Chemical Research in Toxicology

ACS AuthorChoice

Article

pubs.acs.org/crt

Terms of Use

Flavokawains A and B in Kava, Not Dihydromethysticin, Potentiate Acetaminophen-Induced Hepatotoxicity in C57BL/6 Mice

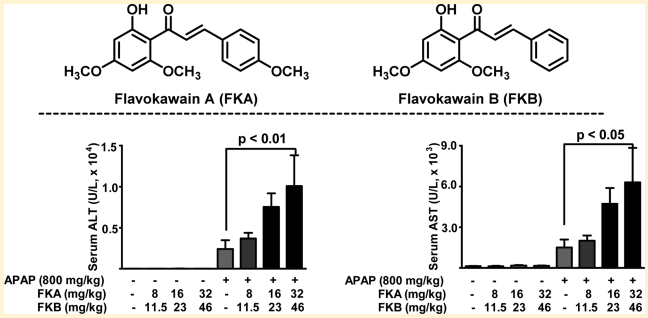
Sreekanth C. Narayanapillai,[†] Pablo Leitzman,[†] M. Gerard O'Sullivan,[‡,§] and Chengguo Xing*,[†]

[†]Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, United States

[‡]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota 55455, United States

[§]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota 55108, United States

ABSTRACT: Anxiolytic kava products have been associated with rare but severe hepatotoxicity in humans. This adverse potential has never been captured in animal models, and the responsible compound(s) remains to be determined. The lack of such knowledge greatly hinders the preparation of a safer kava product and limits its beneficial applications. In this study we evaluated the toxicity of kava as a single entity or in combination with acetaminophen (APAP) in C57BL/6 mice. Kava alone revealed no adverse effects for long-term usage even at a dose of 500 mg/kg bodyweight. On the contrary a three-day kava pretreatment potentiated APAP-induced hepatotoxicity, resulted in an increase in serum ALT and AST, and increased severity of liver lesions. Chalcone-based flavokawains A (FKA) and B (FKB) in kava recapitulated its hepatotoxic synergism with APAP while dihydromethysticin (DHM, a representative kavalactone and a potential lung cancer chemopreventive agent) had no such effect. These results, for the first time, demonstrate the hepatotoxic risk of kava and its chalcone-based FKA and FKB *in vivo* and suggest that herb–drug interaction may account for the rare hepatotoxicity associated with anxiolytic kava usage in humans.



INTRODUCTION

Traditional kava is an aqueous extract of the roots of *Piper methysticum* and serves as a ceremonious and daily beverage or an herbal remedy for South Pacific islanders.[1] Kava had also been used clinically to treat mild and moderate anxiety, based on results of numerous clinical trials.[2−5] Anxiolytic kava was typically prepared as an organic extract of kava root with ethanol or acetone, instead of the traditional aqueous preparation. Anxiolytic kava had been banned in Europe and a few other countries since 2002 because of its risk to induce hepatotoxicity, and it is listed on the USA FDA advisory board,[6,7] but Germany's Federal Administrative Court negated the ban in June 2014.[8]

Various causes have been proposed for kava's hepatotoxic risk, but none have been validated so far. First of all, in response to high demand, anxiolytic kava may have included nonroot toxic plant parts.[9] It has also been postulated that some kava roots were not properly dried, resulting in hepatotoxin contamination.[10] Usage of nontraditional cultivars could be another cause; different kava cultivars have diverse chemical profiles while traditional kava is prepared from only a few of them.[10,11] Due to preparation difference, traditional and anxiolytic kavas have distinct composition profiles,[12,13] which may impose different hepatotoxic risks as well. Furthermore, ∼90% of the purported hepatotoxic cases associated with kava usage involved concomitant consumption of other drugs or dietary supplements,[14,15] suggesting that kava's hepatotoxic risk may be mediated via herb–herb or herb–drug interactions.

In addition to kava's anxiolytic benefit, one epidemiological survey suggested that traditional kava usage may be able to reduce cancer risk,[16] which was supported by results from several laboratory animal tumorigenesis models.[13,17−21] Moreover, despite its ban and being on USA FDA's advisory list, kava consumption has experienced a global resurgence based on the amount of kava exported from the major kava producing nations (The Republic of Vanuatu, Fiji, and Tonga) between 2008 and 2013.[22] With the recent overturn of the kava ban in Germany, its usage is expected to increase further globally. Our recent metabolomics and cellular cytotoxicity analyses of an array of current commercial kava products revealed that they were diverse in chemical profile and cellular cytotoxicity,[22] and likely distinct in their health benefit and risk.

