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Phytochemistry 64 (2003) 673–679
PHYTOCHEMISTRY
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Molecules of Interest

Kava lactones and the kava-kava controversy

Peter A. Whitton[a], Andrew Lau[a], Alicia Salisbury[b], Julie Whitehouse[c], Christine S. Evans[d,*]

[a]*Phyto-Research Ltd, Epinal Way, Loughborough, Leicester LE11 3EH, UK*
[b]*School of Pharmacy, Aston University, Birmingham, UK*
[c]*School of Integrated Health, University of Westminster, 115 New Cavendish St, London W1W 6UW, UK*
[d]*School of Biosciences, University of Westminster, 115 New Cavendish St, London W1W 6UW, UK*

Accepted 5 June 2003

Abstract

Kava-kava is a traditional beverage of the South Pacific islanders and has had centuries of use without major side effects. Standardised extracts of kava-kava produced in Europe have led to many serious health problems and even to death. The extraction process (aqueous vs. acetone in the two types of preparations) is responsible for the difference in toxicity as extraction of glutathione in addition to the kava lactones is important to provide protection against hepatotoxicity. The Michael reaction between glutathione and kava lactones, resulting in opening of the lactone ring, reduces the side effects of the kava kava extracts. This protective activity was demonstrated using *Acanthamoebae castellanii* in which 100% cell death occurred with 100 mg ml^{-1} kava lactones alone, and 40% cell death with a mixture of 100 mg ml^{-1}glutathione and 100 mg ml^{-1} kava lactones. A comparison of kava lactone toxicity with other pharmaceutical products is discussed and recommendations made for safe usage of kava-kava products

Keywords: Kava-kava; Kava lactones; *Piper* methysticum; Hepatotoxicity; Glutathione

1. Introduction

In the Oceanic Islands of the South Pacific, kava-kava is consumed as an intoxicating beverage typically prepared from the roots of the kava plant *Piper methysticum*. (Some islanders also use extracts from peeled stems in preparation of the drink.)

Traditional kava-kava extracts are prepared from macerated roots with water and coconut milk (Norton and Ruze, 1994). Such extracts have been consumed over the last 2000 years without serious effects on health (Steiner, 2000). The intoxicating effects of the beverage have been sought by the Western world as a beneficial alternative to alcohol in reducing anxiety and as a remedy for sleeplessness and menopausal symptoms. Various commercial preparations of kava-kava, such as capsules, tinctures and fluid extracts, have been available in Europe and the USA.

However, the safety of kava-kava products has been questioned due to the reported hepatotoxic side effects of kava-kava extracts in the German medical press. There have been 24 cases of severe liver damage reported to German regulators, including three requiring transplants and one death from use of standardized extracts containing 30–70% lactones (Anon, 2001; Denham et al., 2002). Side effects have only been reported in Europe with standardized extracts (Escher et al., 2001). Epidemiological studies carried out in the Northern Territories of Australia have shown that in a population where traditional extracts of kava have been taken by individuals regularly in quantities equivalent to between ten and 50 times the recommended daily dose of kava lactones, no evidence of liver damage has been seen (Clough, 2002).

Kava lactones are considered to be the active constituents of kava-kava responsible for the pharmacological activity in humans and animals (Siméoni and Lebot, 2002). Kava lactones include kawain, methysticin, yangonin, dihydrokawain (Fig. 1). The different methods of extraction of the kava lactones from the roots may explain the toxicity of Western products compared with the safe use of the traditional beverage.

* Corresponding author. Tel.: +44-207-911-5128; fax: +44-207-911-5087.
E-mail address: evansc2@wmin.ac.uk (C.S. Evans).

