Herbal medicine containing aristolochic acid and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection

Chi-Jen Chen1,2, Yao-Hsu Yang1,3,4,5, Meng-Hung Lin1, Chuan-Pin Lee1, Yu-Tse Tsan6,7, Ming-Nan Lai8, Hsiao-Yu Yang4,9, Wen-Chao Ho10, Pau-Chung Chen4,9,11 and The Health Data Analysis in Taiwan (hDATa) Research Group

1 Health Information and Epidemiology Laboratory, Chang Gung Memorial Hospital, Chiayi County, Taiwan
2 Graduate Institute of Data Science, Taipei Medical University, Taipei, Taiwan
3 Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chiayi County, Taiwan
4 Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan
5 School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan
6 Division of Occupational Medicine, Department of Emergency Medicine, Taichung Veterans General Hospital, Taichung, Taiwan
7 School of Medicine, Chung Shan Medical University, Taichung, Taiwan
8 Department of Statistics, Feng Chia University, Taichung, Taiwan
9 Department of Public Health, National Taiwan University College of Public Health, Taipei, Taiwan
10 Department of Public Health, China Medical University, Taichung, Taiwan
11 Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

It was suspected that aristolochic acid-induced mutations may be associated with hepatitis B virus (HBV), playing an important role in liver carcinogenesis. The purpose of this study was to investigate the association between the use of Chinese herbs containing aristolochic acid and the risk of hepatocellular carcinoma (HCC) among HBV-infected patients. We conducted a retrospective, population-based, cohort study on patients older than 18 years who had a diagnosis of HBV infection between January 1, 1997 and December 31, 2010 and had visited traditional Chinese medicine clinics before one year before the diagnosis of HCC or the censor dates. A total of 802,642 HBV-infected patients were identified by using the National Health Insurance Research Database in Taiwan. The use of Chinese herbal products containing aristolochic acid was identified between 1997 and 2003. Each patient was individually tracked from 1997 to 2013 to identify incident cases of HCC since 1999. There were 33,982 HCCs during the follow-up period of 11,643,790 person-years and the overall incidence rate was 291.8 HCCs per 100,000 person-years. The adjusted hazard ratios (HRs) were 1.13 (95% confidence interval [CI], 1.11–1.16), 1.21 (95% CI, 1.13–1.29), 1.37 (95% CI, 1.24–1.50) and 1.61 (95% CI, 1.40–1.84) for estimated aristolochic acid of 1–250, 251–500, 501–1,000 and more than 1,000 mg, respectively, relative to no aristolochic acid exposure. Our study found a significant dose-response relationship between the consumption of aristolochic acid and HCC in patients with HBV infection, suggesting that aristolochic acid which may be associated with HBV plays an important role in the pathogenesis of HCC.

The consumption of Chinese herbs containing aristolochic acid has been associated with an increased risk of urothelial carcinoma.1,2 The International Agency for Research on Cancer listed that plants containing aristolochic acid are carcinogenic to humans (Group 1) in 20023 and that aristolochic acid is carcinogenic to humans (Group 1) in 2012.4

Key words: herbal medicine, aristolochic acid, hepatocellular carcinoma, hepatitis B virus infection

Abbreviations: ACE: angiotensin-converting enzyme; CI: confidence interval; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HR: hazard ratio; ICD-9: International Classification of Diseases, 9th Edition; NHI: National Health Insurance; NSAIDs: nonsteroidal anti-inflammatory drugs

Chi-Jen Chen and Yao-Hsu Yang share co-first authorship.

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Correspondence to: Wen-Chao Ho, Department of Public Health, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan, E-mail: wcho@mail.cmu.edu.tw; or Pau-Chung Chen, Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, 17 Xuzhou Road, Taipei 10055, Taiwan, E-mail: pchen@ntu.edu.tw
There were a series of studies of Chinese herbs containing aristolochic acid that reported increased risks of chronic kidney disease, kidney failure and urinary tract cancers in Taiwan.\(^5\)\(^–\)\(^7\) In addition, increased risk of kidney failure and urinary tract cancers were also found among Chinese herbalists.\(^8\)\(^–\)\(^11\) Further, Chen et al. investigated 152 patients with upper urinary tract urothelial carcinoma and found the 93 had been exposed to aristolochic acid based on the presence of aristolactam-DNA adducts and AA-mutational signature in the renal cortex.\(^12\)\(^,\)\(^13\) Finally, aristolactam-DNA adducts were also detected in 76% of clear cell renal cell carcinoma (ccRCC) and AA-mutational signature was evident in 6 of 10 sequenced ccRCC exomes from Taiwanese patients.\(^14\)

