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ScienceDirect
Food and Chemical Toxicology 45 (2007) 1293–1300
www.elsevier.com/locate/foodchemtox

Kava feeding in rats does not cause liver injury nor enhance galactosamine-induced hepatitis

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Received 8 July 2006; accepted 15 January 2007

Abstract

Kava, like a number of herbals, has been associated with causing liver damage based on limited evidence. In contrast, the present study found that in rats, 3 mo feedings of two types of kava extracts (an acetone extract and an ethanol extract of the Samoan kava cultivar Ava Laau) at three different doses (31.25, 62.5 and 133 mg/kg diet) produced no liver injury based on serum markers of liver damage (sorbitol dehydrogenase activities, bile acid concentrations, and β-glucuronidase activities) and serum lipid peroxide readings. In fact, for some measurements and some kava doses, the injury marker readings were below control values. Moreover, for these same parameters, kava feeding did not enhance the effects of the hepatotoxin galacatosamine (500 mg/kg ip); some kava doses even showed modest protection against liver injury. Liver histology analysis showed no signs of kava causing or enhancing liver injury. Thus, this study does not support the concept that kava produces or aggravates liver injury.

Keywords: Kava; Liver; Hepatitis; Galactosamine

1. Introduction

Kava (*Piper Methysticum*) is a plant that grows in the southern Pacific islands. The aqueous extract from the root of this herb has been used for centuries in local populations for relaxation and reducing anxiety (Bilia et al., 2002, 2004). The ethanol or acetone extract of kava root has also widely been used in western countries in the last two decades as treatment for anxiety disorders (Bilia et al., 2002; Singh and Singh, 2002). This treatment was regarded to be safe, and if adverse effects were reported, they were mild gastrointestinal discomforts and skin rash (Singh and Singh, 2002; Clouatre, 2004). However, in western countries, reports of 78 cases of hepatotoxicity after consumption of commercial kava extracts led to a sale ban of kava preparation in Europe, Canada and Australia, and caused reduced use of the products in the USA (Clouatre, 2004). Most of the subjects in the hepatotoxicity cases underwent remission after stopping consumption of kava, while those with hepatic failure had to have liver transplantation (Humberston et al., 2003; Stickel et al., 2003; Gow et al., 2003). However, the situation is not clear cut (Anke and Ramzan, 2004). A careful investigation of these reports raises a number of issues. First, the medical information available may not have been sufficient to diagnose kava-induced hepatotoxity (Humberston et al., 2003). Second, most of the cases report simultaneous use of other medicines or herbs that may be associated with liver damage, or may cause interactions with kava that produce compounds that are toxic to the liver (Humberston, 2003; Stickel et al., 2003; Gow et al., 2003); therefore, recovery of liver functions after kava withdrawal may just be due to the existence of these interactions. Third, the reported toxicity cases are often associated with high kava doses, such as above 240 mg/d, which is above those typically recommended (Humberston et al., 2003). Fourth, if kava is hepatotoxic to some people, the toxicity may occur primarily in people who are undergoing an additional liver toxic

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doi:10.1016/j.fct.2007.01.015

stress. Fifth, kava toxicity may vary with different extraction procedures for kava preparations. For example, in southern Pacific islanders, who use aqueous kava extract regularly, the occurrence of hepatic failure is very rare, and when it does occur, the increase of hepatic enzymes is mild and reversible (Clough et al., 2003). These observations may or may not apply to western kava preparations which typically use acetone, ethanol or other organic solvents to extract kavalactones. Comparison of several different extracts shows that water extracts have the lowest concentration of kavalactones, while acetone, methanol, and ethanol extracts have much higher concentration of kavalactones (Humberston et al., 2003). Sixth, kava toxicity may vary with the part of the plant used for herbal preparation. Traditional user of kava use only the underground root, but with the high demand for kava supplements that has arisen in western countries, some suppliers are thought to have resorted to including leaves or stem peelings in the material used for extraction. Along these same lines, pipermethystine, a kava lipophilic alkaloid found in kava leaves, induces death in human hepatoma cell line, while the same or even higher concentration of the root ingredients dihydromethsticin and demethoxyyangonin have no effect on cell viability (Humberston et al., 2003).

