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Extracts of kava (*Piper methysticum*) induce acute anxiolytic-like behavioral changes in mice

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Abstract Rationale: Kava has been used for centuries by Pacific Islanders for its tranquilizing and sedative effects. Recent clinical trials suggest that kava has therapeutic value for the treatment of anxiety. Demonstration of kava's anxiolytic effects in animals under controlled conditions would provide additional support for its clinical potential as an anxiolytic and would facilitate investigation of its mechanism(s) of action. **Objectives:** This study systematically characterized the acute dosage-dependent anxiolytic and sedative effects of kava extract in well established quantitative murine behavioral assays and compared kava- and diazepam-induced behavioral changes. **Methods:** Various doses of an ethanolic extract of kava root or diazepam were administered intraperitoneally to BALB/cByJ inbred mice. Behavioral changes were measured in the mirrored chamber avoidance assay and elevated plus-maze assay. Reduced latency to enter and increased time spent in a normally avoided environment operationally defined anxiolysis. Sedation was defined by a significant decrease in locomotor activity in a circular arena. **Results:** Kava extract produced statistically significant dose-dependent

anxiolytic-like behavioral changes in both assays of anxiolysis. ED₅₀ values for kava-induced increases in time spent inside the mirrored chamber and on the open arms of the plus maze were 125 mg/kg and 88 mg/kg, respectively. Kava extract also caused a profound decrease in locomotor activity (ED₅₀ of 172 mg/kg). Flumazenil, a competitive benzodiazepine receptor antagonist, blocked both the anxiolytic and sedative effects of diazepam, but had no effect on kava's behavioral actions. **Conclusions:** Kava extracts produce significant murine anxiolytic-like behavioral changes and sedation that are not mediated through the benzodiazepine binding site on the GABA_A receptor complex.

Keywords Kava · Anxiety · Sedation · Mice · Benzodiazepines

Introduction

For centuries, an intoxicating herbal beverage prepared from kava (*Piper methysticum* Forst.) has been consumed by people in the South Pacific islands for ceremonial and social purposes. Early descriptions of the psychopharmacological effects of kava reported that the beverage reduced fatigue, relieved anxiety and produced muscle relaxation and sedation (Lembert 1967; Singh 1992). Kava also was used by Oceania natives as an analgesic, sudorific, diuretic, and antipyretic, and to treat venereal diseases, gout and diarrhea (Keller and Klohs 1963). Studies in laboratory animals have shown that kava extracts possess sedative, anticonvulsant, anxiolytic, analgesic, muscle relaxant and neuroprotective activities (O'Hara et al. 1965; Kretschmar et al. 1970; Jamieson et al. 1989; Jamieson and Duffield 1990; Backhaus and Krieglstein 1992; Martin et al. 2000; Smith et al. 2001; Rex et al. 2002). Over-the-counter preparations of kava (e.g. capsules and tinctures) and beverages containing kava have now become widely available in the United States where they are used to reduce stress and anxiety and enhance social interaction (Norton 1998; Kaul and

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Joshi 2001). Kava preparations are used clinically in Europe and have been included in the herbal pharmacopoeia for a number of years. A limited number of clinical trials have been reported, but these generally can be criticized on the basis of subject number, study design and outcome measures of anxiety status. Nevertheless, Pittler and Ernst (2000) concluded from a recent meta-analysis of seven clinical reports that kava does have anxiolytic effects that are significantly greater than placebo. Despite the widespread human usage of kava as a tranquilizing agent, few studies have systematically evaluated the anxiolytic action of kava in well-characterized animal assays responsive to anxiolytic drugs.

Numerous compounds have been isolated from the kava root, and six to eight α -pyrones (kava lactones) have shown to be biologically active (Keller and Klohs 1963; Shulgin 1973; Duffield and Ligdard 1986; Duffield et al. 1986; Singh 1992). The mechanisms of kava's pharmacological effect are not well characterized, nor is it clear which kava lactones are responsible for the various pharmacological effects observed. Kava's anticonvulsant properties have been linked to its interaction with the voltage-gated sodium and calcium channels (Gleitz et al. 1996; Maura et al. 1997; Friese and Gleitz 1998; Schirmacher et al. 1999). Kava's anxiety-reducing effects have been suggested to be due to its interactions with the GABA_A receptor; however, data supporting this conjecture are equivocal (Davies et al. 1992; Jussofie et al. 1994; Boonen and Häberlein 1998; Dinh et al. 2001). Other studies have indicated that kava extracts and kava lactones may alter several neurochemical systems (Seitz et al. 1997; Baum et al. 1998; Feger et al. 1998; Dinh et al. 2001); however, none of these actions have been associated with specific behavioral effects in vivo.

