A Phased Clinical Trial of a Dietary Supplement Kava: Biomarker Changes and Anxiolytic Effects Phase 2: Kava Biomarker

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
5.2	Change in exclusion criteria to remove language that excludes those who are taking psychotropic medication to include them as long as they have been on a stable dose for 6 weeks at the baseline visit	During screening for the study many have been excluded who were on stable medication for anxiety. However, we are finding that they were still experiencing high levels of anxiety. This change will help to evaluate Kava as an adjunct therapy and does not affect the aims of the study or cause additional risk to study participants.

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STATEMENT OF COMPLIANCE

Provide a statement that the trial will be conducted in compliance with the protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP) and applicable state, local and federal regulatory requirements. Each engaged institution must have a current Federal-Wide Assurance (FWA) issued by the Office for Human Research Protections (OHRP) and must provide this protocol and the associated informed consent documents and recruitment materials for review and approval by an appropriate Institutional Review Board (IRB) or Ethics Committee (EC) registered with OHRP. Any amendments to the protocol or consent materials must also be approved before implementation. Select one of the two statements below. If the study is an ***intramural*** *NIH study, use the second statement below:*

1. The trial will be carried out in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP) and the following:

    - United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

    National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.
    OR
2. The trial will be conducted in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the [specify NIH Institute or Center (IC) [Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the funding agency and documented approval from the Institutional Review Board (IRB), and the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, if applicable, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.
    For either option above, the following paragraph would be included:
    The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form(s) must be obtained before any participant is consented. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form(s) will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: A Phased Clinical Trial of a Dietary Supplement Kava: Biomarker Changes and Anxiolytic Effects

Study Description: This study will examine the utility of plasma and urinary based biomarkers for the anxiolytic properties of kava, a natural dietary supplement. We will conduct a one week, double blind, randomized placebo controlled trial of kava, dosed at three 75 mg capsules per day, vs placebo, in adults with generalized anxiety disorder. Clinical measures of anxiety, blood, and urine will be obtained. Biomarkers of interest include PRKACA, cortisol, urinary TCE, and NA-5HT. CYP2D6 testing will be conducted at the screening visit to examine, at the end of the study, whether kava metabolism through this enzyme affects the biomarker responses. Participants will be assessed pre- and post-treatment. They will also be followed for 12 weeks after the end of treatment to identify any potential rare adverse events, particularly liver toxicity, that appear in a delayed fashion.

Objectives:

Primary Objective:	To evaluate the effect of a one week flavokavain A/B-free kava trial in GAD patients on biomarker and anxiety scale changes, and the relationships between these measures, in a double-blind RCT.

Endpoints:

Primary Endpoint:	Plasma PRKACA, cortisol, urinary TCE, urinary NA-5HT and cortisol levels
Secondary Endpoints:	Compliance determined by missed doses and increased dihydromyricetin (DHM) levels

Study Population: We will screen 30 subjects in order to randomize 26 adults with GAD ages 18-50 for entry into the study, with at least 20 of them completing the study. There are no restrictions on ethnicity, social background, or gender. Subjects will only be accepted into the study if they are free of any history of significant medical illness as determined by history taking, physical examination, and routine laboratory tests. Female subjects of child-bearing potential will be given a pregnancy test.

Phase:	2
Description of Sites/Facilities Enrolling Participants:	The University of Florida Clinical Research Center will be used to enroll participants and conduct research procedures. This facility is designed for clinical research.
Description of Study Intervention:	Bottles of flavokavain AB-free kava (75 mg kavalactones/capsule) with the same lot number will be directly purchased from Thorne Research Inc. This product will be used because of its lack of flavokavains A and B (<0.2 mg/capsule) and consistent content of total kavalactones. Participants will take either three 75 mg capsule of kava or three placebo capsule per day for one week.
Study Duration:	65 weeks
Participant Duration:	13 weeks

1.2 SCHEMA

Week/Day (-35 to -1) Screening

- Obtain informed consent
- Obtained demographic information
- Screen potential participants by inclusion and exclusion criteria
- Obtain medical and psychiatric history, document
- Concomitant medication review
- Height, weight, and vital signs
- Lab testing (comprehensive metabolic panel (CMP, PT/INR, direct and indirect bilirubin)
- Urine Pregnancy test (for women of child bearing potential)
- Urine Drug Screen
- Adverse event review and evaluation
- CYP2D6 testing
- Psychiatric evaluaion including mood, anxiety, sleep, and suicidality rating scales (M.I.N.I., MADRS, ISI, C-SSRS, and CGI-S)
- Complete Case Report Forms (CRFs)

Visit 1 /Day 1 Baseline assessments/Study intervention

- Administer study medication
- Concomitant medication review
- Urine pregnancy test (for women of child bearing potential)
- Urine drug screen
- Adverse event review and evaluation
- Biomarker assays (PRKACA, cortisol, c-AMP, and NA-5HT)
- Randomize
- Complete Case Report Forms (CRFs)

Visit 2/Week 1

- Concomitant medication review
- Lab testing (CMP, PT/INR)
- Adverse event review and evaluation
- Biomarker assays (PRKACA, cortisol, c-AMP, and NA-5HT)
- Psychiatric evaluaion including mood, anxiety, sleep, and suicidality rating scales (MADRS, ISI, C-SSRS, CGI-S, and HAM-A)
- Complete Case Report Forms (CRFs)

Visit 3/Week 4

- Concomitant medication review
- Lab testing (CMP)
- Adverse event review and evaluation
- Complete Case Report Forms (CRFs)

Visit 4/Week 8

- Concomitant medication review
- Lab testing (CMP)
- Adverse event review and evaluation
- Complete Case Report Forms (CRFs)

