined as mixed type. For this analysis, $R = ALT_{measured\ value} \times ALP_{ULN} / (ALT_{ULN} \times ALP_{measured\ value})$ (Danan & Teschke, 2015).

2.2.4. Causality of RUCAM score

RUCAM scores were calculated according to the HILI Guidelines, and the causal relationship between drug use and liver injury was determined. According to the updated RUCAM, it was recommended that an RUCAM score $\geq$ 6 should be considered indicative of a correlation between herbal drugs and liver injury (Danan & Teschke, 2015; Teschke et al., 2020). The relationship between liver injury and drug consumption was classified as "excluded" ($\leq$ 0 points), "probably unrelated" (1–2 points), "probably related" (3–5 points), "most likely related" (6–8 points) or "highly related" (> 8 points).

2.2.5. Exclusion of combinational use with synthetic drugs

Information on medical and medication histories were collected using the Medication Use Questionnaire for Drug-induced Liver Injury recommended by the HILI Guidelines to exclude western drugs with known hepatotoxicity or interactions that cause drug hepatotoxicity in practice.

2.2.6. HPLC identification

Samples were transferred into sampling vials before HPLC analysis (Cheng, Yao, et al., 2013; Li et al., 2010; Zhou et al., 2011), which was performed using an Agilent 1200 high-performance liquid chromatograph system with a ZORBAX Eclipse Plus C_{18} analytical column (4.6 mm $\times$ 250 mm, 5 μm), HP-12000 DAD detector and HP chemistry workstation. The column oven temperature was maintained at 35 °C. The mobile phase consisted of solvent A (0.1% phosphoric acid in water) and solvent B (acetonitrile). The flow rate was 0.80 mL/min, with a linear gradient as follows: 0–5 min, 95% A; 5–80 min, 95%–64% A; 80–95 min, 64%–52% A; 95–110 min, 52%–20% A; 110–112 min, 20%–0 A; 112–125 min, 100% B; 125–135 min, 0–95% A; 135–140 min, 95% A. The injection volume was 10 μL, and the detection wavelength was 270 nm.

2.2.7. Chemical addition exclusion

Patient-digested samples were further characterized using liquid chromatography coupled with mass spectrometry (LC/MS) to detect herb powders containing any adulterating drugs or compounds. Screening was performed using the fake-TCM drugs database, which includes 5 620 chemical drugs and compounds. LC-MS analysis was performed with an Agilent Technology 1290 Infinity UHPLC system coupled with an Agilent Technology iFunnel 6550 Q-TOF LC/MS system (UHPLC Q-TOF MS) (Agilent Technologies, Santa Clara, USA). Chromatographic separation was carried out using an Acquity UPLC HSS T3 C_{18} column (2.1 mm $\times$ 100 mm, 1.8 μm); the column temperature was maintained at 30 °C, and the sample temperature was maintained at 4 °C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The flow rate was 0.30 mL/min, with a linear gradient as follows: 0–1 min, 95% A; 1–9 min, 95%–60% A; 9–19 min, 60%–10% A; 19–21 min, 10%–0 A; 19–21 min, 100% B. The injection volume was 4 μL, and detection wavelengths were 245, 360, 420, 225 and 280 nm.

For mass spectrometry analysis, an Agilent 6550 i Funnel Q-TOF LC-MS system with positive and negative electrospray ionization sources (ESI) was used. Data collection was controlled using ESI Continuum acquisition mode, and the data range was 50–1 200 *m/z*. Capillary voltages were set to 2.5 and 2.2 kV in positive and negative modes, respectively. For positive mode and negative modes, the cone voltage was 40 V. The applied source temperature was 130 °C. The cone hole gas flow was set to 50 L/h. The desolvation gas flow was set to 800 L/h, and the desolvation temperature was 350 °C. Nitrogen and argon were used as the collision gas. The high collision energy scan was set at a ramp energy scan from 10 to 55 eV, and the low collision energy scan was set at 4 eV. The scan time for each function was 0.20 s. Leucine enkephalin (100 pg/μL) was applied as the lock mass, generating reference ions of *m/z* 554.261 0 in negative mode and *m/z* 556.277 1 in positive mode. For accurate mass acquisition, reference ions were introduced at 10 mL/min using a lockspray. Sodium formate (1 μmol/L) was used for mass axis calibration.

