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Clinicopathological features of Bu Gu Zhi-induced liver injury, a long-term follow-up cohort study

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Abstract

Background & Aims: Bu Gu Zhi (BGZ) is a Chinese herb consumed mainly for osteoporosis treatment. Only small case series of BGZ-induced liver injury (BGZILI) have been reported. We describe the clinicopathological features and clinical course of BGZILI.

Methods: Patients diagnosed with drug-induced liver injury (DILI) at Beijing Friendship Hospital from 2005 to 2017 were reviewed. Clinical and follow-up data were analysed.

Results: Of the 547 DILI patients, 40 cases (7.3%) were attributed to BGZILI. About 34/40 (85.0%) patients were females with a median age of 63 (range, 54-70) years. The median latency period was 45 (range, 29-90) days. Patients commonly presented with loss of appetite (57.5%), dark urine (57.5%) and fatigue (55.0%). The median level of alanine aminotransferase and aspartate aminotransferase at BGZILI onset was 673.5 and 423.0 U/L respectively. Total bilirubin (TB) and direct bilirubin (DB) were 59.0 and 39.4 μmol/L respectively. The biochemical liver injury pattern was hepatocellular (92.5%), cholestatic (5.0%) and mixed (2.5%). They were categorized into 'mild' (N = 23, 57.5%), 'moderate' (6, 15.0%) or 'severe' (11, 27.5%) according to severity assessment by DILI network. The main histological injury pattern in 9/40 patients with liver biopsy was acute hepatitis with/without cholestasis. Median duration of follow-up was 26.3 months with recovery in 37 patients within 6 months. No patients died or required transplantation.

Conclusions: BGZ-induced liver injury manifested more often as a hepatocellular injury pattern with mild to moderate hepatocellular damage. Most patients recovered after cessation of BGZ within 6 months, and none developed end-stage liver disease or died.

KEYWORDS

Bu Gu Zhi, clinical and pathological features, liver injury, Psoralea corylifolia Linn

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; BMI, body mass index; DB, direct bilirubin; DILI, drug-induced liver injury; DILIN, drug-induced liver injury network; GGT, γ-glutamyl transferase; HDS, herbal dietary supplements; HILI, herb-induced liver injury; IgG, immunoglobulin G; INR, international normalized ratio; LFT, liver function test; RUCAM, Roussel Uclaf Causality Assessment Method; TB, total bilirubin; TBA, total bile acid; TCM, Traditional Chinese Medicine; ULN, upper limit of normal.

Lan Wang and Yan Wang contributed equally to this study.

1 | INTRODUCTION

Consumption of herbal products has increased world-wide in recent years. The annual cost of herbal products has been estimated to exceed US$36.7 billion in the United States of America (USA), and overall use of plant/food supplements in Europe has increased by up to 18%.[1,2] This increase has been attributed to the easy availability of herbs over the Internet and in local Chinese markets, large-scale immigration of Asian people to Europe and USA and the widely held belief that herbs are 'natural; and not toxic.[1-3]

The prevalence of herb-induced liver injury (HILI) has been increasingly reported worldwide.[1,4,5] A recent study from the Drug-induced Liver Injury Network (DILIN) suggested that HILI accounts for 20% of prospectively registered drug-induced liver injury (DILI) cases.[2,6,7] A similar trend has been noted in the Spanish DILIN.[1] HILI is becoming a leading but yet still under-recognized cause of moderately-to-severely abnormal liver biochemistry.[1,2] HILI is reported even more commonly in the Asia-Pacific region because of the widely held belief that herbs are efficacious and safe without side effects. The liver toxicity of a given herb is largely unknown because of the availability and multi-usage of a wide variety of herbs and the lack of systematic investigation.[1-3,8-11]

Bu Gu Zhi (BGZ) is a popular Chinese herbal medicine used widely as a supplement to treat bone fractures and osteoporosis. BGZ is one of the key components in several herbal compounds, such as Gu Kang capsules, Zhuang Gu Guan Jie Wan and Bai-shi pills.[12-17] BGZ and its compounds are recommended by two Chinese traditional medicine practice guidelines, namely, the clinical practice guideline of Traditional Chinese Medicine for primary osteoporosis,[18] and the guideline for the diagnosis and management of primary osteoporosis.[19] It has been estimated that about 59.7% patients with osteoporosis consume herbs containing BGZ for a certain period of time in this country.[20] BGZ is usually taken conjunction with vitamin D and calcium products. Evidence of its individual efficacy on osteoporosis based on randomized control trials is lacking.[19]

Several case reports of liver injury induced by BGZ have been reported worldwide, highlighting the potential hepatoxic effects of this herb.[17,21-29] However, the characteristics of BGZ-induced liver injury (BGZILI) including latency, causality, clinical phenotypes, severity and long-term prognosis after dechallenge (ie, stopping of a drug, usually after an adverse event or at the end of planned treatment) are unknown.[22,28,29] We herein describe the clinical and pathological features of BGZILI in 40 patients. Our study findings can help to increase awareness and improve early identification and diagnosis of BGZILI.

Keypoints

Bu Gu Zhi-induced liver injury (BGZILI) was more often encountered in older women who likely consume the herb as a supplementary treatment for osteoporosis in China. Most patients recovered within 6 months after cessation of BGZ, and none developed end-stage liver disease or died. We summarize the clinical and histological features of BGZILI which could aid in early diagnosis.

