Apparent hepatotoxicity of Giloy (*Tinospora cordifolia*): Far from what meets the eyes

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Abstract

Tinospora cordifolia is a wonder plant that has been prescribed in the ancient Indian medicinal system of Ayurveda as an immunity and hepatic function booster, and modulator for diseases. Pharmacological benefits of *T. cordifolia* has been attributed to the rich collection of phytoconstituents belonging to the broad classes of alkaloids, polyphenols, and sesquiterpenoids. In a recent article, titled 'Herbal Immune Booster-Induced Liver Injury in the COVID-19 Pandemic - A case series' published in the Journal of Clinical and Experimental Hepatology, reports for six patients with drug-induced liver injury following *T. cordifolia* consumption. However, the study lacks a proper recording of the patient medical and medicinal case-histories. Of the 6 patients covered in the study, 5 patients reported pre-existing co-morbidities that were largely under-reported by the authors. The authors also did not report the other medications taken by the patients for their co-morbidities. Medications for some of the co-morbidities have also been associated with observed hepatotoxic effects. Also, the study claim idiosyncrasy without reporting an approximate dose-range for *T. cordifolia* taken by each patient and its correlation to the observed hepatotoxicity. Finally, the study does not present a phytochemical evaluation of the *T. cordifolia*, or analyze the plant–related chemicals present in the liver biopsy and blood serum samples. At the end, the published article presents a far-fetched conclusion masking the beneficial effects of *T. cordifolia* (Giloy/ Guduchi). Hence, case-series deductions should be carefully re-evaluated and more scientific evidence should be presented for linking *T. cordifolia* directly to the observed hepatotoxicity.

Tinospora cordifolia is a wonder plant that has been prescribed in the ancient Indian medicinal system of Ayurveda for augumenting immunity and hepatic disease amelioration. The plant is commonly known as 'Giloy' or 'Guduchi' and is distributed in the tropical parts of Indian sub-continent growing at an altitude of 300-1000 meters above sea-level. *T. cordifolia* has shown good efficacy in ameliorating fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases, chronic diarrhea, and dysentery[1, 2 3]. *T. cordifolia* comprises of several plant components such as alkaloids, diterpenoid, lactones, glycosides, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides such as tinocordiside, tinosporine, tinocordifolioside, tinosinenosides, cordifoliside, berberine, palmatine and magnoflorine[3].

In a recently published article by Nagral *et al.*, the authors have presented 6 patient-related case reports of drug-induced liver injury [DILI] following *T. cordifolia* consumption in the form of boiled extract, tablets, and syrup[4]. However, the presented results are far-fetched based on the presented scientific evidence and contain several lacunae. Proper recording of the medical history for the patients and medicinal dosage of the patients is of utmost importance in any case study and its reporting[5]. Nagral *et al.* have failed to mention the preceding medical histories of the patients along with other prescription or over-the-counter drugs taken by them with the varying doses of *T. cordifolia* extracts[4]. Without a methodical recording and mention of the medical or medication histories of the patients, it is difficult to identify any causative factor for an ongoing disease. Amongst the 6 cases reporting hepatic malfunctions, five were diagnosed with jaundice while the remaining one showed yellow discoloration of urine and eye. A 40-year-old male without any co-morbidities took plant twigs (10 to 12 pieces) boiled with cinnamon and cloves in half-a-glass of water once in two days for 3 months. One 54 years' old female patients took a single *T. cordifolia* twig boiled extract per day for seven months, and another 62 years old female patient took a commercially available syrup containing *T. cordifolia* extract (15 ml/day) every alternate day for a month. Both these patients were

suffering from type-2 diabetes mellitus. A 38-year-old male with Beta-thalassemia minor consumed 3-4 plant twigs boiled extract (15 ml/day) for 6 months. Lastly, two 56 years old females suffering from hypothyroidism respectively took *T. cordifolia* twig boiled extract 2 to 3 days/week for 3 weeks, and a commercially available *T. cordifolia* tablet per day for 3 months. In most of the cases, the medical histories mentioning the severity of the pre-existing diseases and their medications were missing. The study has presented the observed hepatotoxic effects as idiosyncrasy, meaning randomness of the observed toxic effects in few sporadic cases[6]. Without a proper documentation of additional drugs being taken by the patients, it is incorrect to associate the observed hepatic injuries with a single herbal formulation. Besides, an important characteristic of DILI is the long-lag time between the start of the drug and onset of the injury, and specially in case of immunogenic idiosyncratic DILI this time window is further prolonged[7].

