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Evaluation of commercial kava extracts and kavalactone standards for mutagenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells

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Abstract

Kava (*Piper methysticum*) is a member of the pepper family and has been cultivated by South Pacific islanders for centuries and used as a social and ceremonial drink. Traditionally, kava extracts are prepared by grinding or chewing the rhizome and mixing with water and coconut milk. The active constituents of kava are a group of approximately 18 compounds collectively referred to as kavalactones or kava pyrones. Kawain, dihydrokawain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin are the six major kavalactones. Kava beverages and other preparations are known to be anxiolytic and are used for anxiety disorders. Dietary supplements containing the root of the kava shrub have been implicated in several cases of liver toxicity in humans, including several who required liver transplants after using kava supplements. In order to study the toxicity and mutagenicity, two commercial samples of kava, Kaviar and KavaPure, and the six pure kavalactones including both D-kawain and DL-kawain, were evaluated in L5178Y mouse lymphoma cells. Neither the kava samples nor the kavalactones induced a mutagenic response in the L5178Y mouse lymphoma mutation assay with the addition of human liver S9 activation.
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Keywords: *Piper methysticum*; Kava; Kava pyrones; Kavalactones; Mutagenicity; Mouse lymphoma

1. Introduction

The root of the kava plant (*Piper methysticum* G. Forster, Piperaceae) is used to prepare a traditional psychoactive beverage consumed by people in various Pacific Basin countries. The root of the plant is macerated, mixed with water and coconut milk and then strained. More recently, kava in tablets, capsules and tinctures prepared from lipophilic extracts, has become popular in Europe and in the United States (Dentali, 1997) as an alterative to antianxiety drugs. It is claimed that kava promotes relaxation, induces restful sleep, relieves headache and migraine pain, and promotes sociability (Bilia et al., 2002). In 1990, Germany approved kava preparations as nonprescription drugs for the treatment of nervous anxiety disorders such as stress and restlessness. However, with reports of cases of liver

Abbreviations: DMSO, dimethyl sulfoxide; FDA, US Food and Drug Administration; LC–MS, liquid chromatography–mass spectrometry; NCI, National Cancer Institute; NIH, National Institutes of Health; DMBA, 7,12-dimethylbenzanthracene; SIM, single ion monitoring; TK, thymidine kinase; TFT, trifluorothymidine.

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damage associated with the use of kava, the preparations were banned in Germany in 2002 and also removed from the market in several other countries, including Switzerland, United Kingdom, France, Canada, and Australia (Clouatre, 2004). The Centers for Disease Control (2002) reported that 11 individuals using kava products suffered liver failure and had to undergo liver transplants. There have been 78 documented adverse events involving kava ingestion and liver toxicity in various databases (Clouatre, 2004). In 2002, the US Food and Drug Administration (FDA) issued an advisory to consumers about the risk of liver toxicity associated with the use of kava containing products. Additional caution was also recommended for those with or at risk for liver disease (FDA, 2002).

The active components of kava rootstock are contained primarily in the lipid-soluble resin. The compounds of greatest pharmacological interest are the styryl α-pyrones or kavalactones and represent 3–20% of the dried rhizome depending on age of the plant and specific cultivar (Bilia et al., 2002). At least 16 lactones have been isolated from kava and six compounds, namely, yangonin, methysticin, dihydromethysticin, kawain, dihydrokawain and desmethoxyyangonin, account for approximately 96% of the lipid resin (Cote et al., 2004).

In this study, cellular toxicity and mutagenicity of commercially available *P. methysticum* (kava) samples, Kaviar and KavaPure, and six pure kava pyrones were evaluated in L5178Y mouse lymphoma cells in the presence of pooled human liver S9.

