





A Preliminary Assessment of *Tinospora sinensis* on Mice Liver

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Abstract

Objective A preliminary study was conducted to assess the role of *Tinospora sinensis* extract on liver in mice in normal and lipopolysaccharide (LPS)-induced healthcompromised conditions.

Method Mice (n = 3-5) were randomly assigned into groups I to IV for hepatotoxic studies. Group I was assigned normal, group II was given LPS (6 mg/kg, intraperitoneal [ip]), group III was given T. sinensis only (1 g/kg/day for 21 days), whereas group IV was administered T. sinensis (1 g/kg/day per os [po] for 21 days) with LPS (6 mg/kg ip given on 7th day). Group V received monocrotaline (MCT) (200 mg/kg, p.o.) only. Group VI received MCT (200 mg/kg, po) and LPS (6 mg/kg ip). Group VII was given T. sinensis (500 mg/kg/day po for 7 days) followed by MCT (200 mg/kg, p.o.) and LPS (6 mg/kg, ip) on the 7th day. Groups V to VII were used to assess the effect of T. sinensis in MCT + LPSinduced hepatotoxicity model.

Results No elevation in alanine transaminase (ALT) levels was observed in mice treated with T. sinensis in group III or group IV compared with normal (vehicle treated) group (group I). Elevation in ALT levels was observed in group VI (MCT + LPS) and group VII; histopathology showed liver injury. Pretreatment of mice with T. sinensis (group VII) did not show any reduction in the elevated ALT levels.

Conclusions In the preliminary assessment, *T. sinensis* extract was found to exhibit neither hepatotoxicity itself nor the potential to thwart liver damage by a xenobiotic under the given test conditions, dosage, and duration of the study.

Keywords

- ► Tinospora sinensis
- ► extract
- ► lipopolysaccharide
- ► xenobiotic

Introduction

Tinospora sinensis (Lour.) Merr. (Menispermaceae), a large deciduous climber, is distributed in South and South-East Asia, particularly in Pakistan, India, and China. 1-3 The stems

of T. sinensis are used in Chinese and Ayurvedic medicine for treating inflammatory conditions, liver disorders, skin disorders, and urinary tract infections.^{4–7} T. sinensis is often used as a substitute for *T. cordifolia*, ⁸ which is a hepatoprotective and is referred to as the elixir of life for its rejuvenating properties. 9 In

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Fig. 1 Flowchart for preliminary study of Tinospora sinensis on liver in mice. ALT, alanine transaminase; LPS, lipopolysaccharides; MCT, monocrotaline.

Ayurveda, T. sinensis and T. cordifolia are used in a formulation called Satwa (a starchy extract) for the treatment of liver diseases. The hepatoprotective effect of a drug aims to prevent the degeneration to necrosis of hepatocytes and to promote their regeneration. Satwa (starchy extract) of T. sinensis and T. cordifolia has been shown to have a differential hepatoprotective activity. 10 Literature depicting the safety of *T. cordifolia* shows it to be safe. 11-13 In published literature, Satwa made from the stems of T. sinensis has been shown to possess hepatoprotective activity against paracetamol 10,14 and alcohol-induced¹⁵ hepatotoxicity. Moreover, the ethanolic extract of T. sinensis root has been reported to exhibit hepatoprotective activity in carbon tetrachloride-induced hepatotoxicity in rats.¹⁶ Though there are reports that demonstrated the hepatoprotective potential of T. sinensis, at least one report associates consumption of *T. sinensis* with liver toxicity. ¹⁷ A preliminary study was, therefore, conducted to elucidate the hepatotoxic potential of T. sinensis by testing it under inflammatory conditions that was induced in mice by a subtoxic dose of lipopolysaccharide (LPS) (>Fig. 1). 18,19 Second, a preliminary assessment of the protective role of T. sinensis on liver, following exposure to a xenobiotic after pretreatment with T. sinensis, was performed, utilizing the established MCT + LPS induced hepatotoxicity model in mice.²⁰ Monocrotaline (MCT), a naturally occurring pyrrolizidine, is a hepatotoxin.²¹ MCT is not hepatotoxic at a subtoxic oral dose of 200 mg/kg. However, when coadministered at the same dose with LPS, it simulates the intake of a natural product under inflammatory conditions leading to an amplified response, thereby showing its potential hepatotoxicity.²² In a published work, where this model was produced, tissue factor antisense oligonucleotides were tested to block the tissue factor and see if that was associated with the mechanism of hepatotoxicity with $MCT + LPS.^{20}$

Materials and Methods

Chemicals

LPS (from *Escherichia coli*) and Crotaline (MCT) were purchased from Sigma Aldrich (St. Louis, Missouri, United States).