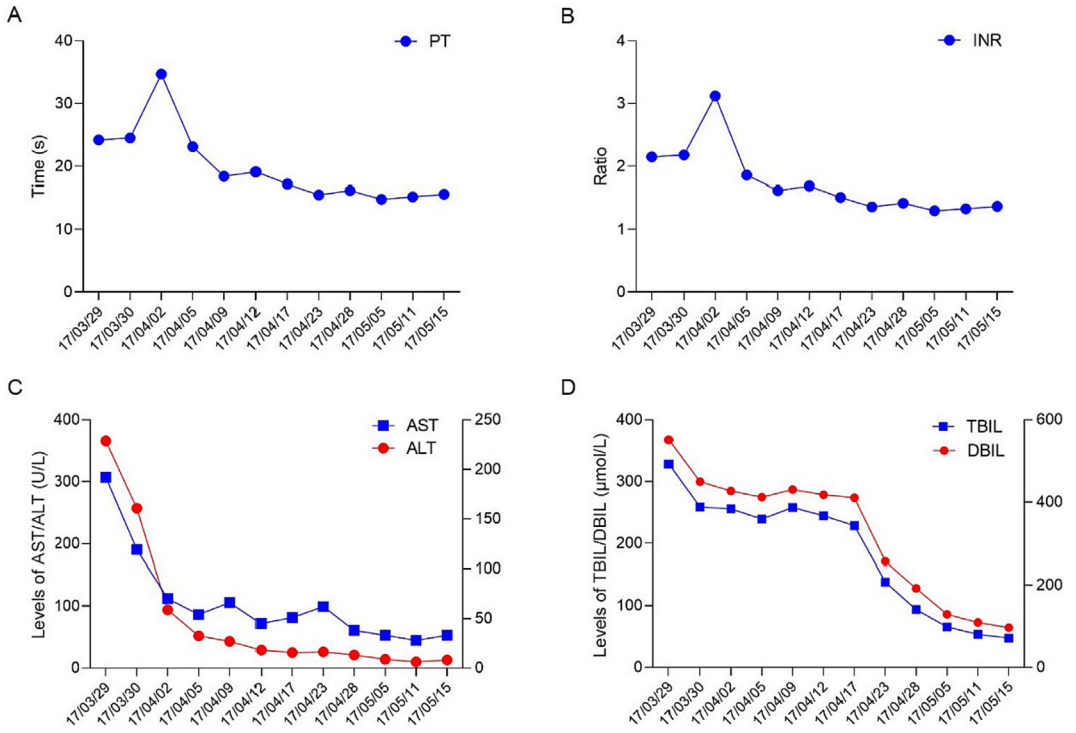

Fig. 1. Blood and biochemical function test during patient's hospital stay. (A) Changes in prothrombin time (PT); (B) Changes in international normalized ratio (INR) for PT; (C) Changes in levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST); (D) Changes in levels of total bilirubin (TBIL) and direct bilirubin (DBIL).

2.2.8. Exogenous toxin contaminants detection

Pesticide residues, heavy metals, and aflatoxins can be identified at qualified institutions or laboratories based on the *Chinese Pharmacopoeia* (2015 edition) (Kong, Li, et al., 2013; Kong, Liu, et al., 2013; Wang et al., 2016) to exclude contamination by harmful substances and similar factors.

