PROTOCOL

1. General Information

1.1 Title:
HSIRB# 126030 5/11/2016 Version 4
Effect of elderberry juice on cognition and inflammation in patients with mild cognitive impairment

1.2 Sponsor:
Mizzou Advantage

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1.6 Associated Sites:
Nutritional Immunology Lab

110B Animal Science Research Center
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Behavioral Neuroscience Lab
340E Life Sciences Center
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2. Background Information

2.1 Name and description of the investigational product(s):

Recent research has revealed potent antioxidant and anti-inflammatory effects of elderberry fruit preparations. Elderberries have traditionally been used for pain relief, swelling/inflammation, urine production, as a laxative, and as a flavoring in food.
This is one of the botanical agents being studied by the MU Center for Botanical Interaction Studies as funded by P50AT006273.

2.2 A summary of findings from studies that potentially have clinical significance and of the known and potential risks and benefits, if any, to human subjects:

Elderberries have been shown in a number of studies to have significant anti-inflammatory and antioxidant effects. It is one of the richest sources of anthocyanins, and is rich in vitamins B2, C, folic acid, biotin, nicotinic acid, beta-carotene, B6, and pantothenic acid (1). Multiple in vitro studies have supported the anti-inflammatory and antioxidant effects of elderberry preparations (reviewed in (1)). Animal studies have also revealed significant anti-inflammatory and antioxidant effects (2). Population studies examining factors that may decrease the risk of Alzheimer's disease, such as the Kame project, have revealed that drinking juices rich in polyphenols is one of the most reliable factors conveying decreased risk for Alzheimer's disease (3). Studies examining the effects of individual vitamins or isolated nutritional factors have yielded mixed results in neurodegenerative diseases such as Alzheimer's disease, but recent evidence suggests that combinations of such factors may yield synergistic benefits (4). Research suggests that combination therapies including multiple dietary supplements and other current therapies in mild dementia patients results in improvements in cognitive performance reported to persist out to 12-24 months (5). Therefore, with the broad range of potential neuroprotective agents contained in elderberry preparations, this seems an ideal agent for utilization in a pilot clinical trial in neurodegenerative patients such as those at risk for Alzheimer's disease. No controlled trial of elderberry has ever been done previously in Alzheimer's or MCI.

In our previous research, we examined the sensitivity of our visuospatial problem solving task for detecting change in cognitive performance with treatment in MCI patients. Twenty-two

subjects with MCI were recruited for our study with a Clinical Dementia Rating (CDR) (22,23) score of 0.5, and a Folstein Mini Mental Status Examination (MMSE) (24) score of at least 24. At the first visit, all subjects were given the MMSE in order to assess global function across several domains including memory, and the Hopkins Verbal Learning Test (25) to assess verbal memory, as well as non-memory tasks including the Boston Naming Test (BNT) (26) (primarily considered as assessing language (naming)), Rey Complex Figure copying (Rey CFT) (27-29) (primarily considered as assessing visuospatial ability), a series of anagrams (primarily considered as assessing verbal and divergent cognitive flexibility) (30) (Rearrange these letters to form an English word: OGRF, RDWO, FALC, LANI, MHBTU, HTRSI, DSLEI, TMLAE), and the visuospatial problem solving battery (VPS) adapted from the "matchstick" problems (15, 31). As with our previous work with these types of tasks (14,30), solution latencies were recorded for performance on both. There was no significant difference between the two groups at baseline. However, at one year, the treated group performed significantly better on their change scores than the not treated group for the VPS task ($p=0.038$). Of interest, there were also trends detected for better change scores in the treated group for the Rey CFT, BNT, anagrams, and importantly, the IADL. As with previous research (13), no effect was observed on the memory task, the HVLT (paper in preparation). We wish to determine the potential effects of elderberry juice on preserving cognitive functioning in a group of people at risk for cognitive impairments in the future.

2.3 Description of and justification for the route of administration, dosage, dosage regimen, and treatment period(s):

Twenty of the subjects will be randomly assigned to receive 5 ml elderberry juice by mouth, diluted in 8oz of water three times daily. The juice will be obtained from River Hills Harvest. Twenty control subjects will receive an equal amount of a flavored liquid with no nutritional content as a placebo control group. The placebo liquid will match the elderberry juice closely in color using commercially available colorants. Subjects will be allowed to mix the juice in 8oz of water if the bitterness of the juice makes it unpalatable. The clinicians will be blinded as to group, as will be the assessor. Neetu Nair, a graduate student in Dr. Beversdorf's lab who is otherwise uninvolved in the study, will keep the unblinding code in case a need arises to break the code for clinical purposes or preliminary data analyses. Subjects will be given sufficient juice at each visit to last until the next visit. They will be provided a liquid medicine measuring spoon and instructed to measure out 5ml per dose. Subjects and caregivers will be asked to check each dose on a dose log, and bring back the used juice containers at each visit to confirm that a proper amount has been taken, as monitored by the assessor. Subjects taking less than 75% of the scheduled dose will be excluded.

