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(Article begins on next page)

Traditional Herbal Medicine Use Associated with Liver Fibrosis in Rural Rakai, Uganda

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Abstract

Background: Traditional herbal medicines are commonly used in sub-Saharan Africa and some herbs are known to be hepatotoxic. However little is known about the effect of herbal medicines on liver disease in sub-Saharan Africa.

Methods: 500 HIV-infected participants in a rural HIV care program in Rakai, Uganda, were frequency matched to 500 HIV-uninfected participants. Participants were asked about traditional herbal medicine use and assessed for other potential risk factors for liver disease. All participants underwent transient elastography (FibroScan[®]) to quantify liver fibrosis. The association between herb use and significant liver fibrosis was measured with adjusted prevalence risk ratios (adjPRR) and 95% confidence intervals (CI) using modified Poisson multivariable logistic regression.

Results: 19 unique herbs from 13 plant families were used by 42/1000 of all participants, including 9/500 HIV-infected participants. The three most-used plant families were Asteraceae, Fabaceae, and Lamiaceae. Among all participants, use of any herb (adjPRR = 2.2, 95% CI 1.3–3.5, p = 0.002), herbs from the Asteraceae family (adjPRR = 5.0, 95% CI 2.9–8.7, p < 0.001), and herbs from the Lamiaceae family (adjPRR = 3.4, 95% CI 1.2–9.2, p = 0.017) were associated with significant liver fibrosis. Among HIV infected participants, use of any herb (adjPRR = 2.3, 95% CI 1.0–5.0, p = 0.044) and use of herbs from the Asteraceae family (adjPRR = 5.0, 95% CI 1.7–14.7, p = 0.004) were associated with increased liver fibrosis.

Conclusions: Traditional herbal medicine use was independently associated with a substantial increase in significant liver fibrosis in both HIV-infected and HIV-uninfected study participants. Pharmacokinetic and prospective clinical studies are needed to inform herb safety recommendations in sub-Saharan Africa. Counseling about herb use should be part of routine health counseling and counseling of HIV-infected persons in Uganda.

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Introduction

Traditional herbal medicines are commonly used for HIV/AIDS and other health conditions in Uganda and sub-Saharan Africa, often in parallel with programs that provide antiretroviral therapy (ART). In the 1990's an estimated 80% of Ugandans living in rural villages used traditional healers for primary health care [1]. A study of 137 HIV-infected Ugandans receiving ART found that 60% used herbs concurrently with ART [2].

In Uganda traditional herbal medicines are usually boiled extracts of herbs taken orally [3]. Some potentially hepatotoxic traditional herbal medicines used in Uganda and sub-Saharan Africa include *Hoodia gordonii* [4], kava [5], *Phytolacca dioica* [6], and herbs from the Asteraceae family [7]. Little is known about the hepatotoxicity of other commonly used herbs or the contribution of herbs to the burden of liver fibrosis and hepatocellular carcinoma in sub-Saharan Africa, including when used concomitantly with ART. Data on the specific types of herbs taken by HIV-infected persons in Uganda is limited, as is information about their components, side effects, toxicities, and ART interactions [8].

In Rakai, Uganda, liver toxicity associated with herbal medicine may be of particular concern given the high prevalence of significant liver disease (17%) among HIV-infected persons in Rakai recently identified by transient elastography (FibroScan[®], Echosense, Paris, France) [9]. In the aforementioned study, reported herbal medicine use was associated with a two-fold increased risk of significant liver disease, defined as a transient elastography score equivalent to METAVIR liver fibrosis stage 2 (portal fibrosis with few septa) or greater [9]. The study presented here follows up on this prior investigation with an in-depth analysis of the herbs used by study participants and their relation to liver fibrosis.

Methods

This cross-sectional study enrolled 500 HIV-infected participants receiving care at five HIV care clinics within the Rakai Health Sciences Program (RHSP) HIV Care Program. 500 HIV-uninfected participants from the Rakai Community Cohort Study (RCCS) were frequency-matched to these participants by age, gender, and community. Begun in 1994 in one of Uganda's hardest-hit regions by the HIV epidemic, the RCCS conducts annual surveys in a population of 10,000–15,000 people aged 15–49 years, and is described in detail elsewhere [10]. Participants underwent a detailed liver-disease focused risk factor questionnaire which included an assessment of herbal drug use, venous blood collection, and transient elastography (FibroScan[®], Echosense, Paris, France) to quantify liver fibrosis.

