Food and Chemical Toxicology 49 (2011) 2820–2829

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Liver toxicity and carcinogenicity in F344/N rats and B6C3F1 mice exposed to Kava Kava

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ARTICLE INFO

Article history:
Received 18 May 2011
Accepted 27 July 2011
Available online 24 August 2011

Keywords:
Kava
Gavage
Liver
Toxicity
Rats
Mice

ABSTRACT

Kava Kava is an herbal supplement used as an alternative to antianxiety drugs. Although some reports suggest an association of Kava Kava with hepatotoxicity , it continues to be used in the United States due to lack of toxicity characterization. In these studies F344/N rats and B6C3F1 mice were administered Kava Kava extract orally by gavage in corn oil for two weeks, thirteen weeks or two years. Results from prechronic studies administered Kava Kava at 0.125 to 2 g/kg body weight revealed dose-related increases in liver weights and incidences of hepatocellular hypertrophy. In the chronic studies, there were dose-related increases in the incidences of hepatocellular hypertrophy in rats and mice administered Kava Kava for up to 1 g/kg body weight. This was accompanied by significant increases in incidences of centrilobular fatty change. There was no treatment- related increase in carcinogenic activity in the livers of male or female rats in the chronic studies. Male mice showed a significant dose-related increase in the incidence of hepatoblastomas. In female mice, there was a significant increase in the combined incidence of hepatocellular adenoma and carcinoma in the low and mid dose groups but not in the high dose group. These findings were accompanied by several nonneoplastic hepatic lesions.

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1. Introduction

The assessment of the efficacy and safety of herbal plants and herbal dietary supplements is important for human health protection (FDA, 2001, 2002; Fong, 2002; Fu et al., 2008a,b; Parkman, 2002). Although the herbal market has been rapidly growing, data on the identification and toxicological characterization of biologically active constituents in many herbs are lacking.

Kava Kava, an extensively used herbal product, is derived from the tropical shrub known as *Piper methysticum*. Traditionally, it has been widely cultivated for its rootstock in three geographic regions of the Pacific: Polynesia, Melanesia, and Micronesia (Norton and Ruze, 1994), where it is used as a ritual beverage to promote relaxation and a sense of well-being. The active principles of Kava Kava rootstock are mostly contained in the lipid-soluble resin. The isolates of Kava Kava resin fall into three general categories: arylethylene–apyrones, chalcones and other flavanones, and conjugated diene ketones. The compounds of greatest pharmacological interest are the substituted α-pyrones or Kava pyrones, commonly known as kavalactones. Fifteen lactones have been isolated from Kava Kava rootstock of which six are present in the highest concentrations and account for approximately 96% of the lipid resin. These are yangonin, methysticin, dihydromethysticin, kavain, dihydrokavain, and desmethoxyyangonin (Shulgin, 1973; Lebot et al., 1992; Dentali, 1997).

Kava Kava extract is one of the most widely used herbals in the United States with estimated sales of $106 million and is readily available at health stores, pharmacies, and super-stores (ABC News, 1998; Mirasol 1998). It is used by approximately 2.2 million people in the United States as a natural alternative to anti-anxiety drugs such as Xanax® and Valium® (Gardiner et al., 2007). It has also been claimed to have diuretic and antiseptic properties, (Norton and Ruze, 1994; JNC Corp., 1998), reported to help children with hyperactivity (Symmetry, 1998), and it is often used as a skin-conditioning agent in cosmetics (Robinson et al., 2009). The recommended oral dose for usage of Kava Kava as an anxiolytic is 50 to 70 mg kavalactones two to four times a day and, as a hypnotic, 150 to 210 mg in a single oral dose before bedtime (Bilia et al., 2002).

The main concern with the use of Kava Kava is hepatotoxicity in humans (Russmann et al., 2001, 2003; Campo et al., 2002; De Smet, 2002; Parkman, 2002; Brauer et al., 2003; Clough et al., 2003; Humberston et al., 2003; Ernst, 2006). However, evidence on the

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0278-6915/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.fct.2011.07.067

hepatotoxic effects of Kava Kava currently remain conflicting with some reports indicating an association of Kava Kava administration with hepatotoxicity including hepatitis, cirrhosis, and liver failure (Escher et al., 2001; Campo et al., 2002; Hefner, 2002; Gruenwald and Skrabal, 2003; Humberston et al., 2003; Teschke et al., 2003), while others demonstrating that it is safe in most individuals at recommended doses (Denham et al., 2002; Kopp, 2003).

The sale of Kava Kava has been suspended in some countries due to potential hepatotoxic effects but it continues to be on the US market although the Food and Drug Administration (FDA) has issued several warnings to consumers about the association between Kava Kava use and serious liver damage (FDA, 2001, 2002). However, whether the dose or duration of use may be correlated with the risk of liver damage remains unknown. It is also unclear if the safety profile of Kava Kava is comparable to other agents used in the management of anxiety. In addition, there are difficulties inherent in causality assessment when dealing with herbal hepatotoxicity such as daily overdose, prolonged therapy, coingestion with up to five other herbals, dietary supplements, and synthetic drugs (Teschke et al., 2008).

Mechanistically, the toxicity of Kava Kava in humans has been partially attributed to the CYP2D6 deficiency seen in 7% to 9% of Caucasian, 5.5% of Western European, almost 1% of Asian, and less than 1% of Polynesian populations (Wanwimolruk et al., 1998; Poolsup et al., 2000; Ingelman-Sundberg, 2005). Reports suggest that genetic differences may constitute significant contributory factors for increased hepatotoxicity in Caucasians (Singh, 2005). Inhibition of these CYPs or a deficiency in CYP2D6 indicates that concomitant exposure to Kava Kava and other drugs and chemical agents has a high potential for causing drug interactions (Jamieson and Duffield, 1990a,b; Mathews et al., 2002, 2005; Unger et al., 2002; Zou et al., 2002, 2004; Raucy, 2003; Teschke et al., 2003; Whitton et al., 2003; Anke and Ramzan, 2004; Bressler, 2005; Hu et al., 2005; Singh, 2005).