Aristolochic acid treatment in rat resulted in DNA adduct formation, and increased mutant frequency in the liver as well as kidneys.\(^15\) It was suspected that aristolochic acid-induced mutations in concert with hepatitis B virus (HBV) play an important role in liver carcinogenesis.\(^16\)\(^,\)\(^17\) Hepatitis B virus (HBV) infection is prevalent in Taiwan, especially before the implementation of the nationwide hepatitis B vaccination program in July 1984.\(^18\)\(^–\)\(^20\) Although plants containing aristolochic acid were prohibited in several countries and in Taiwan in November 2003\(^21\) approximately one-third of people in Taiwan had been prescribed Chinese herbs containing aristolochic acid between 1997 and 2003.\(^22\) The purpose of this study was to investigate the association between the use of herbal medicine containing aristolochic acid and the risk of hepatocellular carcinoma (HCC) among HBV-infected patients.

**Methods**

**Data source**

We used the National Health Insurance (NHI) Research Database. The NHI program provides compulsory universal health insurance, implemented on March 1, 1995, that covers health care services from western medicine to traditional Chinese medicine, from dental care to parturition, and from preventive services to elderly home care in around 99% of the island’s population. We used databases for admissions and outpatient visits, both of which included information on patient characteristics, including sex, date of birth, date of admission, date of discharge, dates of visits and up to five discharge diagnoses or three outpatient visit diagnoses by International Classification of Diseases, Ninth Revision (ICD-9) classification. The data files also contained information on patient prescriptions, including the names of prescribed drugs, dosage, duration and total expenditure. We conducted a population-based cohort study on patients older than 18 years who had a diagnosis of HBV infection (ICD-9 codes 070.2, 070.3 and V02.61) without hepatitis C virus (HCV) coinfection (ICD-9 codes 070.7, 070.41, 070.44, 070.51, 070.54 and V02.62) between January 1, 1997 and December 31, 2010 (Fig. 1).\(^23\)\(^,\)\(^24\) To increase exposure frequency and ensure comparability, we included only patients who had visited traditional Chinese medicine clinics between January 1, 1997 and one year before the diagnosis of HCC or the censor dates in the HBV-infected cohort. Strict confidentiality guidelines were closely followed in accordance with personal electronic data protection regulations; the National Health Research Institutes of Taiwan anonymizes and maintains the NHI reimbursement data as files suitable for research. This study was also approved by the Institutional Review Board of the National Taiwan University Hospital.

**Exposure to herbal medicine containing aristolochic acid**

According to standard prescriptions recommended by the Committee on Chinese Medicine and Pharmacy in Taiwan, herbal medicine produced before new regulations were promulgated in November 2003 might include the following herbs containing aristolochic acid: Guan Mu Tong (\textit{Aristolochia manshurienisis}), Guang Fang Ji (\textit{Aristolochia fangjii}), Ma Dou Ling (fruits of \textit{Aristolochia debils} or \textit{Aristolochia contorta}), Qing Mu Xiang (roots of \textit{A. debils}), Tian Xian Teng (stems and leaves of \textit{A. debils} or \textit{A. contorta}), and Xi Xin (\textit{Asarum heterotropoides} or \textit{Asarum sieboldii}).\(^25\) These herbs were taken as single products or were components of mixed herbal formulas that are recommended by ancient Chinese medicine books. We determined the original amount of herbs, in grams, for each mixture of herbal medicine and the total dose of each aristolochic acid-containing herb during the exposure period from January 1, 1997 to October 31, 2003. To allow a minimal induction time for an exposed subject to develop HCC, we calculated the cumulative dose for each herb prescribed to an individual up to one year before the diagnosis of HCC or the censor dates. We also calculated the estimated cumulative dose of aristolochic acid for each subject using an estimated average dose of aristolochic acid per 1 g. For Guan Mu Tong, Guang Fang Ji, Ma Dou Ling, Qing Mu Xiang, Tian Xian Teng and Xi Xin this was 2.59,
2.04, 0.63, 0.009, 0.026 and 0.042 mg, respectively.\textsuperscript{26–30} The weight of patients was not available through the database; therefore, the AA intakes are presented as the total AA intake in mg, instead of showing it as milligrams per kilogram of body weight.

Diagnosis of hepatocellular carcinoma
Patients with HCC (ICD-9 code 155.0) were identified in the admission files with the first-time diagnosis date as the index date. For the diagnosis of HCC, the American Association for the Study of Liver Diseases Practice Guidelines were recommended by the Bureau of NHI.\textsuperscript{31} Only patients admitted for HCC were included to increase the validity of the diagnosis. Newly diagnosed patients between January 1, 1999 and December 31, 2013 were further analyzed to allow at least 2 years for subjects to accumulate doses of herbal medicine sufficient to induce HCC.