3. Results

3.1. Diagnostic criteria of HILI

The patient reported that she was taking only XLGB Tablets, demonstrating a history of consuming herbal medicines or related preparations before the onset of abnormal liver test. ALT was 366 U/L, meeting the HILI biochemical diagnostic criteria of ALT $\geq 5 \times$ ULN (Teschke et al., 2020).

3.2. Exclusion of non-drug etiologies of liver injury

As shown in Table 1, surface antibody results for hepatitis B were negative, antibodies for hepatitis A, C and E were negative, autoimmune indexes, such as antinuclear and anti-mitochondrial antibodies, were negative, immunoglobulins were normal and thyroid function was normal. Abdominal B-ultrasound indicated that the patient had mild fatty liver and splenomegaly. Therefore, liver injury caused by viral, autoimmune, inherited metabolic, biliary, vascular-related or other systemic dysfunctions could be excluded. The patient had no history of alcohol consumption, so alcoholic liver disease could be ruled out. Non-alcoholic fatty liver can be excluded based on body mass index, blood lipids, and abdominal B-ultrasound.

3.3. Typing according to cell damage

Patient with ALT $\geq 3 \times$ ULN and a calculated R of 3 were typed as hepatocyte injury according to the type of target cells damaged (Teschke et al., 2020).

3.4. RUCAM score

As shown in Table 2, using the updated RUCAM of 2016, the patient's RUCAM score was 9. In other words, the association between liver injury and XLGB was deemed "highly probable" (Teschke et al., 2020).

3.5. Exclusion of combinational use of synthetic drugs

As shown in Table 3, on the basis of suspected HILI diagnosis, the patient's medication use was investigated using the medication history questionnaire, and western drugs with clear hepatotoxicity or drug hepatotoxicity due to interactions were excluded from combined application.

3.6. HPLC identification

As shown in Fig. 2, a fingerprint profile of the preparation taken by the patient was compared to that of a commercially available XLGB preparation. The similarity between the two was 0.99, suggesting that the preparation taken by the patient was indeed XLGB, rather than another drug or drugs. In addition, we analyzed a control sample containing the drug as prescribed and compared it to a chromatogram of the preparation taken by the patient at corresponding retention time positions (Fig. 3). The results confirmed that the preparation taken by the patient was XLGB. Therefore,

Table 2
RUCAM score.

Scoring projects	Items for hepatocellular injury			Results
	Initial medication	Non initial medication	Scores	
Time from taking medicine to onset (d)	5–90	1–15	+2	1
	< 5, > 90	> 15	+1	
Time from drug withdrawal to onset (d)	≤ 15	≤ 15	+1	
Course of disease after drug withdrawal	Decrease of ALT from peak value			3
	Drop within 8 d ≥ 50% ULN		+3	
	Drop within 30 d ≥ 50% ULN		+2	
	Drop more than 30 d ≥ 50% ULN		0	
	Drop more than 30 d < 50% ULN		−2	
Risk factors	Alcohol		+1	0
	No alcohol		0	
Age (years)	≥ 50		+1	1
	< 50		0	
Others drugs	No data related to drug combination or reduction		0	0
	Having concomitant drugs and the time indication		−1	
	Hepatotoxic drugs and the time indication		−2	
	Other drugs with evidence of liver injury (such as reactivation positive reaction)		−3	
Other reasons	Complete exclusion group Ia and IIb		+2	2
	Complete exclusion group I		+1	
	Exclude 4–5 items in group I		0	
	Exclude less than 4 items in group I		−2	
	Highly likely non-drug factors		−3	
Past information	Having relevant records about product description		+2	2
	Having literature reports, but not having records about product description		+1	
	Unknown		0	
Drug reactivation reaction	Positive		+3	0
	Suspicious positive		+1	
	Negative		−2	
	Not done or unable to judge		0	
Total score				9

Note: [a]GroupI: including HAV, HBV, HCV (acute), biliary obstruction, alcoholism, recent hypotension (Hucke's liver); [b]Group II: including CMV, EBV, herpes virus infection.

Table 3
Drug investigation on drug-induced liver injury.