Considering the increasing human exposure and the diverse chemical composition of current kava products, the hepatotoxic risk of kava needs to be clarified and the responsible chemicals need to be identified, which is the focus of this study. Our results showed that kava was safe when given alone but significantly enhanced acetaminophen (APAP)-induced hepatotoxicity in C57BL/6 mice. Chalcone-based flavokawains A (FKA) and B (FKB) recapitulated kava's potentiation of APAP-

Received: August 5, 2014
Published: September 3, 2014

   dx.doi.org/10.1021/tx5003194 | *Chem. Res. Toxicol.* 2014, 27, 1871−1876

induced hepatotoxicity while dihydromethysticin (DHM) lacked such a risk.

MATERIALS AND METHODS

Chemicals and reagents. An ethanolic extract of the wild crafted kava root from Vanuatu was purchased from Gaia Herbs, Inc. (Brevard, NC, standardized to 150 mg/mL total kavalactones). DHM was purified from this kava product using normal phase silica gel chromatography as described earlier.[21] FKA and FKB were synthesized and characterized following an established procedure.[17] Kava and all compounds were completely dried under vacuum to remove any solvent residue. APAP was purchased from Sigma-Aldrich (MO, St. Louis). The desired drug formulations were prepared by mixing kava or pure compounds with PEG-400 and stored at 4 °C until use.

Animal study design. All animal studies were performed in compliance with the Institutional Animal Care and Use Committee at the University of Minnesota guidelines. Six-week-old female C57BL/6J mice (Jackson Laboratories, ME) were housed at specific pathogen-free animal facilities of Research Animal Resources, University of Minnesota, with free access to standard rodent food and water. All mice were acclimatized for 1 week before being used for experiments. Mice were gavaged with dose formulations at the indicated doses and times, and euthanized by CO_2 overdosing with necropsy performed by experienced researchers.

The long-term study was designed to evaluate the hepatotoxicity of kava alone. C57BL/6 mice were randomized (n = 4). Mice in the control group were given PEG-400 (200 μL) on a daily basis via gavage, 6 days a week, for 14 weeks. Mice in the kava treatment group were given kava at a dose of 500 mg/kg bodyweight on a daily basis via gavage, 6 days a week, for 14 weeks. The chosen kava dose was based on the recent safety studies of another kava product performed by the National Toxicology Program.[23] Mouse bodyweight was measured once a week. Upon necropsy, final bodyweight was measured and serum from each mouse was analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), two major biomarkers of liver function.

The short-term combination studies were designed to evaluate the potential synergism of kava and its chemicals to APAP-induced hepatotoxicity. C57BL/6 mice were randomized (8−15 mice per group) and were administered with PEG-400 (200 μL), kava (500 mg/kg bodyweight), DHM or FKA, and FKB in PEG-400 (200 μL) at the indicated doses daily via oral gavage for 2 days. On the third day, mice in the respective groups were coadministered with APAP (800 mg/kg bodyweight) in PEG-400 (200 μL). Bodyweight was recorded daily. Necropsies were performed 24 h after the last gavage by experienced researchers. Serum from each mouse was analyzed for ALT and AST. Livers were collected and preserved in 10% neutral buffered formalin. Appropriately fixed tissues were processed into paraffin blocks using standard histological techniques, and 5 μm sections were cut and stained with hematoxylin and eosin (H&E). Histological slides were examined using light microscopy by an experienced A.C.V.P board certified pathologist (M.G.O'S.) under blinded conditions, with liver lesions graded on a 0 to 4 scale based on the extent of necrosis (0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe).

Statistical analysis. The clinical chemistry data were reported as mean ± SD (n = 4−15). For the long-term kava alone study, the two-tailed Student t-test was used to compare the means between the control and treatment groups. p-value ≤ 0.05 was considered statistically significant. One-way analysis of variance (ANOVA) was used to compare the means among different groups in the short term combination studies. Dunnett's test was used for comparisons of APAP and other treatment groups when the one-way ANOVA analysis was statistically significant. p-value ≤0.05 was considered statistically significant. All analyses were conducted in GraphPad Prism 4 (GraphPad Software, Inc. La Jolla, CA).

RESULTS

Kava alone did not affect mouse growth and induced no signs of hepatotoxicity. At the tested dose (500 mg/kg bodyweight), daily kava treatment did not affect mouse growth (data not shown). There were also no statistically or biologically significant differences between control and kava-treated mice with respect to ALT and AST (Figure 1A and 1B).