2. Materials and methods

2.1. Chemicals

The source of the commercial kava samples and concentration of the kavalactones in these samples are listed in Table 1. The manufacturer/supplier, Chemical Abstracts Service number, formula, formula weight, and purity of each kavalactone standard are listed in Table 2. The compounds were acquired by the FDA and the NIH, and supplied as coded samples to the contract laboratory (BioReliance, Rockville, MD). Stock solutions of each compound were prepared in the appropriate solvent immediately prior to use. The final concentration of solvent in the mutagenicity assay was 1% for both ethanol and dimethyl sulfoxide (DMSO).

2.2. Analysis of kavalactones

Approximately 0.04 g of each kava sample was weighed into a scintillation vial using an analytical balance. Five millilitres of HPLC grade ethanol were pipetted into the vials and they were sonicated at 40 °C for 30 min. The extracts were then filtered using a 25 mm nylon membrane syringe filter with a 0.45 μm pore size, and diluted 1:1000 for KavaPure and 1:250 for Kaviar in 25% methanol:75% water. The internal standard, 8-methoxypsoralen, was added to 1 ml aliquots of the diluted extract to give a final concentration of 0.1 μg/ml. The samples were then analyzed using an Agilent 1100 LC–MS (Agilent Technologies, Palto Alto, CA) with an atmospheric pressure photoionization interface in the positive ionization mode as described by de Jager et al. (2004). Each diluted extract was analyzed three times and the concentrations were calculated using a five point calibration curve over the concentration range of 4.0–0.08 μg/ml. Average concentrations of the kavalactones for the two samples are listed in Table 1.

Table 1
Concentration of kavalactones in commercial kava (*Piper methysticum*) samples

Sample	Kavalactones	Concentration (mg/g)	Source
Kaviar	Methysticin	16	Cosmopolitan Trading (Seattle, WA)
	Dihydromethysticin	18	
	Kawain	36	
	Dihydrokawain	39	
	Yangonin	29	
	Desmethoxyyangonin	11	
	Total	149	
KavaPure	Methysticin	13	Madis Botanicals, Inc. (Hackensack, NJ)
	Dihydromethysticin	25	
	Kawain	38	
	Dihydrokawain	36	
	Yangonin	23	
	Desmethoxyyangonin	16	
	Total	151	

2.3. Human S9 preparation

Human liver S9 was purchased from In Vitro Technologies Inc., Baltimore, MD. Human liver was pooled from 15 individuals of mixed sexes and the S9 was prepared according to the methods described by Guengerich (1989), Easterbrook et al. (2001), and Nomeir et al. (2001). The S9 contains the subcellular fractions where the drug metabolizing cytochrome P450 enzymes reside.

2.4. L5178Y TK+/− mouse lymphoma assay

L5178Y TK+/− 3.7.C mouse lymphoma cells were originally obtained from Ms. Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC or American Type Culture Collection, Manassas, VA. The cells were grown in Fischer's medium for leukemic cells of mice (Irvine Scientific, Irvine, CA) supplemented with 10% horse serum (Gibco, Grand Island, NY) and 0.02% pluronic F-68 (BASF Wyandotte Corp., Wyandotte, MI). Cells were screened for the presence of mycoplasma

Table 2
Chemical information and source for kavalactone standards

Chemical name	CAS number	Formula	Formula weight	Purity (%)	Source
Dihydromethysticin	19902-91-1	$C_{14}H_{16}O_5$	276.29	99.8	PhytoLab GmbH & Co. (Vestenbergsgreuth, Germany)
Desmethoxyyangonin	15345-89-8	$C_{14}H_{12}O_3$	228.25	100.0	PhytoLab GmbH & Co.
Methysticin	20697-20-5	$C_{15}H_{14}O_5$	274.27	99.9	PhytoLab GmbH & Co.
Dihydrokawain	587-63-3	$C_{14}H_{16}O_3$	232.28	99.8	PhytoLab GmbH & Co.
Yangonin	500-62-9	$C_{15}H_{14}O_4$	258.27	100.0	PhytoLab GmbH & Co.
D-Kawain	3155-48-4	$C_{14}H_{14}O_3$	230.26	99.0	ElSohly Laboratories, Inc.(Oxford, MS)
DL-Kawain	3155-48-4	$C_{14}H_{14}O_3$	230.26	99.0	ElSohly Laboratories, Inc.

before and after cryopreservation. New cultures were initiated from cells stored in liquid N_2 at approximately 3-month intervals.