Ethics Statement

Written informed consent was obtained from all participants. Institutional Review Boards of the National Institute of Allergy and Infectious Diseases, the Johns Hopkins Medical Institutions, the Scientific and Ethics Committee of the Uganda Virus Research Institute, and the Uganda National Council for Science and Technology approved this study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and is registered on clinicaltrials.gov (#NCT00782158).

Herb Use Assessment

Participants were asked about any current herb use and then to name the two herbs they used most often. Scientific names were assigned to local herb names in consultation with local traditional medicine practitioners and a member of Makerere University

Botany Department (CKB). The Makerere University Herbarium database was also used to validate herb identities. Some participants reported non-plant substances such as clay and spiritual charms as herb use. Participants reporting use of non-plant based entities were reclassified as non-herb users in this analysis.

Laboratory Assays

HIV-1 serology was determined by two HIV-1 enzyme immuno-assays: Vironostika HIV-1 (OrganonTeknika, Charlotte, North Carolina, USA) and Cambridge Biotech (Worcester, Massachusetts, USA). Participants with discrepant HIV-1 enzyme immune assay results were tested with western blot (HIV-1 Western Blot; Bio-Merieux-Vitek, St. Louis, Missouri, USA). For HIV-infected participants, current CD4 count (within 12 months) and CD4 count nadir were abstracted from the RHSP HIV Care Program database. CD4 counts were measured by FACSCalibur flow cytometer (software version 1.4, Becton Dickinson, San Jose, California, USA). Hepatitis B virus surface antigen (HBsAg) was determined using ELISA (ETI-MAK-2 Plus, Diasorin, Vercelli, Italy). Alanine aminotransferase (ALT) was tested using standard methods (COBAS CII; Roche, Basel, Switzerland), and hepatotoxicity was defined by ALT elevations and classified according to AIDS Clinical Trial Group criteria [11]. The upper limit of normal for ALT was defined as 19 IU/L in women and 39 IU/L in men [12,13].

Transient Elastography

Transient elastography or FibroScan[®] is a novel, validated, noninvasive technology for the evaluation of fibrosis in chronic liver disease [14]. A FibroScan[®] is approximately the size of an ultrasound unit. A probe placed over a patient's abdomen produces vibration and the speed of the responding elastic wave is detected by ultrasound. The propagation of these waves through the liver is directly correlated to the degree of liver stiffness. The results are instantaneously received as a single, quantitative parameter of liver stiffness measurement (LSM, reported in kPa). Each transient elastography scan takes ten liver stiffness measurements in rapid succession over several seconds. The median of the ten measurements is reported as the final liver stiffness measurement. The procedure is non-invasive, painless, has no side effects, and requires only a few minutes to perform. The device requires minimal training and does not need to be performed by advanced medical personnel.

In this study a conservative liver stiffness measurement cutoff of ≥ 9.3 kPa, from a validation study in persons of predominantly African descent, was used to define significant fibrosis equivalent to METAVIR fibrosis stage 2 (portal fibrosis with few septa) or greater [15]. Two study nurses at the Rakai Health Science Program study site conducted all transient elastography scans after receiving certification from the manufacturer. According to manufacturer recommendations, scans with high variability—defined as an interquartile range greater than 30% of the median LSM value from an individual examination—were not considered valid and were excluded from the analysis. Participants with invalid scans on an initial attempt were repositioned and rescanned up to 4 times to achieve a valid scan.

Statistics

Baseline demographic, behavioral and clinical characteristics were compared by HIV status. Differences in continuous variables were assessed using t-tests and Wilcoxon-Mann-Whitney tests. Categorical variables were compared using Pearson's chi squared test.

Table 1. Baseline characteristics of study participants.