Potential confounders
Comorbidities, identified in a systematic way, were treated as potential confounders defined by the following diagnoses recorded between January 1, 1997 and one year before the diagnosis of HCC or the censor dates: liver cirrhosis (ICD-9 codes 571.2, 571.5, 571.6, 572.2, 572.3, 572.4, 572.8 and 573.0), alcohol-related disease (291, 303.0, 303.9, 305.0, 571.0, 571.1, 571.2 and 571.3), nonalcoholic steatohepatitis (571.8 and 571.9), cholelithiasis (574), hypertension (401), diabetes (250), hyperlipidemia (272) and chronic obstructive pulmonary disease (491 and 492). The prescriptions of medications that could confound the association between taking herbal medicine containing aristolochic acid and cancer risk were further identified, including anti-HBV treatments (i.e., interferon, lamivudine, entecavir, adefovir dipivoxil and telbivudine), aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme (ACE) inhibitors (i.e., captopril, enalapril, lisinopril, perindopril, ramipril, quinapril, benazepril, cilazapril and fosinopril), metformin and statins (i.e., simvastatin, lovastatin, atorvastatin, fluvastatin, pravastatin and rosuvastatin). Sociodemographic characteristics (age, sex, income and level of urbanization) were also considered in the model. Urbanization levels in Taiwan are divided into four strata according to the Taiwan National Health Research Institute publications, with level I referring to the most urbanized communities and level IV referring to the least urbanized communities.

Statistical analyses
We used the Kaplan–Meier method to estimate HCC cumulative incidences. The log-rank test was performed to examine differences in the risk of HCC in the cohort. We also used the direct method to adjust the incidence rates according to the age and sex distribution of the general population of Taiwan during the period 1999–2013. Finally, Cox proportional hazards models were used to compute the hazard ratios (HRs) and accompanying 95% CIs before and after adjustment for the variables including sex, age, monthly income, urbanization, liver cirrhosis and diabetes.\textsuperscript{32,33} Deceased patients (with a death date in the admission file) and those from the beneficiaries who were lost to follow-up were censored.

We conducted sensitivity analyses to evaluate the difference and consistency between taking herbal medicine containing aristolochic acid and the risk of HCC and to test for interaction in the subgroup effects. Cox proportional hazards regression models with two-year induction time, competing mortality, propensity score matching and time-dependent covariates were also used to estimate the relationship between estimated aristolochic acid and HCC to avoid potential confounding or time-related biases. A two-tailed \textit{p} value of 0.05 was considered significant. All these analyses were conducted using SAS statistical software (version 9.4; SAS Institute, Cary, NC).

Results
A total of 802,642 HBV-infected patients were included in the study cohort, of whom 59.4% had taken herbal medicine containing aristolochic acid, respectively. Table 1 lists the
Table 1. Demographics and clinical characteristics of the patients with HBV infection