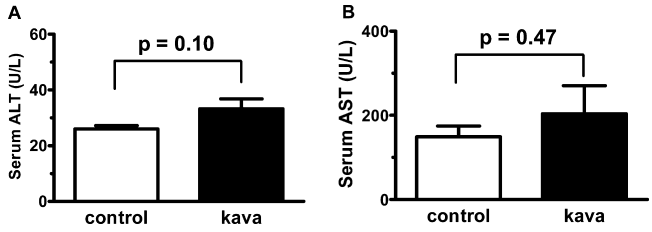


Figure 1. Effect of 14-week daily kava treatment (500 mg/kg bodyweight) via gavage on mouse serum ALT (A) and AST (B). p values were given with comparison between the control group (n = 4) and the kava treatment group (n = 4) using the two-tailed Student t-test.

Kava enhanced APAP-induced hepatotoxicity in C57BL/6 mice. Since ∼90% of the human kava hepatotoxic cases involved concurrent consumption of other medications or dietary supplements,[14,15] herb−drug interactions may contribute to kava's hepatotoxic risk. Based on this and on a recent report that kava enhanced the toxicity of APAP *in vitro*,[24] this study was designed to evaluate the effect of kava on APAP-induced hepatotoxicity *in vivo*. The treatment regimen was designed to mimic potential scenarios in humans—kava was consumed on a daily basis while APAP was used occasionally. As expected, kava treatment alone had no effect on ALT and AST while APAP treatment significantly increased serum ALT and AST activities (Figure 2A). Kava and APAP combination caused further increase in serum ALT and AST activities (∼3-fold increase relative to APAP alone, Figure 2A), and these increases were statistically significant in comparison to APAP treatment alone. Histopathological analyses of the liver tissues revealed no lesions in control and kava treated mice (Figure 2B), confirming the lack of hepatotoxicity by kava treatment alone. The lesions from APAP-treated mice evenly distributed among different severity categories (0 being no lesion and 4 being the highest grade lesion) while kava and APAP combination markedly increased the number of mice with the highest liver lesion (Figure 2B), supporting the notion that the increases in ALT and AST activities were biologically significant. These clinical chemistry data and histopathological findings for the first time demonstrate that kava enhanced APAP-induced hepatotoxicity *in vivo*, and may reflect the purported kava hepatotoxicity cases in humans. The histopathological lesion severity also nicely correlated positively

