

Title: Association between the Concurrence of Pre-existing Chronic Liver Disease and Worse Prognosis in Patients with an Herb- *Polygonum multiflorum* Thunb. induced Liver Injury: A Case-control Study from a Specialized Liver Disease Center in China

Jing Jing^{1, 2†}, Rui-lin Wang^{2†}, Xin-yan Zhao³, Yun Zhu², Ming Niu⁴, Li-fu Wang², Xue-ai Song², Ting-ting He², Yong-qiang Sun², Wen-tao Xu², Si-miao Yu², Li-ping Wang², Yu-ming Guo⁴, Zhao-fang Bai⁴, Xiao-he Xiao^{1, 2*}, Jia-bo Wang^{4*}

¹ Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100853, China;

² Division of Integrative Medicine, Beijing 302 Hospital, Beijing 100039, China;

³ Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing, 100069, China.

⁴ Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039, China.

† Jing Jing and Rui-lin Wang contributed equally to this paper.

* To whom correspondence should be addressed: Jia-bo Wang, Institute of Chinese Herbal Medicine, Beijing 302 Hospital. Email: pharm_sci@126.com; Xiao-he Xiao, Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100039, China; Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039,

China. Email: pharmacy302xxh@126.com. Jia-bo Wang (pharm_sci@126.com) as show in Scholar One. Jia-bo Wang and Xiao-he Xiao contributed equally to this paper.

Conflict-of-interest statement:

The authors declared no conflict of interest.

Grant Support: This work was supported by the National Key Technology R&D Program (no. 2015ZX09501-004-001-008 and 2015ZX09501004-001-002), the National TCM Industry Science and Technology Program (no. 201507004-04), the National Natural Science Foundation of China (nos. 81373984 and 81403126) and Beijing Natural Science Foundation (no. 7152142).

Supplementary materials:

Table S1 Twelve types of Chinese patent medicines with PMT associated with 2 or more patients with HILI

Name of Chinese patent medicines with PMT	Number of cases (HILI with pre-existing CLD/entire HILI)	Constituents	Indications	Suggestion for patients with pre-existing CLD in the label	Hepatotoxic information
Yang-xue-sheng-fa capsule	2/14	<i>Radix rehmanniae preparata, Radix angelica sinensis, Rhizoma et radix notopterygii, Fructus chaenomelis, Rhizoma ligustici chuanxiong, Radix paeoniae alba, Semen cuscutae, Rhizoma gastrodiae, Radix polygoni</i>	Hair loss (n=13); Alopecia areata (n=1)	Following doctor's advice	Reaction labelled in the product instruction

<i>5ultiflora preparata</i>					
Jing-wu capsule/tablet	1/7	<i>Radix polygoni 5ultiflora preparata, Rhizoma 5 ultiflora, Fructus ligustri lucidi, Herba ecliptae</i>	Hair loss (n=4); Alopecia areata (n=1); Amnesia (n=2)	None	Reaction published but unlabeled
Xin-yuan capsule	1/5	<i>Radix polygoni 5ultiflora preparata, Salviae miltiorrhizae; Rehmanniae radix</i>	Coronary heart disease (n=4); Hyperlipoidemia (n=1)	None	Reaction published but unlabeled
Yan-shou tablet	1/4	<i>Polygonum multiflorum Thunb., Cuscutae semen, Eucommiae cortex, Ecliptae herba, Ligustri lucidi fructus, Rehmanniae radix, Achyranthis bidentatae radix, mulberry, Sesami semen nigrum, Sojae semen nigrum</i>	Health improvement (n=3); Hyperlipoidemia (n=1)	None	Reaction published but unlabeled

Qi-bao-mei-ran pill/granule	0/4	<i>Radix polygoni Multiflora preparata, Cuscutae semen, Poria, Radix angelica sinensis, Lycii fructus, Achyranthis bidentatae radix, Psoraleae fructus</i>	Hair loss (n=4)	Following doctor's advice	Reaction published but unlabeled
Jian-yang capsule	1/4	Extracts from <i>Rhizoma Polygonati, Morindae officinalis radix, Lycii fructus, Ganoderma, Radix polygoni Multiflora preparata, Radix Notoginseng</i>	Health improvement (n=4)	None	Reaction unknown
Gu-shen-sheng-fa pill	1/3	<i>Polygonum multiflorum Thunb., Lycii fructus, Notoptergii 6 ultifl et radix, Radix Polygoni 6 hizome 6 ra praeparata, Chuanxiong 6hizome, Chaenomelis fructus, Ligustri lucidi fructus, Radix angelica sinensis, mulberry, Salviae miltiorrhizae; Codonopsis radix,</i>	Hair loss (n=3)	Following doctor's advice	Reaction unknown