| Demographics and clinical characteristics | Patients who took HM containing AA % (n=477,115) | Patients who did not take HM containing AA % (n=325,527) |
|--------------------------------------------|-----------------------------------------------|-------------------------------------------------------|
| Sex                                        |                                               |                                                       |
| Female                                     | 47.5                                          | 39.2                                                  |
| Male                                       | 52.5                                          | 60.8                                                  |
| Age, year                                  |                                               |                                                       |
| 18–29                                      | 32.4                                          | 36.0                                                  |
| 30–39                                      | 30.0                                          | 28.1                                                  |
| 40–49                                      | 21.8                                          | 20.4                                                  |
| 50–59                                      | 9.7                                           | 9.5                                                   |
| ≥60                                        | 6.1                                           | 6.0                                                   |
| Mean                                       | 37.3                                          | 36.6                                                  |
| Standard deviation                          | 12.6                                          | 12.9                                                  |
| Monthly income, NT$                        |                                               |                                                       |
| 0                                          | 16.9                                          | 19.6                                                  |
| 1–15,840                                   | 16.9                                          | 17.2                                                  |
| 15,841–25,000                              | 40.8                                          | 36.6                                                  |
| >25,000                                    | 25.3                                          | 26.7                                                  |
| Urbanization level                         |                                               |                                                       |
| I                                          | 31.1                                          | 31.6                                                  |
| II                                         | 47.7                                          | 47.0                                                  |
| III                                        | 14.8                                          | 14.4                                                  |
| IV (rural area)                            | 6.4                                           | 6.9                                                   |
| Guan Mu Tong, g                            |                                               |                                                       |
| 0                                          | 51.4                                          | 100.0                                                 |
| 1–30                                       | 33.0                                          | 0.0                                                   |
| 31–60                                      | 7.7                                           | 0.0                                                   |
| 61–100                                     | 3.8                                           | 0.0                                                   |
| 101–200                                    | 2.8                                           | 0.0                                                   |
| >200                                       | 1.4                                           | 0.0                                                   |
| Guang Fang Ji, g                           |                                               |                                                       |
| 0                                          | 49.7                                          | 100.0                                                 |
| 1–30                                       | 44.8                                          | 0.0                                                   |
| 31–60                                      | 3.4                                           | 0.0                                                   |
| 61–100                                     | 1.2                                           | 0.0                                                   |
| 101–200                                    | 0.7                                           | 0.0                                                   |
| >200                                       | 0.3                                           | 0.0                                                   |
| Ma Dou Ling, g                             |                                               |                                                       |
| 0                                          | 99.1                                          | 100.0                                                 |
| 1–30                                       | 0.6                                           | 0.0                                                   |
| 31–60                                      | 0.2                                           | 0.0                                                   |
| 61–100                                     | 0.1                                           | 0.0                                                   |
| >100                                       | 0.1                                           | 0.0                                                   |
| Qing Mu Xiang, g                           |                                               |                                                       |
| 0                                          | 54.2                                          | 100.0                                                 |
| Estimated aristolochic acid, mg            |                                               |                                                       |
| 0                                          | 0.0                                           | 100.0                                                 |
| 1–250                                      | 94.2                                          | 0.0                                                   |
| 251–500                                    | 3.9                                           | 0.0                                                   |
| 501–1,000                                  | 1.5                                           | 0.0                                                   |
| >1,000                                     | 0.5                                           | 0.0                                                   |
| Disease                                    |                                               |                                                       |
| Liver cirrhosis                            | 6.6                                           | 7.0                                                   |
| Alcohol-related disease                    | 4.0                                           | 4.5                                                   |
| Nonalcoholic steatohepatitis               | 10.3                                          | 9.9                                                   |
| Cholelithiasis                             | 8.1                                           | 7.7                                                   |
| Hypertension                               | 30.6                                          | 30.0                                                  |
| Diabetes                                   | 20.0                                          | 19.9                                                  |
| Hyperlipidemia                             | 32.1                                          | 30.8                                                  |
| Chronic obstructive pulmonary disease       | 11.1                                          | 8.8                                                   |
| Medication                                 |                                               |                                                       |
| Anti-HBV treatment                         | 5.0                                           | 5.8                                                   |
| Aspirin                                    | 16.4                                          | 15.2                                                  |
| NSAIDs                                     | 92.9                                          | 88.2                                                  |
| ACE inhibitors                             | 15.8                                          | 15.2                                                  |
| Metformin                                  | 10.2                                          | 10.9                                                  |
| Statins                                    | 15.5                                          | 15.5                                                  |

Abbreviations: AA, aristolochic acid; ACE, angiotensin-converting enzyme; HBV, hepatitis B virus; HM, herbal medicine; NSAIDs, nonsteroidal anti-inflammatory drugs; NT$, New Taiwan Dollar.
demographic characteristics, medical conditions and medication use of patients.

There were 33,982 HCCs during the follow-up period of 11,643,790 person-years and the overall incidence rate was 291.8 HCCs per 100,000 person-years. There was a higher risk (adjusted HR, 1.14; 95% CI, 1.12–1.17) of HCC in patients with HBV infection who took herbal medicine containing aristolochic acid (Table 2). Elevated HRs were also found for using >60 g of Guan Mu Tong and 200 g of Guang Fang Ji (Supporting Information, Table S1).

Regarding the cumulative dose of aristolochic acid, there were increasing trends in the age-sex standardized incidences associated with dose from none to >1,000 mg in the HBV-infected cohorts (Fig. 2). The adjusted HRs were 1.13 (95% CI, 1.11–1.16), 1.21 (95% CI, 1.13–1.29), 1.37 (95% CI, 1.24–1.50), and 1.61 (95% CI, 1.40–1.84) for estimated aristolochic acid of 1–250, 251–500, 501–1,000 and >1,000 mg, respectively, relative to no aristolochic acid exposure. There was also a significant dose–response trend \( \left( p < 0.0001 \right) \) when we applied the exposure variable as a continuous variable for 100-mg increase of estimated aristolochic acid (Table 2). The log-rank tests revealed significant observed differences \( \left( p < 0.001 \right) \) over the entire Kaplan–Meier curves (Fig. 3).

