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Toxicity Potential of Aqueous Kava Extracts on Liver Function Tests in Rats

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TOXICITY POTENTIAL OF AQUEOUS KAVA EXTRACTS ON LIVER FUNCTION TESTS IN RATS

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ABSTRACT

Kava, prepared as the traditional aqueous infusion, was tested in the rat for possible effects on liver function tests. Extracts were administered in dosages containing 200 or 500 mg of the active kavalactones per kg for two or four weeks. Sera were assayed for four enzymes that are markers of liver toxicity and liver homogenates for malondiadehyde formation that indicates changes in lipid peroxidation. The data showed that none of the enzymes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, nor malondialdehyde levels were elevated, in fact in some cases they were significantly reduced, suggesting the lack of a toxic effect on the liver by kava.

Key words: kava, kavalactones, hepatotoxicity, liver function tests

INTRODUCTION

Kava (Piper methysticum Forst f., family Piperaceae) is an herb that has been used for ceremonials and rituals by inhabitants of the South Pacific (Singh, 1992; Lebot et al., 1997). It is now sold widely in western countries as an herbal supplement to treat stress, anxiety, insomnia and restlessness (Singh and Singh, 2002). Until recently, it was reputed to cause no severe toxicity apart from a skin condition called kava dermopathy which occurs on prolonged and excessive use. However, in the past few years, about 35 cases of hepatotoxicity apparently associated with kava use have been reported from Europe, mostly in Germany and Switzerland, with some additional cases from the United States (MMWR, 2002; Schmidt, 2002; Waller, 2002). The adverse event reports (AERs) for these incidents included cholestatic hepatitis, jaundice, increased liver enzymes, liver cell impairment, severe hepatitis with confluent necrosis and irreversible liver damage; in a few cases, liver transplantation became necessary. Although much of the data on most of the cases were either incomplete or generally unavailable, relatively detailed information has been published for five of them (Singh and Singh, 2002). The AERs have prompted a number of countries to take regulatory action to either suspend the sale of kava products or issue health advisories on their use.

These incidents of hepatotoxicity are somewhat surprising in light of the experience of South Pacific islanders who have safely consumed kava for many centuries. In those communities, only men consume kava, often habitually and in much larger amounts than used in the west, yet their incidence of liver toxicity is low and similar to that of island women who do not take kava. Furthermore, the commercial products were manufactured using organic solvents that are confirmed hepatotoxins, such as acetone, ethanol, or chloroform, in contrast to the water infusions drunk by the islanders. Whether the extraction procedure introduces some toxic plant components or solvent residue into the final product has not been established (Singh and Singh, 2002).

A number of independent reviews of the reported cases have been done and are revealing. In one analysis of a total listing of 35 AERs from Germany (Schmidt and Nahrstedt, 2002), four were double or triple listed, in four cases had no association with kava, causal relationship with comedications was probable for 14 cases, doubtful but not definitely excluded in four others, not assessable in six cases, and in three others causal relationship with kava was probably due to abuse or excessive intake. Thus only in the one remaining incident was there probable causal relationship in dosages conforming to the German Commission E monograph (Blumenthal *et al.*, 1998). The authors also note that in a majority of the German cases the documentation was "far from complying with the current requirements of the relevant European guidelines" for AER reporting and that "the allocation of kava [to hepatotoxicity] is not logical in the majority of cases and appears arbitrary. Furthermore, in many cases, available information is not taken into account, e.g., with regard to other causes." An extreme example they cite is the one fatality where "it was evidently known ... that the cause of liver failure was due to many years of alcohol abuse and that kava was not involved in the development of liver symptoms – the biopsy showed that cirrhotic processes in the liver had started long before kava was taken!"

Based on another analysis of 30 AERs from Germany and five submitted to the FDA, it was concluded that there is "no clear evidence that the liver damage reported in the U.S. and Europe was caused by the consumption of kava," and that those cases in which there is a possible association between the use of a kava extract and liver dysfunction" appear to have been hypersensitivity or idiosyncratic base responses" (Waller, 2002).

The possibility of an interaction between kavalactones and pharmaceutical drugs resulting in hepatotoxicity has recently received some attention. A major way in which such interaction may occur is by an inhibition of cytochrome P450 (CYP 450) enzymes by kavalactones. Since these enzymes are responsible for the hepatic metabolism of a majority of drugs and xenobiotics, their inhibition could elevate the plasma concentrations of these drugs to toxic levels.