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doi:10.1016/S0031-9422(03)00381-9

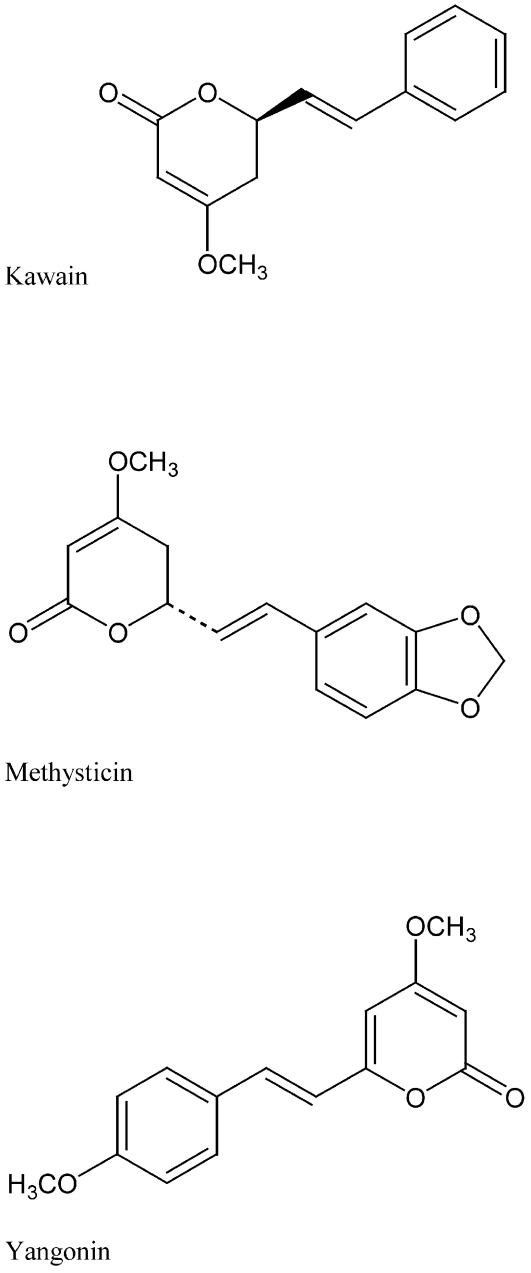

Fig. 1. Structures of kava lactones extracted from roots of *P. methysticum*.

In this paper we describe new experimental data that accounts for the variation in toxicity of the kava-kava extracts, and review recent progress on understanding the mechanism of their hepatotoxicity.

2. Preparation of extracts of kava lactones

In the South Pacific Islands, the older roots of *P. methysticum* are highly prized for producing the best quality beverages. Variation of kava lactone concentrations can be expected depending on the age of the plant, the variety of the species and the harvest procedures. We investigated two samples of imported roots of *P. methysticum*; the localization of the kava lactones within the different root structures was identified. The root samples were pieces of 5-year-old and 8-year-old roots, from which three tissue samples were removed: bark, parenchyma immediately beneath the bark and sclerenchyma. An overall cross-section of each root was also used to measure an average distribution of kava lactones. After extraction of all tissues in 96% ethanol for 24 h at room temperature, kava lactone concentrations were measured by HPLC. Fig. 2 shows the distribution of kava lactones in the root tissues. In both ages of roots, the greatest concentration of kava lactones was found in the bark with relatively lower concentrations in the parenchyma and sclerenchyma tissues. In the cross-sections of the roots, the kava lactone concentration per mg dry wt was highest in the younger root reflecting the lower amounts of parenchyma and sclerenchyma tissues compared with the bark. In the 8-year-old root, more parenchyma and sclerenchyma tissues were present, so a high concentration of kava lactones in the bark tissue was balanced by the low concentrations in the other tissues. These data show that the prized older roots of *P. methysticum,* while containing a higher concentration of kava lactones in the bark, provide a 'diluted' concentration across the root as a whole. It has been reported that kava plants from different islands have differing concentrations of kava lactones (Siméoni and Lebot, 2002).

