Herb-induced Liver Injury—A Guide to Approach. Lessons from the *Tinospora cordifolia* (Giloy) Case Series Story

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Background: ***Tinospora cordifolia*** **(TC) is being increasingly consumed in India for its health and suggested immune-enhancing benefits in preventing and countering COVID-19. We previously published our experience of hepatotoxicity with self-medication of TC in six individuals. Since herb-induced liver injury (HILI) has been described with *Tinospora crispa* (TCR) consumption, it was contested that our patients may have mistakenly self-medicated with TCR which is similar in appearance to TC. *Methods:* We collected the four plant samples and two commercial preparations that were consumed by our patients for further analysis. The six samples underwent high performance thin layer chromatography phytochemical analysis and DNA barcoding studies for the confirmation of the genus and species. The four plant part samples which included stems and leaves were also analysed by a botanist for the characteristic morphological and microscopic features. *Results:* Based on morphological, microscopic, phytochemical and DNA studies, the four plant part samples were identified as TC. The two commercial preparations could not be analysed on phytochemical analysis or DNA barcoding studies due to other ingredients that most likely interfered with the analysis. The herb consumed by our study subjects was confirmed to be *Tinospora cordifolia*. *Conclusion:* We have highlighted the key morphological and phytochemical differences between these two species. We propose an algorithmic approach to accurately identify the implicated herb in cases of HILI. Future studies on causality need to focus on the serological/histopathological identification of active herb/metabolites in human tissues.** **(J Clin Exp Hepatol 2023;13:360–371)**

Tinospora Cordifolia (TC) has been a popular over-the-counter herbal immune boosting supplement for a vast proportion of Indians. TC, popularly known as Guduchi, Moonseed, Giloy and Amrita in Sanskrit, is a popular herb used in many traditional medicinal practices.[1] With the start of the COVID-19 pandemic in March 2020, the self-medicated use of Giloy increased. In July 2021, we published our experience in patients presenting with liver injury following the consumption of TC.[2] We presented 6 cases of patients with acute hepatitis and a history of consumption of TC. After thorough history-taking and acquisition of routine serological parameters, the patients were subjected to a liver biopsy. The details of patient profiles and histological features are in Table 1. We had concluded that the herb TC due to its immune booster properties caused an autoimmune like hepatitis or unmasking of a latent autoimmune liver disease. In a previous paper, Karousatos et al.[3] had described a single patient with Giloy-related possible liver injury toxicity although the patient was not biopsied. Subsequently, others have described their experience with TC-induced hepatotoxicity.[4–10] In an editorial by Bjornsson et al. on our publication of the potential hepatoxicity of TC, the editors analysed the data and concluded that the liver injury of TC was suggestive of a drug-induced autoimmune hepatitis.[11]

In India, herbal products have been widely used for centuries.[12] However, the drugs have not gone through rigorous-phased trials to establish their efficacy and safety. Besides, most of the commercial preparations are mixtures of several herbs and their interactions as well as toxicities have not been well researched. Since *Tinospora crispa* (TCR), a closely related plant has been described to be

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Abbreviations: COVID-19: Coronavirus Disease 2019; DILI: Drug Induced Liver Injury; DNA: Deoxyribonucleic Acid; HILI: Herb-Induced Liver Injury; HPTLC: High Performance Thin Layer Chromatography; RUCAM: Roussel Uclaf Causality Assessment Method; TC: *Tinospora Cordifolia*; TCR: *Tinospora Crispa*
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Table 1 Biochemical, Serological, Histopathological, HPTLC and DNA Profile of Six Patients Consuming *Tinospora cordifolia*.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age/sex	40/M	54/F	38/M	62/F	56/F	56/F
TC preparation consumed	Stem	Stem	Stem	Commercial preparation (Syrup)	Stem	Commercial preparation (Tablet)
Laboratory parameters						
Hb (g/dl)	13.7	10.9	9	12.4	11.5	11.8
TLC (cells/cmm)	16000	6200	9200	8400	8550	6690
Platelets (cells/cmm)	388000	187000	371000	103000	241000	140000
Presentation total bilirubin (direct bilirubin) (mg/dl)	7.9 (6.7)	15.3 (10.9)	7.4 (3.9)	9.1 (5.7)	12.2 (7.5)	9.13 (4.41)
Peak total bilirubin (direct bilirubin) (mg/dl)	45.1 (25)	24.9 (21)	20 (10.7)	15.1 (12.5)	12.2 (7.5)	9.1 (4.4)
Peak AST (IU/L)	1773	1195	1504	2222	1099	455
On presentation ALT (IU/L)	2894	768	560	202	256	207
Peak ALT (IU/L)	3114	768	1482	855	256	472
Time to normalisation of LFT (in days)	95	164	78	111	38	53
Autoimmune profile (pre/post)[a]						
ANA	Negative/–	1:100/–ve	1:100/–ve	1:320/1:320	Negative/–	Negative/–
Anti-SMA	Negative/–	Negative/–	–	Positive/Negative	Negative/–	Weakly positive/Negative
Serum IgG (700–1600 mg/dl)	Normal/–	Normal/–	Normal/–	–	2570/1721	2045/1680
Revised AIH score[b] (pre/post when steroids given)	12	19/21	15	19/22	18/21	18
Liver injury pattern on biopsy	Hepatocellular	Hepatocellular + Cholestatic	Hepatocellular	Hepatocellular	Hepatocellular	Hepatocellular
Updated RUCAM score ref[c]	4	5	4	7	7	4
HPTLC (Band at Rf 0.61)	Seen	Seen	Seen	Not seen	Seen	Not seen
DNA Barcoding	TC	TC	TC	NR	TC	NR

Abbreviations: AIH, Autoimmune hepatitis; ALT, Alanine aminotransferase; ANA, Anti-nuclear antibody; Anti-SMA, Anti-smooth muscle antibody; AST, Aspartate aminotransferase; DNA, Deoxyribo Nucleic Acid; F, Female; Hb, Haemoglobin; HPTLC, High Performance Thin Layer Chromatography; IgG, Immunoglobulin G; LFT, Liver function tests; M, Male; NR, Not recordable; TC, *Tinospora Cordifolia*; TLC, Total leucocyte count.

