

Developing and Applying *In vitro* Liver Models to Address Potential Hepatotoxicity of Botanical Dietary Supplements

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23 April 2020





Outline

- Introduction – liver as a target organ for natural product toxicity
- Complexity of studying botanicals and hepatotoxicity
- Goals of the BSC Hepatotoxicity/ADME Sub-team
- Examples using an *in vitro* liver model to study botanical-drug interactions, and that may be useful for studying hepatotoxicity of botanicals

Liver as a target organ for dietary supplement-induced injury

- The Drug-Induced Liver Injury Network (DILIN) reports that liver injury cases due to botanical-based dietary supplements continues to increase.
- Study results are picked up by media sources.
- Consumers and health care professionals express concern (and confusion) about risk.
- Lack of scientific understanding of risk prevents consumers and health care professionals from making informed decisions about dietary supplement use.



Health / Liver Damage From Supplements Is On The Rise



Liver Damage From Supplements Is on the Rise

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Products withdrawn from the market for confirmed or suspected hepatotoxicity



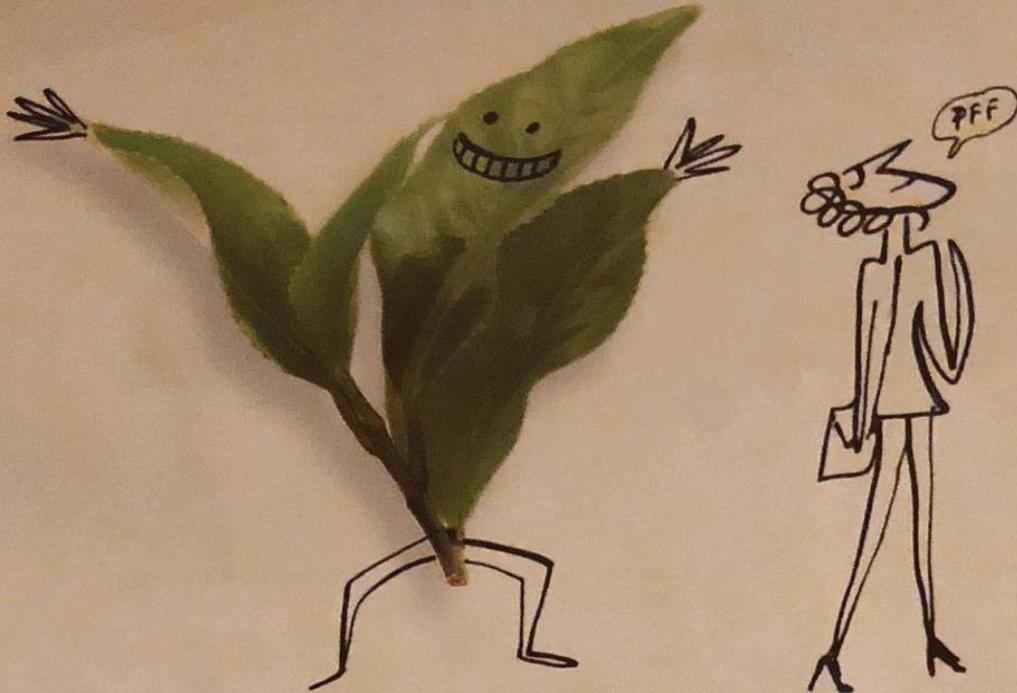
Products withdrawn from the market for confirmed or suspected hepatotoxicity



Products withdrawn from the market for confirmed or suspected hepatotoxicity



Is green tea or the supplement healthy or dangerous? I keep hearing conflicting things.



GTE is so potentially harmful that we include it on our list of 15 supplements to always avoid

- Consumer Reports

Feb, 2018



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Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep



United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts



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USP powdered GTE (PDGTE) Expert Panel conclusions

- Clear occurrence of severe hepatotoxicity from ingestion of GTE in humans albeit with very low frequency...EGCG likely involved in observed hepatotoxicity. Factors that can contribute:
 - Concentration of catechins in certain GTE-containing products
 - Bolus dose by certain dosage forms
 - Intake of GTE under fasting conditions – increases absorption of constituents
 - Genetic susceptibility, idiosyncrasy, underlying liver health may play a role



USP powdered GTE (PDGTE) Expert Panel label recommendation

- The USP PDGTE ingredient monograph label cautionary statement:
 - *Do not take on an empty stomach. Take with food. Do not use if you have a liver problem and discontinue use and consult a healthcare practitioner if you develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the skin or eyes).*
 - Compliance with label caution statement is mandatory only for products that claim compliance with USP quality standards for PDGTE.