Visit 5/Week 12 Final assessments

- Concomitant medication review
- Lab testing (CMP, PT/INR)
- Adverse event review and evaluation
- Complete Case Report Forms (CRFs)

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Phone Screen	Screening Day -35 to -1	Baseline Visit 1, Day 1	Week 1 Study Visit 2 Day 7 +/-1 day	Week 4 Study Visit 3 Day 28 +/- 1 day	Week 8 Study Visit 4 Day 56 +/-1 day	Week 12/Final Visit Study Visit 5 Day 84 +/-1 day
Informed consent		X					
Demographics		X					
Inclusion/exclusion review	X	X					
Medical and psychiatric history	X	X					
Administer study intervention			X				
Concomitant medication review	X	X	X	X	X	X	X
Height and weight		X					
Vital signs		X					
Comprehensive Metabolic Panel		X		X	X	X	X
PT/INR		X		X			X
Direct and Indirect Bilirubin		X					
Urine pregnancy test [a]		X	X				
Urine drug screen		X	X				
Adverse event review and evaluation		X	X	X	X	X	X
Biomarker assays [b]			X	X			
CYP2D6 testing		X					
M.I.N.I		X					
MADRS, ISI, C-SSRS, and CGI-S		X		X			
HAM-A	X			X			
Randomize			X				
Complete Case Report Forms (CRFs)		X	X	X	X	X	X

a: For women of child bearing potential
b: PRKACA, cortisol, urinary TCE, and NA-5HT

2 INTRODUCTION

2.1 STUDY RATIONALE

To validate PRKACA reduction (primary outcome), cortisol, urinary TCE, and NA-5HT reductions (secondary outcomes) as biomarkers for kava-induced anxiolysis in GAD.

2.2 BACKGROUND

There is a critical need for new anxiolytics with novel mechanisms for GAD treatment. GAD is a common, chronic, and impairing psychiatric disorder that affects more than 16 million American adults annually (1). Evidence-based treatments for GAD include selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRI's), serotonin type 1A partial agonists, and benzodiazepines (BZD) (47). Unfortunately, many patients with GAD report only a partial response with these treatments or cannot tolerate their side effects. These patients continue to suffer with high levels of anxiety and impairment throughout their lives. BZDs are of particular concern because they often become the only treatment option for patients with GAD but carry a risk of chemical dependency, tolerance to the therapeutic effects, cognitive impairment, and in the long term, dementia and even death (3). Specifically, there is an unmet clinical need for new anxiolytics with a mechanism different from BZDs and free of the associated adverse effects (4). **Kava is a promising candidate as a mechanistically novel anxiolytic.** Kava is derived from *Piper methysticum,* a shrub that grows in the South Pacific islands. Extracts from the roots of this shrub (kava) are traditionally consumed as a daily beverage to help people relax, socialize, and improve sleep quality. Kava was used to treat anxiety in Europe in the 1990s (7-13). Clinical studies have shown that kava is effective for anxiolysis across a variety of patient groups (7-13). A recent meta-analysis of four randomized placebo-controlled trials that examined kava use for GAD favored improvement of anxiety symptoms with kava over placebo, although the results were not statistically significant (standardized mean difference = -0.99, CI -2.12 to 0.14) (14, 15). *Importantly, current studies demonstrate no signs of addiction or withdrawal from kava, a clear advantage over the BZDs (23).* These results not only suggest that kava is a promising candidate for treatment of GAD, but also clearly indicate that more research is needed to confirm its utility. The mechanisms behind kava's anxiolytic activity are far from established. Several targets have been proposed based on biochemical or cellular results at human irrelevant concentrations without *in vivo* validation, including $GABA_A$ (17-19), dopamine receptors (18), opioid receptors (18), histamine receptors (18), MAO-B (20), or ion channels (21). A few *in vivo* studies indicate that kava's anxiolytic effect is not mediated through $GABA_A$ (24, 25), consistent with its lack of addiction or withdrawal in clinic. Given that most of currently available treatments for GAD work via serotonin reuptake or $GABA_A$, this study has the potential to identify an anxiolytic with a unique mechanism of action. **Kava use as a dietary supplement is increasing.** Kava has long been available as a dietary supplement in the US market with a resurgence recently – the amount of kava exported from Vanuatu, Fiji and Tonga (the major kava producing countries) increased by ~25% annually between 2008 – 2013 and the U.S.A. is a major destination of kava export (30). Quality control and standardization, however, are lacking – we have analyzed the composition of 25 commercial kava products by LC-MS/MS. The abundance of the major kavalactones

varied by 10 – 30 fold (30) despite the fact that these kavalactones have been recommended for standardization (48). These products therefore likely vary significantly in their anxiolytic efficacies. The abundance of the hepatotoxic compounds, flavokavains A and B (32), varies even more (30), such that some products may impose higher risk of hepatotoxicity than others. Rigorous research therefore is needed to characterize kava's pharmacology in conjunction with accurate characterization of its chemical compositions, essential to maximize kava's benefit and to minimize its potential adverse risk.