dx.doi.org/10.1021/tx5003194 | Chem. Res. Toxicol. 2014, 27, 1871−1876

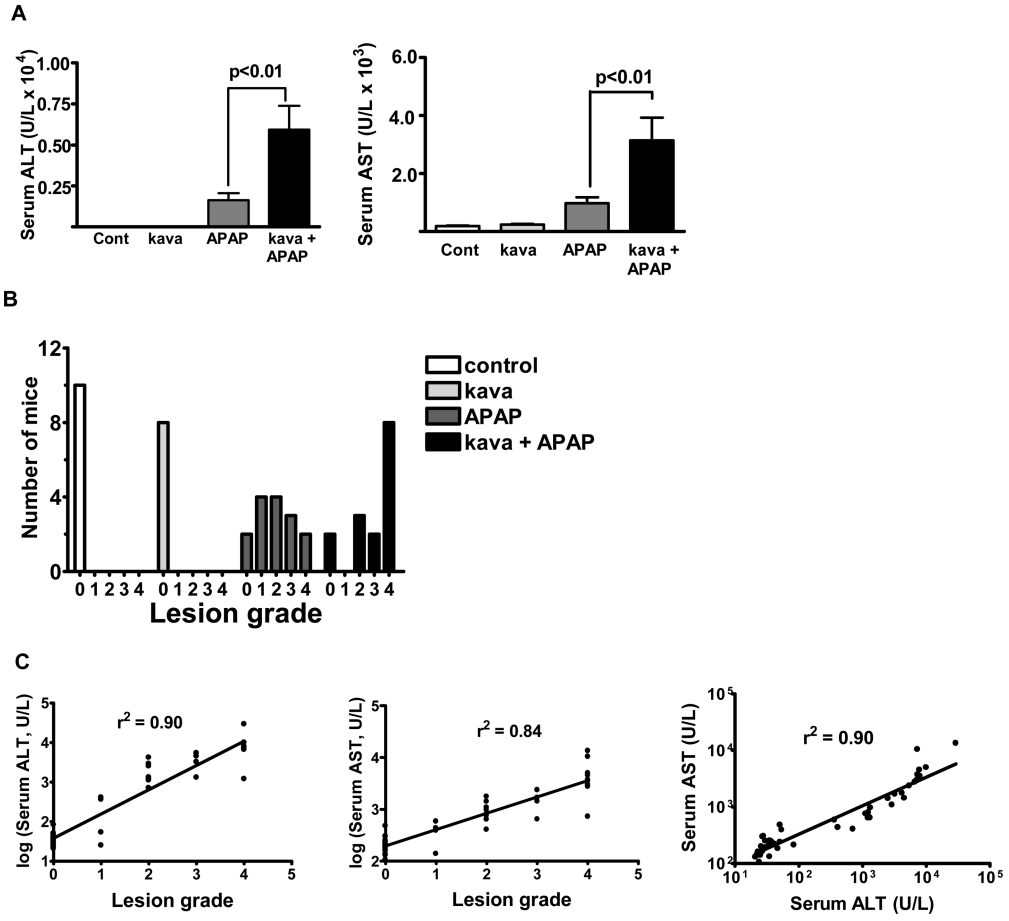


Figure 2. Effect of 3-day daily kava treatment (500 mg/kg bodyweight) via gavage on mouse serum ALT and AST and liver lesions with/without APAP treatment (800 mg/kg bodyweight) via gavage. (A) Serum ALT and AST. (B) The number of mice with different grades of liver lesions. (C) The relationships among serum ALT, AST, and the grades of liver lesions. For A, comparisons were made with the APAP treatment group by Dunnett's test when ONE-WAY ANOVA was statistically significant (n = 8−15).

with the clinical chemistry results (Figure 2C). Therefore, only clinical chemistry was performed in subsequent studies.

DHM did not potentiate APAP-induced ALT and AST while FKB increased both. This experiment was designed to explore the potential of DHM and FKB (Figure 3) to synergize

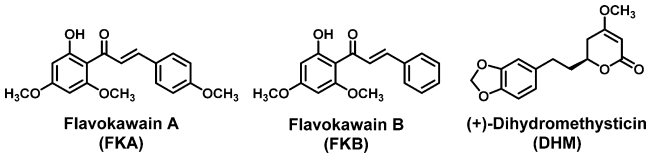


Figure 3. Chemical structures of flavokawains A and B, and dihydromethysticin.

the hepatotoxicity of APAP following the same kava and APAP cotreatment regimen. Thirteen chemicals have been isolated and quantified from the kava product used in this study with no detection of pipermethystine.[13] DHM and FKB were selected for this initial evaluation because they are representatives of kavalactones and chalcones, respectively, two major classes of chemicals in kava. In addition DHM has been recently demonstrated to potently and effectively block NNK-induced lung tumorigenesis in mice[13] while FKB has been identified as the most cytotoxic compound in kava to various cancerous cells.[12,25] The dosages for DHM (37.5 mg/kg) and FKB (11.5 mg/kg) were based on their abundance (7.5% and 2.3%, respectively) in this kava product at a dose of 500 mg/kg.[13] DHM and FKB individually caused no effect on serum ALT and AST (Figure 4). DHM had no effect on serum ALT and

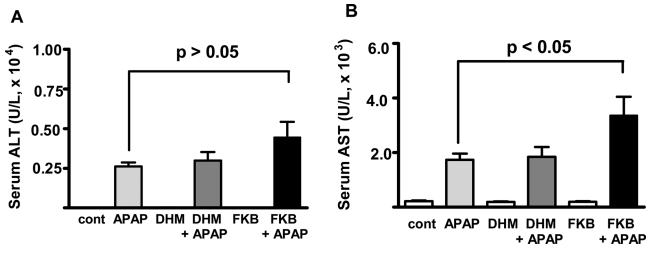


Figure 4. Effect of 3-day daily DHM (37.5 mg/kg) or FKB (11.5 mg/kg) via gavage on mouse serum ALT and AST with/without APAP treatment. Comparisons were made with the APAP treatment group by Dunnett's test when ONE-WAY ANOVA was statistically significant (n = 6−15).