2 | PATIENTS AND METHODS

2.1 | Patients

Individuals diagnosed with DILI at the Beijing Friendship Hospital (Beijing, China) from August 2005 to September 2017 were reviewed retrospectively. Patients whose liver injury was attributed to BGZ were identified for this study. The diagnosis of DILI was based on medical history, causality assessment and clinical course after dechallenge.

The Roussel Uclaf Causality Assessment Method (RUCAM) score was used to evaluate causality between BGZ administration and liver injury. RUCAM scores were grouped into likelihood levels as 'excluded' (0), 'unlikely' (1-2), 'possible' (3-5), 'probable' (6-8) and 'highly probable' (≥9).[10,30,31]

2.2 | Inclusion criteria

Inclusion criteria were patients: (a) for whom a chronological relationship between drug exposure and liver injury could be discerned; (b) who had changes of liver biochemical abnormalities after dechallenge; (c) with a RUCAM score ≥3.[10,30,31]

2.3 | Exclusion criteria

Exclusion criteria were patients: (a) with a RUCAM score <3; (b) infected by hepatitis virus (A, B, C or E) or human immunodeficiency virus; (c) with ethanol consumption of >40 g/day; (d) with other pre-existing liver diseases (eg, steatosis, steatohepatitis, autoimmune liver diseases or metabolic liver disorders and cardiac/renal dysfunction); and (e) with incomplete clinical data.[10,30,31]

2.4 | Clinical parameters

Demographic data and clinical parameters (routine blood indexes, liver biochemical parameters and immunological tests) at baseline and follow-up were retrieved from the electronic database of Beijing Friendship Hospital.

The pattern of liver injury was classified according to criteria set by the Council of International Organizations of Medical Sciences.[10,30-32] The latter uses levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) at DILI onset, expressed as x times of the upper limit of normal (ULN), to determine the ALT: ALP ratio (R). Three patterns of liver injury were defined: 'hepatocellular' (R ≥ 5), 'cholestatic' (R ≤ 2) and 'mixed' (2 < R <5).[10,30-32] Disease severity was categorized

as 'mild', 'moderate', 'severe' or 'fatal/transplantation required' as described by an International Expert Working Group on DILI.[10,30,31]

2.5 | Follow-up

Patients were followed up until their liver biochemical parameters returned to normal or until a period of at least 6 months. Normalization was defined as that the levels of ALT and/or AST were <1×ULN (40 U/L) and total bilirubin (TB) <1.5 × ULN (30.78 μmol/L).[7] 'Chronic DILI' was defined as that liver biochemical parameters failed to normalize within 6 months.[30,33]

2.6 | Histology

Formalin-fixed, paraffin-embedded (FFPE) blocks of liver biopsies from nine patients with BGZILI were retrieved. The tissue cores were recut and stained with haematoxylin and eosin, periodic acid-Schiff with diastase, reticulin, Masson trichrome and cytokeratin (CK)7 or CK19. Three pathologists and one hepatologist trained in liver histopathology used a standardized scoring system to assess each case independently. All four investigators were aware of the diagnosis of BGZILI but were blinded to laboratory data during the slide review. An overall injury pattern was obtained according to criteria proposed by David Kleiner.[6,34-36]

2.7 | Statistical analyses

Statistical analyses were carried out using SPSS v21 (IBM). Median values with 25th and 75th percentiles are given for continuous variables. Frequencies and percentiles are given for categorical variables. Kruskal-Wallis and chi-square tests were undertaken for continuous and categorical variables respectively. $P < .05$ was considered significant.

The study was approved by the Human Ethics Committee of Beijing Friendship Hospital. The requirement of informed consent from patients was waived.

3 | RESULTS

3.1 | Demographics and clinical manifestation of BGZILI

This was a retrospective study. We reviewed the inpatient discharge records system from August 2005 to September 2017 at the Beijing Friendship Hospital. There were 547 inpatients diagnosed with DILI with 377 (68.9%) cases caused by Traditional Chinese Medicine (TCM) or TCM with Chemical drugs/poisons, of which 40 (7.3%) were attributed to BGZ (Figure 1).

Table 1 and Table S1 summarize the demographic and detailed clinical information of patients with BGZILI. About 85.0% of patients (34/40) were women with a median age of 63 (range, 54-70) years and body mass index (BMI) of 24.35 (range, 22.10-26.27) kg/m^2. There was a variety of presenting complaints such as loss of appetite (57.5%), dark urine (57.5%), fatigue (55.0%), nausea (37.5%), jaundice (35.0%), abdominal distension (30.0%), vomiting (20.0%) and pruritus (12.5%).

In all 40 cases, BGZ was ingested as a component of a herbal compound; the other concomitant components are listed in Table S2.