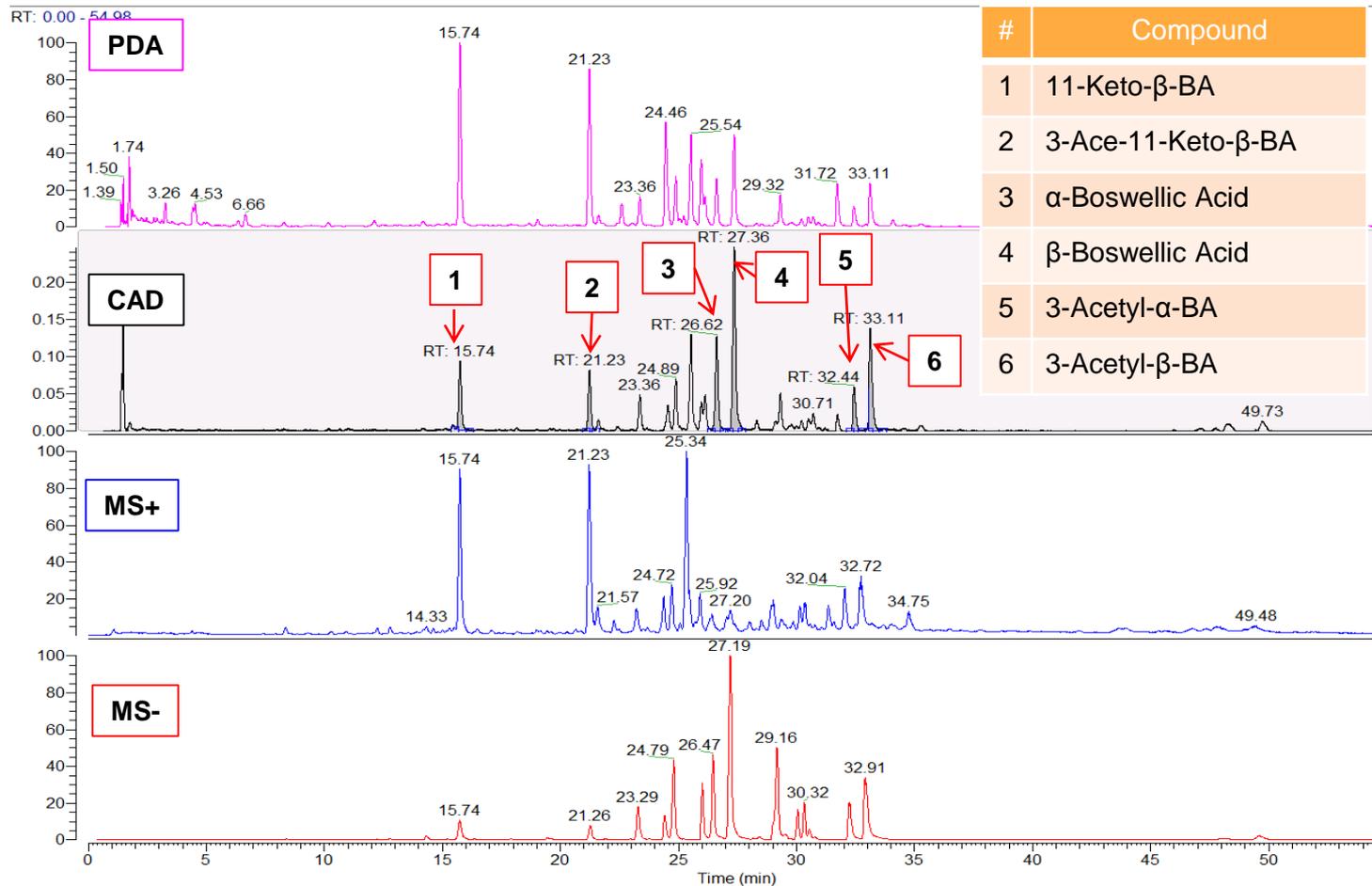


The combined complexity of using botanicals and studying hepatotoxicity is quite challenging

- Botanicals are complex mixtures that can pose challenges to *in vitro* systems
- There is a wide spectrum of liver injury that may be observed clinically
- *In vitro* and animal models have limitations in predicting the diversity of liver injury possible
- How do you study ADME parameters of complex mixtures *in vivo* (what constituents do you monitor)?