To address these issues, a study was done in rats to investigate the effects of consumption of three different doses of two different commercial, organic solvent extracted preparations of kava roots, plus or minus an acute liver toxic stress induced by galactosamine, a known hepatoxin (Newsome et al., 2000). A previous rat study (Singh and Devkota, 2003) has already reported that kava does not impact galactosamine-induced hepatic injury, but that study examined aqueous extract, used fairly short kava intake times (2 or 4 weeks), and considered only very high dosages of kavalactones (200 mg/kg/d or 500 mg/kg/d). The present study considered longer exposure times and used the types of organic solvent extracted kava preparations sold in western countries.

2. Materials and methods

The animal protocol was approved by the Ohio State University Institutional Laboratory Animal Care Committee. Male Sprague–Dawley rats, with an initial body mass of 150–174 g were obtained from Harlan Sprague–Dawley (Indianapolis, IN, USA) and fed a pelleted AIN-93G see mi-purified diet (Dyets Inc, Bethlehem, PA, USA) for 3 mo. Two kava extracts were prepared from the roots of the Samoan kava cultivar Ava Laau by Finzelberg (Andernach, Germany). Preparation A was produced with acetone (75%, m/m), Preparation B with ethanol (96%, m/m). The extraction was done twice by percolation. The herbal drug extract ratio was 11–20:1 (acetone extract) and 13–20:1 (ethanol extract). These preparations corresponded to qualities found in commercially available, German registered kava products. The kava was added to the powdered diet prior to pelleting as follows:

C. Control, No kava addition
A1. Kava A, 31.25 mg/kg diet
A2. Kava A, 62.5 mg/kg diet
A3. Kava A, 133 mg/kg diet
B1. Kava B, 31.25 mg/kg diet
B2. Kava B, 62.5 mg/kg diet
B3. Kava B, 133 mg/kg diet
$N = 10$ rats per diet group.

Galactosamine was purchased from Sigma Chemical Co (St Louis, MO, USA), dissolved in saline, and injected ip at 500 mg/kg. Rats were killed 24 h after injection by decapitation after anesthesia with CO_2. Trunk blood was collected and centrifuged at about 3000*g* to separate the serum, which was then frozen at −80 °C until analyzed. Serum was analyzed for sorbitol dehydrogenase (SDH) activities, bile acid concentrations, β-glucuronidase activities, and malondialdehyde concentrations. SDH and β-glucuronidase activities were assayed by spectrophotometric kinetic assays (Korsrud et al., 1973; Melen et al., 1985), bile acids were determined using an assay kit from BioQuant (Nashville, TN, USA) and malondialdehyde concentrations were done by a kit from Calbiochem–Novachem (LaJolla, CA, USA). Data was analyzed by unpaired *t*-test using Excel from Microsoft (Redmond, WA) with the significance set at $P < 0.05$.

3. Results

Analysis of the kava preparations appear in Table 1. No pipermethystine was detected, which is consistent with this preparation being derived from roots and not leaves; pipermethystine was detected in the leaves from the raw kava plants used for extraction. It should also be noted that the extracts had very low concentrations of residual acetone and ethanol (<0.01%). Final body weights for rats showed no significant differences among groups (Table 2). As seen in Fig. 1, SDH activities, which increase with liver cell membrane injury (Korsrud et al., 1973), do not show high values for kava feeding at various levels compared to controls (no kava feeding). In fact, all kava groups showed lower means than control, though by a two-sided *t*-test, each kava group was just outside of statistical significance ($0.05 < p < 0.10$). For galactosamine injected rats (Fig. 2), kava feeding did not further elevate the high values associated with galactosamine injection. In fact, mean values were a little lower for each kava group versus control, with each of the highest intake groups reaching statistical significance.

The results for serum bile acids, whose values increase with impaired liver function (Mahl, 1998), resembled those of SDH, though patterns of statistical significance were slightly different. For rats not treated with galactosamine, mean bile acid values for all kava groups were below those of the control, with all three of the A groups showing statistical significance by two-sided *t*-test (Fig. 3). For the B group, statistical significance was found for the middle intake level (Fig. 3). For galactosamine-injected rats (Fig. 4), none of the kava injected rats showed bile acid values significantly above control, with one intake group from each type of kava showing somewhat lower values than control with the differences being statistically significant.

For MDA, a marker of oxidant stress (Kehrer, 1993), for both non-injected and injected rats, there was only one statistically significant difference for the kava groups versus control (Figs. 5 and 6). This was for the middle intake of preparation B, galactosamine-injected rats, versus the control, injected group.