| Characteristic | HIV-infected pts (n = 500) | HIV-uninfected pts (n = 500) | p value |
|-------------------------------------|----------------------------|------------------------------|---------|
| | n (% or IQR) | n (% or IQR) | |
| Characteristic | | | |
| Median Age, years | 38 (IQR 31–44) | 37 (IQR 32–44) | 0.025 |
| Female | 312 (67%) | 333 (67%) | 0.89 |
| Heavy Liquor use (>1.25 L/week) | 11 (2%) | 9 (2%) | 0.65 |
| Lifetime occupational fishing | 5 (1%) | 1 (0.2%) | 0.65 |
| HBsAg positive | 23 (5%) | 14 (3%) | 0.10 |
| Valid TE scan | 468 (94%) | 494 (99%) | <0.001 |
| Herb Use | | | |
| Current herb use | 8 (2%) | 33 (7%) | 0.0001 |
| Known herbs | 5 (0.9%) | 16 (3%) | 0.015 |
| Unknown herbs | 4 (0.9%) | 17 (3%) | 0.004 |
| Asteraceae family | 2 (0.4%) | 6 (1%) | 0.16 |
| Fabaceae family | 0 (0%) | 6 (1%) | 0.014 |
| Lamiaceae family | 1 (0.2%) | 4 (0.8%) | 0.18 |
| ACTG Hepatotoxicity Criteria | | | |
| Median ALT (U/L) | 22 (IQR 16–31) | 19 (IQR 15–25) | <0.001 |
| Grade 0 (<1.25×ULN) by ALT | 354 (71%) | 414 (83%) | |
| Grade 1 (1.25–2.5×ULN) by ALT | 122 (24%) | 77 (15%) | |
| Grade 2 (2.6–5×ULN) by ALT | 19 (4%) | 9 (2%) | |
| Grade 3 (5.1–10×ULN) by ALT | 5 (1%) | 0 (0%) | |
| Grade 4 (>10×ULN) by ALT | 0 (0%) | 0 (0%) | |
| CD4 and ART Characteristics | | | |
| Current CD4 count (cells/uL) | 449 (IQR 320–642) | | |
| Nadir CD4 count (cells/uL) | 214 (IQR 130–350) | | |
| Nadir CD4 count <100 cells/uL | 95 (19%) | | |
| On ART | 302 (60%) | | |
| ART duration (months) | 19 (IQR 9–38) | | |

HIV (Human Immunodeficiency Virus), IQR (Interquartile Range), HBsAg (Hepatitis B Surface Antigen), ACTG (AIDS Clinical Trials Group), CD4 (Cluster of Differentiation 4 positive Helper T cells), ART (Antiretroviral Therapy).
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The primary outcome measure was liver fibrosis. Because odds ratios may overestimate the magnitude of association between variables if the outcome of interest is common, adjusted prevalence risk ratios (adjPRR) with 95% confidence intervals (95% CI) were estimated using modified Poisson regression [16]. The multivariable models adjusted for HIV, gender, occupation in the fishing industry, chronic hepatitis B infection (positive hepatitis B surface antigen), and drinking ≥ 1.25 liters per week of liquor, as these risk factors were associated with liver disease in previous analysis of this study population [9]. Age was included in all models and nadir CD4 cell count and ART status were included in models restricted to HIV-infected participants for reasons of biologic plausibility. STATA version 11.0 (STATA Corp, College Station, TX) was used for statistical analysis.

Results

Baseline Characteristics

The HIV-infected and uninfected groups each had 67% females (see table 1). The median age of 38 years in the HIV-infected

group was close to the median age of 37 years in the HIV-uninfected group ($p = 0.025$). Only 2% of both HIV-infected and uninfected participants were heavy liquor drinkers ($p = 0.65$). The prevalence of chronic HBV infection was similar in both groups, 5% in HIV-infected participants and 3% in HIV-uninfected participants ($p = 0.010$). 29% of HIV-infected participants and 17% of HIV-uninfected participants had any grade 1 or higher hepatotoxicity by AIDS Clinical Trial Group (ACTG) criteria ($p < 0.001$). No participants demonstrated grade 4 hepatotoxicity.

At the time of enrolment HIV-infected participants had a median CD4 count of 449 cells/ μ L (IQR 320–642) and 60% were receiving ART with a median duration of 19 months (IQR 9–38). Demographics of the HIV-infected group were also similar to participants in the Rakai Health Sciences HIV Care Program, in which 65% of participants are female, 64% are on ART, and the median CD4 count is 480 cells/ μ L.

468/500 (94%) of HIV-infected participants and 494/500 (99%) of HIV-uninfected participants had valid elastography scans. Those with valid scans were included in the assessment of liver fibrosis and were included in the regression models.