The toxicity of each chemical was determined with pooled human liver S9. S9 mix was prepared following the procedure described by Clive et al. (1979). Cells at a concentration of 6×10^5/ml (6×10^6 cells total) were exposed for 4 h to a range of concentrations of each chemical. The cells were then washed, resuspended in growth medium and incubated at 37 ± 1 °C for 24 h. Cells in the cultures were then adjusted to 3×10^5 cells/ml and incubated at 37 ± 1 °C for an additional 24 h. The rate of cell growth was determined for each of the treated cultures and compared with the rate of growth of the solvent controls. The doses of each chemical selected for testing were within the range yielding approximately 0–90% cytotoxicity or up to the limit of solubility. For each assay there was a solvent control and a positive control of aflatoxin B-1 at 0.5 or 1.0 μg/ml.

The mutagenicity assay was performed as described (Clive and Spector, 1975). A total of 1.2×10^7 cells in duplicate cultures were exposed to the test chemical, positive control and solvent control for 4 h at 37 ± 1 °C, washed twice with growth medium and maintained at 37 ± 1 °C for 48 h in log-phase growth to allow recovery and mutant expression. Cells in the cultures were adjusted to 3×10^5 cells/ml at 24-h intervals. They were then cloned (1×10^6 cells/plate for mutant selection and 200 cells/plate for viable count determinations) in soft-agar medium containing Fischer's medium, 20% horse serum, 2 mM sodium pyruvate, 0.02% pluronic F-68 and 0.23% granulated agar (BBL Inc., Cockeysville, MD) or 0.22% Noble agar (Becton Dickinson, Sparks, MD). Resistance to trifluorothymidine (TFT) was determined by adding TFT (final concentration, 3 μg/ml) to the cloning medium for mutant selection. The 100× stock solution of TFT in saline was stored at −70 °C and was thawed immediately before use. Plates were incubated at 37 ± 1 °C in 5% CO_2 in air for 10–12 days and then counted with a ProtoCOL automated colony counter (Synoptics Ltd., Cambridge, UK). The results from this study were evaluated according to the guidelines in Clive et al. (1995). A data set would be considered to show an indication of mutagenic activity if there was evidence of a dose response with at least one concentration giving an increase in mutant frequency of at least 100 mutants per 10^6 surviving cells above the concurrent solvent control value. Only colonies larger than approximately 0.1 mm in diameter were counted. Mutant frequencies were expressed as mutants per 10^6 surviving cells. Only doses yielding total growth values of $\geqslant 10\%$ were used in the analysis of induced mutant frequency.

The size of mutant mouse lymphoma colonies was also determined using a ProtoCOL colony counter/sizer. An internal discriminator was set to step sequentially to exclude increasingly larger colonies in increments of approximately 0.1 mm in colony diameter. The size range used was approximately 0.2–1.1 mm.

2.5. Statistical analysis

Differences among mutant frequencies were assessed by analysis of variance (Snedecor and Cochran, 1980).

3. Results

Analysis of Kaviar by LC–MS single ion monitoring (SIM) chromatography, revealed the presence of six major kavalactones, namely, methysyticin, dihydromethysticin, kawain, dihydrokawain, yangonin and desmethoxyyangonin (Fig. 1). Similar results were obtained with the KavaPure sample. The average concentrations in mg/g for the kavalactones and the total concentration of the six kavalactones for each sample are shown in Table 1. For the Kaviar sample the total concentration for the six kavalactones was 149 mg/g, while the concentration for the KavaPure sample was 151 mg/g. With the possible exception of dihydromethysticin, for which there was 18 mg/g for the Kaviar sample and 25 mg/g for the KavaPure sample, the amounts of the kavalactones in each sample were comparable (Fig. 2).