We have a long-term interest in establishing the pharmacological mechanism by which kava extracts exert their anxiolytic effects. To initiate these studies it was necessary to first evaluate kava's dose-dependent effects in animal assays selectively sensitive to established, therapeutically useful anxiety-reducing drugs. The present study examined the effects of kava in two well characterized rodent behavioral assays believed to reflect the anxiety state, the elevated plus-maze (Lister 1987; Carola et al. 2002) and the mirrored chamber avoidance assay (Toubas et al. 1990; Seale et al. 1996; Bowers et al. 2000). Sedation is a common side effect of prescribed anxiolytic agents (e.g. diazepam) as well as kava, and can interfere with behavioral changes in animals used as markers for anxiety reduction (Toubas et al. 1990; Seale et al. 1996). Therefore, we also determined the sedative properties of our kava preparations and evaluated their relationship to kava's dose-dependent anxiolytic-like behavioral actions.

Materials and methods

Animals

Six-week-old male BALB/cByJ mice were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). The mice were group housed in a climate controlled, AAALAC-approved facility, with a 12 h light:dark cycle, and provided free access to standard rodent food and water. Mice were given 1 week to recover from the stress of shipping prior to experiments. On the day of the experiments mice were acclimated to the laboratory for one hour before the behavioral experiments were initiated. BALB/c mice were chosen for this study due to their robust response to anxiolytic drugs in the behavioral assays utilized in this study (Toubas et al. 1990; Seale et al. 1995; Griebel et al. 2000), and this strain has been shown to be responsive to kava in several behavioral assays (Jamieson et al. 1989). All protocols were approved by the Institutional Animals Care and Use Committee and followed the NIH "Guidelines for Care and Use of Laboratory Animals" (1996).

Drug sources and administration

Kava root (a generous gift from Nature's Way, Springville, Utah, USA) was ground to a fine powder with a commercial blender. Twenty grams of powdered root was extracted with 200 ml of 95% ethanol using a soxhlet apparatus. After 4 h, the thimble was replaced with a second thimble containing 20 g of powdered root and extracted overnight with the same 200 ml of 95% ethanol. The ethanol extract was evaporated to dryness under vacuum to yield a dark oil. The oil was triturated with petroleum ether and the ether was evaporated to yield 2.6 g of resin.

Kava resin, diazepam (Sigma-RBI) and flumazenil (a generous gift from Hoffmann-LaRoche, Inc.) were dissolved in equal volumes of DMSO (Mallinckrodt) and Alkamuls EL-620 (Rhône-Poulenc). The solutions were diluted with 150 mM NaCl to give a final composition of 15% DMSO, 15% Alkamuls and 70% NaCl. Drugs were administered intraperitoneally at a volume of 0.1 ml/10 g body weight. This vehicle has been widely used by us and others and shown to have no behavioral effects when injection volume is limited to 0.1 ml/10 g body weight. Diazepam was administered at doses from 0.0316 to 5 mg/kg, and kava resin was administered at doses from 32 to 316 mg/kg. Experiments were performed from 0900 to 1600 hours, and doses of drugs were administered in a random order. Eight to ten mice were used for each treatment group. Naive mice were used for all behavioral assays.

HPLC analysis

The concentration of individual kava lactones in the kava resin was determined by HPLC analysis (Ganzera and Khan 1999) using a Waters 2695 Alliance Separations Module equipped with a 996 PDA detector (Waters, Milford, Mass., USA). Kava lactones were separated using a Phenomenex Luna 3 μ C8 column (100 \times 4.6 mm; 3 μ m particle size) (Torrance, Calif., USA) maintained at 40°C. The isocratic mobile phase consisted of 65 nanopure water (A)/20 acetonitrile (B)/15 reagent alcohol (C) at a flow rate of 0.75 ml/min. The run time was 20 min. Each run was followed by a 5 min wash with methanol and an equilibration period of 15 min. Prior to injection, extracts were dissolved in 10 ml of methanol and filtered through a 45 μ m Nylon filter. The detection wavelength was 246 nm and the injection volume was 5 μ l. Peaks were assigned by a comparison of the retention times and the UV-spectra. Data were collected and analyzed by Waters Millennium software and tabulated by Microsoft Excel. All samples were injected in triplicates and standard deviations were less than 2.8%. The kava resin contained 49.3% kava lactones and was composed of 94.6 μ g methysticin/mg resin, 93.7 μ g dihydromethysticin/mg resin, 107.8 μ g kavain/mg resin, 124.2 μ g dihydrokavain/mg resin,

43.7 μg yangonin/mg resin and 28.6 μg desmethoxyyangonin/mg resin.

Behavioral assays

Two well characterized avoidance assays were used to measure anxiolytic activity in mice, the mirrored chamber avoidance assay and the elevated plus-maze. Behavioral effects indicative of anxiety reduction are more likely to be correctly interpreted if similar findings are observed in more than one behavioral paradigm. The effects of kava were compared to the effects of diazepam, a ligand that binds to the GABA_A receptor complex (Mohler and Okada 1977; Squires and Braestrup 1977) and is known to have anxiolytic activity both clinically and in animal assays of anxiety (McDowall et al. 1966; Garrett et al. 1998).