In a study of the actions of the six major kavalactones on cDNA-derived CYP450 enzymes, the most potent inhibition of CYP1A2 occurred with desmethoxyyangonin (DMY); of CYP2C19 with dihydromethysticin (DHM), DMY, and methysticin (M); and of CYP3A4 with DHM and M (Zou *et al.*, 2002). The inhibition of CYP450 enzymes by whole kava extract and individual kavalactones was also investigated in human liver microsomes (Mathews *et al.*, 2002). The extract caused significant inhibition of the activities of CYP1A2, 2C9, 2C19, 2D6, and 3A4 while CYP2A6, 2C8, and 2E1 activities

were unaffected. While kavain did not inhibit CYP2C9, 2C19, 2D6, and 3A4, there was significant inhibition of CYP2C9 by M, DHM, and DMY; of 2C19 by DHM; of 2D6 by M; and of 3A4 by DMY, DHM, and M. These data indicate that kava has a high potential for causing drug interactions through inhibition of CYP450 enzymes responsible for the metabolism of a large number of medications.

Genetic polymorphism of many CYP enzymes, leading to interindividual variation in drug metabolism, may be another factor in the marked discrepancy in hepatotoxic response to kava of Pacific Islanders on the one hand and Caucasians on the other. The enzyme CYP2D6 is thought to cause much of the interindividual variations seen in drug responses, adverse effects, and interactions with drugs (Poolsup *et al.*, 2002). Individuals may be poor (slow), intermediate, extensive (fast), or ultrafast metabolizers. In a Caucasian population 7-9% of individuals are homozygous deficient in CYP2D6 and thus are poor metabolizers (Poolsup *et al.*, 2002). On the other hand, the incidence of CYP2D6 deficiency is almost 0% in persons of pure Polynesian descent (Wanwimolruk *et al.*, 1998). Since this enzyme is a major metabolizer of kavalactones, it is tempting to speculate that the genetic variability between Caucasian kava users and the Pacific Islanders may be a major contributory factor. However, the genetic polymorphism of CYP2D6 for Melanesians of Fiji, Vanuatu, and other kava consuming nations or that of other CYP enzymes in the Pacific island populations is yet to be determined.

The purpose of this research was to evaluate the level of toxicity induced by kava administration on liver function in young Sprague-Dawley rats by measuring its effects on several enzymes that are markers for liver toxicity, and formation of malondialdehyde, a byproduct of lipid peroxidation that may result in liver damage. A significant increase in these enzymes or malondialdehyde is considered an indication of liver toxicity. Rats were treated for two or four weeks, with a placebo or an aqueous infusion of kava in dosages of 200 or 500 mg kavalactones per kg per day. The results showed that there was no significant difference in the activity of the four serum enzymes, or the amount of malondialdehyde formed at either of the two doses, or at each of the two time points compared to the control groups. We believe the work presented provides good indication that kava prepared in the traditional manner and in kavalactones levels up to three times that recommended by Commission E shows no significant indication of liver dysfunction in rats.

MATERIALS AND METHODS

Kava extract preparation

Dried kava root was obtained directly from Fiji where it was collected and authenticated at the South Pacific Herbarium, Suva. A voucher specimen has been stored in Herbarium. Eight grams of the powdered kava was extracted in 50 ml of water by sonication for 20 min at room temperature (~21 ± 1° F) and then centrifuged at 10,000xg for 20 min. The supernatant was removed and the residue was re-extracted with 25 ml of water. The two supernatants were combined, the filtrate frozen and then lyophilized. The dry extract with the natural proportions of the kavalactones was used to make the appropriate doses for rat administration based on the HPLC assay of their kavalactone

composition. For the purposes of reproducibility, the same batch of the kava sample was used for the entire study.

HPLC

The kavalactone content of the powdered extract was assayed by a modification of a previously published and authenticated reversed phase HPLC method (Ganzera and Khan, 1999). A Luna C8 column, 100 x 4.6 mm, 3 _m particle size, with a guard column (from Phenomenex, Torrance, CA) was used. The mobile phase was water:acetonitrile:reagent alcohol (60:25:15 v/v). The reagent alcohol consisted of ethanol 90.7%, methanol 4.6%, and isopropyl alcohol 4.7%. Chromatographic conditions were: flow rate 0.5 ml/min, detection wavelength 246 nm, injection volume 5 _l, and running time 25 min. The kavalactone standards, consisting of kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin, were dissolved in acetone. The samples as well as the standards were filtered through a 0.22 _m membrane filter before injection.