Many medications meant for managing the mentioned co-morbid conditions are associated with hepatotoxicity as per the scientific literature. Type-2 Diabetes Mellitus is established to be a risk factor for the development of non-alcoholic fatty liver disease (NAFLD). Moreover, it is additionally known to accelerate the progression of the disease to non-alcoholic steatohepatitis (NASH) and advanced liver fibrosis[8]. Recently, a large prospective cohort study showed that the patients with type-2 diabetes are at an increased risk of developing hepatocellular carcinoma, acute liver diseases, and failure[9, 10]. Furthermore, hepatotoxicity has been reported with the use of antidiabetic medications *per se* like the first and second-generation sulfonylureas[11-13], alpha-glucosidase inhibitors[14], biguanides[15], and thiazolidinediones[16]. In addition to Type-2 Diabetes Mellitus, hypothyroidism has also been linked to the pathogenesis of NAFLD[17]. Pathogenesis of hypothyroidism-induced NAFLD is driven by intra- and extrahepatic mechanisms. In mice with induced hypothyroidism even mild reduction of thyroid hormones led to developement of NAFLD[18]. Levothyroxine is the standard

drug prescribed for the management of hypothyroidism. While hypothyroidism itself is capable of inducing NASH in patients, Levothyroxine has also been reported to cause liver toxicity in several case studies[19-21]. Hence, it is important to mention whether the patients took any medication for their co-morbid conditions along with *T. cordifolia* and if the patients continued these medications for their co-morbid conditions, once, they were put off *T. cordifolia*. This missing information is crucial for directly co-relating the observed hepatotoxicity to *T. cordifolia* consumption.

In the study, the authors have reported an updated RUCAM (Roussel Uclaf Causality Assessment Method) scoring, a system used to understand the chances of association of any drug with hepatotoxicity[22]. The updated RUCAM scoring is not applicable- A) if it is not individually calculated for each co-administered product, B) when the onset of hepatic injury is before the start of the product use, and C) in case of preexisting chronic liver disease[22, 23]. In this case-series study, 4 out of 6 patients already had chronic liver disease. Moreover, authors have not discussed the co-morbidities associated drugs intake, and also failed to provided any information regarding the baseline liver conditions[4]. Hence, the RUCAM score would be difficult to directly correlate the observed hepatotoxicity specifically to *T. cordifolia* treatment.

In the presented case study, authors have failed to authenticate the plants consumed in 4 of the case reports, where the patients took boiled extracts of *T. cordifolia* twigs[4]. In India, *T. cordifolia* grows locally along with several close phenotypically related plants. In Ayurveda, identification of the correct medicinal plants that go into the preparation of medicine is very important. Consumption of wrong plants due to mistaken identification, lack of knowledge, ambiguity, and seasonal availability leading to the use of substitutes can lead to low efficacy or unwarranted side-effects[24]. Additionally, no chemical analysis of the plants consumed was done for active components or contaminants. To directly associate *T. cordifolia* with the observed hepatotoxicity it is important to correlate the presence of the plant chemical

constituents either in the liver biopsy samples or in the blood serum of the patients. Furthermore, the case series also failed to mention the manufacturing and dose details of the syrup and tablets consumed by the two patients[4]. Therefore, all six patients consumed different amounts of *T. cordifolia* for varying durations[4]. A dose-dependent contribution of *T. cordifolia* to hepatotoxicity (if at all anything like that exists) is not addressable through this study as required for any toxicological profiling.