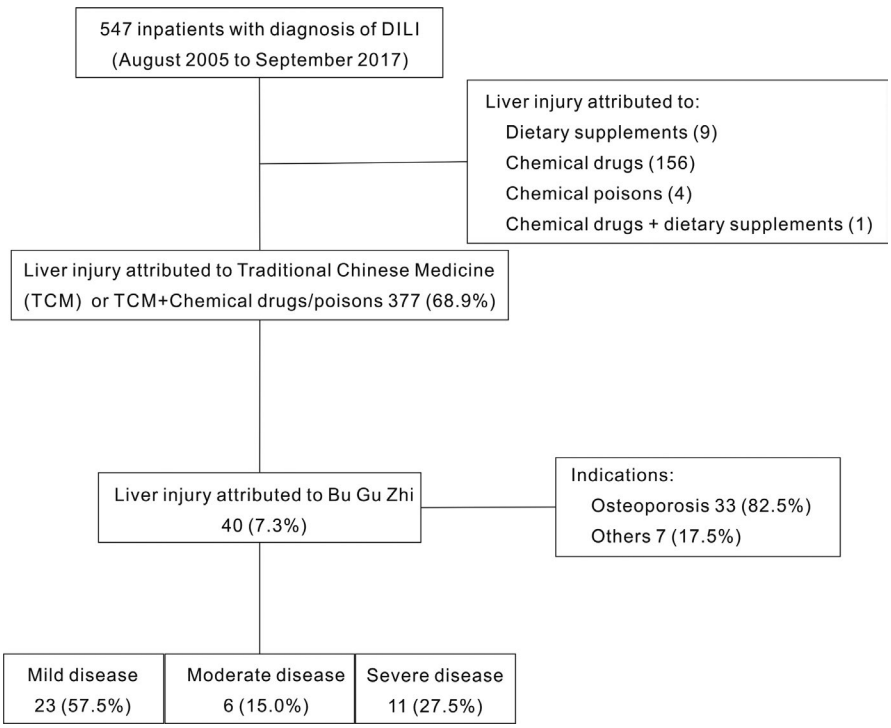

FIGURE 1 Study flowchart. We identified 40 patients whose liver injury was induced by BGZ. Screening of 547 hospitalized patients diagnosed with DILI. Out of 377 (68.9%) cases caused by Traditional Chinese Medicine (TCM) or TCM with Chemical drugs/poisons, 40 cases were induced by herbal compounds containing BGZ. They were divided into three groups according to severity: 'mild' (23), 'moderate' (6) or 'severe' (11)

Treatment of bone diseases (eg, osteoporosis) was the main indication (82.5%); the remaining indications included lung, stomach and prostate diseases. According to the RUCAM score, BGZILI was categorized into possible (10.0%), probable (50.0%) and highly probable (40.0%).

The median latency period between initial consumption of BGZ and symptom onset was 45 (range, 29-90) days. The median duration of BGZ consumption was 49 (range, 30-90) days. The BGZILI pattern was classified as hepatocellular (92.5%), cholestatic (5.0%) and mixed (2.5%).

3.2 | Overall clinical features and derangement of biochemical parameters in BGZILI

Table 2 summarizes the biochemical parameters of patients with BGZILI. The median initial levels (in U/L) of ALT and AST were 673.5 (range, 425.5-1044.0) and 423.0 (range, 202.0-754.0) respectively. Increase in ALT levels was higher than that for AST (median AST: ALT ratio was 0.65 [range, 0.46-0.84]). Levels of ALP and γ-glutamyltransferase (GGT) were mildly elevated, with median levels (in U/L) of 169.0 (range, 117.0-219.0) and 209.0 (range, 101.5-321.5) respectively. Median levels (in μmol/L) of TB and direct bilirubin (DB) were 59.0 (range, 23.6-185.7) and 39.4 (range, 8.6-119.3), respectively. The median peak level (in U/L) of ALT was 742.5 (range, 502.8-1162.2) and that of AST was 587.5 (range, 260.5-763.8). The AST: ALT ratio was 0.73 (range, 0.48-0.87), which was similar to that at disease onset. However, levels of TB and DB at peak levels of aminotransferases were lower than that at onset (39.6 and 25.0 μmol/L respectively). The median value of the international normalized ratio (INR) was 1.05 (range, 0.98-1.13). With regard to antinuclear antibody (ANA), 30/40 (75.0%) patients were negative or borderline-positive, 5/40 (12.5%) moderately positive (1:160) and 12.5% strongly positive (1:320). Also, 9/38 patients had increased levels of immunoglobulin G (IgG) (>1.1 × ULN). Full blood count indices were within normal ranges.

3.3 | Comparison of clinical and biochemical features for different severities of BGZILI

According to severity assessment by DILIN, the 40 patients were categorized into three groups: mild (N = 23; 57.5%), moderate (N = 6; 15.0%) and severe (N = 11; 27.5%) (Tables 1-3). Sex and age showed no significant difference among the groups. BMI was significantly lower according to the severity of liver injury (25.39, 24.47, and 22.27 kg/m^2, respectively; P = .005): mild vs moderate (P = .346), moderate vs severe (P = .269), and mild vs severe (P = .001). There was no significant difference in clinical presentation between each

TABLE 1 Comparison of demographic and clinical features among patients with BGZILI of different severity