In the literature, there are a limited number of animal studies on the toxicity of Kava Kava extract and its constituents. Most of these studies focus on the potential for clinical signs for hepatotoxicity. For example, a study by Singh and Devkota (2003) showed that Kava Kava administered to Sprague Dawley rats by gavage at 200–500 mg/kg/day or 2 and 4 weeks, exhibited no increases in serum markers of hepatotoxicity or malondialdehyde production. In another study, administration of Kava Kava root extract (100 mg/kg) by gavage in male F344/N rats for 2 weeks failed to elicit any significant changes in serum markers of hepatotoxicity (alanine aminotransferase and aspartate aminotransferase) or markers of hepatic lipid peroxidation and apoptosis (Lim et al., 2007). Subchronic studies following oral exposure to Kavain (10–400 mg/kg) in dogs for 91 days revealed the presence of mild toxicity with multicentric liver necrosis in one high-dose dog (Hapke et al., 1971). There are no long-term studies to identify and characterize the potential for chronic toxicity or carcinogenicity following Kava Kava exposure.

Kava Kava continues to be used widely in the United States, although the correlation of dose and duration of use with the risk of liver damage is unclear. Therefore, Kava Kava was nominated for toxicological assessment by the National Cancer Institute (NCI) due to its increasing use as a dietary supplement in the mainstream United States market, reports of liver toxicity among humans, and lack of sufficient toxicity and carcinogenicity data.

The NTP conducted 2-week, 3-month, and 2-year toxicity and carcinogenicity studies in F344/N rats and B6C3F1 mice to address these issues. The current results focus on the toxic effects on the liver, the main target organ of concern following Kava Kava administration. Details of the complete study can be found in the NTP Technical Report (NTP 2011).

2. Materials and methods

2.1. Chemicals and reagents

Kava Kava extract (CAS 9000–38-8), a medium yellow powder was obtained from Cosmopolitan Trading Co., (Seattle, WA). A combination of chromatographic and spectrometric techniques was used to characterize the test article; an authentic standard of kavain was used for quantitation. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) with ultraviolet (UV) light detection was used to determine the composition of the bulk chemical.

The batches of Kava Kava extract used in these studies were consistent with the composition of commercially available Kava Kava extract. The identified kavalactones in the extract included methysticin, dihydromethysticin, kavain, dihydrokavain, yangonin, and desmethoxyyangonin.

The dose formulations were prepared by mixing Kava Kava extract with corn oil to give the required concentrations. The dose formulations were stored at room temperature in clear glass bottles sealed with Teflon®-lined lids enclosed in amber plastic bags for up to 37 days (2-week studies) or 42 days (3-month and 2-year studies) and were stable during this period.

2.2. Animals and exposures

The studies were conducted at Battelle Memorial Institute (Columbus, OH). Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory (Germantown, NY). Rats and mice were quarantined for 14 days and were 5–6 weeks of age at the beginning of the studies. The animals were distributed randomly into groups of approximately equal initial mean body weights and identified by tail tattoo. Male rats were housed five per cage in the subchronic and three per cage in chronic studies, female rats were housed five per cage, male mice were house individually, and female mice were housed three to five per cage. Tap water and NTP-2000 diet (Zeigler Brothers, Inc., Gardners, PA) were made available *ad libitum*. The care of animals on this study was according to NIH procedures as described in the US Public Health Service Policy on Humane Care and Use of Laboratory Animals (National Research Council, 1996). These studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (Food and Drug Administration 1987).

2.3. Study design

In the prechronic studies, groups of male and female F344/N rats and B6C3F1 mice (n = 5/dose/sex in 2-week studies and 10/sex/dose in 3- month studies) were administered Kava Kava extract by gavage in corn oil at concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 g/kg For the subchronic studies, groups of 10 male and 10 female rats and mice were dosed at concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 g/kg. For the chronic studies, groups of 50 male and 50 female rats and mice were dosed at concentrations of 0, 0.3, 1.0 g/kg (rats) and 0, 0.25, 0.5, 1.0 g/kg (mice), respectively.

Body weights were recorded initially, weekly, and at study termination for the subchronic studies. For the chronic studies, body weights were recorded initially, weekly for the first 13 weeks, monthly thereafter, and upon study termination. Clinical findings were recorded once a week beginning on day 1 and at the end of the studies for the subchronic and at 4-week intervals beginning at week 5 until study termination for the chronic studies. Additional groups of 10 male and 10 female rats designated for clinical chemistry evaluations were administered the same doses for up to 23 days in the 3-month studies and up to 18 months in the chronic studies. Blood was collected via the retroorbital plexus (rats) or sinus (mice) of the clinical pathology study rats on days 4 and 23 and from the main study rats and mice at the end of the studies for hematology (only mice at day 4) and clinical chemistry (rats only). For the chronic studies, blood was collected from the retroorbital plexus of clinical chemistry study rats at 6, 12, and 18 months.

Necropsies were performed on all study animals. In the prechronic studies, the heart, right kidney, liver, lung, right testis, and thymus of rats and mice were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all vehicle control and 2.0 g/kg rats and mice and on study animals that died early; tissues were examined to a no-effect level in the remaining core study groups. For the chronic studies, at necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved as described above. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. A complete microscopic examination was performed in all chronic study animals. Additional details regarding the pathology data generation, quality assurance review, and NTP pathology working group are available in the NTP technical report (Hardisty and Boorman, 1986; NTP 2011).