We still observed significantly higher risks of HCC in patients with HBV when using Cox proportional hazards regression with 2-year induction time, competing mortality,

Table 2. Crude and adjusted HRs of HCC associated with herbal medicine containing aristolochic acid during the follow-up period in the patients with HBV infection

| Herbal medicine containing aristolochic acid | No. of patients | No. of person-years | No. of patients with HCC | Incidence (per 10^5) | 95% CI | Crude HR | 95% CI | Adjusted HR^1 | 95% CI |
|----------------------------------------------|-----------------|---------------------|--------------------------|----------------------|--------|----------|--------|--------------|--------|
| All patients                                 | 802,642         | 11,643,790.2        | 33,982                   | 291.8                | 288.7–294.9 | 1.00     | 1.00   |              |        |
| No                                           | 325,527         | 4,732,298.0         | 13,223                   | 279.4                | 274.7–284.2 | 1.00     | 1.00   |              |        |
| Yes                                          | 477,115         | 6,911,492.2         | 20,759                   | 300.4                | 296.3–304.4 | 1.08     | 1.05–1.10 | 1.14    | 1.12–1.17 |
| Estimated aristolochic acid, mg              |                 |                     |                           |                      |        |          |        |              |        |
| 0                                            | 325,527         | 4,732,298.0         | 13,223                   | 279.4                | 274.7–284.2 | 1.00     | 1.00   |              |        |
| 1–250                                        | 449,261         | 6,509,392.1         | 19,235                   | 295.5                | 291.3–299.7 | 1.06     | 1.04–1.08 | 1.13    | 1.11–1.16 |
| 251–500                                       | 18,378          | 266,104.8           | 892                      | 335.2                | 313.2–357.2 | 1.20     | 1.12–1.29 | 1.21    | 1.13–1.29 |
| 501–1,000                                     | 6,940           | 99,870.9            | 422                      | 422.5                | 382.2–462.9 | 1.52     | 1.38–1.67 | 1.37    | 1.24–1.50 |
| >1,000                                        | 2,536           | 36,124.4            | 210                      | 581.3                | 502.7–659.9 | 2.09     | 1.82–2.40 | 1.61    | 1.40–1.84 |
| Each 100 mg increase                          | –               | –                   | –                        | –                    | –       | 1.03     | 1.03–1.04 | 1.02^2  | 1.02–1.03 |

Abbreviations: CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio.

^1Adjusted for sex, age, monthly income, urbanization, liver cirrhosis, and diabetes.

^2\( p < 0.0001 \).
propensity score matching and time-varying changes. Additional covariates in the sensitivity analysis had only a little change in the estimates of the association between estimated aristolochic acid and the risk of HCC by different models. When the data were stratified according to sex, age, liver cirrhosis, alcohol-related disease, nonalcoholic steatohepatitis, diabetes, anti-HBV treatment, aspirin, metformin or statin use, the estimates of the association almost remained significant in the subgroup analysis (Table 3).

**Discussion**

We found higher risks of HCC in patients with HBV infection who took herbal medicine containing aristolochic acid. In addition, there were increasing trends in the adjusted HRs associated with estimated aristolochic acid dose from none to >1,000 mg.

This study has a number of strengths. First, the study population was taken from a large size population-based computerized database in Taiwan. Because the patients were recruited from an insured general population, we can rule out the possibility of selection bias and losses to follow up. Second, because the NHI reimbursement database collects all prescription information prospectively, we can rule out the possibility of recall bias for the intake doses of various herbal medicine and aristolochic acid. Third, we included a well-defined clinical endpoint, HCC, to examine the clinical significance of the research question in the longitudinal analyses. Fourth, we allowed a minimum induction time of one year and calculated the cumulative dose up to one year before diagnosis or the censor dates. Finally, we conducted sensitivity analyses by analytical designs, potential confounders and stratification, and the results revealed no significant changes in the HRs of the different models and subgroups.

In the HBV-infected cohorts, 59.4% of patients had taken herbal medicine containing aristolochic acid before their prohibition in November 2003 (Table 1). Among the top ten Chinese herbal formula prescribed for chronic hepatitis in Taiwan during 2002, three types of herbal medicine—Long Dan Xie Gan Tang and Gan Lou Xiao Du Dan—contained 40.8% and 6% Mu Tong, respectively. More than 84% of Mu Tong herbal formulas contained Guan Mu Tong (Aristolochia manshuriensis), and the estimated average dose of aristolochic acid per 1 g of Guan Mu Tong was 2.59 mg. Therefore, patients with HBV virus infection were more likely to...
Table 3. Sensitivity analysis of adjusted HRs of aristolochic acid consumption in risk estimation for hepatocellular carcinoma during the follow-up period in the HBV-infected cohort