Traditionally kava-kava extracts are prepared by maceration of roots in a water and coconut milk solution (Norton and Ruze, 1994). For commercial herbal extracts of kava-kava the solvents used are either ethanol (60% or above) or acetone (60% or above) in order to specifically extract the kava lactones. These are commonly called 'standardised' extracts as the extract has been concentrated and standardised to contain a certain amount of a particular component. In Western traditional herbal medicine, a 25% ethanol–75% water mixture is the solvent used to make a tincture (British Pharmaceutical Codex, 1934). Depending on the relative solubilities of the kava lactones in the different solvents, the extraction procedure is therefore relevant to the final potency of the extracts and may explain the confusion as to the safety of kava-kava herbal remedies.

We have analysed both types of extracts for major biochemical differences to find an explanation for the non-toxic nature of the traditional extract and the toxicity of the standardised extracts. Various solvents including acetone, ethanol and water were used to extract roots of *P. methysticum* and kava lactones were assayed by HPLC. The extracts were dried, then weighed and the ratio of the absorbance of the kava lactones at 260 nm to the percentage of solids in the dried extracts provided a unit of absorbance with which

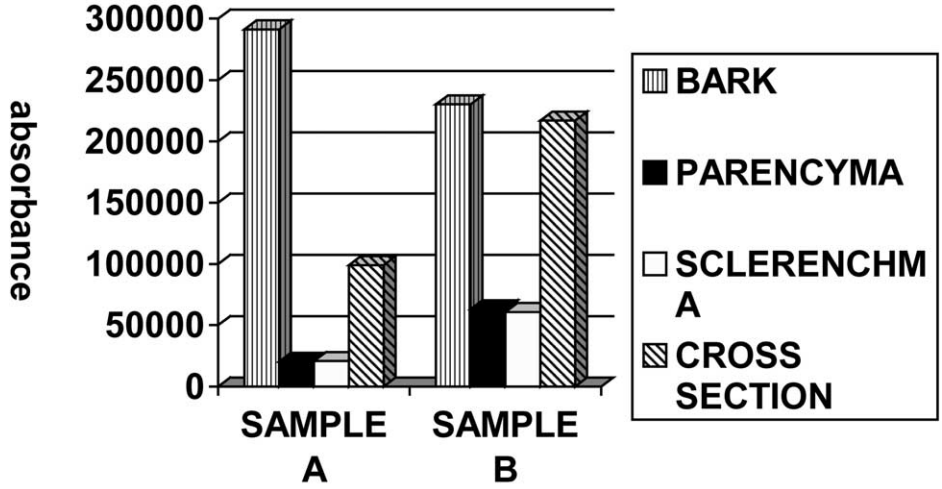

Fig. 2. Kava lactone concentration across different tissues of roots of *P. methysticum*. Sample A = 8-year-old root; Sample B = 5-year-old root.

all extracts could be compared. Table 1 shows the extraction of the kava lactones with the various solvents. Acetone (>80%) extracts contained 100% of kava lactones based on HPLC analysis and ratio of absorbance to% solids on drying. Extraction with 96% ethanol also resulted in 100% kava lactone extracts, while extraction with 25% ethanol gave only 15% kava lactones and the water extract contained <3% kava lactones.

Another compound separated from both the aqueous extract and in 25% ethanol on analysis by HPLC was glutathione. Its identity was confirmed by comparison to an authentic reference sample for its UV absorbance spectrum of the peak (by peak purity analysis) and co-chromatography. Glutathione and kava lactones were separated under the same conditions on HPLC analysis. Commercially available kava-kava tinctures and fluid extracts were analysed and the ratio of kava lactones to glutathione was determined as shown in Table 2. Glutathione is insoluble in ethanol at concentrations higher than 50%, but is increasingly soluble at lower ethanol concentrations (Merck Index, 1996). Analysis by HPLC showed that the tinctures and fluid extracts contained both glutathione and kava lactones, with the amount of glutathione extracted increasing as the polarity of the solvent increased.