ID: 3001225544 This is the first time to see a doctor due to drug discomfort.	
Have you taken drugs or health products for some disease or some reason in the past 6 months? ( ) No (√) Yes	
Whether to provide relevant items to the pharmacist in charge, if yes, please tick "√". (√) Residual drugs ( ) Drug instructions ( ) Prescription (√) Medicine box packaging ( ) Copy of local medical records	
Name of drug or health product	Xianling Gubao Tablets
Source of drug purchase (or reason for drug use)	Local pharmacy
Underlying disease (or medication reason)	Osteoporosis
Start time	December 30, 2016
Last time taken	March 27, 2017
Total administration time/d	87
Whether to take medicine to this visit	No
Usage	Oral
Consumption	1.8 g/d
Time of first discomfort	January 12, 2017
Time from taking medicine to onset/d	72
What discomfort (please describe, such as nausea, vomiting and others)	Loss of appetite, dizziness and nausea
Is there a drug-related rash	No
Is the discomfort improved after drug withdrawal	No

the patient's liver injury was probably caused by the administration of XLGB, rather than other drugs.

3.7. Chemical addition exclusion

The molecular ion peak mass-to-charge ratios of the tested compounds were compared with those of chemical drugs in the database. The results showed that the errors were > 100, suggesting that no other chemical drugs were added to the preparation of XLGB taken by the patient. Therefore, added chemicals can be excluded as a factor influencing liver injury in this patient.

3.8. Detection and exclusion of pesticide residues, heavy metals and aflatoxins

As the preparation of XLGB is not listed in the *Chinese Pharmacopoeia*, its pesticide residue, heavy metal and aflatoxin testing standards are based on the relevant testing standards described for licorice in the *Chinese Pharmacopoeia* (2015 edition). As shown in Table 4, no aflatoxins were detected in the preparation taken by the patient, and levels of pesticide residues and heavy metals did not exceed the limits set for licorice. Therefore, the influence of foreign factors, such as pesticide residues, heavy metals and aflatoxins, on liver injury can be excluded for this patient.

4. Discussion

As shown in Fig. 4, the case was diagnosed according to the diagnostic method and process described in the HILI Guidelines. First, the patient presented with liver test abnormalities that met the biochemical criteria for HILI recommended by interprofessional Spine Assessment and Education Clinics (iSAEC), as well as a history of suspected application of a liver-injuring drug before disease onset, consistent with criterion ①. The patient had no previous history of specific diseases, and post-admission laboratory tests excluded viral, autoimmune, inherited metabolic, biliary, and vascular-related causes of liver injury, as well as liver injury caused by other systemic dysfunction factors. The patient had no history of alcohol consumption, so alcoholic liver disease could