+++: Prominent/Abundant ++: Moderate +: Mild/Occasional –: Not seen/done.

[a]Pre-stopping the drug and post-stopping the drug, – implies not done as previous ANA/ASMA were negative or IgG was normal.

[b]Revised AIH score: Pre-treatment: Definite AIH >15 Probable AIH l0-15 Post-treatment: Definite AIH >17 12 Probable AIH 12–17.

[c]Updated RUCAM score and resulting causality grading: #0, excluded; 1–2, unlikely; 3–5, possible; 6–8, probable; and 9 highly probable.

Table 2 Comparative List of Characteristic Differences Between TC and TCR.

	Tinospora Cordifolia[11,12]	*Tinospora Crispa*[11,12,20]
Distribution in India	Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Goa, Karnataka, Kerala, Maharashtra, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal[21]	West Bengal, Odisha, Arunachal Pradesh and Assam,[22] extending to Southeast Asia
Stem morphology	• Less prominent tubercules • Deciduous climber with seriate stems	• More prominent blunt tubercles • Fleshy old stems—striate and often with aerial roots
Micromorphology of stem	• Transverse section is wedge-shaped • More layers of cork cambium with distinct walls • Protoxylem is not clearly distinguishable in pith • Less prominent pericyclic layer that continues forming a sclerenchymatous cap of lignified cells • Fewer vascular bundles	• Transverse section is circular • Few layers of lenticels with wavy walls. • Pith shows protoxylem • Larger number of vascular bundles • More prominent pericyclic layer
Leaf morphology	• Heart-shaped, 10–20 cm long with a 3–4 cm petiole broadly ovate-cordate, sinuate at base, abruptly cuspidate-acuminate at apex • Leaf surface has characteristic epicuticular waxy coating	• Heart-shaped 6–12 cm long with a 5–15 cm petiole, broadly ovate to oblong-orbicular, shortly or deeply cordate at base and caudate at apex • Epicuticular waxy layer is absent on leaf surface
Flowers	• Inflorescences are usually 5–15 cm and solitary, being pseudo-racemose, axillary, or leafless branches	• Male inflorescences are 5–10 cm or more; female inflorescence are 2–6 cm long with yellowish-green flowers

Botanical terms explained in order of appearance: tubercules—protuberances, seriate—arranged in rows, striate—with parallel lines or grooves, cork cambium—inner layer of the bark/epidermis, Protoxylem—the first formed vascular tissue seen in the central part of the stem, pericyclic layer—cells that surround the vascular bundles, lignified cells—cells with thick cell walls that help water conduction, petiole—stalk, sinuate—wavy margin, cuspidate-acuminate—tapering tip with a sharp point, epicuticular—outermost layer, ovate—egg shaped, oblong-orbicular—rounded, caudate—attenuated to tail-like.

potentially hepatotoxic,[13,14] the Ayurveda community queried whether our patients mistakenly consumed the wrong herb and whether that could be an explanation for the reported herb-induced liver injury (HILI).

The aim of our study was to correctly identify the herbs consumed by our patients. To that end, we have been able to provide an algorithmic approach to a patient who consumes an unknown herb that causes liver injury and which serves as a guide to identify the herb and further management.

METHODS

An institutional ethical clearance (EC/1087/2021 dated 7-6-2021) was obtained. A written informed consent was taken from all patients who participated in our study,[2] and samples of the plant parts consumed by them were collected. There were six patients described to have HILI with TC, four of them had consumed extracts of the stem (n = 4), while two patients consumed commercially available preparations containing TC (n = 2).

The samples of the plant parts (stem and leaves) were preserved by wrapping them in dry paper, which were then examined by a botanist for morphological characteristics. Plant samples (stem pieces) provided by patients were sectioned by hand to obtain the transverse sections of stem. The sections were stained with toluidine blue and safranin for microscopic evaluation of anatomy (both gross anatomy and microscopic tissue anatomy).

All 6 samples, including the 2 commercial preparations, were processed for phytochemical analysis by high performance thin layer chromatography (HPTLC). After being identified by the botanists, controls for the plant parts (TC and TCR) were obtained from plant nurseries maintained at Mahim Nature Park and Sanjay Gandhi National Park, Mumbai, respectively. Methanolic extracts of the specimen were prepared by overnight extraction and were spotted on 20 × 10 cm TLC plate, precoated with silica gel 60 F_{254} (0.2 mm thickness; Cat. No. 1.05554.0001; Merck, Darmstadt, Germany). Samples were spotted using the CAMAG Linomat V Automatic Sample Spotter (Muttenz, Switzerland) fitted with a syringe (100 μL; Hamilton). The plates were developed in a CAMAG glass twin trough chamber (20 × 10 × 4 cm) with mobile phase of chloroform:ethylacetate:ethanol:formic acid (10:15:5:0.5 v/v/v/v). After drying, the plates were derivatised with methanolic sulphuric acid and visualised under 366 nm. Densitometric scanning was performed at 366 nm (mercury lamp) using CAMAG TLC Scanner 4 linked to WINCATS software. Distinctive bands obtained for authenticated specimen were compared with that obtained from the patient samples.

The 6 samples were also processed for DNA analysis for confirmation of the species.

Table 3 Published Data on Liver Injury Related to *Tinospora cordifolia* (Giloy[a]).