Kava is safe for use in clinical populations, especially when properly prepared. Kava was banned for clinical use in Europe from 2001 – 2014 because of concerns about potential hepatotoxicity (26). However, the purported incidence rate of kava-induced hepatotoxicity is extremely low (0.3 – 1 cases per one million daily doses) (48-56), much lower than BZD-based anxiolytics (57, 58). In addition, the WHO found that kava-related hepatotoxicity was likely due to quality problems, adulteration of the root with other parts of the plant, use of ethanol- rather than water-based extraction, and in some cases, interactions between kava and other drugs/herb preparations or chronic alcohol use (5, 49, 50, 59). As a result of these assessments, the ban on kava use was lifted in 2014, and kava is again available for use in Europe (26). We have closely monitored the safety of kava and its ingredients in all of our studies. We detected no signs of hepatotoxicity when kava was evaluated alone, even at elevated dosages and extended exposures (27-29, 32, 36, 60, 61). This was further supported by the results of our recent study, demonstrating that kava was safe unless co-administered with acetaminophen, due to flavokavains A and B (32), two lipophilic chemicals low in aqueous extracts (30). This finding suggests that the already low rate of potential hepatotoxicity with kava can be further mitigated by the use of preparations that have low (or no) levels of flavokavains A and B, and by excluding patients who are taking medications known to have potential hepatotoxicity. The risk of observing an instance of hepatotoxicity according to the WHO is less than 0.03% in the R61 phase and less than 0.9% in the R33 phase of this study; this risk is further mitigated by our use of flavokavain A/B-free kava.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The risks of participating in this project include those incurred during the administration of rating scales, blood drawing, and the administration of study medication (kava). Drawing blood requires venipuncture and is associated with the momentary discomfort of the needle stick. The risks of venipuncture include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection and uncommonly faintness. The amount of blood collected at each time point is less than two tablespoons. Potential side effects from the administration of Kava include: excessive sedation if combined with alcohol, rare allergic reactions and rare liver toxicity. The frequency of liver injury in Kava is not known. Based upon reported cases, liver injury due to kava is less than 1:1,000,000 daily doses, and is likely due to the presence of flavokavains A and B in the kava preparation. A few cases of clinically apparent liver injury have been published in the literature. One of the potential risks with kava is an acute liver injury with severe hepatitis ending in fulminant hepatic failure, requiring liver transplantation, or even leading to death. Preliminary data from a previous human study by our group

did not identify any adverse events due to the consumption of kava supplementation. **In addition, we are using a kava preparation that has been rigorously tested for the removal of flavokavains A and B, which are thought to cause the rare cases of liver toxicity that have been observed.**

2.3.2 KNOWN POTENTIAL BENEFITS

None

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

All study personnel are accustomed to maintaining and ensuring patient confidentiality in the course of their work. In addition, all study personnel are highly trained in research procedures and in issues regarding protection of participants' rights and privacy. All members of the research team, including the PIs, coinvestigators, study coordinator, data collectors, research assistants, data manager, and all students associated with the project will complete UF-mandated human subject training prior to study commencement. Participants' identities will be protected through the following measures: consent forms will be stored behind a locked door and in a locked filing cabinet accessible only by the PI, and all identifying information will be stored in a separate, secured location from data that is collected during the study. Access to locked files will be restricted to essential personnel only. In addition to consent and identification files that are secured, all data files will be coded in a manner that makes identification of the participants extremely unlikely. Indeed, participants will be given a numerical ID code to ensure that inadvertent or unauthorized identification does not occur. Data will receive additional layers of security because the whole building, floor, and office in which locked records are maintained have restricted, keyed access. The anonymous codes assigned to participants will be verified and maintained throughout the study, and codes with names will be provided only to meet federal guidelines applicable to the facility. Data will be entered into a REDCap data base developed for the project. REDCap (Research Electronic Data Capture) is a secure, HIPAA-aligned web-based application designed to support traditional case report form data capture. REDCap's automated export procedure will be utilized to export data into a SAS data set for analysis.

The study coordinator for the proposed project has 6 years of experience and will be trained in all study procedures. She will coordinate the screening of potential participants and will meet with Dr. Mathews weekly to discuss study progress. Dr. Firpi-Morell, a gastroenterologist with expertise in hepatology and liver transplantation, will be responsible for overseeing the medical safety monitoring for this trial. He has extensive experience as a medical monitor for national clinical trials of multiple medications. He will communicate with Dr. Mathews on a regular basis, and will also communicate any and all adverse events to the data safety monitoring board. He will interpret the results of the liver biochemistries (all results will be blinded to treatment condition) and will be responsible for referring participants to appropriate additional clinical evaluation and intervention if needed.

Effective screening will rule out active medical or psychiatric conditions, including supplement or medication use, chronic alcohol use, and evidence of pre-existing liver disease that may prevent someone from participating in the studies. Once the subject enters the study, an experienced research team will closely follow him or her. The research team has developed considerable expertise in monitoring the safety of subjects participating in research studies. An experienced research psychiatrist and the hepatologist is available 24 hours a day, 7 days a week. If at any point in the procedures symptoms become distressing or dangerous, subjects will be withdrawn from the study and, if necessary, treatment instituted.
This project will be conducted in compliance with research statutes outlined in the Health Insurance Portability and Accountability Act. Each participant will be assigned a unique identification number, and this number will be used to link all subsequent information collected. This file will be secured with password access, since it represents a direct link between the name and identification number of a participant. All paper files and data are kept in locked file cabinets to ensure confidentiality. The other procedures to ensure confidentiality follow the regulations and policies of the University of Florida and UF Health. Data file archival and back-up will be performed on a regular basis. The routine monitoring, maintenance, and quality control of the databases will be the responsibility of Dr. Mathews.

Participant distress during evaluations: All evaluations have been designed to minimize burden on patients with respect to fatigue, stress and/or discomfort. Only trained individuals will administer study tests and questionnaires. These individuals will be trained to be sensitive to signs of patient fatigue, stress or discomfort. Breaks during testing will be scheduled, and can be initiated by the patient if they desire. Participants may choose whether or not to receive the results of their clinical interviews. Participants who are assessed as suffering from an untreated psychiatric disorder and choose to obtain the results of their interview will be offered referrals to treatment locally if they choose. Participants will be free to discontinue their participation at any time if they feel that it is causing unacceptable distress or discomfort.