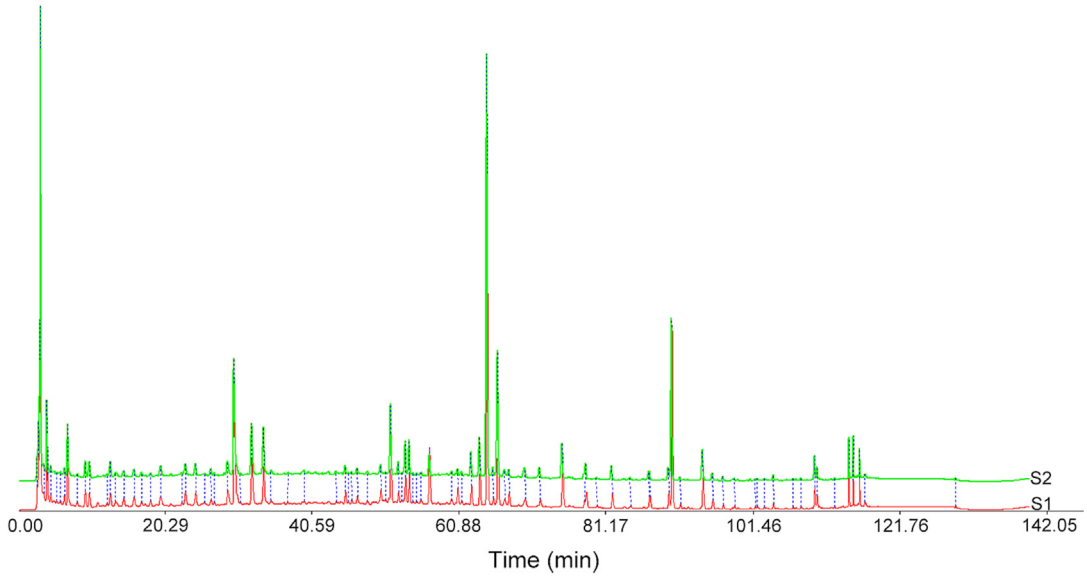

Fig. 2. HPLC fingerprints of XLGB Tablets. HPLC fingerprints showed similar characteristic peaks between saled XLGB Tablets (S1) and patient's digested materials (S2).

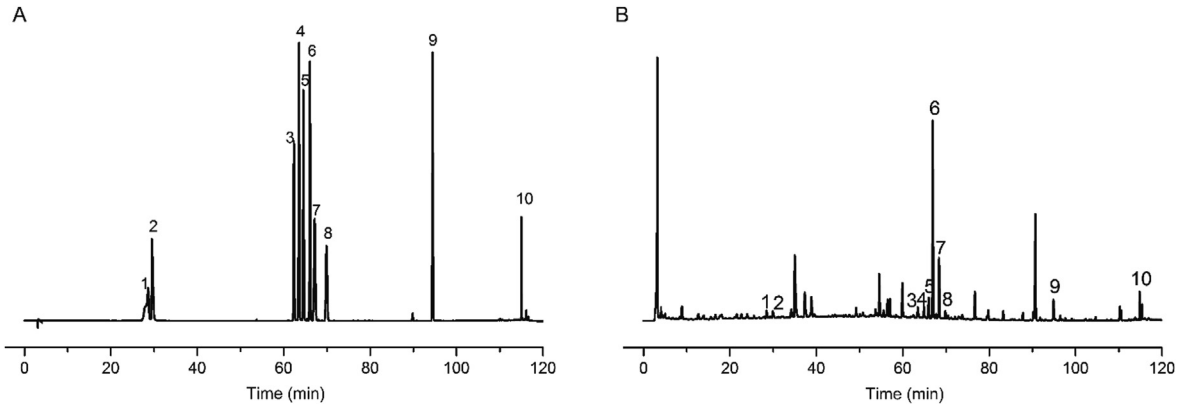

Fig. 3. HPLC identification of digested materials of patient (A) and control sample containing drug as prescribed (B). Characteristic peaks were identified by the reference substances. 1, sweroside; 2, magnoflorine; 3, epimedin A; 4, epimedin B; 5, epimedin C; 6, icariin; 7, psoralen; 8, angelicin; 9, icarisid II; 10, anhydroicaritin.

Table 4
Harmful elements test results of pesticide residues, heavy metals and aflatoxins in drugs taken by patient.

Harmful elements	Test items	Results
Pesticide residues	α-HCH (μg/kg)	< 0.18
	Pentachloronitrobenzene (μg/kg)	5.41
	γ-HCH (μg/kg)	< 0.01
	β-HCH (μg/kg)	< 0.05
	σ-HCH (μg/kg)	< 0.02
	p,p′-DDE (μg/kg)	< 0.01
	o,p′-DDT (μg/kg)	< 0.12
	p,p′-DDD (μg/kg)	< 0.09
	p,p′-DDT (μg/kg)	< 0.32
Heavy metals	Pb (mg/kg)	0.512
[Total heavy metal (in Pb)]	Cd (mg/kg)	0.072 3
	As (mg/kg)	0.047 5
	Hg (mg/kg)	< 0.048 8
	Cu (mg/kg)	2.14
Aflatoxins	AFB_1 (μg/kg)	–
	AFB_2 (μg/kg)	–
	AFG_1 (μg/kg)	–
	AFG_2 (μg/kg)	–