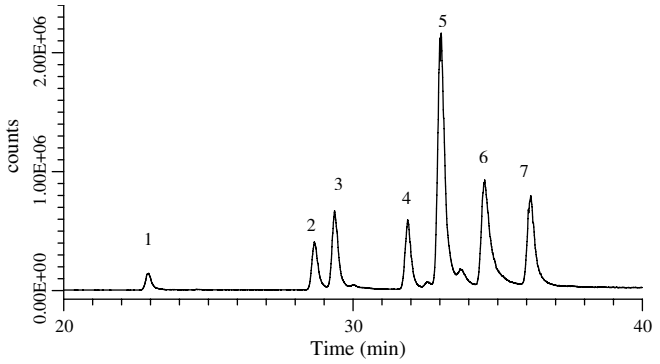

Fig. 1. LC–MS single ion monitoring (SIM) chromatogram of extracts from Kaviar samples. Peak identification is as follows: 1 = internal standard, 2 = methysticin, 3 = dihydromethysticin, 4 = kawain, 5 = dihydrokawain, 6 = yangonin, 7 = desmethoxyyangonin.

The two kava samples, KavaPure and Kaviar as well as the six major kavalactones were tested in L5178Y mouse lymphoma cells in the presence of pooled human liver S9. Kaviar was slightly more toxic than KavaPure based on the dose level that was required and the percentage of relative total growth to the control (Table 3). Following the guidelines of Clive et al. (1995), both compounds were considered to be nonmutagenic, since there was no dose increase and no dose showing at least 100 mutants above the concurrent solvent control. Differences in mutant frequencies for the doses were assessed by analysis of variance for KavaPure and Kaviar, and *P* values are included in Table 3. The results of the assays with the six component kavalactones are shown in Tables 4 and 5. Yangonin and desmethoxyyangonin appeared to be the most toxic kavalactones with dose ranges for testing of 0.5–20 μg/ml and 1.25–20 μg/ml, respectively. The remaining kavalactones were tested within a range of 60–300 μg/ml which

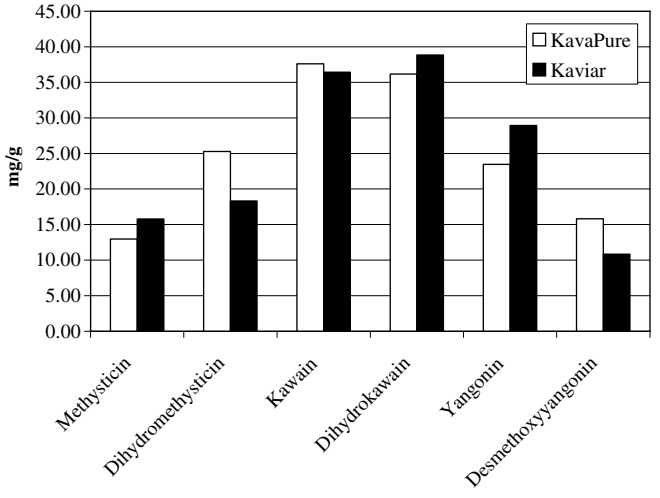

Fig. 2. Comparison of kavalactone concentrations for Kaviar and KavaPure.