Glutathione and kava lactones may interact in a similar way to that reported for sesquiterpene lactones with glutathione (Schmidt et al., 1999). Sesquiterpene lactones react with the sulfide group of glutathione in a reversible pH dependent reaction. There are two potentially reactive groups in sesquiterpenes for reaction with glutathione—the lactone ring and the α-methylene substituent. It can be shown that glutathione binds irreversibly with kava lactones by a Michael type reaction, due to opening of the lactone ring (Fig. 3). This is supported by a decolourisation of the solution of kava lactones in the presence of glutathione, suggesting that the lactone group has reacted with glutathione causing opening of the lactone ring. Unlike sesquiterpene lactones, no α-methylene groups are present in kava lactones. Some indication that there are reaction products between kava lactones and glutathione are seen on HPLC (Fig. 4), and these products need further analysis.

3. Toxicity of herbal medicine products

3.1. Root extracts of *P. methysticum*

High doses of kava lactones have been reported to cause hepatotoxic side effects (Gow et al., 2003). Lactones are usually metabolized in the liver by the cytochrome P450 system (Schmidt et al., 1999) and in the serum by lactone hydrolases (Bargota et al., 2003). As the dose of kava lactones in standardized extracts is

Table 1
Extraction of kava lactones from roots of *P. methysticum* in different solvents[a]

Extract	% kava lactones in dried extract	Unit of absorbance: (A_{260}:% solids)
Acetone extract (standardised method)	100 (0.001)	1
96% ethanol extract (standardised method)	100 (0.001)	1
25% ethanol (traditional method)	15 (0.02)	0.13
Water (traditional method)	2.97 (0.03)	0.015

[a] Data presented as means (and standard deviations) for ten samples in each solvent.

Table 2
Kava lactone:glutathione ratio in extracts prepared according to commercial preparations of *P. methysticum* roots[a]

Sample	Solvent	Kava lactone:glutathione
Kava standardised extract powder (30% kavalactones)	25% ethanol, 75% water	1:0
82% ethanol extraction of kava root	None (this was already a liquid preparation)	1:0.017
Tincture extraction (1 part root to 3 parts solvent)	25% ethanol, 75% water	1:1.15
Fluid extract extraction (1 part root to 1 part solvent)	25% ethanol, 75% water	1:2.2

[a] Data are the means from ten replicate samples of each type.