AST as well when combined with APAP (Figure 4). FKB on the other hand when combined with APAP moderately increased the serum levels of ALT and AST, and the increase in AST was statistically significant (Figure 4), suggesting that FKB contributes to kava's potentiation of APAP-induced hepatotoxicity.

dx.doi.org/10.1021/tx5003194 | Chem. Res. Toxicol. 2014, 27, 1871−1876

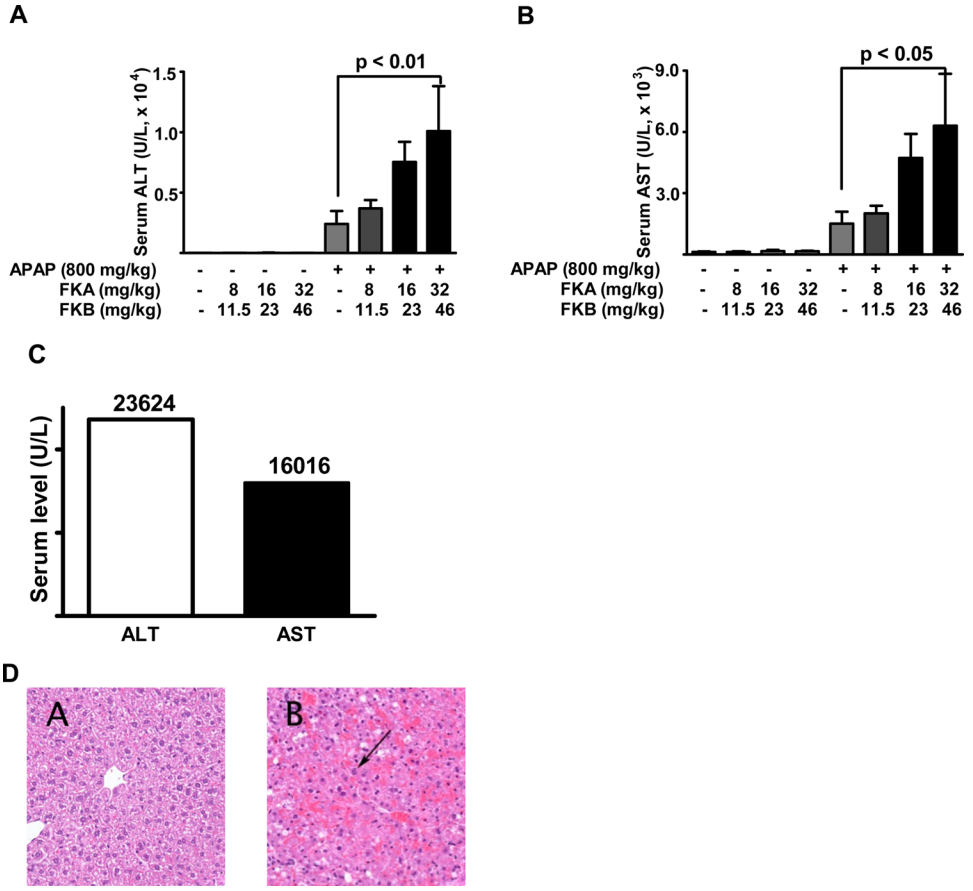


Figure 5. Dose–response effect of 3-day daily FKA and FKB via gavage on mouse serum ALT and AST and livers with/without APAP treatment. (A and B) Serum ALT and AST. Comparisons were made with the APAP treatment group by Dunnett's test when ONE-WAY ANOVA was statistically significant (n = 5). (C) Serum level of ALT and AST from the dead mouse in the high-dose FKA and FKB groups with APAP cotreatment. (D) Photomicrographs of H- and E-stained livers from a control mouse (Panel A) and a mouse treated with FKA and FKB plus APAP (Panel B). Note extensive karyorrhexis (arrow) reflecting acute necrosis of hepatocytes (increased eosinophilia) in mouse treated with FKA, FKB, and APAP (Panel B).

The combination of flavokawain A (FKA) and FKB dose-dependently enhanced APAP-induced hepatotoxicity. Given that the kava product used in this study contains flavokawain A (FKA) of similar abundance as FKB (Figure 3), this experiment was designed to evaluate the dose–response effect of FKA and FKB together on APAP's hepatotoxicity following the same treatment regimen. The final dosages of FKA and FKB were 1, 2, and 4 times their abundance (1.6% and 2.3%, respectively) of a kava dose at 500 mg/kg bodyweight. FKA and FKB together did not induce any changes on serum ALT and AST at the three tested dosages (Figure 5A and B). When combined with APAP, FKA and FKB dose-dependently potentiated the increase in ALT and AST induced by APAP (Figure 5A and B). Of note, one mouse with the treatment of the highest dose of FKA and FKB in combination with APAP died ~0.5–2 h before necropsy (i.e., 22 to 23.5 h after the combined dose of APAP with FKA and FKB). This was the only mouse among all the studies that died before necropsy. Its serum ALT and AST levels were the highest among all mice (Figure 5C), and 2–3 times higher than the next highest values. Histopathological examination revealed multifocal and coalescing acute centrilobular necrosis in the liver of this mouse (Figure 5D, panel B), whereas livers from a control mouse (Figure 5D, panel A) and a mouse treated with FKA and FKB alone (not shown) were histologically within normal limits. These data suggest that severe hepatotoxicity likely contributed to its early death.