Table 3
Mutagenicity and cytotoxicity of KavaPure and Kaviar in mouse lymphoma cells

	Dose (μg/ml)	Human S9	Absolute cloning efficiency[a]	Relative total growth (% of control)	Average number TFT^R colonies	Mutant frequency per 10^6 survivors[b,c]
KavaPure						
Solvent control (DMSO)	0	+	0.93/0.82	100.0	35/26	35
	150.0	+	0.85/0.81	84.0	45/32	45
	200.0	+	0.78/0.74	71.0	65/27	61
	250.0	+	0.64/0.57	47.5	65/47	93
	300.0	+	0.67/0.48	40.0	34/31	59
	400.0	+	0.62/0.70	30.0	78/27	83
Positive control (aflatoxin B-1)	0.5	+	0.41	37.0	114	283
Kaviar						
Solvent control (DMSO)	0	+	0.80/0.63	100.0	56/46	72
	50.0	+	0.77/0.64	73.5	56/31	61
	75.0	+	0.79/0.59	56.0	61/25	60
	100.0	+	0.78/0.85	52.0	39/25	40
	150.0	+	0.77/0.72	27.5	70/59	87
	200.0	+	0.74/0.78	14.5	63/69	88
Positive control (aflatoxin B-1)	0.5	+	0.52	44.0	137	267

[a] Based on the average of three petri dishes each plated with 200 cells.
[b] 1×10^6 cells in a measured volume were plated in each of three plates/culture in the presence of trifluorothymidine (3 μg/ml) after 2 days of expression. The values are for duplicate cultures (four cultures/solvent control).
[c] Differences in mutant frequencies among KavaPure and Kaviar were assessed by analysis of variance (ANOVA). KavaPure P value = 0.455 and Kaviar P value = 0.080.

was 10 times higher than the levels used for yangonin and desmethoxyyangonin. The maximum dose level tested for each of the remaining kavalactones was as follows: dihydromethysticin, 300 μg/ml; methysticin, 150 μg/ml; dihydrokawain, 200 μg/ml; DL-kawain, 160 μg/ml, and D-kawain 160 μg/ml. No mutagenic activity was obtained with these standard kavalactones.

4. Discussion

Traditionally prepared aqueous kava extracts have been used for many years throughout the South Pacific with few reported side effects. There is no evidence of liver damage in kava using populations of the native Pacific Islanders or Australians who have used aqueous kava extracts (Clough et al., 2003). Epidemiological evidence suggests that kava may act as a chemopreventive agent (Steiner, 2000). The cancer rate for countries in the South Pacific was compared with kava consumption and it was found that the more kava consumed the lower the cancer incidence (Steiner, 2000). The results from this study with two commercially available kava samples as well as six kavalactone standards showed no mutagenic responses at the TK locus of L5178Y mouse lymphoma cells in the presence of human liver S9. The most toxic kavalactones were desmethoxyyangonin and yangonin. This is the first in vitro mammalian cell study in which exogenous metabolic activation is provided by pooled human liver S9.

Several solvents have been used for extracting the kavalactones from the roots of *P. methysticum* including acetone, ethanol and water. For commercial extracts 60% or more of ethanol or acetone have been used for obtaining higher yields of kavalactones. The traditional aqueous preparations of the kava rhizome produce an extract that contains a balance between the kavalactones and glutathione that may provide protection against hepatotoxicity (Whitton et al., 2003). Cote et al. (2004) compared the traditional aqueous kava extracts with organic kava extracts and commercial kava caplets and reported differences in the ratio of kavalactones which suggest there could be changes in biological activity. They also compared the extracts for inhibition of the major drug metabolizing P450 enzymes and found that inhibition was more pronounced for the commercial preparation.

There are several possible mechanisms for kavalactone hepatotoxicity. Most evidence that kavalactones are associated with hepatotoxicity is related to the demand on the cytochrome P450 enzymes for clearance by the liver. Kavalactones may influence liver detoxification pathways by inhibition of cytochrome P450 activity and a reduction in liver glutathione. Inhibition of cytochrome P450 was strongly suggested for a patient in Switzerland who developed malaise, loss of appetite, and jaundice, with elevated levels of aminotransferases, bilirubin, and alkaline phosphatase after taking 210 mg of kavalactones daily for 3 weeks together with 60 g of alcohol (Russmann et al., 2001). Kavalactones of commercial kava have been shown to be inhibitors of enzymes of the cytochrome P450 system (Mathews et al., 2002; Zou et al., 2002; Cote et al., 2004). Inhibition of human P450 enzymes is an important pharmacological criterion because it is a primary cause of drug interactions and has been suggested as a possible cause of