UHPLC/UV/CAD/HRMS of *Boswellia serrata* Extract



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Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Follow that botanical: Challenges and recommendations for assessing absorption, distribution, metabolism and excretion of botanical dietary supplements



Suramya Waidyanatha^{a,*,1}, Kristen Ryan^{a,1}, Amy L. Roe^b, Wei Jia^c, Mary F. Paine^d, Stephen Ferguson^a, Bill J. Gurley^e, Kevin Welch^f, Moses S.S. Chow^g, Michael Devito^a, Cynthia Rider^a

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BSC Hepatotoxicity/ADME Working Group

Chairs: Amy Roe and Stephen Ferguson

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Paul Walker

Merrie Mosedale

Yitong Liu

HESI: Michelle Embry and
Connie Mitchell

Goals of the BSC – Hepatotoxicity/ADME Working Group

- To predict clinically-relevant hepatotoxicity and ADME parameters using 21st century toxicology tools:
 - *in silico* methods
 - *in vitro* assays and models
- To make recommendations for the field:
 - Predicting hepatotoxicity (and potential MOAs)
 - Estimating hepatic clearance
 - Predicting botanical-botanical and botanical-drug interactions that may lead to hepatotoxicity
 - How to incorporate the gut into ADME considerations



Proposed Testing Strategies – a Tiered Approach

Early Tier

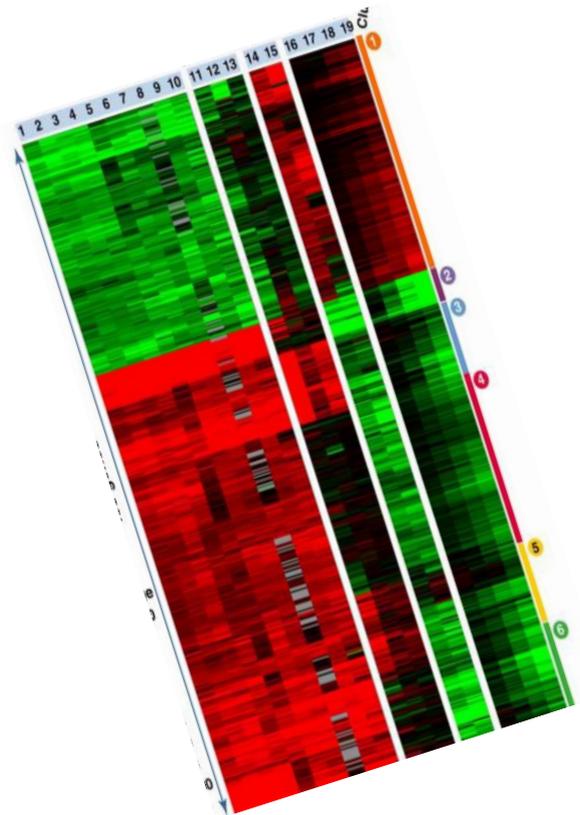
- *In silico*
 - Molecular docking of key liver-related targets
 - QSAR models