DISCUSSION

Kava has demonstrated anxiolytic activity in the clinic and potentially reduces cancer risk in humans. On the other hand, kava usage has been speculated to be associated with rare but severe hepatotoxicity. Various mechanisms have been proposed and different chemicals have been postulated with no confirmation. Given kava's global resurgence and the diverse chemical composition among current kava products, it is urgent and important to recapitulate kava's hepatotoxicity in an *in vivo* model, which can help identify the responsible chemicals and guide the development of strategies to minimize and ideally eradicate such an adverse potential.

The results from this study demonstrated that kava when administered alone via gavage in C57BL/6 mice induced no adverse effect even at a fairly high dose (500 mg/kg bodyweight daily) in a chronic manner, as reflected in mouse growth and serum levels of ALT and AST (Figure 1). These results are consistent with the results from many early studies.[26−29] On the other hand, kava significantly potentiated the hepatotoxicity of APAP in C57BL/6 mice, as indicated by the increase in serum ALT and AST, and the increased severity of liver lesions (Figure 2). The treatment regimen was designed to mimic

dx.doi.org/10.1021/tx5003194 | *Chem. Res. Toxicol.* 2014, 27, 1871–1876

potential circumstances among human kava users that kava would be consumed on a daily basis while other medications, APAP in this case, were used occasionally when needed. Since the majority of kava-associated hepatotoxic cases consumed other medications or dietary supplements concomitantly, the results from this study may have direct indication to the observed hepatotoxicity among kava users. It remains to be determined whether kava usage can potentiate the hepatotoxic risk of other medications or hepatotoxins, such as alcohol consumption. It also remains to be determined whether other kava treatment regimens, such as prolonged kava usage or in a fasted stage (recommended for traditional kava usage), may potentiate its hepatotoxic risk even at lower kava dosages.

With the C57BL/6 mouse model that captures kava's hepatotoxic risk *in vivo*, we investigated the potential responsible compound(s). The results demonstrated that a chalcone-based compound in kava, FKB, moderately potentiated APAP's hepatotoxicity while DHM, a representative of kavalactones in kava, lacked such a risk when they were evaluated at a dose equivalent to kava at a dose of 500 mg/kg bodyweight (Figure 4). As the kava product contains FKA, an analog of FKB, at similar abundance, the combination of FKA and FKB was evaluated, which dose-dependently enhanced APAP-induced hepatotoxicity (Figure 5). Indeed, the one mouse that died early, and which had the highest ALT and AST levels (Figure 5C), reflecting extensive acute hepatocellular necrosis (Figure 5D, panel B), was in the APAP cotreatment group at the highest dose of FKA and FKB. These data overall indicate that FKA and FKB are the responsible compounds in kava that potentiate APAP-induced hepatotoxicity while DHM is free of this risk. Besides FKA and FKB, flavokawain C (FKC) has been reported in other kava products[11] but was not detectable in the kava product used in this study. FKC might be another compound responsible for hepatotoxicity.

Our recent analysis of a set of kava products on the current market demonstrates that the abundance of FKA and FKB can vary ~20-fold.[22] Similarly, a recent study analyzed the abundance of FKA, FKB, and FKC in different kava cultivars.[11] Cultivars not recommended for traditional use were found to contain higher abundance of FKA, FKB, and FKC than the traditionally consumed cultivars.[11] Further studies therefore are warranted to evaluate whether cultivars or kava products with higher content of FKA, FKB, and FKC would impose a higher hepatotoxic risk. Future studies are also needed to elucidate the molecular mechanisms of the observed hepatotoxicity enhancement, such as the depletion of glutathione.[30] Such knowledge will help guide the preparation of kava products for human use with higher health benefit and minimal adverse effects.