Animal Care and Treatment

Sprague-Dawley rats, 4 weeks of age and body weight of 225-250 g, were obtained from Harlan (Indianapolis, IN). They were randomly divided into six groups of six animals each and housed in pairs in a climate-controlled room at 25°C, 12-hour light/dark cycle, and with free access to rodent chow (Purina, Richmond, IN) and tap water. After seven days of acclimatization, the rats were administered by gavage with either kava (200 or 500 mg kavalactones per day) in two divided doses or a saline placebo. These doses of the kava were about 50% and 250%, respectively, higher than the dose recommended by the Commission E monograph. Food and water consumption as well as animal body weight were monitored at the same time of the day every two days throughout the test period. The care and treatment of the rats were in accordance with the guidelines of the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University.

Sample collection

After two or four weeks of treatment, the animals were anesthetized with Phenobarbital (40 mg/kg), the thoracic cavity opened to expose the heart, and the blood drawn by intracardiac puncture. Serum was prepared and frozen until used for the enzyme essays. Portions of the liver were removed and made into homogenates for the lipid peroxidation experiments.

Enzyme assays

Diagnostic kits from Sigma (St. Louis, MO) were used for the assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) by following the accompanying procedures.

Lipid peroxidation (LPO)

A modification of the thiobarbutric acid method was employed for the estimation of malondialdehyde formed (Lee *et al.*, 1991). Briefly, after incubation for 60 min at 37°C, a 1.0 ml aliquot of the liver homogenate was mixed with 1.0 ml 40% trichloracetic acid (TCA), followed by addition of 1.0 ml 2% thiobarbutric acid. The mixture was boiled for 15 min, cooled in an ice bath for 5 min, then 1 ml 40% TCA added to it. After standing for 20 min, the mixture was centrifuged at 800xg for 20 min, and the absorbance of the supernatant read at 532 nm. The protein content of the samples was determined by the Lowry method (Lowry *at al.*, 1951) using a kit from Bio-Rad (Richmond, CA). The extinction coefficient was used to determine the amount of malondialdehyde formed/gram protein.

Statistical analysis

Data are means ± S.E.M. of 5-6 replicates. All statistical analyses were performed using ANOVA followed by Student's *t*-test, with $P<0.05$ being regarded as significant.

RESULTS

There were no overt signs of clinical toxicity or behavioral changes in the rats during either the two or four week treatment period. Compared to the placebo groups, kava administration did not produce significant ($P>0.05$) alterations in food or water intake or in body weight gain (**Figure 1**).

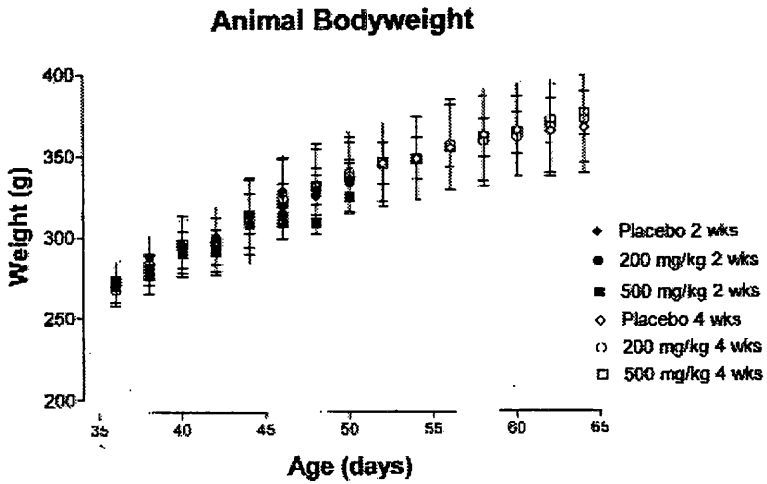

Figure 1. Animal bodyweight during the treatment period

Six major peaks which corresponded to the six major kavalactones were obtained with reversed phase HPLC of the kava infusion. The relative proportions of the compounds were as follows: kavain 39.45%, dihydrokavain 21.12%, methysticin 24.14%, dihydromethysticin 15.35%, yangonin 11.37%, and desmethoxygangonin 5.7%. Furthermore, the percentage of total kavalactones in the dried sample was 8.34 ± 0.62 g/100 g (n = 4) of the kavastock, which is in good agreement with literature values (Young *et al.*, 1966; Duve, 1981; Lebot and Lévesque, 1989).