<i>Sesami semen nigrum</i>					
An-shen-bu-nao liquid	1/2	<i>Cervi cornu pantotrichum, Radix polygoni 7 ultiflora preparata, Epimedii folium, Rhizoma Zingiberis, Radix Glycyrrhizae, Jujubae fructus, Vitamin B₁</i>	Insomnia (n=2)	Following doctor's advice	Reaction unknown
Kun-bao pill	1/2	<i>Ligustri lucidi fructus, Fructus Rubi, Semen Cuscutae, Fructus Lycii, Polygonum multijiorum Thunb., Carapax et Plastrum Testudinis, Cortex Lycii, Radix Adenophorae, Radix Ophiopogonis, Semen Ziziphi Spinosae, Radix Rehmanniae, Radix Paeoniae Alba, Radix Paeoniae Rubra, Radix Angelica sinensis, Caulis Spatholobi, Concha Margaritifera, Herba Dendrobii, Flos</i>	Gynecologic diseases (n=2)	None	Reaction published but unlabeled

		<i>Chrysanthemi, Herba Ecliptae, Folium Mori,</i>			
		<i>Radix Cynanchi Atrati, Rhizoma</i>			
		<i>Anemarrhenae, Radix Scutellariae</i>			
Run-zhao-zhi-yang capsule	2/2	<i>Polygonum multijiorum</i> Thunb., <i>Polygoni</i>	Skin diseases (n=2)	Inappropriate	Reaction published
		<i>Multiflori Radix Praeparata, Radix</i>		use	but unlabeled
		<i>Rehmanniae, Folium Mori, Radix Sophorae</i>			
		<i>Flavescentis, Honghuoma</i>			
Shou-wu-yan-shou tablet	0/2	<i>Polygonum multiflorum</i> Thunb.	Hair loss (n=1); Insomnia (n=1)	None	Reaction labelled in the product instruction

Abbreviations used: CLD, chronic liver disease; HILI, herb-induced liver injury;.PMT, *Polygonum multiflorum* Thunb.

Table S2. Herbs combined with PMT in 6 or more patients with HILI after ingestion of single PMT and its preparations.

Names of herbs	Numbers of patients with HILI (n)	Numbers of patients with pre-existing CLD (n)	Clinical report of liver reaction induced by the single herb	Effect on liver in animal/cellular experiments		Hepatotoxic information by Chinese Pharmacopeia
				Hepatotoxicity	Hepatoprotective effect	
<i>Rehmanniae radix</i>	40	13	No	No	Yes	No
<i>Radix angelica sinensis</i>	31	7	No	No	Yes	No
<i>Cuscutae semen</i>	26	4	No	No	No	No
<i>Ligustri lucidi fructus</i>	21	5	No	No	Yes	No
<i>Paeoniae Radix</i>	20	5	No	No	Yes	No

<i>Alba</i>						
<i>Notoptergii</i>	19	3	No	No	Yes	No
<i>rhizoma et radix</i>						
<i>Chaenomelis</i>	19	3	No	No	No	No
<i>fructus</i>						
<i>Chuanxiong</i>	19	3	No	No	No	No
<i>rhizoma</i>						
<i>Gastrodiae</i>	17	2	No	No	No	No
<i>Rhizoma</i>						
<i>Ecliptae Herba</i>	17	4	No	No	No	No
<i>Lycii fructus</i>	17	5	No	No	No	No
<i>Salviae</i>	15	5	Yes	Yes	No	No
<i>miltiorrhizae</i>						
<i>Polygonati</i>	12	3	No	No	No	No