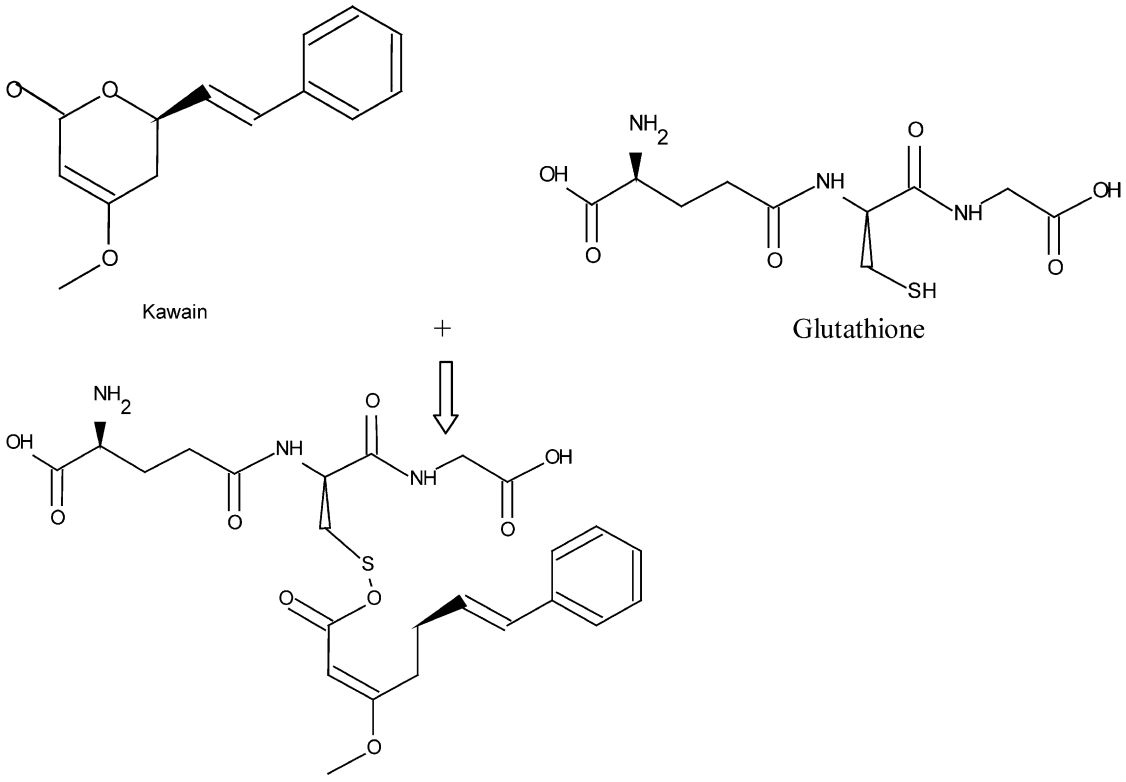

Fig. 3. The Michael reaction between kawain and glutathione.

over 30 times that found in the traditional aqueous extract (Table 1), the enzymatic detoxification pathways are likely to become saturated, leading to hepato-side effects. Glutathione plays an essential role in the phase II conversion of lactones into excretable waste products (Schmidt et al., 1999). Increased side effects due to the lactones may occur on glutathione depletion. The binding of sesquiterpene lactones to glutathione was reported to allow for faster clearance by the lactone hydrolases in the hepatocytes (Schmidt et al., 2001). A similar mechanism is likely to operate with the kava lactones.

Glutathione occurs in most cells of the body in adequate amounts but some individuals show a deficiency linked with cytochrome P450 (Lomaestro and Malone, 1995). In these cases high doses of lactones will lead to rapid depletion in glutathione levels and result in free lactone exposure of the hepatocytes and consequent damage (Zheng et al., 2000). Glutathione supplementation can correct the deficiency (Lautermann et al., 1995). If glutathione is not absorbed intact from the gut, its constituent amino acids will be and glutathione can then be regenerated within the hepatocyte. Glutathione supplements eliminate side effects from other lactones such as sesquiterpene lactones if they are administered at the same time (Lautermann et al., 1997).

In traditional kava-kava extracts, glutathione is extracted in a 1:1 ratio with kava lactones (Table 2) and these extracts have no reported side effects (Steiner, 2000). The only recorded abnormality is a slightly raised γ-glutamyltransferase activity (Barguil, 2001). In contrast to the traditional crude extract, standardized extracts contain no glutathione whilst containing up to 30 times the kava lactone concentration. To avoid potential liver damage, it would therefore seem prudent to limit the organic solvent concentration in extraction