S. no	Study	No. of patients	Herb	Form	Max. Bilirubin (mg/dl)	Max. AST/ALT (IU/L)	Max. INR	Biopsy	Treatment	Outcome
1.	Karousatos CM *et al.*[3]	1	Giloy	Commercial preparation	3.7	1086/1451	1.0	No	Supportive treatment	Complete resolution
2.	Kulkarni AV *et al.*[4]	43	Giloy, giloy containing formulations	Plant parts/local chemists' preparations/commercial preparations	46.6	1637/1269	6	Yes (22/43)	One—liver transplant; Others Supportive treatment	2 mortalities; Others had Complete resolution
3.	Sahney A *et al.*[5]	3	Giloy	Plant part (twig)	23	2300/2150	2.3	Yes	Supportive + Steroids (2)	Complete resolution
4.	Gupta D *et al.*[6]	3	Giloy	Commercial preparation (tablet): 1 Plant part (twig): 2	24.99	1198/1044	Not specified	No	Supportive treatment	Complete resolution
5.	Rastogi M *et al.*[7]	2	Giloy	Plant part	8.9	679/436	1.29	Yes	Supportive + Steroids	Complete resolution
6.	Gupta H *et al.*[8]	2	*Tinospora cordifolia* (TC)	Commercial preparation (juice): 1 Plant part (twig): 1	20.1	697/645	2.8	Yes	Supportive + Steroids	Complete resolution
7.	Parikh P[9]	2	*Tinospora cordifolia*	Commercial preparation (capsule)	9	460/400	1.34	Yes	Supportive + Steroids	Complete resolution
8.	Gupta S *et al.*[10]	2	Tinospora Species not specified	Plant part (stem): 1 Commercial preparation (pellets): 1	Not specified	Not specified	Not specified	Yes	Supportive-1 Liver transplant-1	Complete resolution

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.
[a]Giloy is one of the local names for *Tinospora cordifolia* in India.

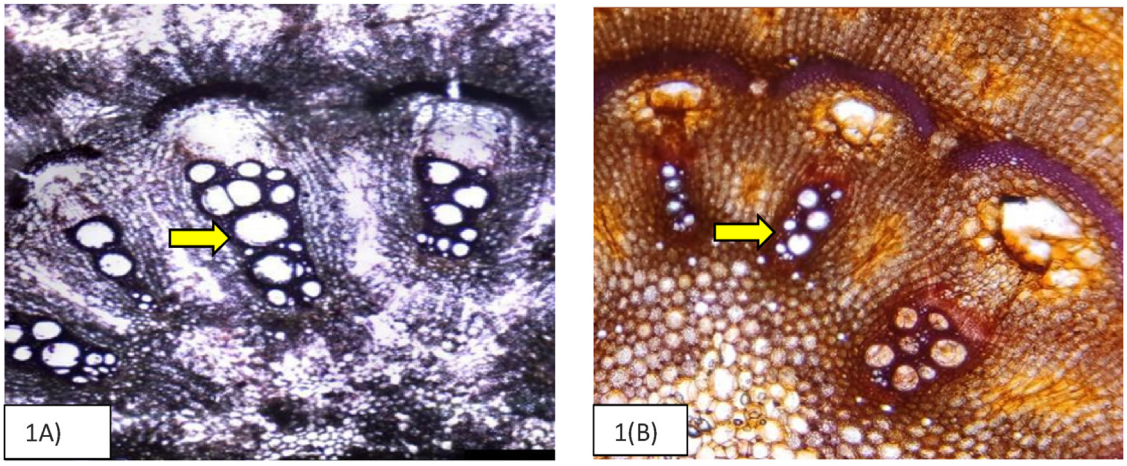

Figure 1 Hand cut transverse sections of stem of *Tinospora cordifolia* (**A**) and *Tinospora crispa* (**B**) stained with Toluidine Blue and Safranin showing distinctive vascular bundles (the conducting vessels in the stem (indicated by yellow arrows) that help in transport of minerals and water) in each species. The sections are viewed under a light microscope at 40×.

1. DNA was isolated from the plant sample provided by the patient while the quality was initially evaluated on 1.0% agarose gel. A single band of high-molecular-weight DNA was observed indicating the purity of the sample.
2. Mitochondria, chloroplast and nuclear genes were amplified using universal markers such as maturase K (matK), ribulose 1,5-biphosphate carboxylase and internal transcribed spacer and the amplified products were purified.
3. Sanger sequencing of amplicons was carried out using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyser.
4. The amplicon gene sequences were used to carry out basic local alignment search tool with the 'nr' database of NCBI GenBank database and Barcode of Life Data system database.

RESULTS

(i) Morphological and micro-anatomical studies

The fresh plant specimens of stem and leaf were evaluated for gross morphological and micro-anatomical features.