Table 4
Mutagenicity and cytotoxicity of kavalactone standards in mouse lymphoma cells

	Dose (μg/ml)	Human S9	Absolute cloning efficiency[a]	Relative total growth (% of control)	Average number TFT^R colonies	Mutant frequency per 10^6 survivors[b]
Dihydromethysticin						
Solvent control (DMSO)	0	+	0.88/0.72	100.0	33/26	37
	100.0	+	0.44/0.65	63.5	18/30	45
	150.0	+	0.44/0.78	54.5	30/34	56
	200.0	+	0.28/0.73	44.0	16/24	45
	250.0	+	0.64/0.43	47.0	50/25	69
	300.0	+	0.56/0.52	42.0	24/21	42
Positive control (aflatoxin B-1)	1.0	+	0.59	44.0	197	334
Desmethoxyyangonin						
Solvent control (DMSO)	0	+	1.09/1.17	100.0	44/50	42
	1.25	+	1.52/1.07	117.0	58/34	35
	2.50	+	0.95/0.93	88.5	46/34	42
	5.00	+	0.90/0.92	89.5	38/47	47
	10.00	+	1.00/0.90	85.0	36/27	34
	20.00	+	1.05/0.92	90.5	41/32	37
Positive control (aflatoxin B-1)	0.50	+	0.70	38.0	198	285
Methysticin						
Solvent control (DMSO)	0	+	0.82/0.92	100.0	46/48	55
	10.0	+	0.85/0.94	92.0	39/61	56
	25.0	+	0.85/0.77	83.0	68/43	68
	44.0	+	0.88/1.06	86.0	60/42	54
	100.0	+	1.08/0.97	45.0	51/43	47
	150.0	+	1.05/0.93	36.5	51/55	54
Positive control (aflatoxin B-1)	0.5	+	0.63	46.0	189	301
Dihydrokawain						
Solvent control (DMSO)	0	+	0.82/0.92	100.0	46/48	55
	60.0	+	0.87/0.86	95.5	49/37	50
	75.0	+	0.93/1.01	102.0	43/46	47
	100.0	+	0.80/1.11	89.5	40/43	44
	150.0	+	0.92/1.03	100.0	48/41	46
	200.0	+	0.80/0.95	21.5	38/50	50
Positive control (aflatoxin B-1)	0.5	+	0.63	46.0	189	301
Yangonin						
Solvent control (DMSO)	0	+	0.82/0.92	100.0	46/48	55
	0.5	+	0.83/1.07	74.5	44/50	50
	1.0	+	1.11/1.10	108.0	63/57	55
	5.0	+	1.07/0.92	105.5	38/68	55
	10.0	+	0.98/1.00	103.5	71/58	66
	20.0	+	0.96/1.04	50.5	45/47	47
Positive control (aflatoxin B-1)	0.5	+	0.63	46.0	189	301

[a] Based on the average of three petri dishes each plated with 200 cells.
[b] 1×10^6 cells in a measured volume were plated in each of three plates/culture in the presence of trifluorothymidine (3 μg/ml) after 2 days of expression. The values are for duplicate cultures (four cultures/solvent control).

kava hepatotoxicity (Cote et al., 2004). This indicates that kava has a potential for causing pharmacokinetic drug interactions with other herbal products or drugs, which are metabolized by the cytochrome P450 enzymes (Anke and Ramzan, 2004). Another possible mechanism for kavalactone hepatotoxicity is the inhibition of cyclooxygenase enzyme activity (Wu et al., 2002; Clouatre, 2004). Because immunologic pathways may be involved in many of the reports of kava induced hepatotoxicity, it would be important to examine the possible role of cyclooxygenase inhibition. The inhibition of cyclooxygenase enzyme activity has been linked in other drugs to irregularities in liver function (Clouatre, 2004). Additional studies are required to examine the possible mechanism of inhibition of cyclooxygenase enzyme activity and kava hepatotoxicity.