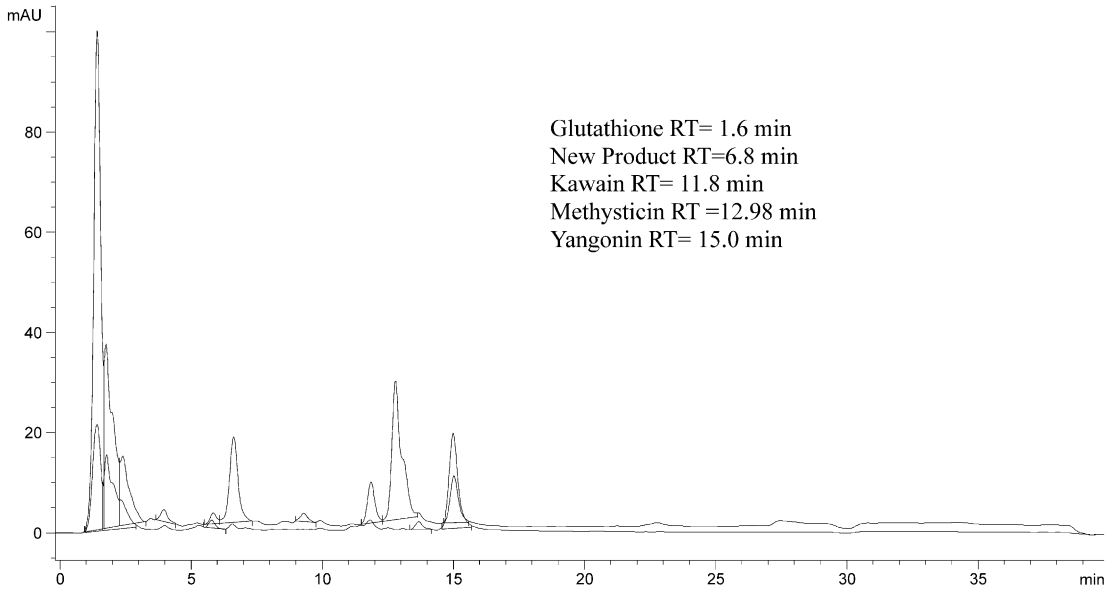

Fig. 4. Separation of kava lactones by HPLC from a 25% ethanol extraction (pH 8) of roots of *P. methysticum*.

of kava-kava to 25% ethanol in order to ensure the preservation of the hepato-protective effect of the glutathione.

3.2. Stem extracts of P. methysticum

There are reported uses of stem rather than root material providing the source of kava-kava extracts in commercial production (Dayton, 2003). Traditionally, if stems are used, they are peeled before preparation of the kava drink, but when demand for kava-kava from Europe and the USA was high (2000–2001), peelings became an important trading material as they provided a cheap, waste product with a high concentration of kava lactones for preparation as kava-kava capsules (Dayton, 2003).

Dragull et al. (2003) have isolated an alkaloid, pipermethysticine, from the stem peelings that is not found in the roots, and suggest that this alkaloid may be responsible for the hepatotoxic side effects caused by consumption of some Western kava-kava capsules.

They also commented that methods of analysis used by some companies could not distinguish between the kava lactones and pipermethysticine, so its presence was undetected.

4. Toxicity of kava lactones to *Acanthamoeba castellanii* cell cultures

To measure the toxicity of kava lactone extracts, their effect on cells of the eukaryotic, amoeba species *A. castellanii* provided a convenient cell culture for investigating toxicity that was easy to use. The biochemical pathway for detoxification of kava lactones is by the cytochrome P450 pathway that is common to all organisms (Shen, 1997)

The effects of kava lactones alone, and a mixture of kava lactones and glutathione were incubated with the amoeba cells in microtitre plates for 7 days at 34 °C. The kava lactone extract was prepared by extracting root of *P. methysticum* in 80% ethanol: 20% water by reflux percolation for 1 h. The percentage of dissolved material was ascertained by the British Pharmacopoeia method (1999) and the kava lactone content analysed by HPLC. Mannitol was added to the extract in the ratio 70% (w/v) mannitol to 30% (w/v) kava lactones, and all solvent removed by rotary evaporation in vacuo. Equal concentrations of kava lactones were added to the amoeba cells, with and without glutathione (dissolved in 25% dimethylsulfoxide and 75% distilled water) in doubling dilutions in the microtitre plates. Cell numbers in each plate well were recorded and the percentage of surviving cells calculated.

Cell death due to the kava lactone preparation was greater than that caused by the mixture of kava lactone with glutathione (Table 3). At 100 mg ml^{-1} the kava lactone extract caused 100% cell death whereas at the same concentration but in the presence of glutathione, cell death was only 40% compared with control cells. These data emphasise the toxic nature of kava lactones to cells and the partial protection provided by glutathione.