Mirrored chamber avoidance assay

The mirrored chamber avoidance assay was performed using the method of Toubas et al. (1990). This assay is based on the image-induced acute changes in behavior that occur when vertebrates observe themselves in a mirror (Gallup 1968; Houry 1986). Briefly, the mirrored chamber consisted of a cubed chamber (30×30×30 cm), made from five pieces of one-sided mirrors, with the mirrors facing the inside of the cube. The mirrored cube was placed in the center of a 40×40×40 cm box made of black Plexiglas walls, a white Plexiglas floor and an open top. The Plexiglas box had a mirror on the wall facing the opening of the mirrored chamber. A mouse at the opening of the mirrored chamber was surrounded by mirrors on six sides. The lighting intensity in the corridors of the mirrored chamber was 200 lux and the intensity within the chamber was 100 lux. Following acclimation to the room, mice were injected with either vehicle or drugs, and 10 min later the mice were placed at a specific starting point in the corner of the corridor between the containment box and the mirrored chamber. Each mouse was allowed to move freely around the corridor during a 5-min period. Two independent indices for anxiety, total time spent in the mirrored chamber and latency to the first entry, were measured by an observer approximately 1 m from the apparatus. The criterion for entry into the mirrored chamber was that all four feet were on the floor of the mirrored chamber. Anxiolytic effects of a drug were operationally defined as a statistically significant decrease in the latency to first entry into the mirrored chamber and a statistically significant increase in the time spent inside the mirrored chamber.

Elevated plus-maze assay

The elevated plus-maze assay was performed using the methods of Lister (1987) which was based on the studies of Pellow et al. (1985), Montgomery (1958) and Handley and Mithani (1984). The elevated plus-maze was made of two sets of black Plexiglas runways, each 61 cm long and 5 cm wide constructed at 90° to each other. The arms of one runway were open and the arms of the other were surrounded by clear Plexiglas walls 15 cm high. The runways were mounted on a clear Plexiglas base, 38.5 cm from the floor. Immediately following testing in the mirrored chamber, mice were placed in the center of the elevated plus-maze. The lighting conditions used were the same as those used in the mirrored chamber. We have previously determined that evaluation in the mirrored chamber prior to testing in the plus-maze did not alter the effects of diazepam in the plus-maze (Garrett et al. 1998). Mice were evaluated for a 5-min period, and the latency to first entry on an open arm of the plus-maze and the total time spent on the open arms was scored by an observer. Latency to the first entry was used to have a second, independent measurement of anxiolytic activity. Criteria for entry onto the open arm of the plus-maze were when all four feet of the mouse were on the open arm. Anxiolytic activity of a compound was defined as a statistically significant decrease in the

latency to enter an open arm and a statistically significant increase in the total time spent on the open arms. The mirrored chamber and the elevated plus-maze were washed with water after each test to remove any potential cues left by the previous mouse. The observer scoring both the mirrored chamber avoidance assay and the elevated plus-maze was not blind to the experimental condition. The small number of crossings into the closed arm of the plus-maze and the high variability precluded the use of this measure as an index of locomotor activity.

Locomotor activity

Effects of drugs on locomotor activity were determined by measuring open field activity in an automated, computerized circular arena (Garrett et al. 1998). The circular arena was 60 cm in diameter, the walls were 25 cm high, and the apparatus was equipped with two infrared beams and detectors placed on the outside of the arena. The infrared light was directed through 1.5 cm holes to the detectors on the opposite side of the arena. The two beams were oriented 90° to each other. Light beam breaks (crossings) were recorded using a Rockwell AIM 65 microprocessor system. Dim light was supplied to the apparatus by a 25 W light on the outside of the arena. Mice were placed in the circular arena 10 min after administration of drugs, and data were collected for 10 min. This time period corresponded to the same post-injection period used to evaluate the mirrored chamber avoidance assay and the elevated plus-maze assay. Locomotor activity is expressed as the number of beam crossings per 10 min.

The time course for kava-induced inhibition of locomotor activity was determined by injecting the mice with 316 mg/kg kava and measuring the locomotor activity for 10 min at 1-h intervals after injection. Each mouse was assessed at only one time period, and ten mice were used per time period. Control values were determined by injecting mice with vehicle and 10 min following drug administration their locomotor activity was measured for 10 min. The half-life of the sedative effects of kava was calculated by plotting the locomotor activity values as the percentage of control locomotor activity versus time and fitting the data to a polynomial equation. The equation was solved for the time that intersected the 50% control activity to calculate the half-life of the inhibition of locomotor activity.

The pharmacological specificity of kava was tested by attempting to block its anxiolytic and locomotor activity effects with flumazenil, a selective benzodiazepine antagonist (Darragh et al. 1982; Patel et al. 1982). Flumazenil or vehicle was administered to mice 15 min prior to administration of the test compound, and mice were tested 10 min after injection of the test compound. To insure that any observed effects of flumazenil were not due to the injection per se, all treatment groups received two injections, one at t_{-15} min and one at t_0 . Control groups included vehicle-vehicle and flumazenil-vehicle treatments. Test groups included vehicle-diazepam, flumazenil-diazepam, vehicle-kava and flumazenil-kava treatments. For antagonism experiments in the elevated plus-maze, mice were administered 10 mg/kg flumazenil and an ED₁₀₀ dose of diazepam (1 mg/kg) or kava (133 mg/kg). Experiments examining the antagonism of locomotor activity used 15 mg/kg flumazenil and doses of diazepam (5 mg/kg) and kava (215 mg/kg) that reduced locomotor activity by 70–90% in the dose-response experiments. Doses of flumazenil greater than 15 mg/kg could not be used due to effects on locomotor activity.