| Model                                      | Estimated aristolochic acid | 1–250 mg | 251–500 mg | >500 mg | p for interaction |
|--------------------------------------------|------------------------------|----------|------------|---------|------------------|
|                                            | HR 95% CI                    | HR 95% CI| HR 95% CI  |         |                  |
| Main model                                 | 1.13 (1.11–1.16)             | 1.21 (1.13–1.29) | 1.44 (1.33–1.56) | 1.44 (1.33–1.56) | -                |
| Analytical designs                         |                              |          |            |         |                  |
| Model with 2-year induction time           | 1.14 (1.12–1.17)             | 1.22 (1.14–1.31) | 1.40 (1.29–1.52) | 1.22 (1.14–1.31) | -                |
| Model with competing mortality             | 1.12 (1.10–1.15)             | 1.21 (1.13–1.29) | 1.43 (1.32–1.55) | 1.21 (1.13–1.29) | -                |
| Model with propensity score matching       | 1.14 (1.11–1.16)             | 1.18 (1.05–1.33) | 1.46 (1.25–1.71) | 1.18 (1.05–1.33) | -                |
| Model with time-varying changes            | 1.32 (1.25–1.39)             | 2.15 (1.72–2.69) | -           | -       |                  |
| Additional covariates                      |                              |          |            |         |                  |
| Main model + alcohol-related disease       | 1.15 (1.12–1.17)             | 1.25 (1.17–1.34) | 1.49 (1.38–1.62) | 1.25 (1.17–1.34) | -                |
| Main model + nonalcoholic steatohepatitis | 1.13 (1.11–1.16)             | 1.21 (1.13–1.29) | 1.44 (1.33–1.56) | 1.21 (1.13–1.29) | -                |
| Main model + hypertension                  | 1.13 (1.10–1.15)             | 1.19 (1.11–1.27) | 1.41 (1.30–1.52) | 1.19 (1.11–1.27) | -                |
| Main model + hyperlipidemia                | 1.14 (1.12–1.17)             | 1.21 (1.13–1.30) | 1.44 (1.33–1.56) | 1.21 (1.13–1.30) | -                |
| Main model + chronic obstructive pulmonary disease | 1.15 (1.13–1.18)   | 1.24 (1.16–1.33) | 1.48 (1.37–1.61) | 1.24 (1.16–1.33) | -                |
| Main model + anti-HBV treatment            | 1.13 (1.10–1.15)             | 1.21 (1.13–1.29) | 1.42 (1.31–1.54) | 1.21 (1.13–1.29) | -                |
| Main model + aspirin                       | 1.14 (1.12–1.17)             | 1.22 (1.14–1.30) | 1.44 (1.33–1.56) | 1.22 (1.14–1.30) | -                |
| Main model + NSAIDs                        | 1.23 (1.20–1.26)             | 1.28 (1.20–1.37) | 1.48 (1.37–1.61) | 1.28 (1.20–1.37) | -                |
| Main model + ACE inhibitors                | 1.14 (1.11–1.16)             | 1.21 (1.13–1.29) | 1.43 (1.32–1.55) | 1.21 (1.13–1.29) | -                |
| Main model + metformin                     | 1.14 (1.11–1.16)             | 1.21 (1.13–1.30) | 1.45 (1.34–1.57) | 1.21 (1.13–1.30) | -                |
| Main model + statins                       | 1.13 (1.10–1.15)             | 1.20 (1.12–1.29) | 1.41 (1.31–1.53) | 1.20 (1.12–1.29) | -                |
| Subgroup effects                           |                              |          |            |         |                  |
| Sex                                        |                              |          |            |         |                  |
| Female                                     | 1.09 (1.04–1.15)             | 1.14 (0.98–1.33) | 1.63 (1.36–1.95) | 1.14 (0.98–1.33) | <.0001            |
| Male                                       | 1.14 (1.12–1.17)             | 1.23 (1.14–1.33) | 1.40 (1.28–1.53) | 1.23 (1.14–1.33) |                  |
| Age, years                                 |                              |          |            |         |                  |
| 18–39                                      | 1.15 (1.10–1.20)             | 1.32 (1.16–1.50) | 1.75 (1.51–2.04) | 1.32 (1.16–1.50) | -                |
| ≥40                                        | 1.14 (1.11–1.17)             | 1.17 (1.08–1.27) | 1.35 (1.23–1.49) | 1.17 (1.08–1.27) | -                |
| Liver cirrhosis                            |                              |          |            |         |                  |
| No                                         | 1.13 (1.10–1.16)             | 1.15 (1.06–1.25) | 1.40 (1.27–1.55) | 1.15 (1.06–1.25) | -                |
| Yes                                        | 1.15 (1.11–1.20)             | 1.32 (1.17–1.49) | 1.48 (1.30–1.69) | 1.32 (1.17–1.49) | -                |
| Alcohol-related disease                    |                              |          |            |         |                  |
| No                                         | 1.11 (1.08–1.15)             | 0.98 (0.88–1.11) | 1.06 (0.92–1.24) | 0.98 (0.88–1.11) | <.0001            |
| Yes                                        | 1.19 (1.15–1.23)             | 1.48 (1.36–1.60) | 1.80 (1.64–1.98) | 1.48 (1.36–1.60) |                  |
| Nonalcoholic steatohepatitis               |                              |          |            |         |                  |
| No                                         | 1.13 (1.10–1.15)             | 1.18 (1.09–1.26) | 1.44 (1.32–1.57) | 1.18 (1.09–1.26) | 0.0192             |
| Yes                                        | 1.21 (1.12–1.29)             | 1.50 (1.24–1.83) | 1.53 (1.22–1.92) | 1.50 (1.24–1.83) |                  |
| Diabetes                                   |                              |          |            |         |                  |
| No                                         | 1.14 (1.11–1.17)             | 1.20 (1.11–1.30) | 1.47 (1.34–1.61) | 1.20 (1.11–1.30) | 0.2652             |
| Yes                                        | 1.10 (1.05–1.15)             | 1.21 (1.06–1.38) | 1.33 (1.14–1.56) | 1.21 (1.06–1.38) |                  |
| Anti-HBV treatment                         |                              |          |            |         |                  |
| No                                         | 1.13 (1.10–1.16)             | 1.19 (1.11–1.28) | 1.40 (1.29–1.52) | 1.19 (1.11–1.28) | 0.1660             |
| Yes                                        | 1.10 (0.99–1.22)             | 1.48 (1.11–1.98) | 1.80 (1.28–2.52) | 1.48 (1.11–1.98) |                  |
take herbal medicine containing aristolochic acid than general population.\textsuperscript{19}