Table 2. Characteristics of participants reporting current herb use.

| Characteristic | Using Herbs (n = 42) | Not Using Herbs (n = 958) | p value |
|---------------------------------|----------------------|---------------------------|---------|
| | n (% or IQR) | n (% or IQR) | |
| Median Age, years | 39 (32–44 IQR) | 38 (31–44 IQR) | 0.61 |
| Female | 27 (64%) | 643 (67%) | 0.15 |
| Heavy Liquor use (>1.25 L/week) | 3 (7%) | 17 (2%) | 0.015 |
| Lifetime occupational fishing | 1 (2%) | 5 (0.5%) | 0.13 |
| HBsAg positive | 2 (5%) | 35 (4%) | 0.73 |
| HIV infected | 9 (20%) | 491 (50%) | 0.0001 |

L (Liters), HBsAg (Hepatitis B Surface Antigen), HIV (Human Immunodeficiency Virus).
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Characteristics of Herb Users

42/1000 (4%) of all participants reported current use of traditional herbal medicines, including 9/500 (2%) of HIV-

infected participants and 33/500 (7%) HIV-uninfected participants (table 1). 21/42 (50%) of participants could name at least one herb they were taking. 4/46 (9%) of participants reporting

Table 3. Characteristics of known herbs in the Asteraceae, Fabaceae, and Lamiaceae families.

| Family (n taking) | Scientific Name | Local Names (n taking) | English Name | Taken in Rakai for: | Known Pharmacology | Known Liver Toxicity and/or ART Interaction |
|-------------------|------------------------------------|---|----------------|--|--|---|
| Asteraceae (8) | <i>Vernonia amygdalina</i> | Mululuza (4) | Bitter leaf | Fever, fever with jaundice | Contains alkaloids, saponins, tannins, flavonoids, steroid glycosides, sesquiterpine lactone [32] | Hepatotoxic at high doses (750 mg/kg) [24]. One herb from the Veronia genus (<i>V. lasiopus</i>) was hepatotoxic in an <i>in-vitro</i> rat precision cut liver slice model [33]. Many herbs in the Asteraceae family contain pyrrolizidine alkaloids, which are associated with veno-occlusive liver disease [17] |
| | <i>Vernonia</i> genus Kiluluza (2) | | | | | |
| | <i>Microglossa densiflora</i> | Kafugankande, Akafugankande (1) | | Fever, indigestion, loose stools, parasites | Microglossa family contains clerodane diterpenoids [34] | A similar herb from the Microglossa family (<i>M. pyrifolia</i>) was hepatotoxic in an <i>in-vitro</i> rat precision cut liver slice model [33]. Many herbs in the Asteraceae family contain pyrrolizidine alkaloids, which are associated with veno-occlusive liver disease [17] |
| | <i>Aspilia africana</i> | Makaayi (1) | Wild sunflower | Fever with jaundice | Contains saponins, tannins, terpenoids, Sesquiterpenes, monoterpenes [35,36] | No hepatotoxicity in rat <i>in vivo</i> model [38]. Many herbs in the Asteraceae family contain pyrrolizidine alkaloids, which are associated with veno-occlusive liver disease [17] |
| Fabaceae (6) | <i>Pseudarthria hookeri</i> | Bikakala, Kikakala, Omukakala, Mukakala (4) | | Fever, fever with jaundice, allergy, cough, wounds | May have estrogenic activity [37] | Many herbs in the Fabaceae family contain pyrrolizidine alkaloids, which are associated with veno-occlusive liver disease [17] |
| | <i>Indigofera congesta</i> | Namasumi (2) | Indigo | Fever with jaundice, antenatal health | Indigofera family members contain flavonoids, saponins, quinones, sterols/ triterpenes, tannins, gallic acid, caffeic acid, rutin and myricetin [38] | Many herbs in the Fabaceae family contain pyrrolizidine alkaloids, which are associated with veno-occlusive liver disease [17] |
| Lamiaceae (5*) | <i>Ocimum gratissimum</i> | Mujaaja, Omujaaja (3) | African basil | Epigastric pain | Contains tannin phlobaphenes, flavones, flavonols, xanthones, chalcones, aurones, terpenes, flavononols, leucoanthocyanidins, catechins [39] | Herb from <i>Ocimum</i> genus (<i>O. lamiifolium</i>) was hepatotoxic in an <i>in-vitro</i> rat precision cut liver slice model [33], <i>Ocimum gratissimum</i> caused hepatotoxicity <i>in-vivo</i> rabbit liver model [25] |
| | <i>Hoslundia opposita</i> | Kamunye (3) | | To replace blood, postnatal health, vomiting during fever and jaundice | Contains sesquiterpenes and sesquiterpene alcohols [40] | |