Data analysis

Potency of kava and diazepam in the behavioral assays were determined by calculating the ED₅₀ values by fitting the data to a logistic equation (Kaleidagraph 3.5). For biphasic dose-response curves, the plateau of the curve was fitted to the maximal response, and the ED₅₀ was calculated to be the dose that produced the half-maximal response. The potency ratios of the sedative:anxiolytic effects were calculated by dividing the ED₅₀ of the locomotor

activity by the ED₅₀ for time spent in the open arm of the plus-maze. Statistical significance of overall effect of drug dosage was determined by a one-factor ANOVA. Dunnett's test was used to compare differences between individual doses and the vehicle control. The Tukey/Kramer test was used to compare across multiple groups for the antagonism experiments. Statistical significance was defined as $P < 0.05$.

Results

Experiment 1: effects of diazepam and kava on anxiolytic-like behaviors

Mirrored chamber avoidance assay

Diazepam produced biphasic, dose-dependent, anxiolytic-like effects in the mirrored chamber avoidance assay. The overall effect of diazepam dosage was significant for both changes in the latency to enter the mirrored chamber [$F(6,63)=3.08$, $P < 0.01$] and changes in the duration of time spent in the mirrored chamber [$F(6,63)=6.05$, $P < 0.0001$]. Diazepam caused a decrease in latency to enter the mirrored chamber, which plateaued at doses from 0.1 to 3 mg/kg (Fig. 1A). The time spent in the mirrored chamber increased with increasing doses of diazepam and reached the maximal effect at 3.2 mg/kg. The ED₅₀ for diazepam to reduce latency to enter the mirrored chamber was 0.05 mg/kg, and the ED₅₀ for increasing time spent in the mirrored chamber was 0.54 mg/kg. Kava administration also produced anxiolytic-like activity in the mirrored chamber assay (Fig. 1B). The overall effect of kava was significant for both latency to enter [$F(7,66)=3.21$, $P < 0.01$] and time spent in the mirrored chamber [$F(7,67)=4.20$, $P < 0.001$]. The kava dose-response curve was biphasic with a decrease in the latency to enter the mirrored chamber and an increase in the time spent in the mirrored chamber. Both responses peaked at 178 mg/kg and returned to control values at 316 mg/kg. Kava was less potent than diazepam in the mirrored chamber assay. The ED₅₀ for kava to reduce the latency to enter the mirrored chamber was 71 mg/kg, while the ED₅₀ to increase time spent in the mirrored chamber was 125 mg/kg. The dose of individual kava lactones at the ED₅₀ to increase time in the mirrored chamber was 11.75 mg/kg methysticin, 11.71 mg/kg dihydromethysticin, 13.48 mg/kg kavain, 15.53 mg/kg dihydrokavain, 5.46 mg/kg yangonin and 3.58 mg/kg desmethoxyyangonin.

Elevated plus-maze assay

Both diazepam and kava produced biphasic, dose-dependent, anxiolytic-like effects in the elevated plus-maze assay (Fig. 2). A significant overall effect of diazepam dose was found for latency to enter the open arm of the plus-maze [$F(6,36)=3.56$, $P < 0.005$] and time spent on the open arm [$F(6,61)=4.11$, $P < 0.002$]. Diazepam's ED₅₀ for decreasing latency to enter the open arm was 0.05 mg/kg,

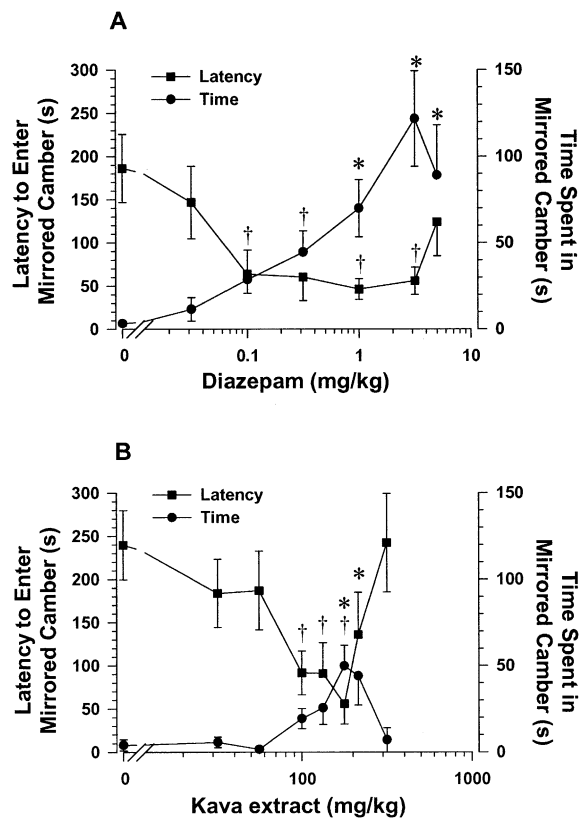


Fig. 1A, B Anxiolytic responses to diazepam and kava extract in the mirrored chamber avoidance assay. Mice were administered varying doses of diazepam (A) and kava extract (B) and latency to enter the chamber and time spent in the mirrored were measured for five min. Values are the mean \pm SE, with $n=8$ for each data point. † Differences from vehicle at a given dose of drug for latency to enter chamber, $P < 0.05$. * Differences from vehicle at a given dose of drug for time spent in chamber, $P < 0.05$