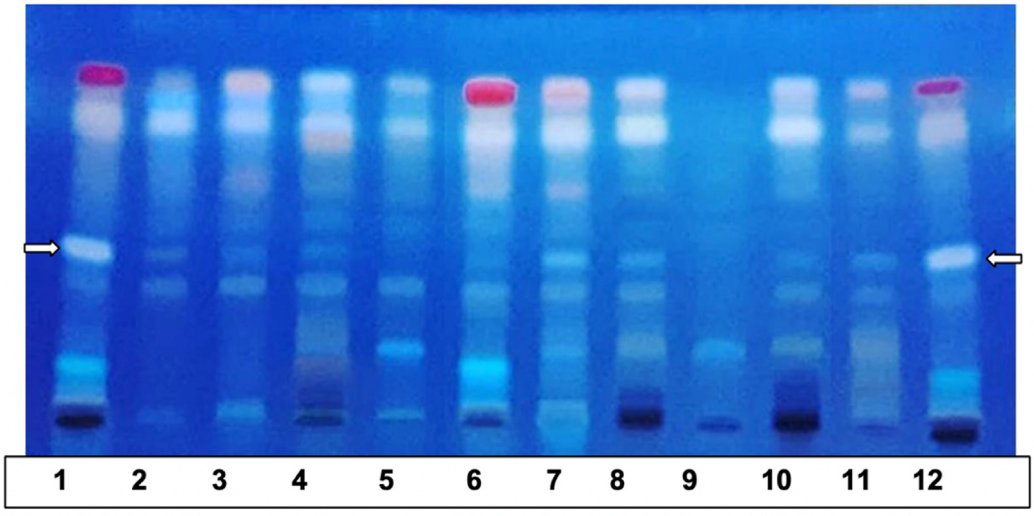

Figure 2 HPTLC fingerprint of various *Tinospora* samples along with sample of *Tinospora crispa*, visualised at 366 nm after derivatisation with 10% methanolic sulphuric acid. Track Details: Track 1—*Tinospora cordifolia* leaf (control sample); Track 2—Patient 1 sample stem; Track 3—Patient 2 sample leaf; Track 4—Patient 2 sample stem; Track 5—Patient 3 sample capsule; Track 6—*Tinospora crispa* leaf (control sample); Track 7—Patient 4 sample leaf; Track 8—Patient 4 sample stem; Track 9—Patient 5 sample syrup; Track 10—Patient 6 sample stem; Track 11—*Tinospora cordifolia* stem (control sample); Track 12—*Tinospora cordifolia* leaf (control sample). Note the distinct band at Rf 0·61 (arrows) seen in all *Tinospora cordifolia* samples and in patient samples (as seen in earlier Figure 1). Note the absence of the band at Rf 0·61 in *Tinospora crispa* (Track no. 6). The general pattern of bands seen in *T. crispa* is different from those in *T. cordifolia* (Tracks 1, 6, 11 and 12). HPTLC, high performance thin layer chromatography.

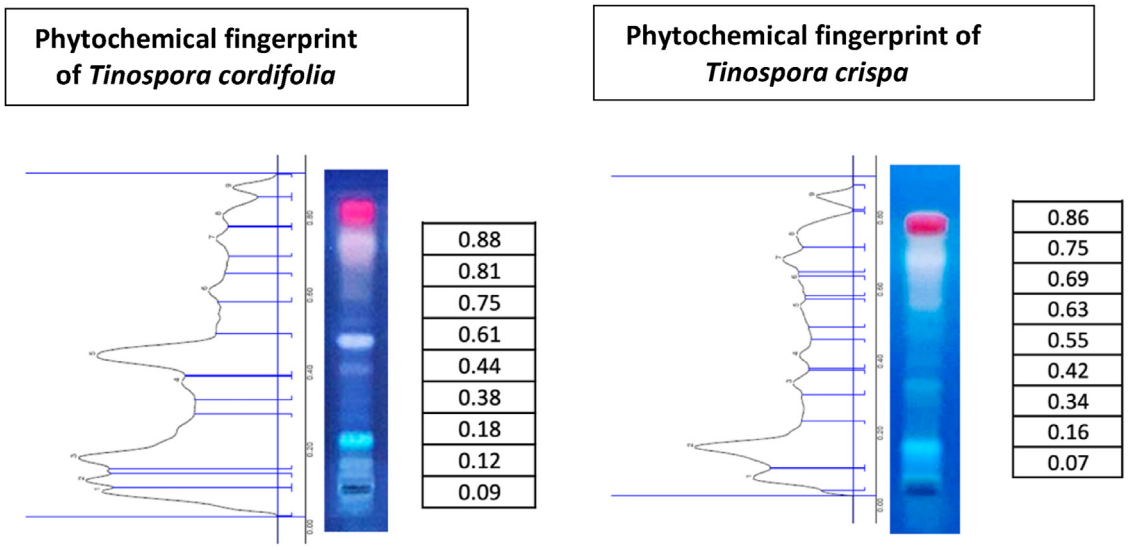

Figure 3 The thin layer chromatography (TLC) fingerprint of phytochemicals from *Tinospora cordifolia* and *Tinospora crispa*. The TLC chromatograms are shown along with the densitometric scan at 366 nm and the Rf values of respective bands in a table. The distinct absence of bright band at Rf 0·61 is absent in the *Tinospora crispa*.

The observations made are in concurrence with those reported earlier.[15,16] *Tinospora* species appear similar in gross observation, especially in their external morphologies. They all have broadly cordate leaves (heart-shaped leaves are attached at the tip of a stalk [petiole] within a notch) and cylindrical stems often with lenticels (raised pores or openings present on the outer surface of the stem, responsible for gaseous exchange). On closer evaluation, distinctive morpho-anatomical features of stem and leaves can be observed. The broad differences have been elaborated in Table 2. Leaves are smooth in both TC and TCR. The stem of TC is mainly used as the crude drug. The young stems in both TC and TCR are green with smooth surfaces but swollen at nodes (points on the stem from where leaves or branching twigs arise). Older stems are light brown in colour and their surface shows wart-like outgrowths that clearly protrude out. These protuberances are due to circular lenticels. A transverse section of the stem taken by hand shows a single-layered epidermis in both TC and TCR as seen in Figure 1.