<i>rhizoma</i>							
<i>Sesami semen</i>	12	4	No	No	No	No	
<i>nigrum</i>							
<i>Achyranthis</i>	10	1	No	No	Yes	No	
<i>bidentatae radix</i>							
<i>Poria</i>	9	2	Yes	No	Yes	No	
<i>Mulberry</i>	8	2	No	No	No	No	
<i>Sojae Semen</i>	7	1	No	No	No	No	
<i>Nigrum</i>							
<i>Glycyrrhizae</i>	7	2	No	No	Yes	No	
<i>radix et rhizoma</i>							
<i>Morindae</i>	6	2	No	No	No	No	
<i>officinalis radix</i>							

Abbreviations: CLD, chronic liver disease; HILI, herb-induced liver injury; PMT, *Polygonum multiflorum* Thunb.

Table S3. The clinical characteristics of all HILI patients with pre-existing CLD.

Characteristics	HILI patients with pre-existing ALD (n=17)	HILI patients with pre-existing NAFLD (n=8)	HILI patients with pre-existing chronic viral hepatitis (n=5)	HILI patients with pre-existing autoimmune liver disease (n=3)
Males (%)	15(88.2%)	4(50.0%)	3(60.0%)	0(0.0%)
Age (years, mean±SD)	45.10±11.51	41.12±8.55	48.94±22.47	54.81±12.52
BMI (kg/m ² , mean±SD)	24.13±3.60	26.71±2.19	24.66±2.49	21.91±6.33
Latency (day, median [IQR])	45.00(29.50,69.50)	29.00(21.00,156.00)	72.00(36.00,601.00)	133.00(45.00,133.00)
Peripheral eosinophilia (×10 ⁹ /L, mean±SD)	0.23±0.09	0.25±1.45	0.19±0.16	0.10±0.06
Positive autoantibody	2(11.8%)	5(62.5%)	1(20.0%)	3(100.0%)
Peak values of laboratory index				
ALT (U/L, mean±SD)	1469.36±810.41	1336.90±494.26	1328.60±515.81	264.67±220.76

AST (U/L, mean±SD)	924.75±586.88	978.36±481.11	855.40±493.82	331.67±243.55
ALP (U/L, mean±SD)	185.63±62.21	169.64±98.69	202.00±68.02	235.67±88.12
GGT (U/L, median [IQR])	160.00(113.00,187.00)	209.00(196.25,257.25)	141.00(76.00,240.00)	231(175.00,231.00)
TB (mg/dL, mean±SD)	18.96±11.54	17.63±9.83	16.00±13.56	13.27±8.46
INR (median, [IQR])	1.11(1.02,1.40)	1.21(0.97,1.40)	0.91(0.90,2.13)	1.20(0.91,1.20)
TC (mmol/L, mean±SD)	3.62±1.52	4.39±1.27	3.50±0.95	4.17±2.15
TG (mmol/L, mean±SD)	2.61±1.12	3.32±1.99	2.68±1.10	2.20±0.67
Pattern of liver injury				
HC/Chol/Mixed	17/0/0	8/0/0	4/1/0	1/1/1
RUCAM score (mean±SD)				
Possible/probable/highly probable	1/16/0	0/7/1	0/5/0	1/2/0
Severity of Liver Injury[†]				

Mild/Moderate/Severe/Liver failure/Fatal	0/2/12/2/1	0/0/8/0/0	1/0/3/0/1	0/0/2/0/1
MELD score (mean±SD)	16.88±5.97	17.13±5.94	13.80±4.38	17.00±4.36
Liver cirrhosis	3/17	0/8	1/5	1/3
Prognosis				
Recovery (n)	10	5	4	1
Chronic (n)	6	3	0	1
Fatal (n)	1	0	1	1

† The severity assessments of HILI were graded as follows^{31,32}: mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl and international normalized ratio (INR) <1.5; moderate elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and/or ALP levels and TB ≥5 mg/dl, with or without INR≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1 mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy, or other organ failure related to DILI; death or liver transplantation because of DILI.

Abbreviations: ALD, alcoholic liver disease; ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; DILI, drug-induced liver injury; GGT, gamma-glutamyl transpeptidase; HC, hepatocellular; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB, serum total bilirubin; TC, total cholesterol; TG, total glyceride

Table S4. The comorbidities of patients with HILI.