Parameter	Total number of patients (40)	Mild injury (23)	Moderate injury (6)	Severe injury (11)	*P* value
Female	34 (85.0%)	19 (82.6%)	5 (83.3%)	10 (90.9%)	.812
Age (years)	63 (54-70)	60 (54-66)	65 (44-75)	64 (54-71)	.590
BMI (kg/m^2)	24.35 (22.10-26.27)	25.39 (23.88-27.61)	24.47 (19.89-26.78)	22.27 (21.08-23.44)	.005†
Symptom					
Loss of appetite	23 (57.5%)	10 (43.5%)	3 (50.0%)	10 (90.9%)	.030*,#
Dark urine	23 (57.5%)	8 (34.8%)	4 (66.7%)	11 (100%)	.001*,#
Fatigue	22 (55.0%)	10 (43.5%)	3 (50.0%)	9 (81.8%)	.106
Nausea	15 (37.5%)	7 (30.4%)	4 (66.7%)	4 (36.4%)	.263
Jaundice	14 (35.0%)	1 (4.3%)	3 (50.0%)	10 (90.9%)	<.001*,#
Abdominal distension	12 (30.0%)	4 (17.4%)	4 (66.7%)	4 (36.4%)	.055
Vomiting	8 (20.0%)	3 (13.0%)	3 (50.0%)	2 (18.2%)	.129
Pruritus	5 (12.5%)	0 (0.0%)	1 (16.7%)	4 (36.4%)	.011*,#
RUCAM					
Possible (3-5)	4 (10.0%)	3 (13.0%)	0 (0.0%)	1 (9.1%)	.848
Probable (6-8)	20 (50.0%)	11 (47.8%)	4 (66.7%)	5 (45.5%)	
Highly probable(≥9)	16 (40.0%)	9 (39.1%)	2 (33.3%)	5 (45.5%)	
Latency (days)	45 (29-90)	45 (28-90)	60 (19-270)	60 (30-100)	.885
Duration of drug consumption (days)	49 (30-90)	45 (30-90)	55 (28-300)	60 (35-90)	.819

Abbreviations: BMI: body mass index; RUCAM: Roussel Uclaf Causality Assessment Method.

*The mild group vs the moderate group (P < .05).

†The mild group vs the severe group (P < .05).

#The moderate group vs the severe group (P < .05).

TABLE 2 Comparison of liver biochemical tests and laboratory data among patients with BGZILI of different severity

Parameter	Total (40)	Mild (23)	Moderate (6)	Severe (11)	*P* value
ALT onset (U/L)	673.5 (425.5-1044.0)	631.0 (436.0-1029.0)	390.0 (210.5-578.5)	756.0 (664.0-1491.0)	.043[#]
AST onset (U/L)	423.0 (202.0-754.0)	359.5 (236.3-591.8)	173.5 (116.5-257.3)	927.0 (708.5-1106.0)	.002[*,†,#]
AST/ALT onset	0.65 (0.46-0.84)	0.49 (0.43-0.76)	0.58 (0.20-0.90)	0.88 (0.70-1.36)	.014
ALP onset (U/L)	169.0 (117.0-219.0)	145.5 (112.0-209.0)	162.0 (111.3-408.3)	173.0 (146.0-237.0)	.417
GGT onset (U/L)	209.0 (101.5-321.5)	139.0 (99.5-307.5)	165.0 (82.0-373.5)	321.0 (100.0-495.0)	.480
TB onset (μmol/L)	59.0 (23.6-185.7)	23.8 (13.5-27.6)	74.9 (38.0-92.2)	192.2 (171.9-274.3)	<.001[*,†,#]
DB onset (μmol/L)	39.4 (8.6-119.3)	10. (3.6-14.9)	47.0 (23.2-70.7)	140.6 (112.9-181.7)	<.001[*,†,#]
ALT max (U/L)	742.5 (502.8-1162.2)	689.0 (486.0-1029.0)	764.0 (422.0-938.0)	900.0 (664.0-1491.0)	.403
AST max (U/L)	587.5 (260.5-763.8)	405.0 (243.5-674.0)	518.0 (214.8-735.8)	927.0 (708.5-1106.0)	.022[†,#]
AST/ALT max	0.73 (0.48-0.87)	0.53 (0.43-0.76)	0.79 (0.61-1.02)	0.88 (0.70-1.36)	.008[†]
ALP max (U/L)	173.0 (122.0-231.0)	154.0 (112.0-209.0)	224.5 (179.8-408.3)	173.0 (146.0-237.0)	.048*
GGT max (U/L)	224.0 (121.3-339.3)	164.0 (103.0-293.0)	330.0 (203.5-596.0)	298.0 (152.0-495.0)	.043*
TB max (μmol/L)	39.6 (19.6-145.3)	19.9 (16.5-26.4)	63.3 (52.7-87.5)	188.5 (145.3-274.3)	<.001[*,†,#]
DB max (μmol/L)	25.0 (6.9-112.4)	7.1 (3.9-14.0)	38.9 (27.7-66.6)	140.6 (112.9-181.7)	<.001[*,†,#]
INR	1.05 (0.98-1.13)	1.04 (0.97-1.08)	1.03 (0.97-1.31)	1.14 (1.05-1.24)	.069
CHOL (mmol/L)	4.58 (3.87-5.07)	4.77 (4.10-5.08)	4.35 (3.11-5.73)	3.68 (2.94-4.80)	.105
TG (mmol/L)	1.52 (1.02-2.35)	1.22 (1.01-1.61)	1.48 (0.84-2.33)	2.27 (1.57-3.75)	.040[†]
HDL-C (mmol/L)	1.16 (0.81-1.54)	1.38 (1.12-1.59)	1.01 (0.91-1.89)	0.47 (0.30-0.95)	.004[†,#]
LDL-C (mmol/L)	2.39 (1.94-3.02)	2.71 (2.20-3.04)	2.11 (1.69-3.35)	2.14 (1.62-2.51)	.141
GLU (mmol/L)	4.95 (4.53-5.30)	4.95 (4.57-5.64)	4.46 (3.99-5.01)	5.01 (4.77-5.34)	.137
AFP	5.26 (3.78-15.47)	4.83 (3.40-9.29)	6.98 (3.93-35.66)	17.08 (6.74-33.45)	.102
ANA					
Negative	11 (27.5%)	9 (39.1%)	1 (16.7%)	1 (9.1%)	.333
1:80	19 (47.5%)	10 (43.5%)	4 (66.7%)	5 (45.5%)	
1:160	5 (12.5%)	2 (8.7%)	0 (0.0%)	3 (27.3%)	
1:320	5 (12.5%)	2 (8.7%)	1 (16.7%)	2 (18.2%)	
IgG (mg/dL)	1395.0 (1192.5-1762.5)	1380.0 (1140.0-1740.0)	1480.0 (1082.0-2330.0)	1395.0 (1197.5-1783.0)	.819
IgG > 1.1 × ULN (1760)	9 (22.5%)	5 (21.7%)	2 (33.3%)	2 (18.2%)	.395
WBC ($\times 10^9$/L)	4.80 (4.28-6.10)	4.80 (4.30-5.80)	4.75 (4.43-7.31)	4.84 (3.70-6.61)	.839
LY% (%)	34.9 (29.0-40.4)	37.0 (31.9-44.3)	33.1 (24.1-38.3)	29.0 (25.0-30.7)	.007[†]
EO% (%)	2.8 (1.6-5.4)	2.9 (1.8-5.0)	5.2 (1.7-6.0)	1.5 (1.0-3.5)	.115
HGB (g/L)	132.0 (121.0-141.0)	133.0 (125.0-144.0)	122.0 (111.5-142.0)	130.0 (119.8-139.8)	.386
PLT ($\times 10^9$/L)	185.0 (151.0-226.0)	197.0 (156.0-234.0)	203.5 (139.0-323.3)	167.5 (130.5-207.3)	.407