Middle Tier

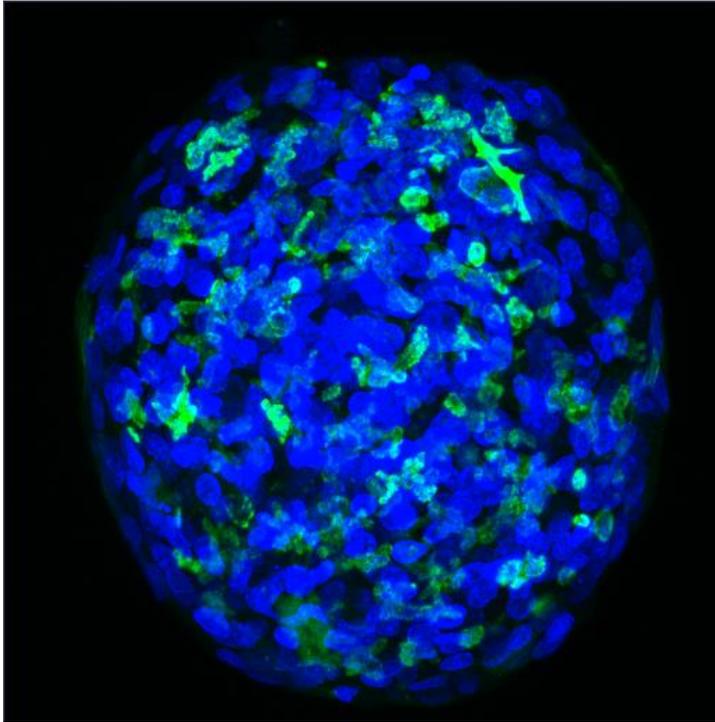
- *In vitro*
 - Receptor screens
 - Transcriptomics
 - Liver models and cytotoxicity endpoint

Later Tier

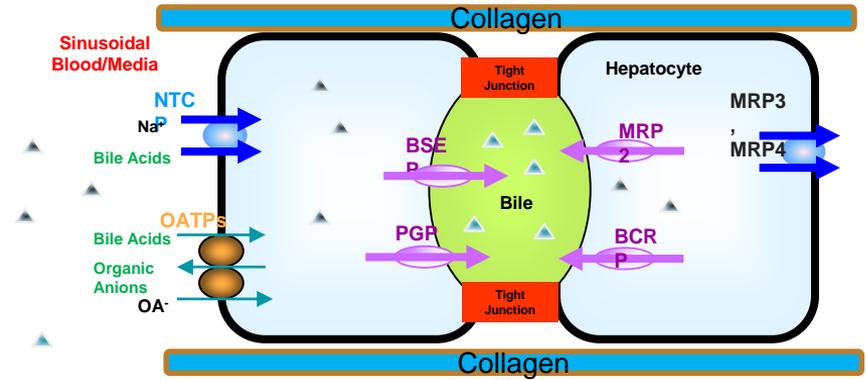
- *In vitro*
 - integrated human hepatocyte models (2D, 3D)
 - Co-culture models (e.g. gut-liver)



Use of human-based advanced liver models to predict hepatotoxicity



3D HepaRG cells



2D Sandwich-cultured hepatocytes

An *in vitro* hepatic clearance approach can accurately predict *in vivo* botanical – drug interactions

1521-009X/45/9/1019–1026\$25.00

DRUG METABOLISM AND DISPOSITION

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<https://doi.org/10.1124/dmd.117.075408>

Drug Metab Dispos 45:1019–1026, September 2017

Prediction of Clinically Relevant Herb-Drug Clearance Interactions Using Sandwich-Cultured Human Hepatocytes: *Schisandra spp.* Case Study

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Christopher B. Black, Kenneth R. Brouwer, and Amy L. Roe

Qualyst Transporter Solutions, Durham, North Carolina (J.P.J., K.M.F., W.W.F., A.G.H., C.B.B., and K.R.B.) and Procter & Gamble,
Cincinnati, Ohio (A.L.R.)

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Schisandra chinensis & *Schisandra sphenanthera*

- Wide range of pharmacological activities (antioxidant, anti-inflammatory, antibacterial, cardio-protective, etc.)
- *In vitro* and clinical data available on interaction potential (CYP3A4 and P-glycoprotein)
- Available clinical literature data used to “validate” our *in vitro* model



Schisandra sphenanthera

Effects of *Schisandra sphenanthera* extract on the pharmacokinetics of tacrolimus in healthy volunteers. Hua-Wen Xin *et al.*; *British Journal of Clinical Pharmacology*, (2007) 64:4:469-475