Names of the comorbidities	Total (n=145)	HILI with pre-existing CLD (n=33)	HILI without pre-existing CLD (n=112)	p value
Gastritis	21 (14.5%)	6 (18.2%)	15 (13.4%)	0.332
Hypertension	17 (11.7%)	4 (12.1%)	13 (11.6%)	0.573
Cholecystic diseases	9 (6.2%)	3 (9.1%)	6 (5.4%)	0.335
Infectious diseases	8 (5.5%)	4 (12.1%)	4 (3.6%)	0.079
Cardiovascular disease	6 (4.1%)	4 (12.1%)	2 (1.8%)	0.024
Diabetes mellitus	5 (3.4%)	0 (0.00%)	5 (4.5%)	0.269
Connective tissue diseases	4 (2.8%)	2 (6.1%)	2(1.8%)	0.223
Kidney diseases	3 (2.1%)	1 (3.0%)	2 (1.8%)	0.542
Thyroid diseases	3 (2.1%)	1 (3.0%)	2 (1.8%)	0.542
Osteonosis	2 (1.4%)	2 (6.1%)	0 (0.0%)	0.051

Andrology	1 (0.7%)	0 (0.0%)	1 (0.9%)	0.772
Otopathy	1 (0.7%)	0 (0.0%)	1 (0.9%)	0.772

Abbreviations: CLD, chronic liver disease; HILI, herb-induced liver injury

Table S5. Comparison of the clinical characteristics in the overall PMT-related HILI

patients between the recovery group and the chronic group.

Characteristics	Recovery group (n=119)	Chronic group (n=23)	<i>p</i> value
Males (%)	55(46.2%)	10(43.5%)	0.809
Age (years, mean±SD)	42.45±14.06	47.25±10.08	0.121
BMI (kg/m ² , mean±SD)	22.46(20.51,25.39)	24.03(22.22,28.01)	0.021
Latency (day, median [IQR])	49(31,78)	57(30,121)	0.803
Duration of drug use (day, median [IQR])	36(30,72)	31(19,122)	0.708
Alcohol use [†] (%)	21(17.6%)	6(26.1%)	0.249
Pre-existing CLD	22(18.5%)	9(39.1%)	0.028
Peak value of ALT (U/L, median [IQR])	1247.00(874.00,1583.00)	1000.00(739.10,1477.40)	0.075
Peak value of AST (U/L, median [IQR])	739.00(493.00,1050.00)	800.00(547.00,1294.00)	0.585
Peak value of ALP (U/L, median [IQR])	176.00(141.00,214.00)	193.00(141.00,221.10)	0.958
Peak value of GGT (U/L, median [IQR])	165.00(105.00,242.00)	174.00(71.00,257.00)	0.539
Peak value of TB (mg/dL, median [IQR])	10.67(6.89,17.74)	14.57(5.18,24.86)	0.448
Peak value of INR (median	1.05(0.98,1.13)	1.15(1.01,1.46)	0.022

[IQR])			
Lowest serum albumin (g/L, median [IQR])	35.00(32.00,38.00)	30.00(25.00,37.00)	0.003
Lowest cholinesterase (U/L, mean±SD)	5199.61±1579.26	3938.26±2055.55	0.010
TC (mmol/L, median [IQR])	3.79(2.94,4.35)	3.78(2.57,4.86)	0.771
TG (mmol/L, median [IQR])	2.51(1.71,3.45)	2.03(1.22,3.17)	0.080
Laboratory index in DILI recognition			
WBC (×10 ⁹ /L, median [IQR])	5.35(4.40,6.42)	5.26(4.12,6.79)	0.998
HGB (g/L, mean±SD)	136.67±18.10	129.04±16.40	0.063
PLT (×10 ⁹ /L, mean±SD)	222.11±66.04	176.96±78.20	0.004
peripheral eosinophilia (×10 ⁹ /L, median [IQR])	0.16(0.10,0.28)	0.18(0.11,0.27)	0.831
IgA (g/L, median [IQR])	2.41(1.64,2.57)	2.57(2.01,3.25)	0.197
IgG (g/L, median [IQR])	12.70(10.00,13.30)	13.18(11.56,18.48)	0.011
IgM (g/L, median [IQR])	0.89(0.52,1.01)	0.84(0.63,1.11)	0.538
Pattern of liver injury			
HC/Chol/Mixed (%)	115/1/3	21/1/1	0.250
RUCAM score (median [IQR])	8(7,8)	7(6,8)	0.113
Possible/probable/highly	7/99/13	2/18/3	0.729

probable

Severity of Liver Injury[†] (%)

of column total)