Injectable sterile normal saline was purchased from Hospira, Inc (Lake Forest, Illinois, United States).

Preparation of Plant Extract

Stems of *T. sinensis* (NCNPR# 17003) were obtained and authenticated by Dr Vijayasankar Raman and deposited at the Botanical Repository in the National Center for Natural Products Research, University of Mississippi, Mississippi, United States. A methanolic extract was obtained by the percolation method. The solvent was evaporated under reduced pressure at 40°C and the resulting extract was freeze dried for in vivo study. The test samples were prepared as a suspension in water with less than 1% of gum acacia.

Animals

Male ND-4 mice weighing 15 to 20 g were obtained from Envigo (Indianapolis, Indiana, United States), housed in microinsulator cages with corncob bedding, fed on Purina chow and water *ad libitum*. They were maintained at a relative humidity of 35 to 50% at 22°C on 12 hours light/dark cycle. The mice were fasted for 12 hours before treatment. Food was made available after the administration of the dose. The animal experimental protocol (Protocol # 16–009) was approved by the Institutional Animal Care and Use Committee, IACUC, at the University of Mississippi.

Experimental Design

To assess the hepatotoxic potential, the *T. sinensis* extract was administered for the duration of 3 weeks to healthy and LPS-induced health compromised mice. Health compromised implies presence of an underlying disease state such as inflammation which was caused by a single dose of LPS in the present study undertaken. Animals (n=5) were randomly assigned to different groups and were given the following treatments: group I (normal control) was given the vehicle (100 μ L/10 g), group II was given LPS (6 mg/kg, intraperitoneal [ip]), group III was given *T. sinensis* extract (1 g/kg/day, per os [po]), group IV was given *T. sinensis* extract (1 g/kg/day,

po), and a single dose of LPS (6 mg/kg, ip) on day 7 post-initiation of treatments. In the second experiment, T. sinensis role on liver was preliminarily assessed against LPS-mediated potentiation of MCT-induced hepatotoxicity in a 7-day study. In this study, (n = 3-5), group V was given a single dose of MCT (200 mg/kg, po), group VI was given a single dose of MCT (200 mg/kg, po), and a single dose of LPS (6 mg/kg, ip) to induce liver damage as described by Abdel-Bakky et al. Croup VII was given T. sinensis (500 mg/kg/day) extract for 7 days followed by exposure to MCT+LPS induced hepatotoxicity on the 7th day. This was achieved by administering MCT followed by LPS after an interval of 45 minutes. All mice were sacrificed at the termination of experiments by CO2 intoxication as per guidelines of the University of Mississippi, IACUC.

Clinical Chemistry and Histopathological Studies

Blood was collected by cardiac puncture in heparinized micro tubes and analyzed for clinical chemistry by Vet Scan dry chemistry analyzer with comprehensive diagnostic profiles (Abaxis Union City, California, United States). For histopathological studies, liver specimens were fixed in 10% formalin, embedded in paraffin and sections 5 µm thick were stained with hematoxylin and eosin as per standard protocol.²³ The stained sections were visualized microscopically for hepatotoxicity evaluation.

Statistical Analysis

The data were analyzed by one-way analysis of variance test followed by Tukey-Kramer multiple comparisons using Graph Pad prism software (La Jolla, California, United States); p < 0.05 was considered statistically significant.

Results

The clinical chemistry of the test groups (III and IV) showed results that were not demonstrative of a hepatotoxic or nephrotoxic effect by *T. sinensis* under the LPS-induced health compromised conditions in mice. The alanine transaminase (ALT) levels were comparable with control group, group II. The preliminary results for the potential hepatoprotective action of *T. sinensis* against MCT+LPS-induced hepatotoxicity (**Table 1**) did not show an alleviating role on the liver, rather a potentiation of the hepatotoxicity is observed (**Table 1**). A single mouse mortality was also observed in group VII.

Histopathological analysis of mice treated with LPS, *T. sinensis*, or *T. sinensis* + LPS and MCT alone showed ballooning of cytoplasm that is due to accumulation of glycogen. This is not considered abnormal histological architecture and was observed in groups I to V (**Figs. 2A–D** and 3A). The histopathological results of the liver specimens from groups VI and VII (**Fig. 3B** and **C**) showed areas of necrosis (cell death) and neutrophilic infiltration. Disintegration of the cellular structure with focal lesions was observed in group VI (**Fig. 3B**). **Fig. 3C**

Table 1 Results of clinical chemistry parameters for T. sinensis in 21-day and 7-day study