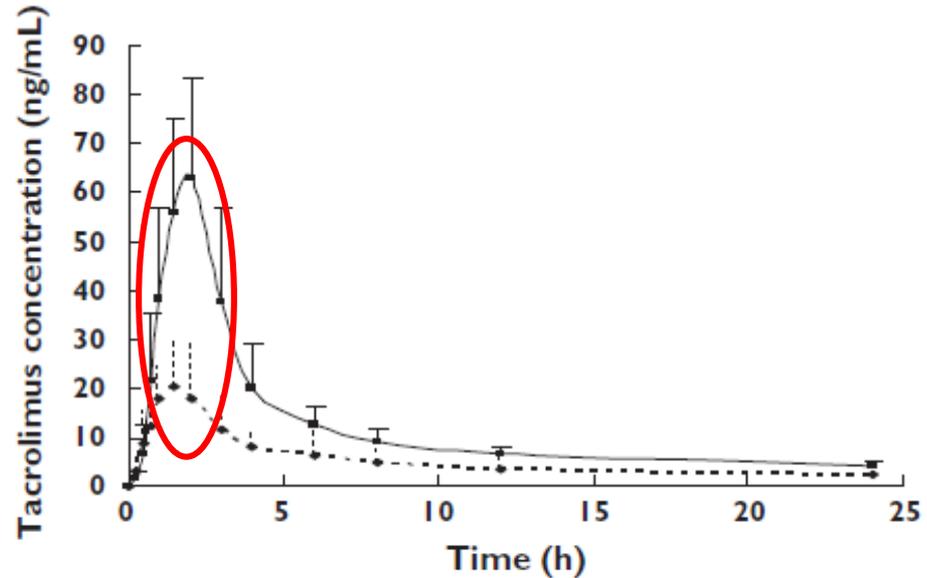
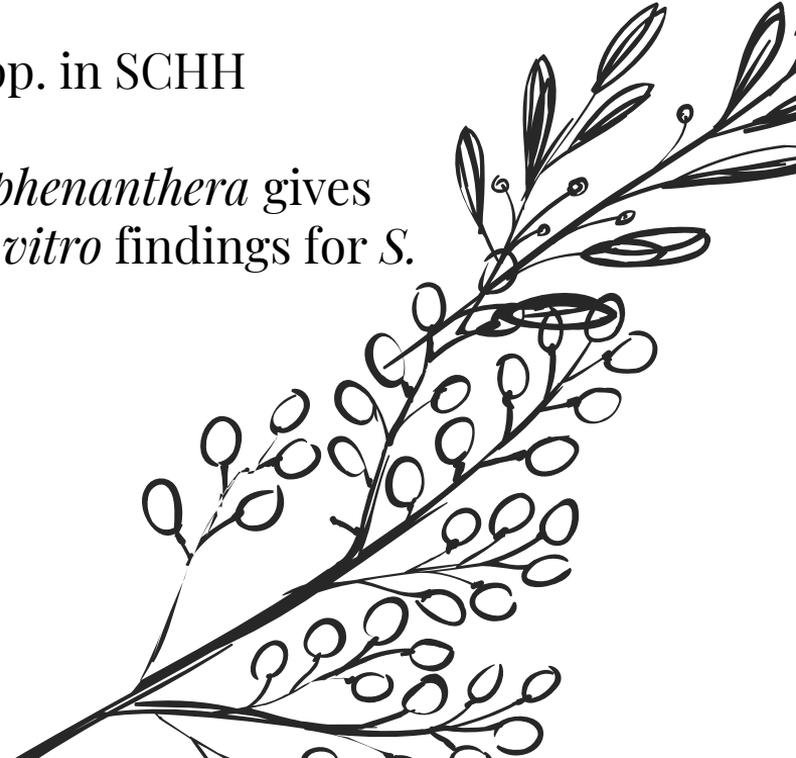


Figure 1

Mean blood concentration–time curves of tacrolimus in 12 healthy volunteers. Before SchE (◆), with SchE (■)

Can we mimic clinical findings in sandwich-cultured human hepatocytes (SCHH)?