Note: Data are expressed as median (25th ,75th). 'Onset' indicates the first biochemical results after the onset of BGZILI. 'Max' indicates biochemical results when the aminotransferases reached the maximum value during the clinical course.

Abbreviations: AFP, alpha foetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; CHOL, cholesterol; DB, direct bilirubin; EO, eosinophil granulocyte; GGT, gamma-glutamyl transferase; GLU, glucose; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IgG, immunoglobulin G; INR, international normalized ratio; LDL-C, low-density lipoprotein cholesterol; LY, lymphocyte; PLT, blood platelet; TB, total bilirubin; TG, triglyceride; WBC, white blood cell.

*The mild group vs the moderate group ($P < .05$).

†The mild group vs the severe group ($P < .05$).

#The moderate group vs the severe group ($P < .05$).

group, except for loss of appetite, dark urine and jaundice ($P = .030$, 0.001 and <.001 respectively).

Comparison of serum liver biochemical parameters among groups with different disease severity is shown in Figure S1. Compared with mild and moderate groups, the severe group had a significantly higher level of peak AST (or AST: ALT ratio) and bilirubin levels (Figure S1A-D). There was a significant difference in peak levels of ALP and GGT among the three groups (mild vs moderate

TABLE 3 Comparison of clinical course among patients with BGZILI of different severity

Parameters	Total (40)	Mild (23)	Moderate (6)	Severe (11)	*P* value
Injury patterns					
Hepatocellular	37 (92.5%)	22 (95.7%)	5 (83.3%)	10 (90.9%)	.410
Cholestatic	2 (5.0%)	0 (0.0%)	1 (16.7%)	1 (9.1%)	
Mixed	1 (2.5%)	1 (4.3%)	0 (0.0%)	0 (0.0%)	
Time for ALT decrease to 50% at peak ALT (days)	8 (5-11)	8 (5-16)	9 (6-11)	7 (4-12)	.808
Time for ALT normalization (days)	31 (24-47)	37 (26-59)	31 (16-42)	29 (22-40)	.343
Time for TB decrease to 50% at peak TB (days)	11 (7-17)	9 (7-11)	7 (4-17)	14 (11-18)	.219
Time for TB normalization (days)	26 (13-49)	9 (7-11)	22 (10-32)	47 (26-49)	.056
Duration of hospitalization (days)	11 (7-15)	8 (7-12)	12 (7-18)	16 (12-23)	.005†
Back to normalization within 6 months	37 (92.5%)	21 (91.3%)	6 (100.0%)	10 (90.9%)	.751
Follow-up time (months)	26.3 (3.5-38.4)				

Abbreviations: ALT, alanine aminotransferase; TB, total bilirubin.

†The mild group vs the severe group ($P < .05$).