(ii) HPTLC

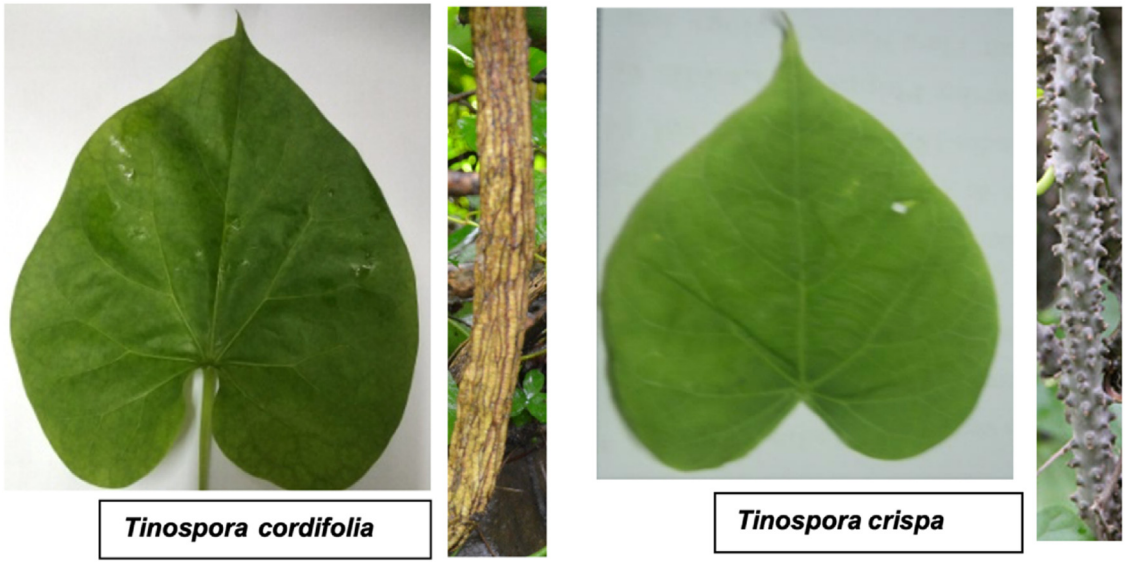

Figure 4 External morphology of leaf and stem of *Tinospora cordifolia* and *Tinospora crispa*. Note the prominent warty protuberances on the stem surface in *T. crispa*.

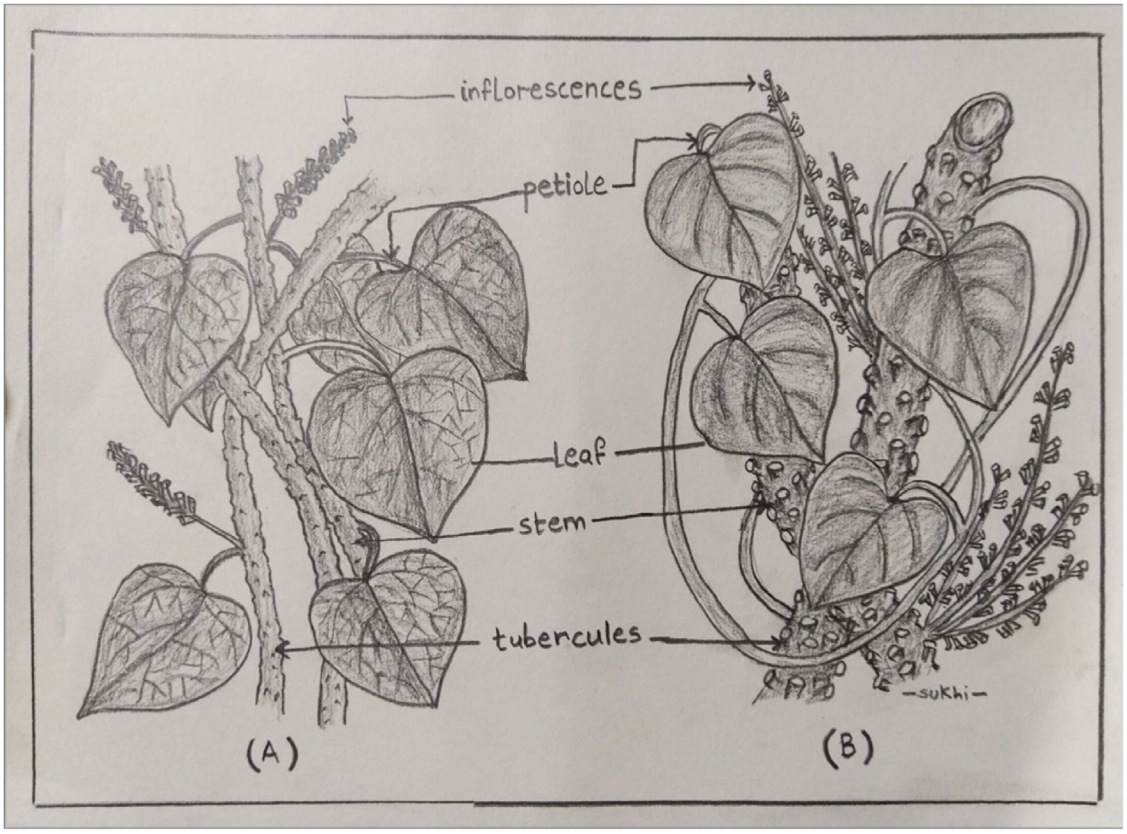

Figure 5 Illustrations of *Tinospora cordifolia* (**A**) and *Tinospora crispa* (**B**) showing morphological differences in the plant species.

The 4 specimens and 2 commercial preparations were evaluated chromatographically using HPTLC at Ramnarain Ruia College Laboratory and phytochemical fingerprints obtained were compared with those of authenticated plant samples. The distinctive band seen in the TC at Rf 0.61 (Figure 2) is absent in the TCR *s*pecimen (Figure 3), where Rf is Resolution Front, which is the ratio of distance travelled by the analyte to the distance travelled by the solvent. The distinctive band at Rf 0.61 of TC shown in Figure 2 is seen in all plant material reportedly consumed by the patients. The 2 marketed formulations of TC consumed by the patients (syrup and capsule) did not show the distinctive HPTLC band at Rf 0.61, although the labels on these commercial preparations mentioned only Giloy as the content and with no other constituent. The findings for each patient have been mentioned in Table 1.

(iii) DNA Barcoding

All six samples underwent DNA barcoding for identification of the species at Barcode Biosciences. As observed in HPTLC, the two commercial preparations of the tablet and syrup did not show up as TC on the DNA analysis also.