Loss of Confidentiality: All research material will be treated in a confidential manner, and only research staff will have access to the data. Confidentiality will be maintained by assigning identification numbers for all aspects of the project; all data entered into computer databases will use only this identifier. Data are kept in separate coded REDCap data sets and in locked cabinets in locked rooms at UF with access limited to a small number of study personnel. All clinical and other records have identifying names of participants and relatives removed. Electronic data will be password protected and stored in a database on a secure server that is also password protected, with no patient identifying information. No identifiable individual data are presented in scientific publications. Only research personnel will have access to this data.

Risks of Treatments of Unknown Efficacy: Participants will be informed by the investigators and through the written consent that few studies exist for kava treatment, and kava for GAD in particular has not been adequately tested.

Emergence of Depressive or other Psychiatric Symptoms or Suicidality: The research team will receive training in the rapid assessment and management of distress or emergence of psychiatric symptoms that may arise during the PK protocol. If a participant's status raises concern, Dr. Mathews will offer a clinical evaluation and, when appropriate, participation in the study will be discontinued, and the participant will be referred for treatment according to clinical indication (hospitalization, partial hospitalization, other psychosocial interventions, pharmacotherapy, etc.). If a participant's psychiatric state worsens at any point such that they are no longer able to participate in the treatment, the participant will be withdrawn from the research protocol and referred for the appropriate treatment according to clinical indication. The same procedures apply to any participant who reports suicidal intent or plan or makes a suicide attempt at any point in the study. Dr. Mathews is a licensed psychiatrist and will provide clinical oversight over assessments of suicidal ideation and safety.

Management of psychiatric emergencies: We believe that the risk of psychiatric emergencies occurring during the execution of this study is low, based on the pilot data and our findings of the patient population. However, should such an emergency arise (such as an acute decompensation, statement or demonstration of active suicidal ideation, violence, etc), we have procedures in place to ensure the safety of the participant and the staff.

Liver function abnormalities: We will use a kava product that has removed the potentially liver toxic elements, flavokavain A and B, and has been rigorously tested for quality. However, we will nevertheless closely monitor serum liver biochemistries (AST, ALT, ALP, bilirubin (total, direct, and indirect) and albumin) at baseline, and at weeks 1, 4, 8 and 12. Although the timing of any potential liver toxicity with kava is not known, the timing of the few reported cases in the NIH liver tox database suggests that the onset of symptoms related to hepatotoxicity (including nausea, fatigue) is likely to be between 2 weeks and 12 weeks following initial ingestion. We will also encourage participants to contact the study team for any concerning symptoms that they may have for up to 6 months post-treatment to ensure that no delayed reactions, no matter how unlikely, are missed. We will carefully educate participants about symptoms to watch for throughout the study and following treatment.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the effect of an 8-week flavokavain A/B-free kava trial in GAD patients on biomarker and anxiety scale changes, and the relationships between these measures, in a double-blind RCT.	• Plasma changes in PRKACA, cortisol, urinary TCE, and urinary NA-5HT and cortisol levels • Changes in HAM-A score pre to post treatment	• Biomarkers of interest • Measure of anxiolysis
Compliance	• Liver function tests • Number of missed doses	• Standard measures of liver function • Quantified measure of compliance
Tertiary/Exploratory		
N/A	N/A	N/A

4 STUDY DESIGN

4.1 OVERALL DESIGN

Aim: To validate PRKACA reduction (primary outcome), cortisol, urinary TCE, and NA-5HT reductions (secondary outcomes) as biomarkers for kava-induced anxiolysis in GAD.

Study design: We will recruit and screen potential participants until we are able to randomize and enroll 26 individuals with GAD, with the expectation that 20 will complete the study (10 per arm) based on a conservative estimate of a 25% drop-out rate (the drop-out rate for our pilot study was 19%). We will build on our prior work with smokers by evaluating the utility of PRKACA as a biomarker for kava-induced anxiolysis among individuals with GAD. A one week double-blinded randomized, parallel, placebo-controlled trial of kava in GAD patients will be conducted. To minimize the risk of adverse events, particularly liver dysfunction, we will use flavokavain A/B-free kava in this study. Participants will be randomized, stratified by sex, to one of two conditions—placebo dosed three times a day or 75 mg of kava dosed three times a day. All pills will be taken with meals. Potential adverse effects will be evaluated via serum liver biochemistries and assessment of clinical symptoms in all participants at baseline, post treatment (day 7) and every 4 weeks for 12 weeks following discontinuation of treatment. Blood and urine for biomarker assessments will be collected at baseline and post treatment (day 7). CYP2D6 testing will be conducted at the screening visit to examine, at the end of the study, whether kava metabolism through this enzyme affects the biomarker responses.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Exploring the potential of plasma PRKACA, and in an exploratory fashion of cortisol, urinary TCE and NA-5HT, as surrogate biomarkers of kava's anxiolytic potential.

4.3 JUSTIFICATION FOR DOSE

We have isolated the major chemicals in kava and determined their benefits (66) and risks (32) in vivo. With such knowledge, we screened twenty-five commercially available kava products and quantified the major beneficial kavalactones, including kavain, dihydrokavain, methysticin, and dihydromethysticin (DHM, the chemopreventive ingredient), and the hepatotoxic compounds – flavokavains A and B (30). We identified one dietary kava product from Gaia Herbs (Kava Root) for our pilot human trial that has a high DHM content and low flavokavain A and B content. To ensure consistency, we characterized the content of three batches of this product by HPLC and NMR. Each capsule contained 75 ± 3 mg of kavalactones (the anxiolytic ingredients), consistent with the labeled 75 mg kavalactone per capsule. Each capsule also contained ~20 mg DHM. With three capsules per day, the human daily dose of kava is within the proposed anxiolytic range (120-280 mg daily (14)); the amount of DHM would be comparable

to its in vivo chemopreventive dose. This kava product also contained minimal flavokavains A and B (<1.0 mg/capsule).