Clinical chemistry		Clinical chemistry parameters in normal and LPS-induced health compromised condition in 21-day study			Clinical chemistry parameters in 7-day study		
	Normal	LPS	Tinospora sinensis only	T. sinensis + LPS	MCT	MCT + LPS	T. sinensis + MCT + LPS
	(Group I)	(Group II)	(Group III)	(Group IV)	(Group V)	(Group VI)	(Group VII)
Alanine transaminase (U/L)	42.00 ± 2.3	25.33 ± 1.3	29.20 ± 0.96	22.00 ± 1.7	54.00 ± 5.0	338±64	790 ± 107 ^a
Albumin (g/dL)	2.5 ± 0.08	2.7 ± 0.39	3.8 ± 0.13	3.6 ± 0.11	3.5 ± 0.10	2.3 ± 0.27	2.6 ± 0.17
Total bilirubin (mg/dL)	0.26 ± 0.033	0.30 ± 0.0	0.30 ± 0.0	0.32 ± 0.02	0.30 ± 0.0	0.30 ± 0.0	0.30 ± 0.0
Blood urea nitrogen (mg/dL)	32.33 ± 2.3	14.33 ± 0.88	21.80 ± 0.73	17.60 ± 0.97	20.50 ± 1.5	103.6 ± 15.01	106.3 ± 39.93
Creatinine (mg/dL)	0.26 ± 0.033	0.20 ± 0.0	0.20 ± 0.0	0.22 ± 0.02	0.20 ± 0.0	0.52 ± 0.22	0.46 ± 0.13
Alkaline phosphatase (U/L)	126 ± 2.1	73 ± 19	98.60 ± 6.8	102 ± 4.5	139 ± 11	46.20 ± 6.34	50.00 ± 5.1
Total proteins (g/dL)	5.0 ± 0.05	5.7 ± 0.06	5.8 ± 0.21	5.5 ± 0.06	5.1 ± 0.35	5.4 ± 0.13	5.6 ± 0.12
Globulin (g/dL)	1.8 ± 0.15	2.4 ± 0.12	2.1 ± 0.30	1.6 ± 0.08	3.0 ± 0.35	3.3 ± 0.12	3.0 ± 0.25
Phosphorus (mg/L)	9.3 ± 0.30	7.4 ± 0.76	5.0 ± 0.41	7.2 ± 0.33	9.6 ± 0.05	12 ± 1.9	13.63 ± 3.8
Sodium (mmol/L)	152 ± 0.0	148 ± 2.6	149 ± 1.0	150 ± 1.2	149 ± 1.5	143 ± 2.6	146.7 ± 2.9
Calcium (mg/dL)	11.17 ± 0.17	10.97 ± 0.27	11.16 ± 0.19	10.76 ± 0.97	11.80 ± 0.30	11.40 ± 0.16	11.93 ± 0.29
Potassium(mmol/L)	6.3 ± 0.17	7.3 ± 0.21	6.9 ± 0.20	6.7 ± 0.11	6.9 ± 0.35	7.6 ± 0.41	7.1 ± 0.70

Abbreviations: LPS, lipopolysaccharides; MCT, monocrotaline; SE, standard error.

Each value represents mean \pm SE, n = 3-5, $^{a}p < 0.05$, compared with the corresponding value for normal group.

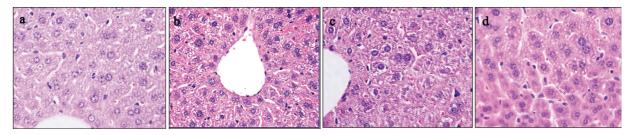


Fig. 2 Photomicrograph (400X magnification) of liver sections from mice given different treatments. (A) Vehicle, (B) lipopolysaccharides (6 mg/kg intraperitoneal [ip]), (C) Tinospora sinensis only (1g/kg/day oral for 21 days), (D) T. sinensis (1g/kg/day oral for 21 days) + LPS (6 mg/kg, ip).

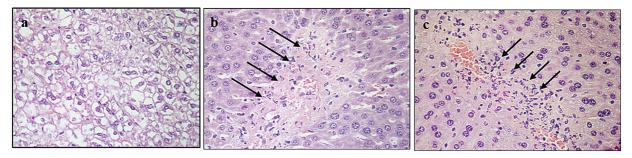


Fig. 3 Photomicrograph of liver sections of mice showing pathological lesions induced by MCT and lipopolysaccharides (LPS). (A) Monocrotaline (MCT): 200 mg/kg oral, (B) MCT + LPS (MCT: 200 mg/kg oral and LPS: 6 mg/kg intraperitoneal [ip]), (C) Tinospora sinensis + MCT + LPS (500 mg/kg/day oral for 7 days; MCT: 200 mg/kg oral and LPS: 6 mg/kg ip given on 7th day). Arrows indicate area of necrosis.

clearly showed further liver damage following exposure to MCT + LPS-induced liver toxicity in group VII.