AUTHOR INFORMATION

Corresponding Author

*Phone: 612-626-5675. E-mail: xingx009@umn.edu.

Funding

This work was supported by Grant R01 CA142649 from the National Cancer Institute, National Institutes of Health.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Junxuan Lu, Dr. Joseph Betz, Dr. Bill Gurley, Dr. Rick Kingston, and Dr. Chi Chen for valuable advice.

ABBREVIATIONS

APAP acetaminophen; FKA flavokawain A; FKB flavokawain B; DHM dihydromethysticin; ANOVA analysis of variance; ALT alanine aminotransferase; AST aspartate aminotransferase; PEG-400 polyethylene glycol-400

REFERENCES

(1) Gounder, R. (2006) Kava consumption and its health effects. *Pacific Health Dialogue 13*, 131−135.

(2) LaPorte, E., Sarris, J., Stough, C., and Scholey, A. (2011) Neurocognitive effects of kava (Piper methysticum): a systematic review. *Hum. Psychopharmacol. 26*, 102−111.

(3) Sarris, J., Kavanagh, D. J., Deed, G., and Bone, K. M. (2009) St. John's wort and Kava in treating major depressive disorder with comorbid anxiety: a randomised double-blind placebo-controlled pilot trial. *Hum. Psychopharmacol. 24*, 41−48.

(4) Sarris, J., Kavanagh, D. J., Byrne, G., Bone, K. M., Adams, J., and Deed, G. (2009) The Kava Anxiety Depression Spectrum Study (KADSS): a randomized, placebo-controlled crossover trial using an aqueous extract of Piper methysticum. *Psychopharmacology (Berl.) 205*, 399−407.

(5) Pittler, M. H., and Ernst, E. (2000) Efficacy of kava extract for treating anxiety: systematic review and meta-analysis. *J. Clin. Psychopharmacol. 20*, 84−89.

(6) Teschke, R., and Wolff, A. (2009) Kava hepatotoxicity: regulatory data selection and causality assessment. *Dig. Liver Dis. 41*, 891−901.

(7) Teschke, R., Sarris, J., and Lebot, V. (2013) Contaminant hepatotoxins as culprits for kava hepatotoxicity–fact or fiction? *Phytother. Res. 27*, 472−474.

(8) Carreño, I., Laurenza, E., Martelloni, A., Salas, B., Simões, B. G., and Vergona, P. R. (2014) German court repeals the withdrawal of marketing authorisation of kava-containing medicinal products. *Trade Perspectives 13*, 2−5.

(9) Schulze, J., Raasch, W., and Siegers, C. P. (2003) Toxicity of kava pyrones, drug safety and precautions—a case study. *Phytomedicine 10*, 68−73.

(10) Anke, J., and Ramzan, I. (2004) Kava Hepatotoxicity: Are we any closer to the truth? *Planta Med. 70*, 193−196.

(11) Lebot, V., Do, T. K., and Legendre, L. (2014) Detection of flavokavins (A, B, C) in cultivars of kava (Piper methysticum) using high performance thin layer chromatography (HPTLC). *Food Chem. 151*, 554−560.

(12) Shaik, A. A., Hermanson, D. L., and Xing, C. (2009) Identification of methysticin as a potent and non-toxic NF-kappaB inhibitor from kava, potentially responsible for kava's chemopreventive activity. *Bioorg. Med. Chem. Lett. 19*, 5732−5736.

(13) Leitzman, P., Narayanapillai, S. C., Balbo, S., Zhou, B., Upadhyaya, P., Shaik, A. A., O'Sullivan, M. G., Hecht, S. S., Lu, J., and Xing, C. (2014) Kava Blocks 4-(Methylnitrosamino)-1-(3-pyridyl)-1-Butanone-Induced Lung Tumorigenesis in Association with Reducing O6-methylguanine DNA Adduct in A/J Mice. *Cancer Prev. Res. (Phila.) 7*, 86−96.

(14) W. H. Organization. (2007) Assessments of the risk of hepatotoxicity with kava products. WHO Document Production Service.

(15) Teschke, R., Schwarzenboeck, A., and Hennermann, K. H. (2008) Kava hepatotoxicity: a clinical survey and critical analysis of 26 suspected cases. *Eur. J. Gastroenterol. Hepatol. 20*, 1182−1193.

(16) Steiner, G. G. (2000) The correlation between cancer incidence and kava consumption. *Hawaii Med. J. 59*, 420−422.