- *In vivo* data (human clinical) available for effects of *S. sphenanthera* on CYP3A4 inhibition
- Evaluate CYP3A4 inhibition by *Schisandra* spp. in SCHH
- Good *in vitro* to *in vivo* correlation with *S. sphenanthera* gives confidence in using model to extrapolate *in vitro* findings for *S. chinensis* to predict clinical relevance



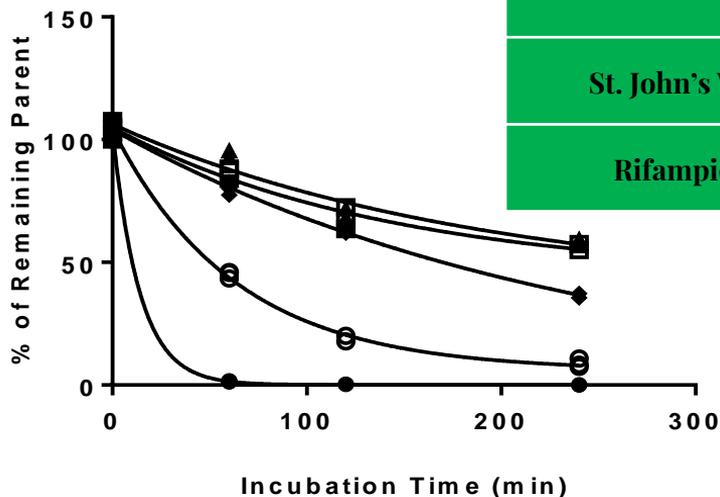
Study Design

- Mechanistic Studies
 - Evaluated inhibition of CYP_{3A4} activity by monitoring 1'-OH midazolam formation in SCHH
 - Evaluated induction of CYPs by *Schisandra* spp. using SCHH including CYP_{3A4} mRNA content
- Assessed impact on “Intrinsic Clearance” of clinical probe substrate in SCHH exposed to *Schisandra* spp.
 - Disappearance of midazolam following exposure
- Monitored intra-cellular concentrations of select phytochemical constituents



Disappearance of MDZ in SCHH Following Exposure to *Schisandra* spp.

Extract	MDZ Clearance (<i>in vitro</i>)	MDZ Clearance (Clinical)	TAC Clearance (<i>in vitro</i>)	TAC Clearance (Clinical)
<i>Schisandra sphenanthera</i>	49% [‡]	55% [‡]	65%	50%
St. John's Wort	198% [‡]	209% [‡]	131%	Not Available
Rifampicin	1180% [‡]	2400% [‡]	Not Available	Not Available

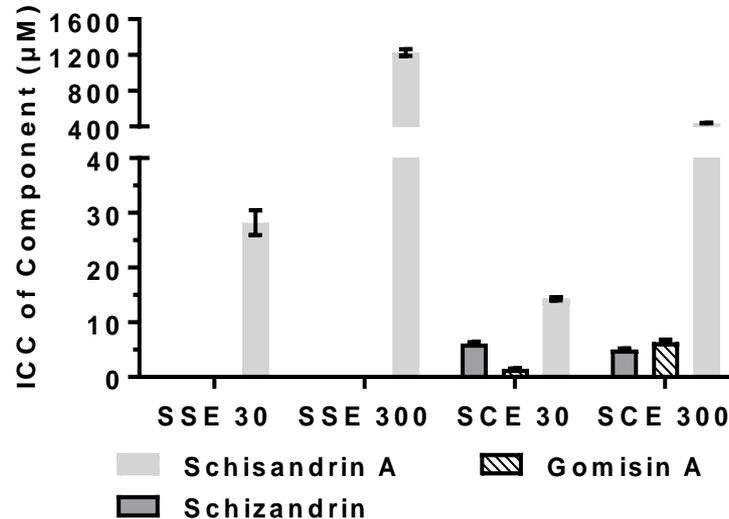


Jackson, JP et al. DMD 45:1019–1026, September 2017

- ◆ DMSO 0.1%
- ▲ SSE 30 μg/mL
- RIF 10 μM
- SJW 20 μg/mL
- SCE 30 μg/mL

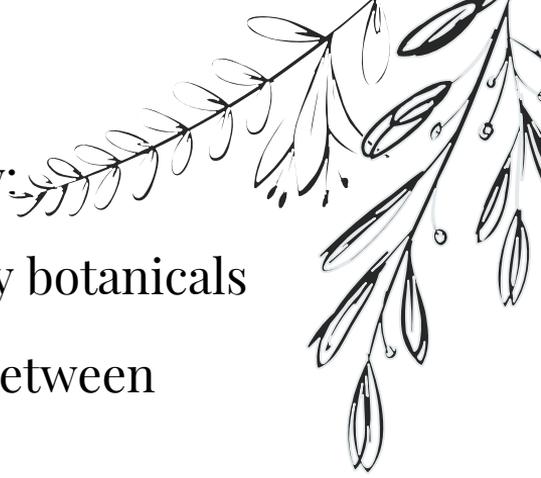
Intracellular concentrations of *Schisandra* spp. phytochemical constituents