According to our analysis, all four samples of stems provided for plant DNA analysis were of TC. This can be interpreted from the sequence similarity table, phylogenetic tree and distance matrix. The sequence similarity table shows us the length of query coverage (which should be 100% for identifying a species). The percentage identity for the 100% query covered sequence when is more than 98% can be concluded as same species. Always the highest percentage of matched species is concluded as the species.

Similarly, the distance matrix and phylogenetic tree also can show the closest species. The sample shares one branch with the nearest species in the phylogenetic tree whereas in the case of the distance matrix, the distance among the species should be less than 0·002. The lesser the distance, the closest the species. Further, the control samples of TC and TCR were also confirmed on DNA analysis. The DNA findings of the 6 patients have been stated in Table 1.

DISCUSSION

The COVID pandemic saw an increase in the use of Ayurvedic products, unsupervised use of plant parts and renewed interest in wellness products.[17] We published our experience with Giloy related histologically proven hepatotoxicity in 6 patients,[2] following which many authors published similar experiences (Table 3). It has been suggested that a closely related species, TCR, which is morphologically similar to TC but known for its hepatotoxicity, could

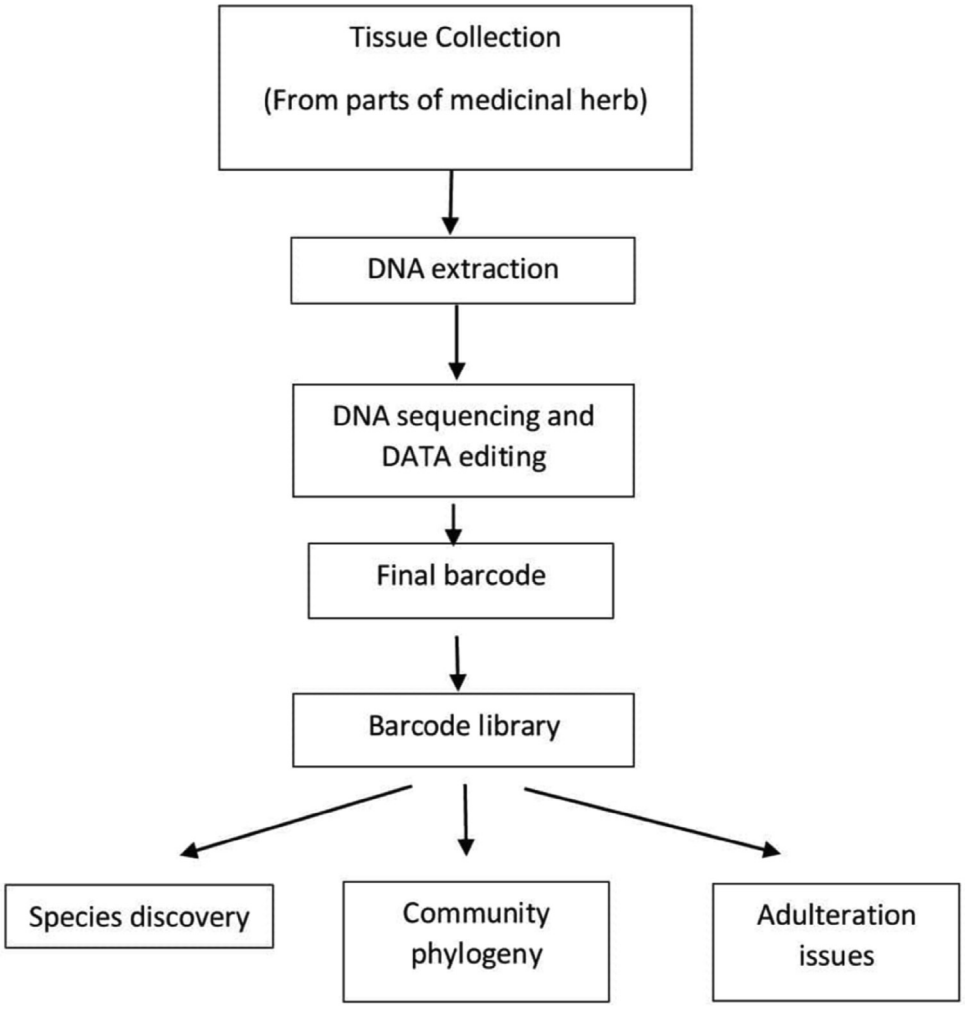

Figure 6 Flow chart depicting simple procedure and use of DNA barcoding in medicinal plants.

have been mistakenly consumed by our patients.[18] Hence, a study to provide further insights into the characterisation of these plant species was planned. It is not only important for knowing the purity of original plant species in the preparations consumed by patients but also for providing a platform for biochemical and phylogenetic relationship studies in similar-looking herbs.

Owing to the time in the pandemic when laboratories were not functioning at full capacity, analysis of the plant and plant parts was not readily possible. The samples, provided by the patients, have now been analysed morphologically, phytochemically and by DNA barcoding. The results prove beyond doubt that the patients did in fact, consume TC. The fact that we analysed pure plant parts in four of our patients helped in the identification of the species as opposed to the commercial preparations (capsule and syrup) which could not be reliably analysed. It has been estimated that over 35% of websites have wrongly identified TC and TCR.[19] This experience convinced us that accurate species identification is imperative for implicating a causative herb in HILI.

TC is a large, glabrous, perennial, deciduous, climbing shrub, of the genus Tinospora, belonging to the family Menispermaceae.[20] The genus Tinospora has 34 species, of which nine are found in various parts of India.[21] Of these, TC and *T. Sinensis* have therapeutic applications.[22] TCR, is a similar-looking plant of the family Menispermaceae, and of known hepatotoxic potential.[14,19] The differences in morphology can be appreciated in Figures 4 and 5.