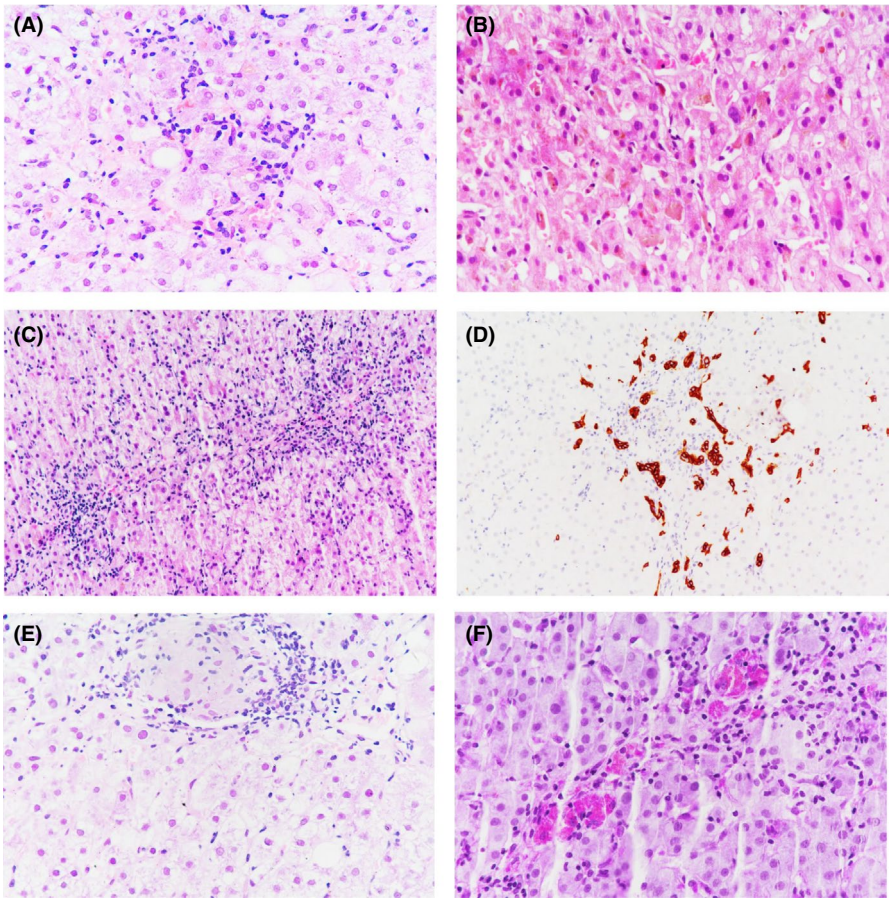

FIGURE 2 Typical histological features of BGZILI (200 × magnification). (A) Mild lobular activity with cholestasis (case 30, H&E). (B) Bland cholestasis around zone 3 (case 30, H&E). (C) Moderate interface hepatitis (case 37, H&E). (D) Mild mixed portal inflammation with moderate ductular reaction (case 36, CK19). (E) Epithelioid granuloma (case 30, H&E). (F) Pigmented ceroid-laden macrophages in perivenular areas (case 39, PAS-Diastase)

group: $P = .023$ for ALP and $P = .041$ for GGT) (Figure S1E-F). The injury pattern and RUCAM score showed no significant difference among the three groups. Neither was there any difference in the median latency period or median duration of consumption of BGZ. No significant differences were observed among the time needed for aminotransferases and TB to decrease from peak to half of their peak value. In the severe group, the time required for normalization of TB levels was longer than that for the mild and moderate groups, but no significant difference was observed. However, the duration of hospitalization was significantly longer in the severe group.