Note: HCH, α-hexachlorocyclohexane; p,p′-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene; o,p′-DDT, 1,1,1-trichloro-2-(p-chlorophenyl)-2-(o-chlorophenyl) ethane; p,p′-DDD, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane; p,p′-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG1, aflatoxin G_1; AFG_2, aflatoxin G_2.

be ruled out, consistent with criterion ②. The patient's RUCAM score was > 6 points, consistent with criterion ③. In conclusion, criteria ① + ② + ③ were met, and a diagnosis of HILI was suspected. Second, the patient had only taken XLGB for 72 d before disease onset, so combinatorial effects of other liver-damaging drugs could be ruled out, and criterion ④ was met. According to the specific herbal medicine prescription and remaining drugs provided by the patient, we determined that the XLGB taken by the patient did not contain any other herbal medicine adulterants or harmful substances, consistent with criteria ⑤ and ⑥. In conclusion, the criteria for clinical diagnosis of HILI were met. In addition, the patient had no previous history of HILI, and the occurrence of a re-excitation event was not considered in this attack. The patient did not meet criterion ⑧, so a clinical diagnosis of HILI was considered. Finally, the patient was diagnosed with HILI of hepatocyte injury type, acute and severe.

RUCAM is a commonly used tool for causality assessment of suspected drug induced liver injury (DILI) and HILI cases quantitatively (Danan & Teschke, 2015). However, RUCAM shows its restricted application to HILI causal inference for the reason that some confounding factors should be taken into consideration, such as complex constituents of herbal products, unknown types of herbal origins and toxin-contaminated plants during evaluating the causal-effect between drug use and the onset of HILI (Wang