4.4 END OF STUDY DEFINITION

The study will come to an end when the data for 13 controls and 13 subjects with GAD has been collected and analyzed.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

1) Adults ages 18-50 who meet DSM-5 criteria for GAD as the primary psychiatric diagnosis. **2)** No more than one failed therapeutic trial of an FDA approved medication for the treatment of GAD. **3)** Score of >14 on the Hamilton Anxiety Rating Scale at screening. **4)** At least a 4 (moderate) on the Clinical Global Impressions Severity Scale at screening. **5)** Females of potential childbearing status must use adequate contraceptive precautions.

5.2 EXCLUSION CRITERIA

1) Any change in current SSRI, SNRI, or other non-benzo anxiolytic medication within 6 weeks of baseline visit. **2)** Inability to refrain from acetaminophen, alcohol or other potentially hepatotoxic substances. **3**) History of liver disease or current liver disease or clinically significant elevation in serum liver chemistries. **4)** Unstable medical or neurological condition. **5)** Positive urine drug screen for substances of abuse. **6)** Active substance abuse/dependence. **7)** Lifetime history of a psychotic disorder, bipolar disorder, PTSD or OCD. **8)** Any significant risk for self-harm or suicidality as determined by the principal investigator or suicide attempt within the last 6 months. **9)** Psychotherapy newly instituted during the 6 weeks leading up to enrollment in the study. Subjects established in psychotherapy without change during the course of the study may participate. **10)** Montgomery-Asberg Depression Rating Scale (MADRS) > 20 (moderate or severe depressive symptoms).

5.3 LIFESTYLE CONSIDERATIONS

This study will exclude any individual who is a current tobacco smoker and anyone who is not able to abstain from alcohol consumption for the duration of the study.

5.4 SCREEN FAILURES

Subjects who do not meet inclusion and/or meets criteria for any of the exclusion criteria will be deemed a screen fail.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Subjects will be recruited from the population of patients seen in clinics staffed by the UF Department of Psychiatry, through advertisements and referrals in the community. These advertisements will be in the form of flyers and newspaper ads. When study referrals are received, a member of the research team will make contact within 24 hours to conduct a pre-screening phone interview. The study will be described to the potential participant and a brief set of questions will be asked to determine eligibility in the trial. Those who meet the inclusion/exclusion criteria below and are interested in participation will then be scheduled for an in-person screening visit. At that screening visit the informed consent will be reviewed in detail and the participant will be given the opportunity to ask questions prior to signing. Participants who are eligible at both screening and baseline will be randomized to receive study drug

and given instructions on taking it. At the end of this visit the participant will be scheduled for the next study visit.

Compliance will be insured via the following methods:

- Close monitoring during participation
- Weekly reminder phone calls to confirm study visits
- 24/7 access to study coordinator and study doctor during participation
- Establishing appropriate rapport such that the participant feels cared about by the research team and is motivated to return.
- Providing ease of access to the research facilities—participants will be given parking or bus vouchers to facilitate their visits. Visits will be scheduled to accommodate the participants' schedules as much as possible within the limitations of the study parameters.

Participants will be compensated $25 for each assessment visit, and for the long term follow-up visits. Money will be loaded onto a prepaid visa card remotely, and will be provided at two time points 1) the end of the treatment (or at the time that the participant drops out) and 2) at the end of the 12 week follow up. Providing compensation at these delayed time points rather than after every visit encourages participation in the assessment visits. Note that participants are not compensated for participating in treatment, rather they are compensated for participating in the research assessments.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Bottles of flavokavain AB-free kava (75 mg kavalactones/capsule) with the same lot number will be directly purchased from Thorne Research Inc. This product will be used because of its lack of flavokavains A and B (<0.2 mg/capsule) and consistent content of total kavalactones. Participants will take either one 75 mg capsule of kava or one placebo capsule three times a day for one week.

6.1.2 DOSING AND ADMINISTRATION

Optimal dosing regimen to be determined in phase 1 (75 mg three times a day or 225 mg once a day).

A trained study team member will retrieve the study supplements from IDS Pharmacy and deliver them to the participant at the UF Health Outpatient Psychiatry Clinic. Instructions will be provided in writing on supplement administration and storage of supplements. Participants will be asked to keep track of any missed doses. Unused supplements will be returned to IDS via the study personnel at the end of the trial and will be destroyed by IDS.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Participants will be randomized to receive kava or placebo and given bottles with the correct number of pills and instructions on how to take the medication.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Bottles of flavokavain AB-free kava (75 mg kavalactones/capsule) with the same lot number will be directly purchased from Thorne Research Inc. This product will be used because of its lack of flavokavains A and B (<0.2 mg/capsule) and consistent content of total kavalactones. For ongoing quality control, three capsules from each bottle will be analyzed via our established method to ensure adequate and constant content of total kavalactones and no flavokavains A and B. The Investigational Drug Service (IDS) pharmacy will package the investigational kava. Supplies with the appropriate number of capsules for each subject will be prepared and bottled for administration.

6.2.3 PRODUCT STORAGE AND STABILITY

Product will be stored according to manufacturer recommendations. All unused supplement will be returned to IDS via study personnel and destroyed by IDS.