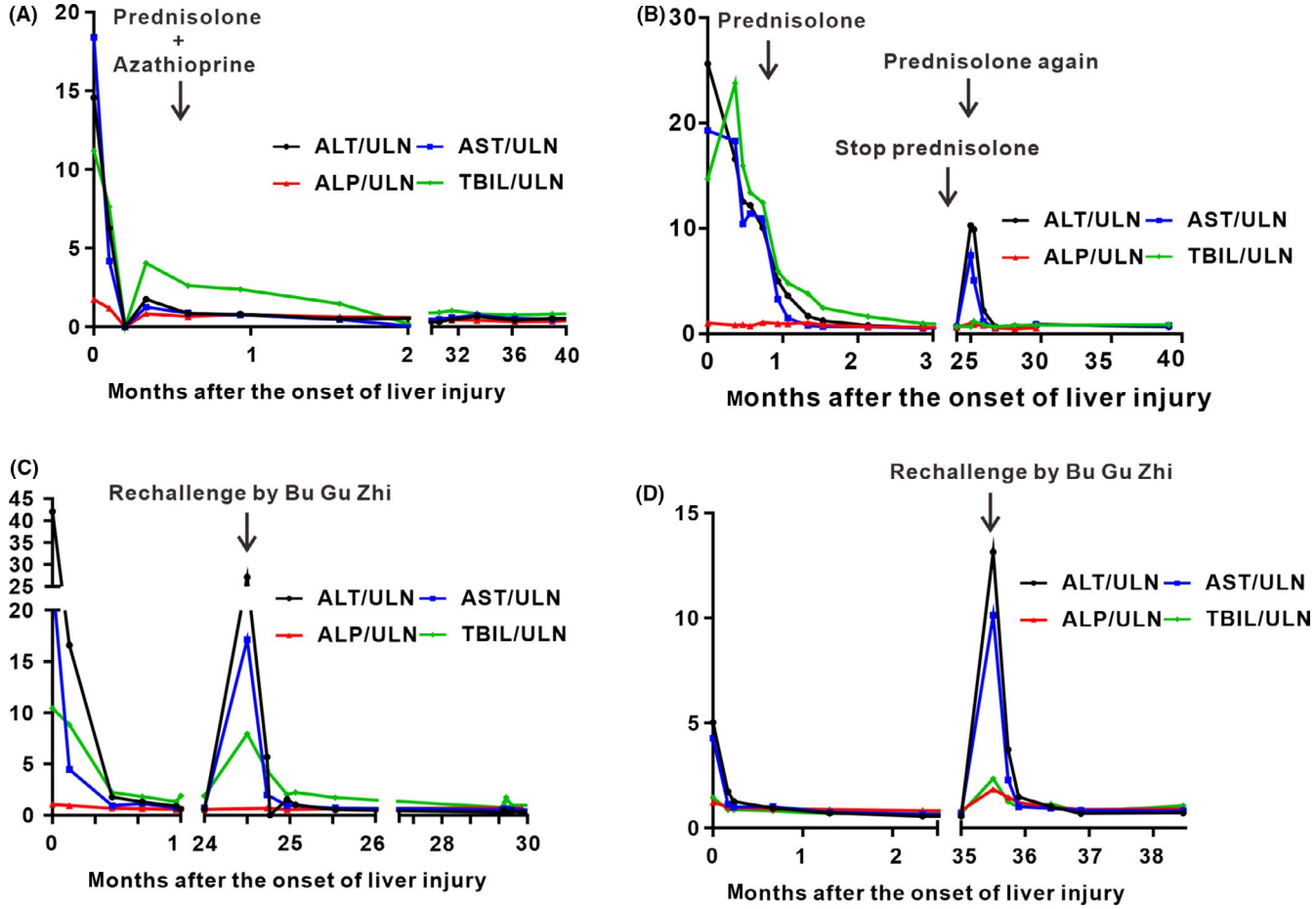

FIGURE 3 Changes in liver biochemistry of two patients who developed AIH and two patients who suffered from DILI because of rechallenge. (A) BGZ-unmasked AIH. Use of prednisolone and azathioprine 18 days after disease onset with current maintenance therapy (case 35). ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin. (B) BGZ-induced AIH. Institution of prednisolone 22 days after disease onset. Liver enzymes elevated after cessation of therapy. Normalization of serum ALT levels after reuse and maintenance of immunosuppresive therapy for the next 25 months (case 33). ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin. (C) Rechallenge with herbal compounds containing BGZ resulted in elevation of ALT levels that returned to normal after stopping BGZ use (case 30). ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin. (D) Another patient with rechallenge after unintended re-exposure to BGZ (case 22). ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin

3.4 | Comparison of clinical features between different indications of BGZ usage (treatment of bone diseases vs treatment of stomach/lung/prostate diseases)

All 40 patients took BGZ according to prescription dosages for various diseases: 82.5% (33/40) for osteoporosis/bone fractures, 2/7 for abdominal discomfort, 2/7 for respiratory diseases and 3/7 for prostate diseases. According to the different indications the cases were categorized into two groups: bone-disease and non-bone-disease groups. No significant difference was found in age between the two groups (Table S3). More male patients were affected in the non-bone-disease group. The onset and maximum ALT, AST, TB and INR were lower in the bone-disease group than that of the non-bone-disease group, even though no significant difference was shown. The time required for normalization of TB levels was significantly shorter in the bone-disease group (Table S3).

3.5 | Histology of BGZILI

Liver biopsies were performed in 9/40 BGZILI patients (Table S4). The median time from peak aminotransferase levels to liver biopsy was 9 (5-18) days. The indication for biopsy was when patients showed a tendency to have severe liver injury and/or took a longer time for resolution of biochemical indices. Characteristics of patients with and without biopsy were summarized and compared in Table S5.

Of the nine patients, four had both hepatitis and hepatocellular cholestasis ('cholestatic hepatitis injury pattern') (Figure 2A), one had cholestatic injury pattern (Figure 2B) and the remaining four had acute hepatitic injury pattern. The patient with cholestatic histological pattern had a corresponding cholestatic biochemical profile.

Mild to moderate mixed portal infiltration of lymphocytes, some plasma cells and eosinophils occurred in most cases, along with mild

interface hepatitis. Two patients with severe portal inflammation and moderate interface hepatitis (Figure 2C) were subsequently diagnosed with autoimmune hepatitis (AIH) (Figure 3). Interlobular bile ducts were preserved but reactive changes (eg irregularity or tortuosity) were observed frequently. Ductular reaction was noted in seven biopsies (Figure 2D).

Different extents of lobular activity were observed (7/9), ranging from spotty necroinflammation to obvious confluent (4/9) or bridging necrosis (3/9), the latter two corresponding to those with more clinical severity. Granulomas (Figure 2E), mild steatosis and ceroid-laden macrophages (Figure 2F) were also observed.