*one patient took both *Ocimum gratissimum* and *Hoslundia oopposita*. mg (milligrams), kg (kilograms).
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Table 4. Characteristics of known herbs in remainder of plant families.

| Family (n taking) | Scientific Name | Local Names (n taking) | English Name | Taken in Rakai for: | Known Pharmacology | Known Liver Toxicity and/or ART Interaction |
|--------------------|--|------------------------------------|--------------------|--|--|---|
| Anacardiaceae (2) | <i>Rhus vulgaris</i> or <i>Rhus natalensis</i> | Olukansokanso, akakwansokwanso (1) | | Antenatal health, aphrodisiac, gastrointestinal ulcers, back pain | No published information about contents | |
| | <i>Mangifera indica</i> | Mango Tree Bark (1) | Mango tree bark | | Leaves contains flavonoids [41], Peel contains phenolic compounds and carotenoids [42] | |
| Primulaceae (2) | <i>Maesa lanceolata</i> | Oluwongwa, Oluwongo (2) | | Neonatal jaundice | Contains Saponins [43], benzoquinone [44] | |
| Euphorbiaceae (2) | <i>Sapium ellipticum</i> | Musasa, Omusiisa, Musanvuma (2) | Jumping tree seed | Hypertension, antenatal health, sexually transmitted infections, epigastric pain | Contains phenols [45] | |
| Amaryllidaceae (1) | <i>Allium sativum</i> | Garlic (1) | Garlic | — | Contains diallyl disulfide [46] | Induces CYP3A4 and Pgp and should not be taken with the following antiretrovirals: APV, ATV, AZT, EFV, IDV, LPV, NFV, NVP, SQV [27] |
| Bignoniaceae (1) | <i>Spathodea campanulata</i> | Ekifabakazi (1) | African tulip tree | Dysmennorrhea, antenatal health | Contains 3β-acetoxyoleanolic acid, siarsinolic acid, oleanolic acid, others [47] | |
| Solanaceae (1) | <i>Solanum incanum</i> | Akatengotengo (1) | Sodom apple | Cough, chest pain | Contains alkaloids, saponins, solanine; High concentrations cause hemolysis of erythrocytes [48] | Herbs containing pyrrolizidine alkaloids are associated with veno-occlusive liver disease [17]. |
| Vitaceae (1) | <i>Cyphostemma adenocaula</i> | Kamombo (1) | | Peptic ulcers | Contain carotenoids (carotenes), xanthophylls, Vitamin C, Tocopherols, and Tocotrienols [49] | |
| Myrtaceae (1) | <i>Callistemon citrinus</i> | Bottlebrush (1) | Bottlebrush tree | Rhino-sinusitis | Contains 1,8-cineole, apha-pinene [50] | |
| | | Kagulukandayi, Katangulucumu (1) | | Epilepsy | | |
| | | Kakubamusolo (1) | | | | |

APV (amprenavir), ATV (atazanavir), AZT (zidovudine), EFV (efavirenz), IDV (indinavir), LPV (lopinavir), NFV (nelfinavir), NVP (nevirapine), SQV (saquinavir). doi:10.1371/journal.pone.0041737.t004

herb use were reclassified as not taking herbs because they only reported use of inert, non-plant substances including clay. Herb users did not differ by age (p = 0.61) or gender (p = 0.15) from those who did not report herb use (see table 2). Herb users were not more likely to work in the fishing industry (p = 0.13) or have chronic hepatitis B infection (p = 0.73). 7% of participants reporting herb use drank liquor heavily (≥1.25 L/week), compared to 2% of participants who did not report herb use (p = 0.015). 19 unique herbs from 13 families were used, and are characterized in tables 3 and 4. The most common families were Asteraceae, Fabaceae, and Lamiaceae, which were used by eight, six and five participants, respectively.

Herb Use and Liver Fibrosis

Among the 137/962 (14%) subjects with significant liver fibrosis, 12/137 (9%) reported herb use. Of the 825/962 (86%) subjects without significant liver fibrosis, 29/825 (4%) reported current herb use (p = 0.005). 56/494 (11%) of HIV-uninfected participants had significant fibrosis, compared to 81/468 (17%) of HIV-infected participants (p = 0.008).