In our study, aristolochic acid may increase the risks for HCC in HBV-infected patients in a dose-dependent manner (Table 2). Mutational signature of AA exposure in humans, cell culture models and animals, suggests that AA mutagenesis and carcinogenesis are mediated by the formation and persistence of aristolactam(AL)-DNA.\textsuperscript{35} Although aristolochic acid treatment resulted in significant increases in DNA adduct formation, mutation frequency, and tumors in rat kidneys, the same treatment did not produce tumors in rat liver, while it did induce DNA adducts and mutations in this tissue at lower levels than in the kidney.\textsuperscript{15} However, Poon \textit{et al.} screened 93 HBV-positive HCC genomes/exomes and identified aristolochic acid-like mutational signatures and about twice as many A \textgreater{} T mutations on the nontranscribed strand in 11 probable AA-exposed patients.\textsuperscript{17} Rossiello \textit{et al.} reported that a single non-necrogenic dose of AA initiated liver cell carcinogenesis which was then promotable to form hepatic foci and nodules.\textsuperscript{36} Ng \textit{et al.}\textsuperscript{37} sequenced the whole exomes of 98 hepatocellular carcinomas (HCCs) from two hospitals in Taiwan and found that 78\% showed the distinctive mutational signature of aristolochic acid (AA) exposure. Asia, especially Taiwan, appears to be much more extensively affected by AA exposure in the world. Thus, it became clear that AA carcinogenesis is not limited to the upper urinary tract.

Consumption of \textgreater{}60 g of Guan Mu Tong or \textgreater{}200 g of Guang Fang Ji was suggested to be associated with an increased risk of HCC in patients with HBV infection (Supporting Information, Table S1). Similarly, \textgreater{}60 g of Mu Tong was also associated with an increased risk of developing urinary tract cancer\textsuperscript{7} and \textgreater{}60 g of Mu Tong or Fang Ji was associated with an increased risk of developing kidney failure.\textsuperscript{7} Moreover, Mu Tong and Fang Ji contain much higher aristolochic acid than the other medicinal herbs such as Ma Dou Ling, Qing Mu Xiang, Tian Xian Teng and Xi Xin.\textsuperscript{24–28}

We also found higher incidences of chronic kidney disease and upper tract urothelial and bladder cancers in patients with HBV infection who took herbal medicine containing aristolochic acid as well as in patients without HBV infection (Supporting Information, Table S2). This evidence is in agreement with current knowledge\textsuperscript{1–4} and confirm our previous studies.\textsuperscript{5–11} Other than hepatorenal syndrome in individuals with cirrhosis or fulminant liver failure, aristolochic acid related nephropathy explains in part that HBV infection was associated with the increased risk of chronic kidney disease among patients in Taiwan\textsuperscript{16,39} and there were high incidences of urinary tract cancers in HCC patients subsequently.\textsuperscript{40} In addition, exposure to aristolochic acid can also partially explain that high incidence of HCC in dialysis patients with HBV infection in the opposite direction.\textsuperscript{41}