In HPTLC, a technique of separation of the phytochemicals, both TC and TCR showed distinct bands that help in differentiating one from the other. TC showed a light blue fluorescent band at Rf 0·61 which was characteristically absent in TCR. HTPLC performed on commercial samples labelled giloy did not yield any result possibly due to the low concentration of the extract or the interference of other additives in the commercial preparation. DNA barcoding is a method of species identification using a DNA sequence from a specific gene. Individual sequences (bar code loci) can be used to discriminate the closely related species and identify new cryptic species also.[23] Depending on the taxon and complexity of the species, different

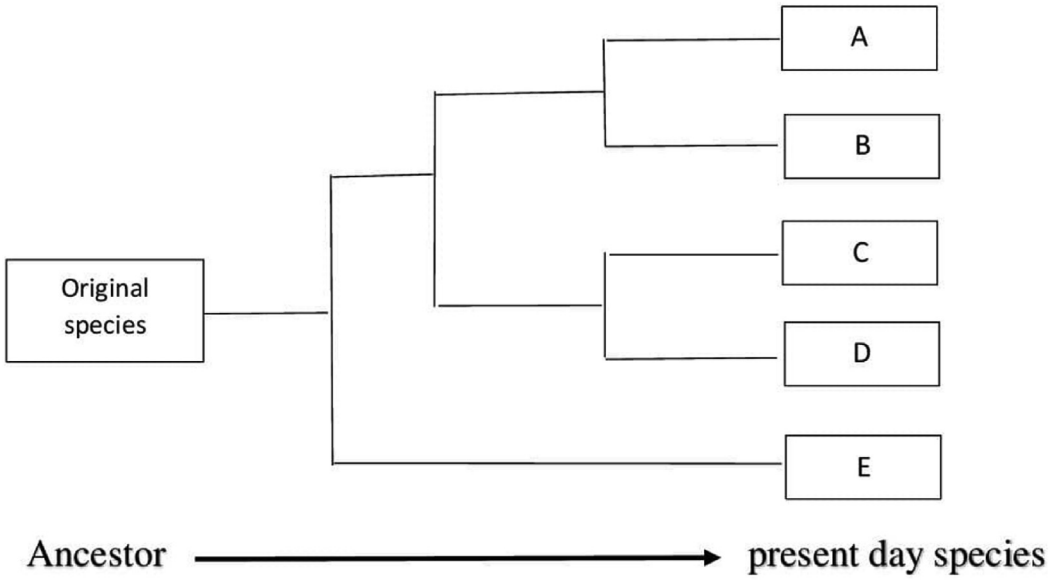

Figure 7 Simplified phylogenetic tree to show how present species evolve from original plant species.

barcode loci are used for the purpose. For example, TC belongs to the Menispermaceae family which includes more than 400 plant species and various subspecies that may have similar appearances. The simple flow chart in Figure 6 shows the procedure and the use of DNA barcoding in plants. Figure 7 demonstrates the phylogenetic tree of a plant species. As the original i.e. ancestral plant evolves and diversifies into various species (A to E), it is important to differentiate these species from each other as they may look similar or have very closely related properties. This is important in validating the purity of medicinal herb species in a formulation. Hence along with phytochemical study, we also undertook DNA studies.

TC has been reported to contain phytoconstituents like terpenoids, alkaloids, lignans, carbohydrates, bitters, steroids, glycosides, sesquiterpenoids, aliphatic compounds, essential oils, fatty acids and polysaccharides.[24] It is a *Rasayana* drug, widely used in the Ayurvedic system of medicine as an immune modulator for improving the body's resistance to infections.[25] The stems of TC, contains various constituents like phenyl propanoid glycosides like cordifolioside A and B, syringin, Phytosterol; 20-β-hydroxyecdysone, diterpenoids like tinosporaside, tinosporin, tinosporidine, tinocordifolioside and alkaloids like columbin, isocolumbin, berberine and magnoflorine.[24,26–28] Cordifolioside A has been reported to have significant immunostimulant with cardio and radioprotective activities.[29] 20-β-hydroxyecdysone has been reported to be a potent immunoprotective wound-healing agent.[30] Columbin has anti-inflammatory, anti-cancer and antioxidant properties.[30] Based on the published potent immunomodulatory properties of phytochemicals, TC could augment the immune response in patients with autoimmune problems, leading to clinically significant complications as experienced in our patients.[29]

Studies of TCR extracts and isolated compounds of TCR have shown a broad range of pharmacological activities such as anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, antimalarial, cardioprotective and anti-diabetic effects.[31] However, studies in rats and humans have indicated hepatotoxic effects of ethanolic extracts of TCR.[13,14]

A multicentre study from India further reiterated the findings of our study by highlighting the liver injury associated with the use of Giloy in commercial pure and multiherbal preparations.[4] In their study, in patients with a history of consumption of commercial preparations of Giloy, they analysed the product for heavy metal contaminants, potential hepatotoxic organic compounds and residual pesticides.[4] Twenty-five patients consumed Giloy in a pure or extracted form, while eighteen consumed it as a part of a multiherbal formulation. Heavy metal contamination with mercury, arsenic and lead above prescribed limits was found in two samples, while others noted multiple phytochemicals with potential immunomodulatory effects and hepatotoxic potential.[3] We did not perform the analysis for any heavy metals in the two commercial preparations as the main aim of our study was to identify the correct herb. However, in the commercial preparations analysed by us, there seemed to be multiple constituents as the main constituent on the label 'Giloy' could not be identified in both HPTLC and DNA studies.