In this connection, it is very important to specify the reported non-clinical safety profiles of *T. cordifolia*. The available literature in the public domain strongly establishes the biosafety of *T. cordifolia* through pre-clinical acute and sub-acute toxicological assessments up to 3.6 g/kg/day for 28 days of repeat dosing in rats. The aqueous extract of *T. cordifolia* has been reported to be non-toxic up to a dose of 2000 mg/kg in an acute toxicity evaluation in Wistar rats[25]. In an alternative acute toxicity assessment, no remarkable adverse effect and no mortality were reported in rats that were orally administered a dose of 3000 mg/kg of *T. cordifolia*[26, 27]. Further, in another sub-acute toxicity assessment, a formulation, made from the stem of *T. cordifolia*, was administered to Wistar rats at the doses of 360, 1800, and 3600 mg/kg/day for 28 days[28]. These studies concluded that *T. cordifolia* preparations were devoid of any observable adverse effects up to the highest dose tested, which is more than ten times the intended human therapeutic dose. In humans, a safety assessment study of *T. cordifolia* at the 500 mg/day did not induce any adverse affect upto 21 days treatment[29]. Taken together, *T. cordifolia* was not found to induce any observable toxicity in these studies, particularly in the liver. Most importantly, *T. cordifolia* has been associated with hepato-protective effects. *T. cordifolia* has demonstrated superior hepatoprotective effects in cadmium-induced[30], arsenic-induced[31], and lead-induced hepatotoxicity in experimental animals[32]. Nagarkatti *et al* have shown the modulation of Kupffer cell activity by *T. cordifolia* in liver damage[33]. *T. cordifolia* is effective as a hepato-protector and immuno-modulator in CCl_4 intoxicated albino rats[34]. It is

also effective in preventing anti-tuberculosis drug-induced hepatotoxicity [17,21]. In addition, *T. cordifolia* has been identified as a potential therapeutic in managing obstructive jaundice-like conditions[35].

Clinical studies have proven that stand-alone therapy of *T. cordifolia* ameliorated alcoholism induced hepatic and gastrointestinal toxicity, by effectively increasing the intestinal absorption of multivitamins, and by restoration of the power of liver[36]. In a case study, involving HIV-positive patients, *T. cordifolia* extract treatment showed immuno-stimulative effects by significantly reduced eosinophil counts and hemoglobin percentage[37]. In this study, 60% of the treated patients also showed a reduction of other HIV-associated symptoms[37]. In another clinical study, rhinitis patients were given *T. cordifolia* extract for 8 weeks and were observed for inflammation-related clinical symptoms[38]. The rhinitis patients showed relief from sneezing, nasal discharge, nasal obstruction, and nasal pruritus. In addition to blood inflammatory symptoms, the rhinitis patients showed increased TLC, reduced eosinophil and neutrophil count in blood following treatment with *T. cordifolia*. In these clinical studies with several patients for longer durations, not a single incidence of adverse effects was observed, suggesting an optimal clinical safety profile of *T. cordifolia*.

The current case series lacks proper designing of the study and has missed describing important critical parameters (medication history, the composition of the formulation, identification of *T. cordifolia* components in livers, etc.), and poorly interpreted the results which have significantly affected the credibility of the conclusions. The current case study should have included the details of twigs/tablets/syrup taken by the patient and the presence of chemical marker of *T. cordifolia* in the liver biopsy and blood serum of case participants. So, the observed hepatotoxicity in this case series should not be linked directly to the consumption of *T. cordifolia*.

Conclusions:

In conclusion, based on the lack of strong connectivity between *T. cordifolia* medication taken by the case-study patients and the observed hepatotoxicity, it is difficult to associate both together. Furthermore, there is a lack of any scientific backing from other published studies regarding liver damage induced by the *T. cordifolia*. The plant has been associated with hepatoprotection based on international peer-reviewed scientific literature. In the end, the inferences deduced from this case series require to be carefully re-analysed and re-interpreted with further scientific validation before reaching any conclusion.

Author Contribution

AB provided a broad direction and identified the gaps, generated resources, and gave final approval for the manuscript. KB, SS, RD, JS, PS, and SH identified the lacunae in the presented toxicological profiling case studies and addressed them in the Editorial. AV supervised overall analysis, reviewed, and finally approved the Editorial.

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