The serum levels of all four enzymes tested, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase ALP), and lactate dehydrogenase (LDH), were almost twice as much in the kava-free rats after two weeks compared to

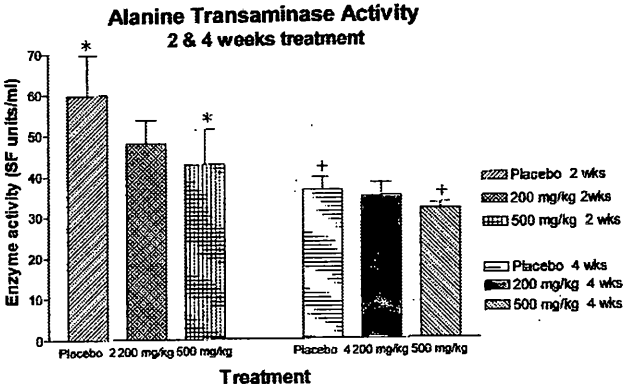

Figure 2a. Kava effects on alanine transaminase activity after 2 and 4 weeks of treatment (*+P<0.05)

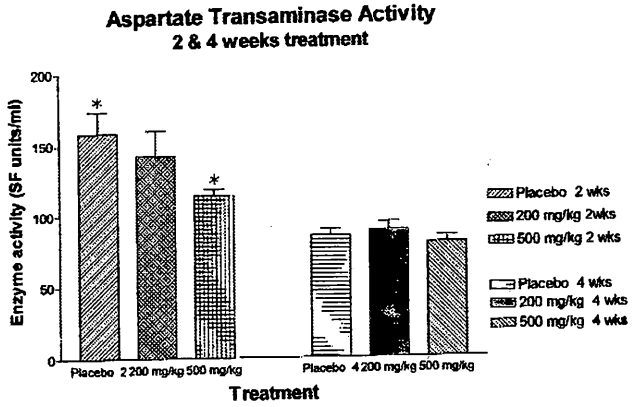

Figure 2b. Kava effects on aspartate transaminase activity after 2 and 4 weeks of treatment (*P<0.05)

four weeks of treatment (**Figure 2**). After two weeks, all enzymes were lower with both doses of kava. The change was significant ($P<0.05$) at 500 mg/kg kavalactones for ALT and AST and at 200 mg/kg for ALP. The reduction in ALT was also significant ($P<0.05$) after 4 weeks with 500 mg/kg.

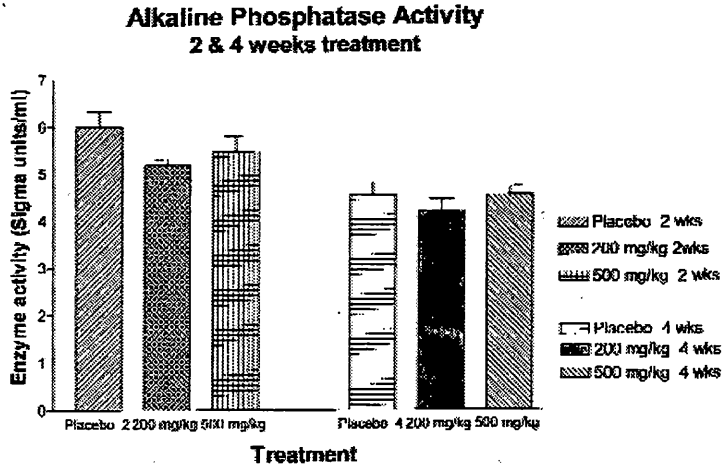

Figure 2c. Kava effects on alkaline phosphatase activity after 2 and 4 weeks of treatment

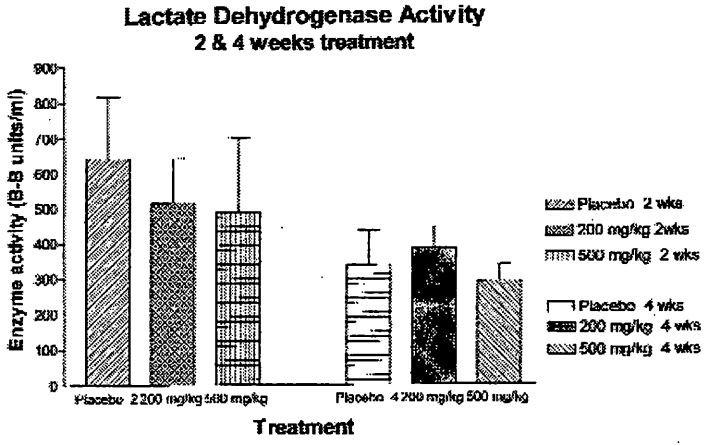

Figure 2d. Kava effects on lactate dehydrogenase activity after 2 and 4 weeks of treatment