Herbs are easily consumed without medical supervision with the impression that they are completely safe. In a patient with documented liver injury and a history of consumption of an herb, we propose an algorithm to

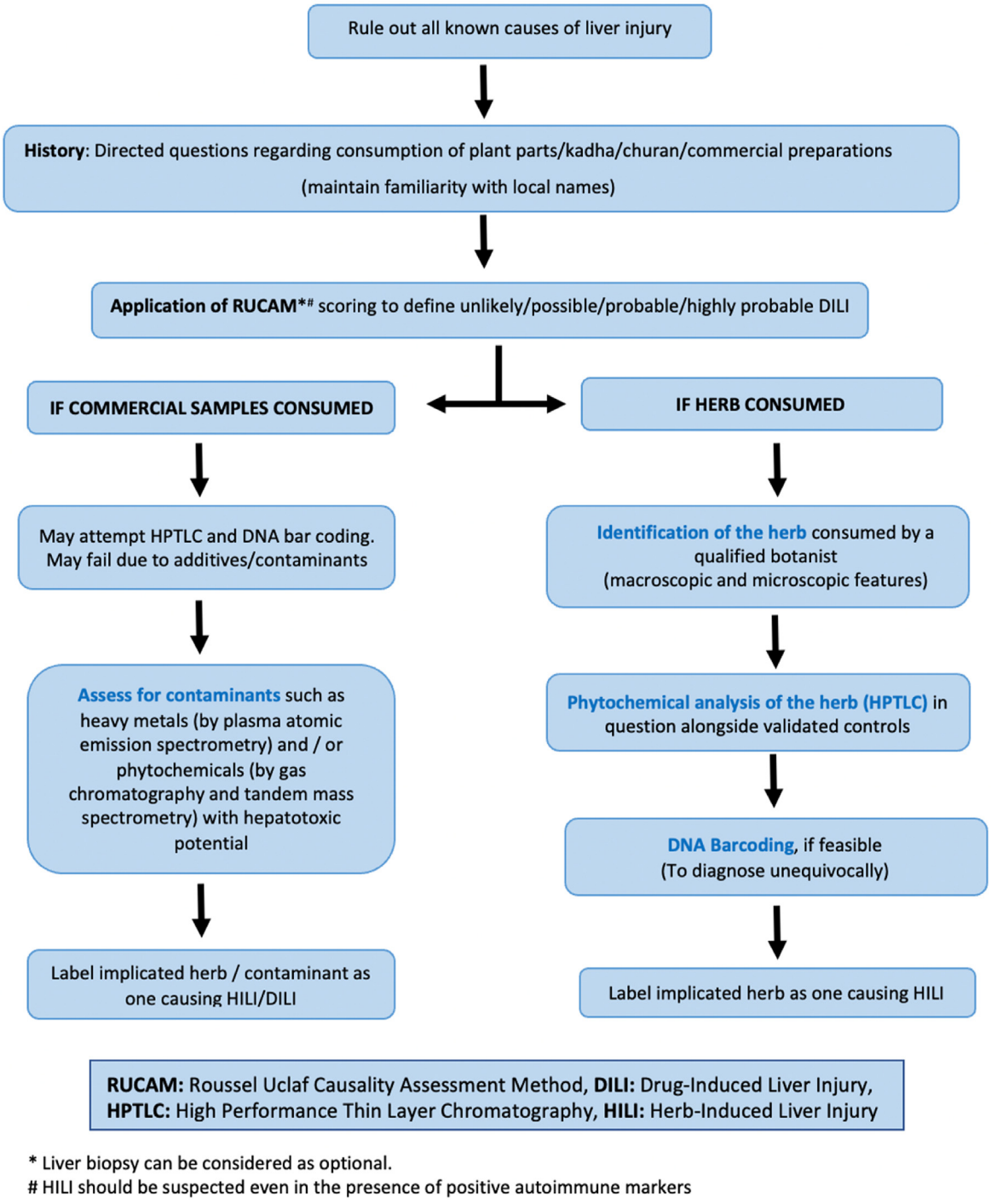

Figure 8 Algorithm to detect herb-induced liver injury (HILI) in patients with history of herb consumption and liver injury.

accurately identify the herb (Figure 8). Documentation of such observations will aid in establishing the hepatotoxic potential of herbs. Further, a positive autoimmune profile or raised immunoglobulin G does not rule out HILI and a detailed drug/herb history needs to be taken. Liver biopsy although optional, would be prudent to rule out alternate etiologies or suspected dual etiologies eg. NAFLD and HILI or autoimmune hepatitis and HILI.

We did not estimate the heavy metals in the commercial preparations of the herbs and this could be considered a limitation of the study although the patients took 'pure' TC. Further, HPTLC and DNA barcoding are expensive

and not available in commercial labs, making its utilisation difficult in the identification of the herb implicated in HILI.

In conclusion, we have described our methods—botanical, HPTLC and DNA barcoding to identify a herb and suggested an algorithmic approach. Using these methods, we have been able to confirm the self-medicated herbs by our patients as being that of TC. Patients presenting with HILI, represent only a fraction of patients with this problem. Complementary and alternative medicines (CAMs) are widely consumed by the Indian population but not all develop HILI. Data regarding the denominator of patients consuming herbs and the numerator i.e. those developing HILI is unknown. Besides data on patient characteristics, dosages at which one develops HILI, interactions with other drugs and herbs is also lacking and may be an area of study in the future. Besides, identifying specific phytochemicals in the blood samples of the patient for direct identification of the implicated herb may be an area of future research and potentially become an inexpensive, serum-based test for the identification of HILI.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

A Nagral contributed to the conception and design of the article, editing and critical revision of the manuscript. *O S Rudra* contributed to the design of the article, acquisition of the data and writing of the manuscript. *S Menezes* contributed to the design of the article and editing of the manuscript. *S Menon* contributed to drafting the manuscript and analysis and interpretation of the HPTLC data. *S Shailajan* contributed to acquiring and editing the botanical data. *S Mallakmir* was responsible for interpreting the DNA studies. *R Reddy* contributed to the critical revision of the manuscript. All authors have read, edited, and approved the final document prior to submission.

CONFLICTS OF INTEREST

All authors have none to declare.

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