5. Abuse of kava-kava

Kava-kava has long been used as a substance of abuse as it can produce feelings of euphoria and relaxation similar to alcohol abuse. In the islands of Oceania where

Table 3
Effects of kava lactones and glutathione on cells of *Acanthamoeba castellanii*

Conc. of kava lactones mg ml^{-1}	+ Glutathione[a] Mean% surviving cells±S.D.		−Glutathione Mean% surviving cells±S.D.	
6.25	82.5	±16.9	57.2	±14.8
12.5	85.3	±22.5	30.7	±12.6
25.0	97.2	±9.4	11.4	±16.1
50.0	72.1	±7.2	9.8	±0.01
100.0	67.2	±8.1	0	±0

[a] + Glutathione at an equal concentration to that of kava lactones.

the fermentation of ethanol did not evolve in their cultures, kava-kava was used ceremonially and also socially as an intoxicant. In Hawaii, and more recently in other parts of the USA, 'kava bars' have opened where the traditional kava-kava extracts are sold for their intoxicating properties. In the 1980s kava-kava was introduced to the aboriginal populations of the Northern Territories in Australia in order to reduce the reliance on alcohol amongst the population, and to try to improve mortality in a population where alcohol abuse was rife. Professor Barrie of Menzies University Medical School has studied these populations over a number of years and has not recorded any evidence of hepatotoxicity in his study group even though they regularly imbibe between ten and 50 times the recommended daily dose of kavalactones in European standardized extracts (Clough, 2002).

6. Compatibility of kava-kava with pharmaceutical products

In data supplied by the Medicines Control Agency, out of 60 cases reported to the authorities concomitant medication or a pre-existing liver complaint were present in all bar two cases (Anon, 2001) Practically all psychoactive drugs are hepatotoxic in the same way as shown for kava-kava, that is via the cytochrome P450 enzymatic pathways (Shen, 1997). If kava lactones are to have a psychoactive effect then their ability to react with concomitant medication must involve inhibition of these enzymatic pathways.

As discussed in this paper, the adjunct of glutathione to kava lactones via a Michael reaction renders the lactones non toxic to eukaryotic cells and therefore bypasses the cytochrome P450 pathways, implying that kava lactones would normally be detoxified through the Phase II pathway via CYP2D6 (Shen, 1997) which conjugates glutathione to the lactone. This would imply that if an individual with a deficiency in CYP2D6 (either congenital or due to overloading of the enzymatic system with other substrates) were to ingest kava lactones then the result could be liver damage. This could be avoided if the kava lactones were taken with an excess of glutathione, which would pass through the stomach into the duodenum (or at least its cysteine moiety would), where it would conjugate with kava lactones in the duodenum at a pH that would allow the Michael reaction to occur. It has long been accepted that interactions between drugs occurs when both are required to pass through the cytochrome P450 Phase I and Phase II pathways in the liver. Therefore there is no difference between the actions in the reported cases of kava lactone- induced hepatotoxicity and that which can be induced by other psychoactive drugs such as valproic acid, Fluoxetine, Paroxetine, Sertraline, Fluvoxamine, Imiprine, Codeine and many others (Shen, 1997). The major difference in the case of kava-kava is that in populations where these drugs and alcohol are also taken along with traditional kava preparations, the same reported incidence of hepatotoxicity has not occurred.

7. Safe use of kava-kava

Based on the evidence discussed in this paper, the reason that kava lactones have been linked to hepatotoxicity is due to their dependence on the cytochrome P450 enzymes for clearance by the liver. The safe use of kava-kava has continued for many years and has been documented. In all traditional preparations of the kava root, the kava lactones are balanced by the availability of glutathione in the preparation. In the tablet and capsule forms of standardized extracts that relate to the reported cases of hepatotoxicity, only the kava lactones have been present in the products and no additional glutathione taken with the product.

Based on the dosages reported to cause problems by the Medicines Control Agency (Anon, 2001) and those reported by Clough (2002), the only difference is the glutathione levels which would explain the differences in toxicity.

Acknowledgements

The authors wish to thank Michael Volchok, Blessed Herbs, Massachusetts,USA, for providing samples of

kava kava roots; Professor Jack Woolley and Dr Randolph Arroo of de Montfort University, Leicestershire, UK for constructive discussions.

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