3.6 | Outcome of patients with BGZILI

Median duration of follow-up was 26.3 (range, 3.5-38.4) months. The time interval for levels of aminotransferases and TB to decrease from peak to half of peak level was 8 (range, 5-11) and 11 (range, 7-17) days respectively. The time required for normalization of ALT and ALP levels was 31 (range, 24-47) (Figure S2A,B) and 14 (range, 13-49) days respectively (Figure S2C). The time required for normalization of GGT and bilirubin levels was 67 (range, 41-113) (Figure S2D) and 26 (range, 13-49) days, respectively (Figure S2E-F).

It took a longer duration for the patients with severe BGZILI to recover. Follow-up data available in 37/40 (92.5%) patients revealed that they all recovered within 6 months after cessation of BGZ treatment. The liver biochemical parameters of the two AIH-like cases normalized after standard immunosuppressive therapy. During follow-up, one patient had disease recurrence after attempts at cessation of corticosteroid therapy, while the other patient who was compliant with therapy remained disease-free. These data suggest that these two cases may have BGZ-triggered AIH (Figure 3A,B). However, it is difficult to ascertain whether it is BGZ-unmasked authentic AIH or BGZ-induced AIH owing to the lack of baseline data. Both patients are well on low-dose immunosuppressive maintenance therapy. An additional two cases (5.0%) had flare of liver enzymes after the first episode of BGZILI, which was discovered to be because of an unintentional rechallenge using different herbal compounds containing BGZ (Figure 3C,D). They recovered fully after dechallenge. There was no mortality in this cohort.

4 | DISCUSSION

We summarize the clinicopathological features of BGZILI in a group of Chinese patients. The main symptoms were poor appetite, dark urine and fatigue. The predominant clinical phenotype was hepatocellular injury defined by R > 5 in 37/40 patients. Three-quarters of the cases had mild to moderate liver injury with a quarter experiencing severe injury; no mortality occurred. The severe cases had significantly more complaints such as fatigue, dark urine and jaundice; significantly higher onset/peak AST, AST: ALT ratio and onset/peak TB levels; a slower course of normalization of serum bilirubin; and longer duration of hospitalization.

Recent studies from the USA and Europe[1] have revealed that middle-aged women are more likely to be affected by herbal and dietary supplement (HDS)-induced liver injury. A significant proportion of them have a hepatocellular injury pattern (R > 5) with jaundice and greater disease severity.[1,4,8,37-39] BGZ is a HDS and, until now, 37 cases of BGZILI have been reported, mainly in the Chinese literature.[23,28,29,40] In accordance with our study, most investigations have shown disease remission and normalization of biochemical parameters after cessation of BGZ administration, with the exception of one fatal case reported from Korea.[14]

In China, the main indications for BGZ treatment include osteoporosis, abdominal discomfort, respiratory diseases and benign prostatic hyperplasia. Comparison of patients taking BGZ for bone diseases and those for non-bone diseases revealed that the former tended to have mild liver injury in terms of lower levels of ALT, AST, TB and INR, though no significant difference was observed. Although the bone-disease group tended to exhibit a more rapid initial increase of liver biochemical parameters, they recovered more rapidly. Conversely, the non-bone-disease group had a more protracted clinical course. It is uncertain if the different underlying diseases may have contributed to this slight but meaningful difference. Our current data suggest that patients taking BGZ for non-bone diseases tended to have more severe liver injury, so intensive monitoring is warranted in this patient population. This finding needs to be validated by larger cohort studies.

Nine out of 40 patients underwent a liver biopsy, with eight biopsied within 20 days of BGZILI onset. This group of patients tended to have severe liver injury with longer recovery time. In these cases, the rationale for the biopsy was to help exclude other potential causes of liver injury. In our study, two patients showed histological features of AIH comprising moderate interface hepatitis with prominent plasma cells. Differentiation between AIH and DILI can be difficult. Suzuki et al[41] found that no single feature was specific for AIH or DILI. They suggest that prominent intralobular lymphocytes, hepatocanalicular cholestasis and prominent portal neutrophils favour DILI, whereas higher degree of portal inflammation with predominant plasma cell infiltrates favour AIH. In addition, clinical course after dechallenge is helpful in differential diagnosis between DILI and AIH. Disease remission was achieved through treatment with corticosteroids and azathioprine in these two cases. One patient who had a relapse after cessation of immunosuppressive therapy, is currently doing well with normalization of ALT and IgG levels after 2 years of treatment. These data suggest that BGZ triggered or unmasked underlying AIH.

This study is the largest reported series of BGZILI using international criteria for DILI. We summarize the clinical and histological features of BGZILI which could help aid early diagnosis. However, our study has two main limitations. Firstly, as a single-centre study, our data cannot be generalized to all patients with BGZILI. Secondly, all the enrolled patients took compounds containing other herbs, which may have had contributed partly to the resulting liver injury.

5 | CONCLUSIONS

The main clinical pattern of BGZILI was a hepatocellular injury pattern, which tended to be mild to moderate in severity. Most patients recovered after stopping BGZ, and no patients had end-stage liver disease or died.

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CONFLICT OF INTEREST

All authors declare that we have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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