Johnson et al. (2003) investigated possible mechanisms of kava hepatotoxicity by examining electrophilic intermediates generated after metabolic activation. Using an

Table 5
Mutagenicity and cytotoxicity of DL-kawain and D-kawain in mouse lymphoma cells

	Dose (μg/ml)	Human S9	Absolute cloning efficiency[a]	Relative total growth (% of control)	Average number TFT^R colonies	Mutant frequency per 10^6 survivors[b]
DL-Kawain						
Solvent control (DMSO)	0	+	1.23/0.97	100.0	79/55	62
	75.0	+	1.05/0.85	90.0	49/68	64
	100.0	+	0.96/0.99	90.5	55/69	64
	120.0	+	1.02/1.57	95.0	72/79	60
	140.0	+	1.05/1.01	71.5	63/81	70
	160.0	+	0.99/0.86	60.0	65/69	73
Positive control (aflatoxin B-1)	0.5	+	0.83	65.0	228	275
D-Kawain						
Solvent control (DMSO)	0	+	1.23/0.97	100.0	79/55	62
	75.0	+	0.99/0.95	93.5	48/55	54
	100.0	+	0.86/1.02	92.5	64/47	60
	120.0	+	0.97/0.93	80.0	43/40	44
	140.0	+	0.91/1.20	74.5	46/52	47
	160.5	+	0.76/0.97	66.5	60/67	74
Positive control (aflatoxin B-1)	0.5	+	0.83	65.0	228	275

[a] Based on the average of three petri dishes each plated with 200 cells.
[b] 1×10^6 cells in a measured volume were plated in each of three plates/culture in the presence of trifluorothymidine (3 μg/ml) after 2 days of expression. The values are for duplicate cultures (four cultures/solvent control).

in vitro MS-based screening assay, two novel electrophilic metabolites of kava, 11,12-dihydroxy-7,8-dihydrokavain-*o*-quinone and 11,12-dihydroxykavain-*o*-quinone were identified. Mercapturic acid of these quinoid species was not found in the urine of a human volunteer after taking kava, but corresponding catechols were metabolized to glucuronic acid and sulfate conjugates. Johnson et al. (2003) concluded that quinoid metabolites are not formed in substantial quantities, but the formation of electrophilic quinoid metabolites might contribute to hepatotoxicity in humans when metabolic pathways are altered, such as a drug interaction.

Nine cases of liver damage occurring with ingestion of kava extracts were reported in Germany and symptoms occurred between 3 weeks and 4 months after starting the kava extracts. The doses ranged from 60 to 210 mg kavalactones/day (Stoller, 2000; Stevinson et al., 2002). In several of these cases, other medications with hepatotoxic potential were being taken at the same time. It is interesting to note that the majority of cases involved acetone extracts.

Dragull et al. (2003) isolated an alkaloid, pipermethystine, in relatively high concentrations from the aerial parts or the stem peelings and leaves of kava plants. This compound is not found in the roots. They report that stem peelings were used as a source of kavalactones in the kava dietary supplement industry because of the high demand by the pharmaceutical industry and shortage of appropriate raw material. They suggested that this alkaloid may play a role in the liver toxicity associated with the consumption of kava containing products. In a recent study, Jhoo et al. (2006) found that organic solvent fractions produced stronger cytotoxicity in HepG2 cells than water fractions of all parts of the kava plant. They identified flavokavain B from the hexane fraction and suggested that it may be the compound responsible for cytotoxicity (Jhoo et al., 2006).

These possible mechanisms for hepatotoxicity associated with kava consumption have been reported following ingestion of commercially prepared kava but none have been documented for the traditional aqueous extract preparation. Hepatotoxicity associated with kava consumption is rare and in most documented cases idiosyncratic.

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