and its ED₅₀ for increasing duration on the open arm was 0.13 mg/kg. Kava's dose-response profile in the elevated plus-maze assay was similar to the responses in the mirrored chamber avoidance assay. A significant dose-dependent decrease in latency to enter the open arm [$F(7,67)=4.85$, $P < 0.001$] and a significant increase in time spent on the open arm of the elevated plus-maze [$F(7,65)=2.69$, $P < 0.02$] were observed following kava administration. The maximal response for kava for both latency to enter and time spent on the open arm of the plus-maze occurred at the 133 mg/kg dose. These behavioral changes diminished above 178 mg/kg and returned to control levels at 215 mg/kg. Kava also was less potent than diazepam in the elevated plus-maze assay. The ED₅₀ of kava for decreasing latency to enter (52 mg/kg) and increasing time (88 mg/kg) on the elevated plus-maze were greater than the ED₅₀ for diazepam. In addition, these values were similar to kava's ED₅₀ in the mirrored chamber avoidance assay. The dose of individual kava lactones at the ED₅₀ to increase time in the elevated plus-maze was 8.32 mg/kg methysticin, 8.25 mg/kg dihydromethysticin, 9.49 mg/kg kavain,

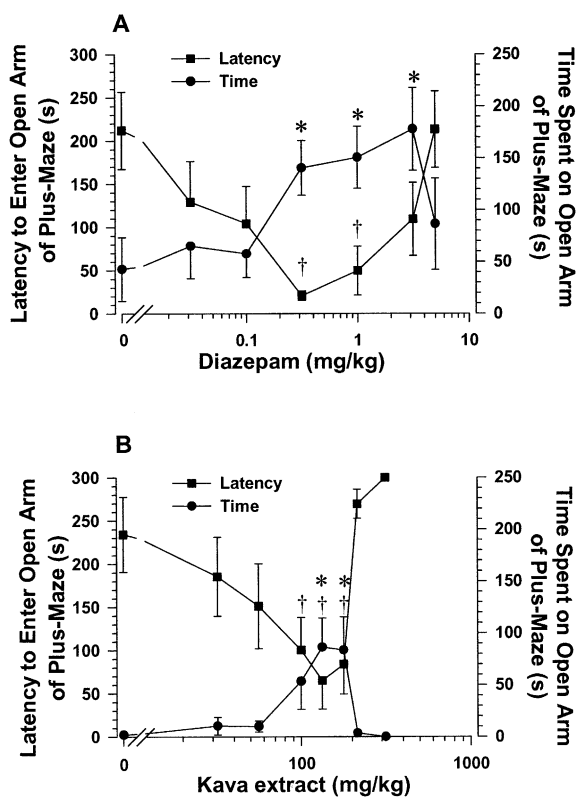


Fig. 2A, B Anxiolytic responses to diazepam and kava extract in the elevated plus-maze assay. Mice were administered varying doses of diazepam (**A**) and kava extract (**B**) and latency to enter the open arm of the plus-maze and time spent on the open arm of the plus-maze were measured for 5 min. Values are the mean \pm SE, with $n=8$ for each data point. † Differences from vehicle at a given dose of drug for latency to enter the open arm, $P<0.05$. * Differences from vehicle at a given dose of drug for time spent on the open arm, $P<0.05$

10.93 mg/kg dihydrokavain, 3.85 mg/kg yangonin and 2.52 mg/kg desmethoxyyangonin.

Experiment 2: effects of diazepam and kava on locomotor activity

The sedative effects of kava and diazepam were assessed by an examining locomotor activity in an independent experiment using a circular arena assay. Diazepam caused a dose-dependent decrease in locomotor activity (Fig. 3A) [$F(6,52)=5.79$, $P<0.001$], and had an ED_{50} of 3.7 mg/kg. Locomotor activity also was significantly decreased by kava in a dose-dependent manner [$F(7,56)=38.90$, $P<0.0001$]. Increasing the dose of kava caused a reduction in locomotor activity between 133 mg/kg and 316 mg/kg (Fig. 3B) with an ED_{50} of 172 mg/kg. The dose of individual kava lactones at the ED_{50} to reduce locomotor activity was 16.27 mg/kg methysticin, 16.12 mg/kg dihydromethysticin, 18.54 mg/kg kavain, 21.36 mg/kg dihydrokavain, 7.52 mg/kg yangonin and 4.92 mg/kg desmethoxyyangonin.