6.2.4 PREPARATION

Bottles of flavokavain AB-free kava (75 mg kavalactones/capsule) with the same lot number will be directly purchased from Thorne Research Inc. This product will be used because of its lack of flavokavains A and B (<0.2 mg/capsule) and consistent content of total kavalactones.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Eligible study participants will be randomly assigned to one of two groups: kava supplement or placebo. The pharmacist will use a computerized program SAS PROC PLAN procedure to generate the randomization scheme. Sequentially numbered, sealed, opaque envelopes containing group assignment (kava supplement or placebo) will be prepared by the pharmacist and numbered for each group 1 to 20. When a participant meets criteria to be randomized the study coordinator will open the next (sequentially numbered) appropriate envelope for participant assignment. Because this is a double-blinded study, neither the investigators nor the participants will know whether participants have received the kava supplement or placebo. The placebo supplement will be provided by identical capsules that contain methylcellulose, an inactive ingredient. The pharmacist will distribute identical capsules (kava and placebo); thus, no distinguishing characteristics will be present. The list of prepared supplements will be maintained by the pharmacist who will distribute the appropriate supplements for the next consented, eligible study participant.

6.4 STUDY INTERVENTION COMPLIANCE

Secondary Endpoint: Compliance will be measured as number of missed doses (self-reported) and defined by the detection of DHM at least 3 times above its limit of quantification via the LC-MS/MS methods at post-treatment in at least 80% of participants who complete the kava arm (e.g., 8/10).

6.5 CONCOMITANT THERAPY

6.5.1 RESCUE MEDICINE

25 mg of Hydroxyzine every 6 hours

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial. If at any time during the study the participants are found to be at excessive risk, the study will be terminated. All key research personnel have completed the required human subjects training course. Key personnel include all individuals responsible for the design and conduct of the study.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Study subjects will be informed that they are welcome to withdrawal or discontinue the study at any time should they feel this is not their best interest, does not meet their time availability, or for any other reason they see fit. Subjects will be asked to speak with the study coordinator or the investigator to indicate their request for withdrawal and all study related activity will stop.

7.3 LOST TO FOLLOW-UP

NCCIH considers lost to follow-up as a research subject who was participating in the study at a certain point in time and subsequently missed two consecutive study visits and is unresponsive to study contact, or is no longer participating in study activities. The anticipated lost to follow-up rate for this study will be 20% of total enrollment (e.g., N=2).

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

N/A

8.2 SAFETY AND OTHER ASSESSMENTS

Built upon our understanding of kava's hepatotoxic risk, a flavokavain A/B-free kava dietary supplement will be used in this trial, which is expected to reduce/eliminate kava's potential hepatotoxic risk. We will exclude individuals with elevated risk for hepatotoxicity, and will closely monitor liver function and other potential adverse events during and for 12 weeks following treatment.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event (AE) is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value), or any combination of these regardless of relationship to participation in the study.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

Any undesirable and unintended (although not necessarily unexpected) effect occurring as a result of interventions, interactions, or collection of identifiable private information in research. In medical research, any untoward physical or psychological occurrence in research, including abnormal laboratory

finding, symptom, or disease temporally associated with the use of (although not necessarily related to) a medical treatment or procedure. Adverse events involving drugs are also referred to as adverse drug experiences. The PI will review and access for any adverse event classification in this study.

8.3.3.1 SEVERITY OF EVENT

The following scale will be used to grade adverse events:

1. Mild: no intervention required; no impact on activities of daily living (ADL)
2. Moderate: minimal, local, or non-invasive intervention indicated; moderate impact on ADL
3. Severe: significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

To assess relationship of an event to study intervention, the following guidelines are used:

1. Related (Possible, Probable, Definite)
    1. The event is known to occur with the study intervention.
    2. There is a temporal relationship between the intervention and event onset.
    3. The event abates when the intervention is discontinued.
    4. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    1. There is no temporal relationship between the intervention and event onset.
    2. An alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The Study PI and Independent Monitoring Committee will be responsible for determining whether an SAE is expected or unexpected. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Unanticipated problems will be recorded in the data collection system throughout the study.
The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At

each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

AEs documented during the course of the trial will be reported to NCCIH on an annual basis by way of inclusion in the annual report and in the annual AE summary which will be provided to NCCIH and to the Independent Monitors. The Independent Safety Monitor(s) Report will state that all AEs have been reviewed.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the Independent Monitors(s), IRB, FDA, and NCCIH in accordance with requirements. For the IND:

- 7-day IND Safety Report (unexpected fatal or life-threatening AEs related to the intervention); a copy of the report sent to the FDA will be submitted to the NCCIH Program Officer and Independent Safety Monitor(s) within 24 hours of FDA notification.
- 15-day IND Safety Report (any other serious and unexpected AE related to the intervention); a copy of the report submitted to the FDA will be submitted to the NCCIH Program Officer and Independent Safety Monitor(s) within 24 hours of FDA notification.

All other AEs documented during the course of the trial will be reported to NCCIH on an annual basis by way of inclusion in the annual report and in the annual AE summary which will be provided to NCCIH and to the Independent Monitors. The Independent Safety Monitor(s) Report will state that all AEs have been reviewed

8.3.7 REPORTING EVENTS TO PARTICIPANTS

In the case of abnormal lab values (liver functions), the PI will address these findings with the study subjects and provide the subject with a copy of the result for their current physician.

8.3.8 EVENTS OF SPECIAL INTEREST

None

8.3.9 REPORTING OF PREGNANCY

All individuals of child bearing age range will be required to provide a urine sample for pregnancy testing at the screening visit. If they are determined to be pregnant they will be excluded from the study.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for unanticipated problems require the creation and completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB:

- Appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- A detailed description of the adverse event, incident, experience, or outcome;
- An explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

To satisfy the requirement for prompt reporting, unanticipated problems will be reported using the following timeline:

- Unanticipated problems that are serious adverse events will be reported to the IRB, Independent Safety Monitor(s), and NCCIH within 7 days of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the IRB, Independent Safety Monitor(s), and NCCIH within 14 days of the investigator becoming aware of the problem.