ICC of schisandrin A, gomisin A, and schizandrin determined – most abundant and representative of other lignans



Conclusions (on BDI work)

- Our work represents a proof of concept on a novel strategy:
 - Capable of analyzing complex mixtures represented by botanicals
 - Encompasses synergistic/additive/competing effects between constituents
 - captures clinically-relevant liver pathways to directly predict clinical outcome (e.g., hepatic clearance)
- Relies on experience in predicting DDI to develop process for predicting BDI
- Fully-integrated, whole-cell models with full complement of metabolic enzymes, uptake and efflux transporters, and regulatory pathways
 - Generates physiologically-relevant intracellular concentrations (cellular exposure)



Hepatotoxicity evaluation of *Schisandra* spp. in sandwich-cultured hepatocytes

- Lack of non-clinical toxicology studies available despite long history of use.
- A 90-day repeated dose oral toxicity study in rats indicated changes in liver enzymes (ALT, AST), increased liver weight, and changes in endogenous compounds regulated by the liver (cholesterol, albumin)
- Our objective was to compare potential hepatotoxicity in *in vitro* rat and human liver hepatocytes.



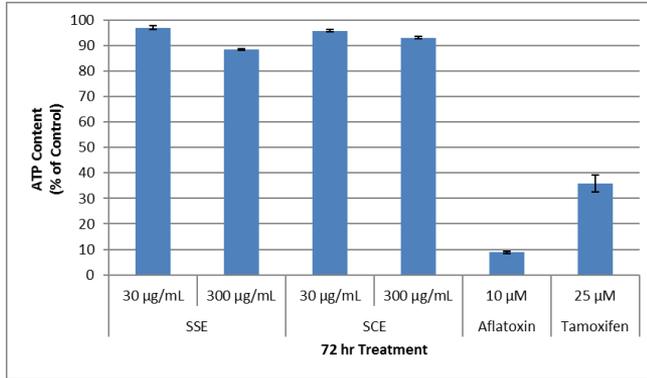


Methodology

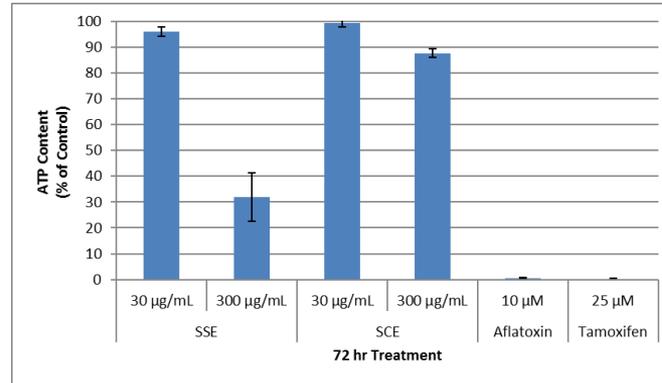
- Tested both *Schisandra* spp. at similar concentrations as in the BDI studies in both rat and human SCHs
- Cytotoxicity assessment: cell morphology (shape, cytoplasmic alterations, accumulation of vacuoles), and cell viability (ATP depletion).
- Treatment compared to positive controls (aflatoxin and tamoxifen)
- Measured intracellular concentrations of 4 of the major phytochemical constituents (schisandrin A, schisantherin A, gomisin A, schisandrin)

Results

SCHH (HUMAN)