	7/14/91/7/0	1/4/14/3/1	0.119
Mild/Moderate/Sever/Liver failure/Fatal			
MELD score (mean±SD)	14.41±4.77	15.61±7.20	0.451

[†] Patients with a history of alcoholism (alcohol intake of >2 drinks per day in women and >3 drinks per day in men) did not drink during 3 months prior to the onset of liver injury.

[‡] The severity assessments of HILI were graded as follows^{31,32}: Mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl, and international normalized ratio (INR) <1.5; moderate, elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and (or) ALP levels and TB ≥5 mg/dl, with or without INR ≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy, or other organ failure related to DILI; fatal, death or liver transplantation because of DILI.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; CLD, chronic liver diseases; DILI, drug-induced liver injury; HC, hepatocellular; HGB, hemoglobin; HILI, herb-induced liver injury; INR, international normalized ratio; IQR,

interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; PLT, platelets;
RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB,
serum total bilirubin; TC, total cholesterol; TG, total glyceride; WBC, white blood cell

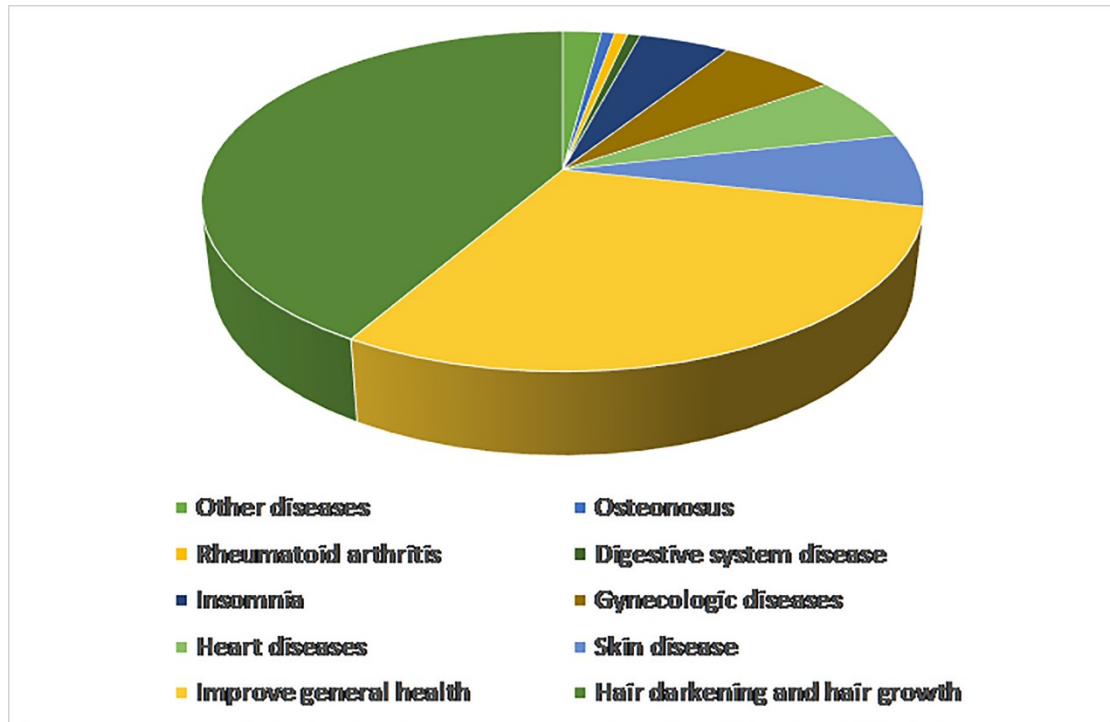


Figure S1. The indications for single PMT and its herbal products in total patients with HILI

Abbreviations: HILI, herb-induced liver injury; PMT, *Polygonum multiflorum* Thunb.