All unanticipated problems should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

During this study we will follow the NIH and UF IRB guidelines on reporting unanticipated problems to study participants.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):
  - Mean PRKACA reduction will be 30% higher in kava group than placebo group
  - Mean PRKACA and HAM-A score reductions in placebo group will be correlated by at least 0.3
- Secondary Efficacy Endpoint(s):
  - Measurable DHM levels will be detected in 8/10 participants in kava arm

9.2 SAMPLE SIZE DETERMINATION

We will recruit and screen potential participants until we are able to randomize and enroll 26 individuals with GAD, with the expectation that 20 will complete the study (10 per arm) based on a 75% success rate (the retention rate for our pilot study was 81%). Of particular interest is determining the utility of measuring changes in PRKACA as a marker of kava-induced anxiolysis. To provide context, the effect size seen in our pilot work was .794 (Cohen's ***f***) for PRKACA reductions on kava from baseline. Based on the proposed design, with 10 subjects per arm, we have 0.80 power to detect an effect size (Cohen's ***f***) of 0.73 (82, 83). While this represents a large effect size, it is consistent with what was found in our pilot work.

9.3 POPULATIONS FOR ANALYSES

Individuals with GAD ages 18-50

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

In this aim, we will explore the effect of kava supplement on changes in PRKACA, cortisol, urinary TCE, and NA-5HT. Separate 2 x 2 (Condition (Placebo / Kava) x Time (Baseline and Post-treatment, within condition) generalized linear mixed models (GLMM) will be analyzed for (a) PRKACA, (b) cortisol, urinary TCE, and NA-5HT outcomes. Of primary interest is exploring effect sizes for the interaction effect (difference in slopes) for those outcomes over weeks 0 (baseline) to 1 under placebo and kava conditions. Simple main effects will provide information about the within-subjects slope for each condition. GLMM analyses allow for missing data for an intent-to-treat approach, and can accommodate measurement of study subjects at different time points during the study, incorporate time varying covariates, flexible covariance structures, and a variety of dependent variable distributions (including counts and dichotomous) (85, 86). The statistical model will contain main effects for condition and time and the condition by time interaction. Of primary interest is the treatment by time interaction effect, as that will reflect the effect of the kava intervention.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

See above

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

No analysis needed- endpoint is simple measurement.

9.4.4 SAFETY ANALYSES

First, we will rigorously analyze the kava product prior to use to ensure its consistent quality with no flavokavains A and B. Second, we have implemented stringent exclusion criteria and will provide education to the enrolled subjects to minimize potential drug-herb interactions. Third, we will monitor participants throughout the study and for 12 weeks following the end of treatment to ensure that any potential acute or delayed adverse effects are identified and addressed. Serum liver chemistries (AST, ALT, ALP, bilirubin (total, direct, and indirect) and albumin, Prothrombin Time and International Normalized Ratio) will be drawn at baseline, post treatment, and every 4 weeks following treatment up to 12 weeks. The adverse events checklist will be completed at each of these visits as well.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Gender, age, baseline HAM-A scores, education, race, and baseline biomarker levels.

9.4.6 PLANNED INTERIM ANALYSES

None

9.4.7 SUB-GROUP ANALYSES

None

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

N/A

9.4.9 EXPLORATORY ANALYSES

N/A

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study. The consent process will be documented in the clinical or research record. To complete the informed consent process at the end of study participation, study staff will inform the subject when his/her participation has come to an end and will document the discussion in the study record.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

All consented subjects will be provided a copy of their fully executed informed consent document.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Each subject will review the informed consent document with the study coordinator and will be provided time to ask and receive answers to any questions. The name of the person obtaining consent will be documented along with date and time consent was obtained.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial."

If at any time during the study the participants are found to be at excessive risk, the study will be terminated. All key research personnel have completed the required human subjects training course. Key personnel include all individuals responsible for the design and conduct of the study.

10.1.3 CONFIDENTIALITY AND PRIVACY

Subject confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to subjects. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records. All research material will be treated in a confidential manner, and only research staff will have access to the data. Confidentiality will be maintained by assigning identification numbers for all aspects of the project; all data entered into computer databases will use only this identifier. Data are kept in separate coded REDCap data sets and in locked cabinets in locked rooms at UF with access limited to a small number of study personnel. All clinical and other records have identifying names of participants and relatives removed. Electronic data will be password protected and stored in a database on a secure server that is also password protected, with no patient identifying information. No identifiable individual data are presented in scientific publications. Only research personnel will have access to this data.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

These data may be combined with those of future large RCTs. The data may also be disseminated in the format of seminar, abstract, peer-reviewed manuscript, and other forms. The remaining samples may be used for appropriate research upon approval, including the discovery of additional biomarkers.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	Medical Monitor
Chengguo Xing	Roberto Firpi
Carol Mathews	

10.1.6 SAFETY OVERSIGHT

The Data and Safety Monitoring Plan for this project will also include monitoring by an independent Data Safety Monitoring Board (DSMB), consisting of an anxiety disorders expert (Dr. Carr), a hepatologist

(Dr. Morelli), a statistician (Dr. Wu), and a pharmacologist (Dr. Law). These individuals will have no affiliation to the proposed study. The DSMB will review any serious adverse event that is reported within 48 hours. They will also convene annually and at the end of the study to review all adverse events. Adverse events that are serious and unexpected will be immediately reported to the IRB, NIH, and FDA (if warranted). Non-serious unanticipated problems shall be described in annual reports to the IRB and NIH. The DSMB will review the adverse events and discuss any concerns regarding them with the Principal Investigators.