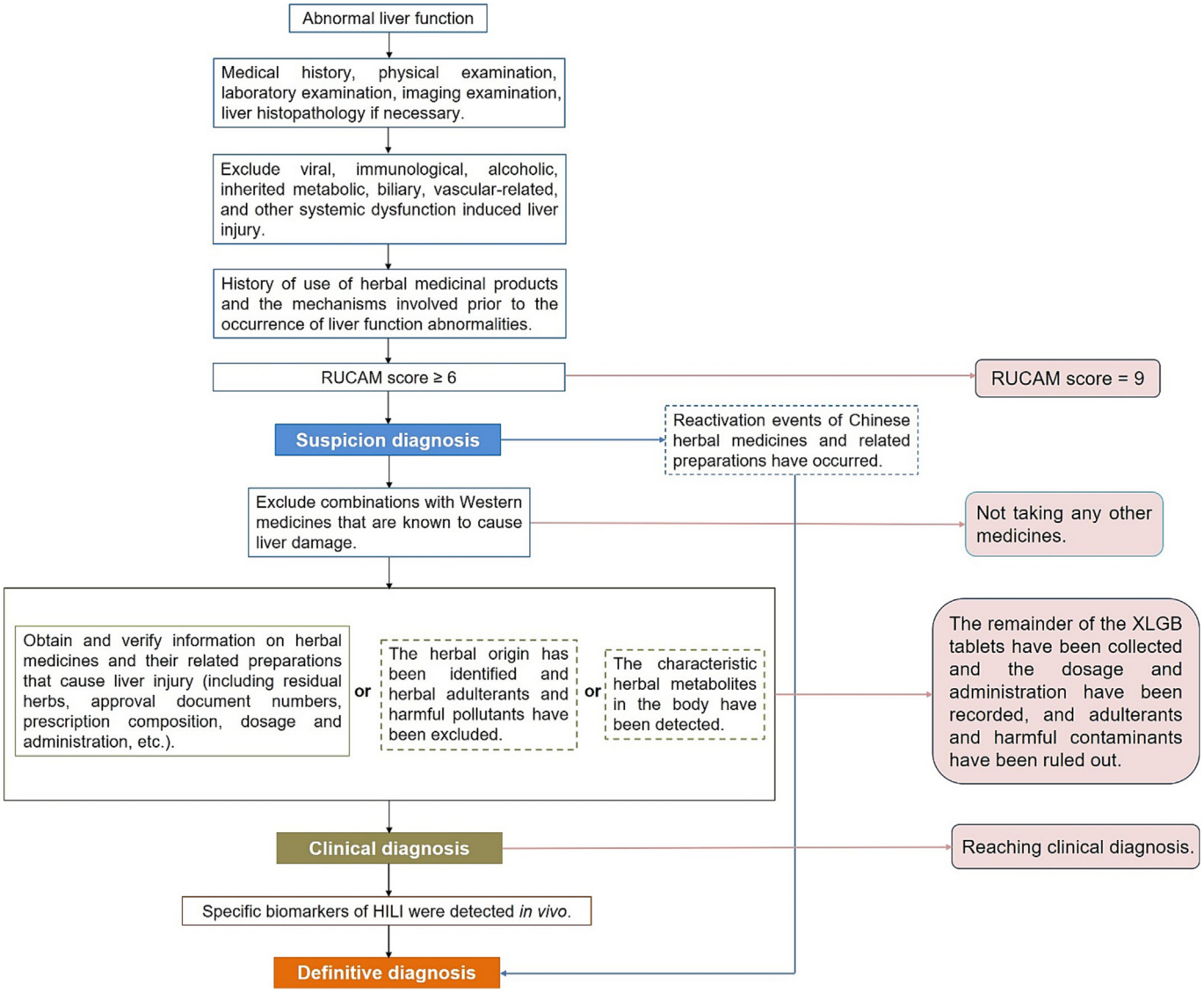

Fig. 4. Diagnosis process and results for XLGB Tablet-induced liver injury.

et al., 2015). In this study, we found that the HILI case with total score above 6 according to RUCAM scoring system was graded "probable", while it was reevaluated as "highly probable" using iEC for complementing elaborate evidence chain. Hence, we collected medication information of HILI case and incorporated herbal constitute identification by HPLC into iEC assessment. From our findings, chemical additions, pesticide residues, heavy metallic elements, and aflatoxins scarcely contributed to HILI, which could not only support original authentication of herbs but also provide supplementary clues to complete the causal grading of RUCAM based on iEC.

The National Medical Products Administration released the seventy-second "Adverse Drug Reaction Information Bulletin" on December 2016, prompting concern about liver injury caused by oral preparations of XLGB (Administration, 2017). As a result, liver injury caused by XLGB has attracted widespread attention across all walks of life, and cases of XLGB-induced liver injury have been subsequently reported. Wu et al. suggested that the mechanism of XLGB-induced liver injury may the combined result of immunostimulants indirectly inhibiting FXR regulation and XLGB inducing CYP7Al expression, suggesting that clinical use of the drug should be avoided in combination with drugs that can affect bile acid metabolism (Wu et al., 2019). Ding et al. showed that continuous administration of XLGB to mice for 27 weeks caused liver injury characterized by infiltration of liver tissue by inflammatory cells (Ding et al., 2019). Lin et al. investigated the cellular hepatotoxicity of XLGB extract, and their results showed that the primary hepatotoxic components contained in XLGB prescriptions were *Epimedii Folium* and *Psoraleae Fructus* extracts (Lin et al., 2020).