In multivariable analysis that adjusted for age, fishing occupation, HIV infection, positive HBsAg, gender, and heavy liquor use, herb use was associated with two to five fold increases in significant liver fibrosis (see table 5). Among all participants, use of any herb (adjPRR = 2.2, 95% CI 1.3–3.5, p = 0.002), herbs from the Asteraceae family (adjPRR = 5.0, 95% CI 2.9–8.7, p < 0.001), and herbs from the Lamiaceae family (adjPRR = 3.4, 95% CI 1.2–

Table 5. Association of herbs with significant liver fibrosis in all participants.

| Herb (n taking) | Univariate | | | Multivariate | | |
|---------------------------|------------|----------|---------|--------------|-----------|---------|
| | PRR | 95% CI | P value | adjPRR | 95% CI | P value |
| Any current herb use (41) | 2.2 | 1.3–3.6 | 0.003 | 2.2 | 1.3–3.5 | 0.002 |
| Asteraceae (8) | 5.5 | 3.6–8.4 | <0.001 | 5.0 | 2.9–8.7 | <0.001 |
| Fabaceae (6) | 1.2 | 0.19–7.1 | 0.86 | 1.6 | 0.26–10.3 | 0.60 |
| Lamiaceae (5) | 2.8 | 0.96–8.4 | 0.060 | 3.4 | 1.2–9.2 | 0.017 |
| Unknown herb (21) | 1.0 | 0.35–2.9 | 0.995 | 1.2 | 0.40–3.3 | 0.79 |

Multivariate model for all participants adjusts for: age, occupational fishing, HIV infection, positive Hepatitis B surface antigen, gender, heavy liquor use (≥ 1.25 L/week). Only participants with a valid TE scan (962/1000) were included in the model. CI (Confidence Interval). doi:10.1371/journal.pone.0041737.t005

9.2, $p = 0.017$) were associated with increased significant fibrosis. Use of herbs from the Fabaceae family was not associated with significant liver fibrosis (adjPRR = 1.6, 95% CI 0.26–10.3, $p = 0.60$).

Of 81 HIV-infected subjects with significant liver fibrosis, 4 (5%) reported herb use (see table 6). Among 387 HIV-infected subjects without significant liver fibrosis, 4 (1%) reported herb use ($p = 0.014$). In the multivariable analysis of HIV-infected participants adjusted for age, occupational fishing, positive HBsAg, gender, heavy liquor use, ART, and CD4 nadir, the associations between herb use and significant liver fibrosis were similar to findings among all participants. Among HIV-infected participants the use of any herb (adjPRR = 2.3, 95% CI 1.0–5.0, $p = 0.044$) and the use of herbs from the Asteraceae family (adjPRR = 5.0, 95% CI 1.7–14.7, $p = 0.004$) were associated with increased liver fibrosis.

Among all participants as well as HIV-infected participants, herb use was not associated with increased hepatotoxicity. 8/41 (20%) of participants reporting herb use had ACTG grade 1–4 ALT elevations, compared to 216/961 (23%) who did not report herb use ($p = 0.56$). Among HIV-infected participants reporting herb use, 6/33 (18%) had grade 1–4 ALT elevations, compared to 79/461 (17%) who did not report herb use ($p = 0.88$).

Table 7 shows the proportion of participants who took individual herbs in the Asteraceae, Lamiaceae, and Fabaceae families who had significant liver fibrosis. 6/8 participants taking herbs in the Asteraceae family had significant liver fibrosis. 4/6

Table 6. Association of herbs with significant liver fibrosis in HIV-infected participants.

| Herb (n taking) | Univariate | | | Multivariate | | |
|--------------------------|------------|----------|---------|--------------|----------|---------|
| | PRR | 95% CI | P value | adjPRR | 95% CI | P value |
| Any current herb use (8) | 3.0 | 1.4–6.2 | 0.003 | 2.3 | 1.0–5.0 | 0.044 |
| Asteraceae (2) | 6.0 | 4.9–7.3 | <0.001 | 5.0 | 1.7–14.7 | 0.004 |
| Unknown herb (5) | 1.2 | 0.20–6.8 | 0.87 | 1.0 | 0.15–6.7 | 0.998 |

Multivariate model for HIV-infected participants adjusts for: age, occupational fishing, positive Hepatitis B surface antigen, gender, heavy liquor use (≥ 1.25 L/week), ART, and CD4 nadir. Only participants with a valid TE scan (468/500) were included in the model. CI (Confidence Interval). doi:10.1371/journal.pone.0041737.t006