Study progress and safety will be reviewed monthly (and more frequently if needed). Progress reports, including patient recruitment, retention/attrition, and AEs will be provided to the Independent Monitor(s) semi-annually. An Annual Report will be compiled and will include a list and summary of AEs. In addition, the Annual Report will address (1) whether AE rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The Annual Report will be sent to the Independent Monitor(s) and will be forwarded to the IRB and NCCIH, FDA, and sponsoring industry collaborator. The IRB and other applicable recipients will review progress of this study on an annual basis.

10.1.7 CLINICAL MONITORING

The study team will generate Study Reports for the Independent Monitor and will provide information on the following study parameters: liver function, serious adverse events, and biomarker data.

Study Report tables will be generated only from aggregate (not by group assignment) baseline and aggregate safety data for the study population.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The site SC will ensure that all study personnel have completed all required institution-specific and protocol-specific trainings and that these trainings are documented appropriately on the Training Log. The site SC will also ensure that new personnel are appropriately documented on the Delegation of Responsibilities Log (DOR). While training should be completed and documented in real time, the Lead SC will verify that all training is current and appropriately documented. All study staff will complete CITI or NIH Human Subjects training prior to commencement of study. The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study subjects, including accurate case report forms (CRFs), and source documentation. All data will be entered into a REDCap (Research Electronic Data Capture) database developed for this project. REDCap is a secure, HIPAA-compliant web-based application designed to support traditional case report form data capture. Data will be exported into a

SAS data set for analysis. Data integrity will be evaluated using descriptive statistics (e.g., means, standard deviations, frequencies, percent, range) appropriate for measurement levels. Checks for implausible or out-of-range values, distributional forms, and missingness will be performed. Data transformations (e.g., Box-Cox family of transforms) and/or cross product terms will be incorporated, if required, based on evaluation of tenability of statistical model assumptions and model fit to the data. All analyses will be conducted using SAS version 9.4 or later.

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

10.1.9.2 STUDY RECORDS RETENTION

During this study we will follow NIH, UF IRB, and FDA regulations on study record retention. Should the retention durations differ, we will recognize the longer duration for record retention.

10.1.10 PROTOCOL DEVIATIONS

All protocol deviation reporting will follow NIH and UF IRB guidelines.

10.1.11 PUBLICATION AND DATA SHARING POLICY

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We will publish the results of our work in appropriate psychiatric and pharmacological journals. After the publication of the results of the study, clinical data (without identifiers) will be made available to other investigators, if requested. As required by the IRB at UF, participants in our study have the option to choose whether or not they agree to allow their data to be shared with other investigators. In our experience, the vast majority of participants agree to have their information shared with other investigators who are interested in studying neuropsychiatric disorders. We also welcome collaboration with others to further explore kava's clinical potential, its active ingredient(s) and detailed mechanism of action.

Our plan includes the following:

- Presentations at scientific meetings
    - From the projects, it is expected that approximately two to three presentations at scientific meetings would be appropriate in the field of anxiety, biomarker or pharmacokinetics.
- Peer-reviewed publications

- The research results will be prepared for peer-reviewed publication. It is expected that the results from the proposed studies will be the basis for 2 – 4 manuscripts in the R61 phase, including 1) pharmacokinetic characterization of kava and its major ingredients; 2) biomarker changes in response to kava treatment and other clinical results.

- Protocol sharing
  - We agree to share our experimental protocols and data with other researchers who are PIs and co-PIs of NIH grants. The sharing can be arranged through the following approaches: 1) on-line data base with a password that can be released to other researchers through the discussion with the program director; and 2) teleconference with other researchers through the arrangement of the program director.

- Department websites
  - Both multi-PI's department maintain departmental websites that have "News" section and websites for individual faculty. The general clinical trial information will be posted both at the "News" site and the multi-PI's individual faculty website that further detailed information may be requested from the multi-PI.

- Clinicaltrials.gov
  - The trial will be registered at clinicaltrials.gov and will be updated as the trial proceeds.

10.1.12 CONFLICT OF INTEREST POLICY

During this study we will follow the NIH policy pertaining to any current or possible conflicts of interest.

10.2 ADDITIONAL CONSIDERATIONS

None

10.3 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event

EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
3	03/02/2021	Added CYP2D6 testing to the study description and schedule of activities.	This was already included in other sections, but we wanted to make sure it was included in every relevant section.
4	03/30/2021	Corrected the discrepancies between the schema and SOA.	There should be no discrepancies so that everyone is on the same page.
5	04/21/2021	Corrected exclusion/inclusion criteria to reflect that we are doing the HAM-A during screening and not at baseline. Also corrected overall design to reflect that participants will be coming in for their week one visit on day 7, not day 8.	There is no point in doing the HAM-A at baseline if we already completed it during the screening process. Participants will be coming in on day 7 because the sample for pharmacokinetic testing must be taken on the last day of their medication dosing.
6	3/31/2022	Change in MADRS exclusionary criteria to reflect correct scoring of mild, moderate, and severe classification.	Previous scoring was not consistent with the scales scoring guidelines for mild, moderate, and severe symptoms of depression. Exclusion was changes to >20 to only exclude moderate and severe cases of depression as mild does not indicate need for exclusion in this study.
7	10/4/2022	Change in exclusion criteria to remove language that excludes those who are taking psychotropic medication to include them as long as they have been on a stable dose for 6 weeks at the baseline visit	During screening for the study many have been excluded who were on stable medication for anxiety. However, we are finding that they were still experiencing high levels of anxiety. This change will help to evaluate Kava as an adjunct therapy and does not affect the aims of the study or cause additional risk to study participants.

11 REFERENCES