Discussion

ALT is produced by the hepatocytes and any injury thereof causes its elevation in blood.

Elevation of ALT is, therefore, a parameter that indicates a liver abnormality.²⁴ Administration of *T. sinensis* extract for 3 weeks to mice with or without sensitization with LPS did not alter any of clinical chemistry parameters; all of which stayed within normal limits compared with those of normal (vehicle treated) mice. The histopathological analysis of the liver specimen from group I shows a normal architecture of liver tissue (>Fig. 2A); Group II (LPS treated), group III (T. sinensis), and group IV (T. sinensis + LPS) showed variable degree of vacuolization in the cytoplasm of hepatocytes (>Fig. 2B-D). No liver necrosis was observed. These results, taken together, indicate that T. sinensis does not appear to cause liver injury under the given experimental conditions. The results further denote the absence of the role of an inflammatory condition to modulate the behavior of the T. sinensis extract to affect a hepatotoxic response for T. sinensis, which therefore eliminates the possibility, for T. sinensis, to cause direct liver damage. However, our experimental study is in mice and was limited to 3 weeks.

The 7-day preliminary study was performed to determine the role of T. sinensis on the liver following exposure to a potential hepatotoxin, that is, from MCT and LPS-induced hepatotoxicity in the mouse model. Group VI showed an elevation of the ALT levels and histopathological changes showed liver damage. However, no alleviating effect on liver damage was observed following the 7-day pre-administration of T. sinensis (500 mg/kg body weight, oral) in group VII. Elevation of ALT levels and an altered histological architecture with damaged hepatocytes was observed confirming liver damage (>Fig. 3B and C). Liver necrosis and disintegration of cellular structure were observed similar to that seen in group VI. Our preliminary results in mice do not support the hypothesis that T. sinensis has a potential role in protecting the liver. Our observation is supported by the clinical report on T. sinensis wherein two cases, male aged 55 to 65 years, experienced hepatotoxicity associated with T. sinensis when consumed for over 3 months to treat "liver fire" and edema. 17 Tinospora species (T. sinensis and T. cordifolia) are popular in traditional medicine for their hepatoprotective activities. Safety evaluation of T. sinensis for its role on liver becomes crucial since the herbal medicine is often substituted for T. cordifolia. While T. cordifolia has been reported to be safe, the safety of T. sinensis remains to be elucidated. The Satwa from T. sinensis has been suggested to have a strengthening effect on the function of liver against alcohol-induced hepatotoxicity.¹⁵ However, the role of the whole extract of T. sinensis, as restorative or liver protective, could not be established in our preliminary assessment under the given experimental conditions for hepatoprotective efficacy. Our results further negate the hepatoprotective assertions of Chavan et al for T. sinensis in rats. A closer analysis of their literature revealed that the ALT levels for normal groups (154.67 ± 2.4) and groups treated with hepatoprotective agent, silymarin (174.77 \pm 1.0) and *T. sinensis* (131.58 \pm 2.9), were inconsistent to assert a hepatoprotective role for T. sinensis, since the established range for the normal ALT levels in healthy rats from a variety of sources lies between 35 and 80 U/L. Elaborate toxicological and mechanistic studies with whole extracts of *T. sinensis* for extended duration need to be undertaken to qualify its use in herbal medicine.

It must be borne in mind that the use of traditional medicines in conjunction with over-the-counter and prescription medicine is quite common and that most consumers selfprescribe herbs and natural products.²⁵ Moreover, substitution of one Tinospora species for the other demands serious consideration and vigilance, as each species has phytochemical, pharmacological, and toxicological variability that warrants caution and increased awareness with regard to their use. Further studies at different dosage and extended time period would enable to establish its safety profile and use among herbal consumers and practitioners alike. While preclinical studies on single plant extracts are important to evaluate their effect on organ pathology, it is equally important to study these products in combination with commonly used over-the-counter medicines as well as other herbal products to better understand herb-drug and/or herb-herb interactions.

Conclusion

Our study showed that T. sinensis did not elevate ALT levels in normal and LPS-induced health compromised mice, indicating that T. sinensis does not qualify as a potential hepatotoxin. On the other hand, pretreatment of mice with extract of T. sinensis for 7 days did not show any protective role on liver. The elevation in ALT levels was persistent with liver injury as a result of MCT + LPS treatment. Therefore, a detailed investigation needs to be undertaken to explore further in this direction.

Conflict of Interest None.

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