Cases of XLGB-induced liver injury reported in the literature and the National Adverse Reaction Center were retrospectively analyzed using the iEC method (Xiao et al., 2021). The results showed that most reported cases of XLGB-induced liver injury described in the literature were incomplete, making it difficult to accurately determine causal relationships. From 2012 to 2016, the National Adverse Reaction Center collected 55 388 cases of drug-induced liver injury, of which 63 cases were related to XLGB. Of these, 36 cases of liver injury were caused by XLGB alone, and the doses of these patients fell within stated limits. Liver injury was not dose- or time-dependent and accounted for a low case rate of 0.06% (36/55 388) (Huang et al., 2021; Li et al., 2020). These results suggest that XLGB-induced liver injury may be idiosyncratic.

According to the patient's reports, she had been consuming XLGB preparations for more than one year. During the period of medication, she had a medical examination without evidence of no liver injury. Liver injury occurred less than three months after taking XLGB, and there were no significant changes in dosage or daily life during the period of medication. Based on these facts, organic factors are important and cannot be ignored. Our research group found that the main effects of XLGB are to nourish the liver and kidneys, activate blood circulation, and strengthen tendons and bones. According to the prescription composition and the theory of traditional Chinese medicine, XLGB should be used for deficiency arthralgia dominated by deficiency of the liver and kidneys. However, clinical use of XLGB may not always be appropriate. For example, for rheumatoid arthritis patients, the treatment should act to dispel wind, dispel cold and remove dampness. However, if XLGB is used for treatment, the *Epimedii Folium* and *Psoraleae Fructus* present in the prescription can tonify the liver and kidneys and enhance body immunity (Jiang et al., 2004; Lu et al., 2019), which will promote the activation of body immunity in such patients, greatly increasing the risk of liver injury. These findings suggest that immune stress may be an important causative factor in idiosyncratic liver injury caused by XLGB. Therefore, our group evaluated a previously described animal model of immune stress and found that while XLGB has no effect on liver injury in normal rats, it induced significant liver injury in an immune-stress model, experimentally confirming the idiosyncratic liver injury properties of XLGB (Li et al., 2020). The results of this study on XLGB showed that both *Epimedii Folium* and *Psoraleae Fructus* can cause liver injury under immune stress conditions. Teschke et al. presented a case report on HILI caused by Indian Ayurvedic herbal products containing extracts from *Psoraleae Fructus* leaves with psoralens as ingredients by causality assessment with the RUCAM (Teschke & Bahre, 2009). The degree of liver injury was more severe in combination compared to when the two compounds were applied individually (Gao et al., 2020), and the severity was stronger than that of the whole formula, suggesting that four other herbs in the formula (*Dipsaci Radix*, *Salviae Miltiorrhizae Radix* et *Rhizoma*, *Anemarrhenae Rhizoma* and *Rehmanniae Radix Praeparata*) can be used in combination with *Epimedii Folium* and *Psoraleae Fructus* to reduce the formula's toxicity. The four herbs in the formula were used in combination with *Epimedii Folium* and *Psoraleae Fructus* individually, and the results showed that all four herbs had reduced liver injury caused by *Epimedii Folium* and *Psoraleae Fructus*, with *Salviae Miltiorrhizae Radix* et *Rhizoma* exhibiting the strongest toxicity-reducing effects (Huang et al., 2021).

Based on our search of the literature, this study is the first report of XLGB Tablets-induced liver injury diagnosed by the HILI Guidelines. The phenomenon of XLGB Tablets-induced liver injury and its idiosyncratic properties have been recognized, but further research is still needed regarding the mechanisms of idiosyncratic liver injury. Future analysis of factors associated with XLGB Tablets-induced idiosyncratic liver injury and the identification of predisposing factors will be valuable to subsequent studies of the risks of XLGB Tablets-induced liver injury.

CRediT authorship contribution statement

Chunyu Li: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Funding acquisition. **Yingying Li:** Methodology, Formal analysis, Data curation, Writing – original draft. **Zhaofang Bai:** Methodology, Formal analysis. **Jiabo Wang:** Methodology, Resources. **Guohui Li:** Conceptualization, Writing – review & editing. **Xiaohe Xiao:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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