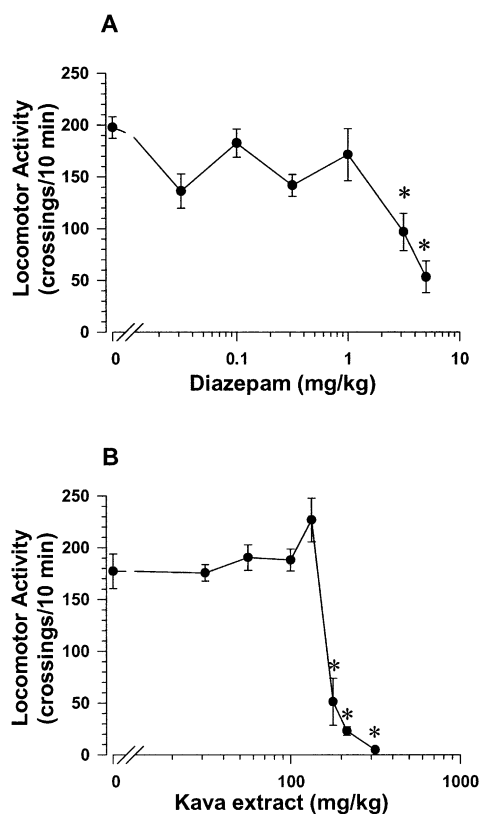


Fig. 3A, B Sedative effects of diazepam and kava. Mice were administered varying doses of diazepam (**A**) or kava extract (**B**) and locomotor activity was measured in the circular arena for 10 min. Values represent mean \pm SE, with $n=8$ for each data point. * Signifies differences from vehicle, $P<0.05$

Experiment 3: time course of kava's effect on locomotor activity

Mice injected with 316 mg/kg of kava became severely ataxic within minutes and developed a Straub tail-like response. By 10 min after injection, the mice were motionless and lay on their side, but only occasionally lost righting reflex. Activity levels were significantly lower than the vehicle treated group for up to 3 h after administration ($P<0.01$), and returned to control values by 4 h (Fig. 4). The half-life of the inhibition of locomotor activity was 2.70 h.

Experiment 4: flumazenil antagonism of kava-induced behavioral responses

Since kava and diazepam have similar pharmacological profiles, experiments were performed to determine whether kava's behavioral effects were mediated through the benzodiazepine binding site on the $GABA_A$ receptor complex. The responses for the vehicle-vehicle group in both the elevated plus-maze and the locomotor activity assays were not statistically different from the responses of the single injection vehicle control groups in the dose-

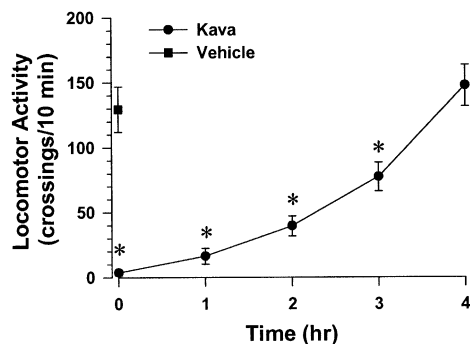


Fig. 4 Time course of sedative activity of kava. Mice were administered 316 mg/kg kava extract, and locomotor activity was measured for 10 min at varying times post-dosing. Locomotor activity for mice administered vehicle was measured for 10 min immediately following injection. Values represent mean \pm SE, $n=10$. * Signifies differences from mice administered vehicle, $P<0.05$

response experiments performed 9 months earlier (Figs. 2 and 3). Also, the responses of the vehicle-diazepam groups and the vehicle-kava groups were not statistically different from the responses from a single drug injection of the same dose in the previous dose-response experiments in the elevated plus-maze and locomotor activity assays (Figs. 2 and 3). The flumazenil-vehicle group was not statistically different from the vehicle-vehicle group in either the elevated plus-maze or the locomotor activity assay. Diazepam (1 mg/kg) administration caused a significant increase in the time spent on the open arm of the plus-maze, and prior administration of flumazenil (10 mg/kg) significantly inhibited this effect ($P<0.05$) (Fig. 5A). Flumazenil also blocked the diazepam-induced decrease in latency to enter the open arm of the plus-maze (data not shown). Flumazenil (10 mg/kg) had no effect on the kava-induced anxiolytic activity. Pretreatment with flumazenil did not antagonize the kava-induced (133 mg/kg) increase in time spent on the open arm of the plus-maze (Fig. 5A), nor did flumazenil affect kava's ability to decrease the latency to enter the open arm (data not shown). Diazepam (5 mg/kg) significantly reduced locomotor activity and this response was inhibited by flumazenil (15 mg/kg) (Fig. 5B). In contrast, inhibition of locomotor activity elicited by kava administration (215 mg/kg) was not affected by pretreatment with 15 mg/kg flumazenil (Fig. 5B).