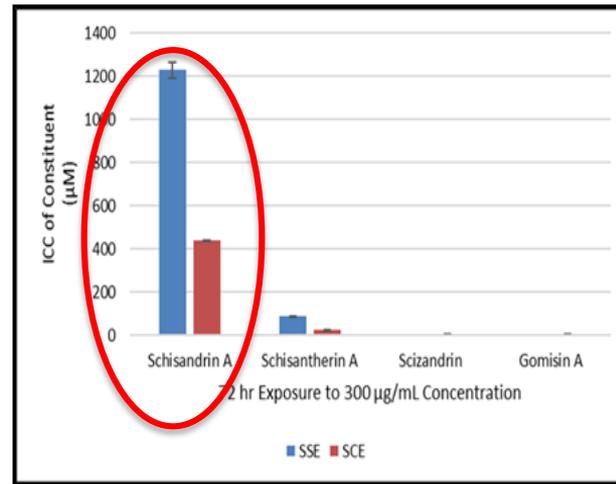
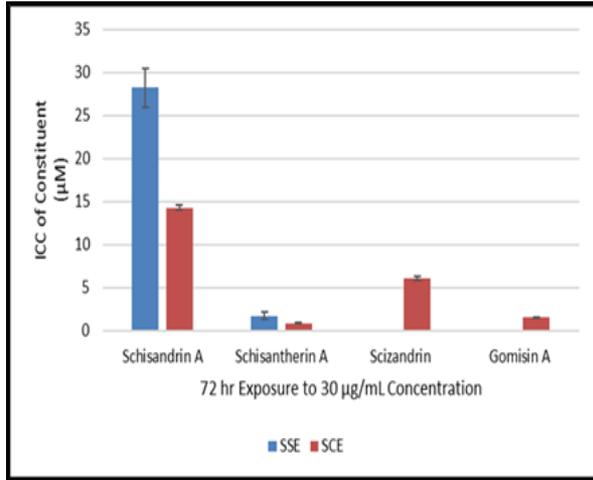
SCRH (RAT)



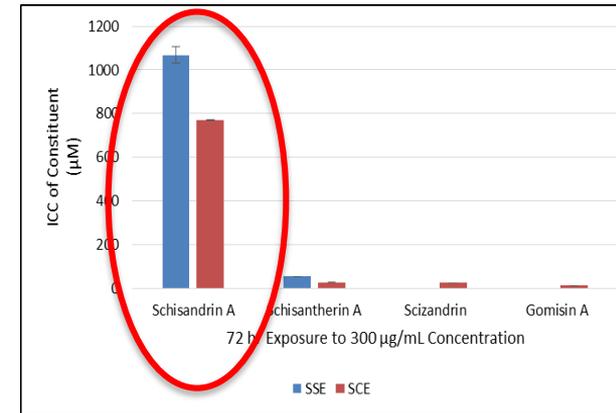
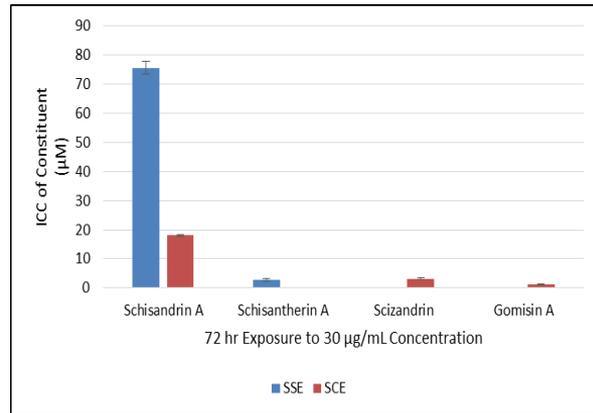
S. sphenanthera significantly more toxic in rats than humans, *S. chinensis* slightly more toxic in rats at the highest dose tested.

Intracellular phytochemical constituents of *Schisandra* spp.

SCHH (Human)



SCRH (Rat)





Summary



- *S. chinensis* exposure to SCH resulted in more toxicity in rat hepatocytes than human hepatocytes at the highest dose tested.
- *S. sphenanthera* was significantly more toxic to rat hepatocytes than human hepatocytes
- In general, intracellular constituent profiles of representative *Schisandra* spp. lignans were qualitatively similar between the two species, but differed quantitatively, with significantly higher intracellular concentrations observed in rat hepatocytes.
- Additional studies are needed to understand whether these constituents accumulate and/or whether concentrations may be associated with markers of hepatotoxicity.



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BSC

Steering Committee,
Hepatotoxicity Working
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For a listing of our publications related to botanicals safety please contact Amy L. Roe (roe.al@pg.com)

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Scientific Publications
and Presentations