(17) Johnson, T. E., Hermanson, D., Wang, L., Kassie, F., Upadhyaya, P., O'Sullivan, M. G., Hecht, S. S., Lu, J., and Xing, C. (2011) Lung tumorigenesis suppressing effects of a commercial kava extract and its selected compounds in A/J mice. *Am. J. Chin. Med. 39*, 727−742.

(18) Johnson, T. E., Kassie, F., O'Sullivan, M. G., Negia, M., Hanson, T. E., Upadhyaya, P., Ruvolo, P. P., Hecht, S. S., and Xing, C. (2008) Chemopreventive effect of kava on 4-(methylnitrosamino)-1-(3-

pyridyl)-1-butanone plus benzo[a]pyrene-induced lung tumorigenesis in A/J mice. *Cancer Prev. Res. (Phila.) 1*, 430−438.
(19) Triolet, J., Shaik, A. A., Gallaher, D. D., O'Sullivan, M. G., and Xing, C. (2012) Reduction in colon cancer risk by consumption of kava or kava fractions in carcinogen-treated rats. *Nutr. Cancer 64*, 838−846.
(20) Zi, X., and Simoneau, A. R. (2005) Flavokawain A, a Novel Chalcone from Kava Extract, Induces Apoptosis in Bladder Cancer Cells by Involvement of Bax Protein-Dependent and Mitochondria-Dependent Apoptotic Pathway and Suppresses Tumor Growth in Mice. *Cancer Res. 65*, 3479−3486.
(21) Narayanapillai, S. C., Balbo, S., Leitzman, P., Grill, A. E., Upadhyaya, P., Shaik, A. A., Zhou, B., O'Sullivan, M. G., Peterson, L., Lu, J., Hecht, S. S., and Xing, C. (2014) Dihydromethysticin (DHM) from kava blocks tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis and differentially reduces DNA damage in A/J mice. *Carcinogenesis,*.
(22) Martin, A. C. J. E., Xing, C., and Hegeman, A. D. (2014) Measuring the chemical and cytotoxic variability of commercially available kava. *PLoS One,*.
(23) National Toxicology Program. (2012) Toxicology and carcinogenesis studies of kava kava extract (CAS No. 9000-38-8) in F344/N rats and B6C3F1 mice (Gavage Studies). *Natl. Toxicol. Program Tech. Rep. Ser.*, 1−186.
(24) Yang, X., and Salminen, W. F. (2011) Kava extract, an herbal alternative for anxiety relief, potentiates acetaminophen-induced cytotoxicity in rat hepatic cells. *Phytomedicine 18*, 592−600.
(25) Jhoo, J. W., Freeman, J. P., Heinze, T. M., Moody, J. D., Schnackenberg, L. K., Beger, R. D., Dragull, K., Tang, C. S., and Ang, C. Y. (2006) In vitro cytotoxicity of nonpolar constituents from different parts of kava plant (Piper methysticum). *J. Agric. Food Chem. 54*, 3157−3162.
(26) DiSilvestro, R. A., Zhang, W., and DiSilvestro, D. J. (2007) Kava feeding in rats does not cause liver injury nor enhance galactosamine-induced hepatitis. *Food Chem. Toxicol. 45*, 1293−1300.
(27) Guo, L., Li, Q., Xia, Q., Dial, S., Chan, P. C., and Fu, P. (2009) Analysis of gene expression changes of drug metabolizing enzymes in the livers of F344 rats following oral treatment with kava extract. *Food Chem. Toxicol. 47*, 433−442.
(28) Guo, L., Shi, Q., Dial, S., Xia, Q., Mei, N., Li, Q. Z., Chan, P. C., and Fu, P. (2010) Gene expression profiling in male B6C3F1 mouse livers exposed to kava identifies—changes in drug metabolizing genes and potential mechanisms linked to kava toxicity. *Food Chem. Toxicol. 48*, 686−696.
(29) Sorrentino, L., Capasso, A., and Schmidt, M. (2006) Safety of ethanolic kava extract: Results of a study of chronic toxicity in rats. *Phytomedicine 13*, 542−549.
(30) Zhou, P., Gross, S., Liu, J. H., Yu, B. Y., Feng, L. L., Nolta, J., Sharma, V., Piwnica-Worms, D., and Qiu, S. X. (2010) Flavokawain B, the hepatotoxic constituent from kava root, induces GSH-sensitive oxidative stress through modulation of IKK/NF-kappa B and MAPK signaling pathways. *FASEB J. 24*, 4722−4732.