Discussion

Ethnopharmacological and clinical studies have reported that kava produces a calming effect and relieves anxiety in humans. This study in laboratory animals supports the clinical findings, and has systematically quantified the potency of the anxiolytic and sedative properties of kava using multiple, mammalian assays of established clinical relevance. The two anxiolytic assays employed in this study, the mirrored chamber avoidance assay and the elevated plus-maze, have different pharmacological pro-

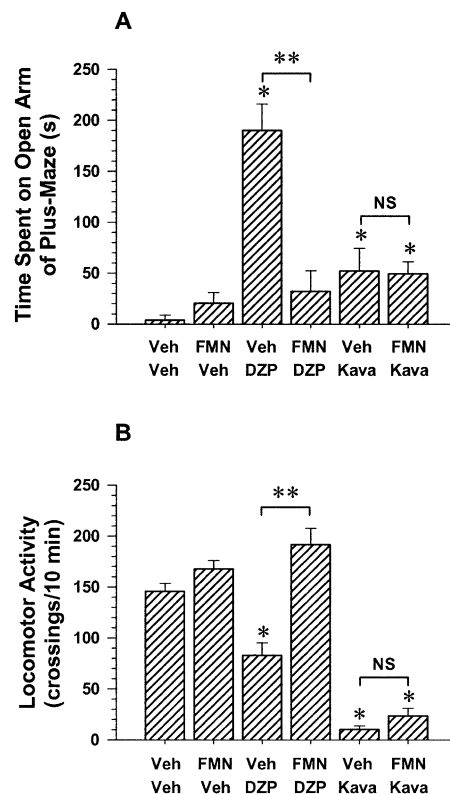


Fig. 5A, B Antagonism of drug-induced anxiolytic and sedative activities by flumazenil. Mice were administered either vehicle or flumazenil followed 10 min later by administration of either vehicle, diazepam or kava extract. Anxiolytic activity was measured for 5 min on the elevated plus-maze (A) or sedative activity was measured for 10 min in the circular arena (B). For anxiolytic activity, mice were administered vehicle or flumazenil (10 mg/kg) and either diazepam (1 mg/kg) or kava extract (133 mg/kg). For sedative activity, mice were administered vehicle or flumazenil (15 mg/kg) and either diazepam (5 mg/kg) or kava extract (216 mg/kg). * Signifies differences between vehicle-vehicle treatment, $P<0.05$. ** Signifies differences between vehicle-diazepam and flumazenil-diazepam groups, $P<0.05$. NS signifies no statistical difference between vehicle-kava and flumazenil-kava groups. For both elevated plus-maze and locomotor activity assays, $n=10$ per group

files. A wider range of clinically effective anxiolytic drugs, such as buspirone, are active in the mirrored chamber avoidance assay compared to the elevated plus-maze assay (Seale et al. 1996). Kava was shown to have dose-dependent anxiolytic-like properties in both behavioral assays. Furthermore, the potency of kava was similar between the two assays. Positive results in both assays supports the hypothesis that the kava has generalized anxiolytic actions and that its behavioral effects are not due some irrelevant action unique to a particular assay.

The potency of the kava extract is significantly less than those of diazepam in both anxiolytic assays. The ED₅₀ values for the crude kava extract for increasing time spent in the aversive environment were 230 and 680 times higher than diazepam in the mirrored chamber and plus-maze, respectively. The maximal time spent in the aversive environment by mice receiving kava was

approximately half the maximal responses of those animals injected with diazepam in both the mirrored chamber and plus maze assays. Two other studies in laboratory animals have reported that kava has anxiolytic-like activity. Rex et al. (2002) reported that oral administration of kava (120 and 180 mg/kg) to rats increased the time spent in the open arms of the X-maze. The maximal time spent in the X-maze by the animals administered kava was less than the responses of rats dosed with diazepam (15 mg/kg). Smith et al. (2001) have reported that kava extract and dihydrokavain produced anxiolytic-like responses in the chick social separation-stress paradigm, and the magnitude of the responses are similar to those of chlordiazepoxide; however, those authors used only single doses of chlordiazepoxide (5 mg/kg), kava extract (30 mg/kg) and dihydrokavain (30 mg/kg). Thus, the anxiolytic effects of kava have been shown to generalize across three vertebrate species and three behavioral paradigms. These findings suggest that further behavioral characterization of kava's anxiolytic actions are warranted in other behavioral paradigms such as the thirsty rat conflict test (Vogel et al. 1971), the light-dark avoidance assay (Crawley 1981) and with multiple endpoints within the elevated plus-maze assay (Rodgers and Johnson 1995).

The sedative actions of kava were studied for three reasons. First, sedation is a reported pharmacological effect of kava in both humans and animals (Keller and Klohs 1963; O'Hara et al. 1965; Jamieson et al. 1989; Singh 1992). Thus, the measurement of sedation served as a positive control demonstrating that our kava preparation was behaviorally active. Second, locomotor effects can confound interpretation of behavioral changes that are used as indices of anxiety reduction (Cao et al 1993; Garrett et al. 1998).