The amount of malondialdehyde produced after 4 weeks was significantly greater (*P<0.05)* than after 2 weeks for each type of treatment, namely placebo and the two doses of kava extract. However, at each time point kava did not significantly (P>0.05) alter the level of lipid peroxidation compared to the corresponding placebo **(Figure 3)**.

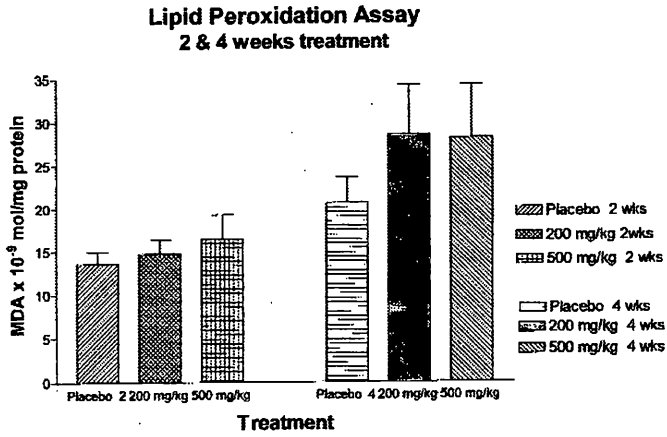

Figure 3. Kava effects on lipid peroxidation after 2 and 4 weeks of treatment

DISCUSSION

Aqueous infusions of kava have been used by South Pacific islanders for hundreds of years without any major adverse effects except for kava dermopathy, a scaly skin condition, which only occurred on prolonged and excessive intake. Thus it came as a surprise when cases of hepatotoxicity were associated with kava in reports from Europe and later, the U.S. Since the herbal supplement is extracted with organic solvents in contrast to the relatively safe water infusion used by the South Pacific consumers, it became important to establish whether the aqueous extract manifested any signs or symptoms of liver dysfunction.

The enzyme assay data clearly indicate that there was no significant increase in any of the four major marker enzymes for hepatotoxicity. In actuality, there usually were reductions in the enzyme activities, especially at the lower kava dose. Furthermore, these changes were significant ($P<0.05$) in four of the cases, which would suggest that rather than aqueous kava being hepatotoxic, it might have a hepatoprotective effect, something that many Pacific islanders have long believed (the faculty mentor is a native of Fiji).

The lipid peroxidation studies were done to determine whether kava constituents promote any free radical activity that may lead to liver injury. The data show that there

was no significant increase in the formation of malondialdehyde, indicating the lack of hepatotoxicity arising from lipid peroxidation.

Although the findings of the present study indicate the absence of a causal relationship between kavalactones and the apparent kava-induced hepatotoxicity, there are other considerations that need to be addressed. The doses of kavalactones used were up to only about three times higher than the recommended maximum whereas toxicity studies in rodents are normally performed in doses that are up to 10 times larger. It is also possible that some, but not all, of the kavalactones cause liver injury and hence the composition of the extracts in this study and in herbal supplements is highly relevant. In the present study, kavain and methysticin constituted about 63.6% of the total lactone content. Chemotypes rich in these two compounds find favor with kava drinkers apparently because of the desirable psychoactive effects they produce (Lebot and Lévesque, 1989) and also the flavor and taste they impart to the beverage. On the other hand, chemotypes high in dihydrokavain and dihydromethysticin are shunned as they appear to lack these properties. These attributes obviously led to a selection by the islanders of which chemotypes to utilize. The liver toxicity profile of these two compounds or of the other four lactones presently is not known; however, should kavain and methysticin prove to be relatively innocuous in this regard, then this would support the results of the present study.

The relative proportions of the kavalactones in the commercial products are established, but will probably be different from that in the aqueous infusion because of their extraction with organic solvents. Whether some or all of the other kavalactones are hepatotoxic, or larger amounts of them or other noxious compounds are extracted remains to be resolved.

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