Table 7. Use of specific herbs and significant liver fibrosis.

| Herb used | Proportion of participants taking with significant liver fibrosis |
|-----------------------------------|---|
| Asteraceae Family | 6/8 |
| <i>Vernonia amygdalina</i> | 2/4 |
| <i>Vernonia</i> , species unknown | 2/2 |
| <i>Microglossa densiflora</i> | 1/1 |
| <i>Aspilia Africana</i> | 1/1 |
| Fabaceae Family | 2/6 |
| <i>Pseudarthria hookeri</i> | 1/4 |
| <i>Indigofera congesta</i> | 1/2 |
| Lamaceae Family | 2/5 * |
| <i>Ocimum gratissimum</i> | 1/3 |
| <i>Hoslundia opposita</i> | 1/3 |

*One participant took both *Ocimum gratissimum* and *Hoslundia opposita*. Only participants with a valid TE scans are shown. doi:10.1371/journal.pone.0041737.t007

subjects who used herbs in the *Vernonia* genus of the Asteraceae family had significant liver fibrosis.

Discussion

This study indicates that traditional herbal medicine use may contribute to liver disease in Uganda. Use of traditional herbal medicines was independently associated with two to five fold increases in significant liver fibrosis. Herbs from the Asteraceae family were the most often used and showed the strongest association with significant liver fibrosis: a five-fold increase in all participants ($p < 0.001$) and HIV-infected participants ($p = 0.004$).

Six of eight participants who took herbs in the Asteraceae family had significant liver fibrosis (see table 5). Many plants in the Asteraceae and Fabaceae families contain pyrrolizidine alkaloids, a known risk factor for veno-occlusive liver disease [7,17]. Although none of the alkaloid-containing herbs used by participants in this study have been confirmed to contain pyrrolizidine alkaloids, ingestion of plants containing pyrrolizidine alkaloids caused outbreaks of veno-occlusive liver disease in Jamaica, India, Egypt, and South Africa [17,18]. No outbreaks of veno-occlusive liver disease associated with pyrrolizidine alkaloids have been reported to our knowledge in East Africa. Pyrrolizidine alkaloids are inert until dehydrogenation by cytochrome P450 3A4 (CYP3A4) in the liver [19], where reactive toxic pyrrolic and N-oxide metabolites directly damage liver sinusoidal endothelial cells and hepatocytes (zone III of the liver acinus) [20]. Pyrroles cause chromosomal damage in a dose-dependent manner, resulting in an inflammatory response that culminates in fibrin deposition [17,20,21].

Although plants in both the Asteraceae and Fabaceae families ingested by study participants may contain pyrrolizidine alkaloids, our data shows a strong association between significant liver fibrosis and use of herbs in the Asteraceae family but not the Fabaceae family. The literature about African traditional herbal medicines is limited and does not explain why this difference might exist. Traditional herbal medicine remedies used in Rakai and throughout Uganda are often mixtures containing multiple herbs [8,22]. It is possible that herbs in the Asteraceae family are taken at high doses, or potentiate the toxicity of other herbs or hepatotoxins.

Two participants with fibrosis reported use of *Vernonia amygdalina* in the Asteraceae family. This particular herb is commonly used in Africa is thought to have hepatoprotective properties [23]. However, animal studies show that at higher doses, this member of the Asteraceae family may be hepatotoxic. In an *in-vivo* rat CCl₄ liver injury model, low doses (250–500 mg/kg) of *Vernonia amygdalina* were hepatoprotective, but a high dose (750 mg/kg) caused increased hepatotoxicity [24].

Herbs from the Lamiaceae family were associated with a 3.4 fold increase in significant liver fibrosis among all participants in our study ($p=0.017$). Herbs in the Lamiaceae family have been associated with hepatotoxicity in an *in-vivo* rabbit model [25]. In addition, Aloe, taken by two participants in our study, has been linked in case reports to severe hepatitis [26]. However, data about the potential hepatotoxicity of many herbs used by participants in this study do not exist, or come from animal model studies only that should be interpreted cautiously.

The risk of significant fibrosis associated with herb use was similar in the overall and HIV- infected study populations. Data on herb use was limited in the HIV-infected population, and plant family specific analysis was only possible for the Asteraceae family. Only two HIV- infected participants reported using herbs in the Asteraceae family.