However, the increased risk shown in the analysis could be due to confounding by disease severity, as patients with more severe forms of hepatitis and thus a higher risk of liver cancer would be more likely to receive these herbs. First, although laboratory data were not available in the claim database, there were similar in the baseline laboratory data of the patients with HBV infection in the subcohort study between January 2001 and October 2003 (Supporting Information, Table S3). Second, the dose-dependent manner of aristolochic acid remained either in noncirrhotic or in cirrhotic subgroups (Table 3). Third, aristolochic acid consumption was assessed between 1997 and 2003, the year of market withdrawal, cases of HCC were identified from 1999 until 2013, that is, 10 years after market withdrawal. Therefore, the

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### Table 3. Sensitivity analysis of adjusted HRs of aristolochic acid consumption in risk estimation for hepatocellular carcinoma during the follow-up period in the HBV-infected cohort (Continued)

| Model      | Estimated aristolochic acid | HR (95\% CI) |
|------------|-----------------------------|--------------|
|            | 1–250 mg                    | 251–500 mg   | >500 mg       | p for interaction |
|            | HR (95\% CI)                | HR (95\% CI) | HR (95\% CI)  |               |
| Aspirin    |                             |              |               | 0.3658         |
| No         | 1.15 (1.12–1.17)            | 1.25 (1.16–1.35) | 1.44 (1.32–1.57) |
| Yes        | 1.11 (1.05–1.17)            | 1.05 (0.89–1.25) | 1.41 (1.17–1.70) |
| Metformin  |                             |              |               | 0.1176         |
| No         | 1.15 (1.12–1.18)            | 1.21 (1.12–1.30) | 1.48 (1.36–1.61) |
| Yes        | 1.07 (1.01–1.13)            | 1.25 (1.06–1.48) | 1.29 (1.03–1.60) |
| Statins    |                             |              |               | 0.3637         |
| No         | 1.13 (1.11–1.16)            | 1.21 (1.12–1.29) | 1.41 (1.30–1.53) |
| Yes        | 1.07 (0.99–1.15)            | 1.17 (0.93–1.48) | 1.52 (1.16–2.00) |

Abbreviations: ACE, angiotensin-converting enzyme; CI, confidence interval; HBV, hepatitis B virus; HR, hazard ratio; NSAIDs, nonsteroidal anti-inflammatory drugs.

\textsuperscript{1}Adjusted for sex, age, monthly income, urbanization, liver cirrhosis and diabetes.

\textsuperscript{2}Subject number = 651,054.

\textsuperscript{3}The estimated aristolochic acid was divided into only two categories of use (1–250 and \textgreater{}250 mg) in the model with time-varying changes.

\textsuperscript{4}The models were adjusted for covariates in the main model and each additional listed covariate.

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confounding by disease severity, if existed, would tend to be trivial.

Other potential limitations of this study should be considered. Because the data on drug prescriptions were not complete in 1996, we included herbal medicine containing aristolochic acid after 1997 so that the use of these drugs before 1997 would not be captured in our analysis. This could have underestimated the actual ingested dosage. In addition, we presumed that all prescribed medications were actually taken by patients as prescribed, which may overestimate the actual ingested dosage because some degree of noncompliance is always expected. Furthermore, there were additional sources of uncertainty in estimating aristolochic acid exposure, for example, varying concentration of aristolochic acid in a given herbal medicine, possible exposure through mistakenly identified herbs, and possible exposure though herbs obtained through channels other than licensed clinics. These could result in bias to the null hypothesis. Finally, several unmeasured confounders, including body mass index, smoking, alcohol intake and exposure to aflatoxin B1 which are associated with HCC, were not included in our database. We used alcohol-related disease, nonalcoholic steatohepatitis, hypertension, hyperlipidemia and chronic obstructive pulmonary disease as additional covariates in the sensitivity analyses. The estimates did not change significantly, that is, no apparent confounding effects were found (Table 3).

Our study first found significant dose–response relationships between the consumption of aristolochic acid and HCC in patients with HBV infection, suggesting that aristolochic acid which may be associated with HBV plays an important role in the pathogenesis of HCC. Further mechanistic research is needed. Although herbal medicine containing aristolochic acid has largely been banned in Taiwan and many other countries, patients with HBV infection, who have taken those herbs, should be closely followed up.

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