Third, the potency ratio for the anxiolytic and sedative effects impacts the therapeutic value of a potential anxiolytic agent. Our kava preparation had both anxiolytic and sedative activities. Behavioral changes indicative of anxiolysis (reduction in latency and increased time spent in the aversive environment) occurred initially at doses of kava that had no effect on locomotor activity. Even at kava's ED₅₀ dose for inhibition of locomotor activity, anxiolytic-like changes in behavior were still evident. At high doses of kava, profound depression of locomotor activity interfered with performance in the two anxiolytic assays because the animals were ataxic. This also occurred at the highest dose of diazepam that we tested. The sedative effects of kava also have been shown to interfere with the evaluation of its antipsychotic activities (Duffield et al. 1989). One of the major limitations in using benzodiazepines to treat anxiety is the sedative actions of these compounds. It has been suggested that kava is a natural alternative to benzodiazepines because of its low sedative properties. Our data on acute responses in laboratory animals would suggest otherwise. The potency ratio of the sedative:anxiolytic effects for diazepam was 6.85, whereas the potency ratio for kava was 1.38. This finding indicates that the sedative

effects for crude kava extracts occur at doses less than 2-fold greater than the effective anxiolytic doses. Thus, the separation between the doses that produce the anxiolytic and sedative effects of kava are no better than diazepam in these clinically relevant murine behavioral assays. If these data from animal studies have direct relevance to therapeutic application of kava, then they suggest that crude extracts may have little value in treating acute anxiety unless sedation is desirable.

The onset of both sedative and anxiolytic-like behavioral changes we observed have the same rapid time course as those reported by others for kava extracts and kava lactones (Kretzschmar et al. 1970). We found that the onset of the anxiolytic and sedative effects occurred within 10 min after injection. Jamieson et al. (1989) also reported that kava resin-induced sedation, loss of muscle control and analgesia occurred from 5 to 10 min post-injection. Jamieson et al. (1989) stated that mice regained muscle tone and spontaneous activity within 3 h after administration of kava resin. In our study, the sedative effects produced by 316 mg/kg of kava fully recovered by 4 h and the response had a half-life of 2.70 h. The rapid onset of the behavioral activities correlates well with pharmacokinetic profile of kava lactones. Keledjian et al. (1988) showed that mouse brain levels of kava lactones peaked from 5 to 15 min after administration of 120 mg/kg kava resin. If the time of onset and duration of action of kava's behavioral effects in mice is analogous to the therapeutic time course, then reduction of anxiety in patients should occur rapidly after administration, but be of relatively short duration. The short latency of onset of behavioral effects in animals contrasts with those reported in humans. In clinical studies, administration of 100–400 mg of WS1490 two to three times a day significantly reduced anxiety symptoms only after 1–8 weeks of treatment (Pittler and Ernst 2000). To date, no clinical studies have examined the acute effects of kava, nor have any animal studies examined the anxiolytic action of chronic low-dose kava administration. Further studies are needed in humans and laboratory animals to determine the onset and duration of action of the various pharmacological effects of kava.

The similarity in the pharmacological profiles between kava and benzodiazepines led several laboratories to investigate the interaction of kava with the GABA_A receptor. Conflicting results have been reported on the effects of kava and kava lactones on binding of ligands to the GABA_A receptor. Jussofie et al. (1994) and Boonen and Häberlein (1998) reported that kava extracts and individual kava lactones increase binding of agonists and antagonists to the GABA binding site on the GABA_A receptor complex. On the other hand, Dinh et al. (2001) showed that kava extracts inhibit muscimol binding to the GABA binding site on the GABA_A receptor. In a third study, Davies et al. (1992) concluded that kava has no effect on the GABA_A receptor complex. The data from these studies led us to examine the potential interaction of kava at the benzodiazepine site on the GABA_A receptor in our behavioral models. Flumazenil, a competitive benzo-

diazepine antagonist (Hunkeler et al. 1981), totally blocked the anxiolytic effects of diazepam, but had no effect on kava's anxiolytic activity. Our data support the findings of Davies et al. (1992) that kava has no effect on benzodiazepine binding to the GABA_A receptor and the conclusion that the anxiolytic effects of kava are not mediated through this receptor. A flumazenil dose that completely blocked diazepam's inhibition of locomotor activity also was without effect on kava-induced motor depression. Thus, neither the anxiolytic nor the sedative effects of kava appear to be mediated through the benzodiazepine receptor. A caveat to this interpretation of the failure of flumazenil to antagonize the depression of locomotor activity by kava is that the behavioral effect of the kava dose was greater than that of the diazepam dose.

Kava extracts contain a number of kava lactones (Ganzera and Khan 1999; Dharmaratne et al. 2002), and these compounds have been shown to vary in their pharmacological activities. Kretzschmar et al. (1970) showed that the rank order of potency of four kava lactones varied between loss of righting reflex and antagonism of strychnine-induced lethality. Dihydrokavain, dihydromethysticin, kavain and methysticin produce analgesia (Jamieson and Duffield 1990); however, of these four compounds, only methysticin and dihydromethysticin protect against ischemia-induced brain damage (Backhaus and Kriegelstein 1992). Smith et al. (2001) showed that out of the six kava lactones tested, only dihydrokavain demonstrated anxiolytic activity in the chick social separation-stress assay. These authors also did not observe any sedative effects of the kava extract or kava lactones. Therefore, further studies of the kava constituents are needed to determine the anxiolytic/sedative indices in murine behavioral assays. Establishment of a murine model to study the anxiolytic and sedative properties of kava provides a means to begin to determine which of the kava lactones in the kava extracts are responsible for the anxiety reducing effects and whether the anxiolytic and sedative effects can be separated from each other.

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