Despite the small number of HIV-infected participants in this study who reported herb use, it is important to note that ART may alter the toxicity profile of co-administered herbs. CYP3A4 is a major pathway for metabolism of a wide range of chemically distinct foreign compounds including phytochemicals and antiretroviral drugs [27]. Antiretroviral drugs of the non- nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitor (PI) classes are also inducers or inhibitors of CYP3A4 activity [28,29]. Therefore, these drugs have potential to influence phytochemical detoxification or detoxification pathways in the liver. For example, commonly used NNRTI in initial ART regimens in Uganda (efavirenz and nevirapine) are inducers of CYP3A4 and therefore have potential to increase generation of toxic metabolites of pyrrolizidine alkaloids [27,28]. Inhibitors of CYP3A4 may lead to accumulation of phytochemicals or their metabolites in the liver which may also result in toxicity.

Conversely, herbs may potentiate ART toxicities by influencing antiretroviral drug disposition in the liver, kidney, and gut. Herbs may affect NNRTI and PI metabolism by CYP3A4 and alter activity of cellular drug transporters and glucuronidation pathways [27]. Existing evidence from Africa about herb-ART interactions is limited to two herb families commonly used in South Africa: *Hypoxis* (African potato) and *Sutherlandia*, neither of which were taken by participants in this study. *Hypoxis* causes a dose-dependent inhibition of CYP3A4 up to 86% of the normal activity of CYP3A4 and 50% reduction of the expression of P-glycoprotein. *Sutherlandia frutescens* also causes a dose dependent inhibition of CYP3A4 up to 96% of CYP3A4 activity [30]. One participant in this study reported garlic use, which is known to significantly reduce concentrations of a PI (saquinavir), most likely by induction of CYP3A4 [31]. Since nevirapine and efavirenz are also eliminated by CYP3A4, garlic may reduce plasma levels of these drugs, but there are no clinical data on these interactions.

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Limitations

This study had limitations. The study was cross-sectional and only information about current herb use was available for analysis. Only 4% of participants in this study reported using herbs, compared to other studies in Uganda in which 60% of HIV-infected persons reported concurrent use of ART and herbs [2]. Some misclassification of herb exposure could have occurred due to a social desirability or reporting bias, especially among HIV-infected persons on ART who are counseled to avoid herbs in the communities around Rakai. Only 2% of HIV- infected participants reported herb use. While this lower number of HIV-infected participants reporting herb use could represent effective counseling, the difference in herb use among those on ART and those not on ART was not significant (1% vs. 2%, $p=0.42$). The small number of participants reporting herb use limited many comparisons (e.g., herb-ART interactions) and suggests that our findings should be interpreted cautiously.

An important limitation of this study is the potential for reverse causality. Although the most frequently used families of herbs in this study contain known hepatotoxins (see table 3), it is possible that the association of fibrosis with herb use could represent reverse causality, or persons with symptomatic liver disease being more likely to use herbal medicines. According to consultations with local traditional practitioners, some of the herbs in Asteraceae and other families are sometimes prescribed for “fever with jaundice”. However, none of the study participants had been previously diagnosed with liver disease within the formal medical system or by traditional healers. Most herbs used in this study to treat fever are usually taken for general fever (“fever” or “malaria”), not fever with jaundice (“yellow fever”).

Conclusions

More studies are needed to assess the impact of traditional herbal medicines in sub-Saharan Africa. Phytochemical, pharmacokinetic and prospective clinical studies are needed to investigate herb contents, benefits, side effects, direct toxicity, and herb-ART interactions. Plants in the Asteraceae family reported in this study should be prioritized for these investigations.

The risk of liver disease associated with herb use was similar in the overall study population and among HIV-infected participants. Given the potential of at least additive risk of hepatotoxicity with long term use of some antiretroviral drugs, as well as the potential for herbs to alter the pharmacology of antiretroviral drugs, it may be prudent to counsel HIV-infected persons against herb use in sub-Africa until there is data about the safety of specific herbs. Counseling about herb use should be part of routine health counseling and counseling of HIV- infected persons in sub-Africa.

Author Contributions

Conceived and designed the experiments: LS SJR TCQ PO GDK. Performed the experiments: LS SJR IB PO. Analyzed the data: BJA LS GDK AN. Contributed reagents/materials/analysis tools: CKB. Wrote the paper: BJA LS. Provided additional technical assistance and contributed to interpretation of the data: BJA CKB ML CM AS PO VK IB FN AN MJW RHG DLT.

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