After initiation of elderberry juice or control, subjects will be retested at 12 weeks, visit 2, (the timeframe that revealed significant effects with other anthocyanin-rich fruit preparations (33,34), 6 months, visit 3, and (pending resubmission for second year of support) 12 months, visit 4. The

need for pursuing follow-up at 12 months is to maximize the sensitivity for detecting incidence of progression to dementia with the CDR. Additionally at visits 2, 3, and 4 subjects will also be assessed with the Clinical Global Impression of Change (CGIC), which is a valid and reliable research tool for overall assessment of salient changes in patients (39), in which the blinded clinician rates the degree of change in the patient from the beginning of the study. The subject/caregiver will be given time to ask any questions and the clinician will be sure the subject/caregiver understands all instructions before dispensing liquid.

2.4 A statement that the trial will be conducted in compliance with the protocol:

All researchers and clinicians taking part in this study will conduct all practices in compliance with this protocol, proceed under Good Clinical Practice (GCP), and follow all regulatory requirements outlined by University, State, and Federal agencies.

2.5 Description of the population to be studied:

When examining the impact of an agent that acts on inflammation and has antioxidant properties, targeting neuroprotection, it is important to identify and treat patients at the earliest stage. Therefore, we propose to investigate the effects of elderberry juice in patients with mild cognitive impairment, MCI. MCI is characterized by impaired memory and preserved activities of daily living (6,7). Patients with this diagnosis are at significantly increased risk for developing dementia (8-10). Most researchers propose MCI as the mildest endpoint on the spectrum of Alzheimer's disease (9). With the recent advances in treatment options for Alzheimer's disease, much attention has been directed at intervention at earlier stages. Recent research has focused on whether or not agents may delay the progression from MCI to dementia (11, 12). In addition to any delay in or prevention of progression, it would also be of interest to determine whether agents result in any cognitive benefits for MCI patients. Studies have examined to effects of cholinesterase inhibitors, the first type of drug on the market for Alzheimer's disease, on cognition and global assessment outcomes in MCI, and have revealed effects on global assessment, psychomotor speed, and attention, but not on specific memory tasks (13).

Inclusion criteria: CDR score of 0.5 and an MMSE of at least 24, as well as have no known sensitivity to elderberry products, or presence of any health condition that in the clinical experience of the investigators might impair their ability to complete the study. Recruited subjects will be aged 50 or more.

Exclusion criteria: Known history of sensitivity to elderberry products. Known allergy to honeysuckle. Current diagnosis of diabetes. Bleeding disorder. Currently pregnant. Currently making changes to other drugs that might affect cognitive performance (subjects showing the greatest cognitive decline and other signs of Alzheimer's disease may be prescribed cholinesterase inhibitors as this is a standard of care for Alzheimer's disease but not MCI). Presence of any condition the health professional believes will impair ability to complete

study procedures (ex. terminal illness, comorbid major psychiatric disorders such as schizophrenia, or drug abuse). Potentially confounding neurodegenerative diseases (e.g. MS).

Between Dr Shenker and Dr Beversdorf's clinics at the University of Missouri Hospital, there are at least 80 patients with MCI seen on an annual basis. Most of these patients have expressed a willingness to participate in research. We may need to enroll up to 60 subjects to obtain a sufficient set of 40. This project also harnesses the skills of Dr Beversdorf and Dr Shenker. Both have completed fellowships in Behavioral and Cognitive Neurology, resulting in special skills in the neurocognitive assessment of patients with memory loss. Should the need arise, we will also recruit from other aging clinics in the area.

2.6 References:

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3. Trial Objectives and Purpose

Our aim is to begin to explore the potential translational impact of elderberry juice, a botanical agent. To achieve this, we will perform a pilot trial of elderberry juice in patients with mild cognitive impairment (MCI). It is critical to determine whether the course of development of Alzheimer's can be altered in this high-risk population. Our primary outcome measure will be visuospatial problem solving, as this has been shown to be sensitive to drug effects in MCI. We will also track other cognitive outcomes as well as the incidence of progression to dementia. Furthermore, we will examine inflammatory markers to begin to explore the potential mechanism of its effect. The results of this study will yield pilot data for publication and for development of a larger clinical trial for which external funds will be sought.

4. Trial Design

4.1 A specific statement of the primary endpoints:

Subjects will be assessed for changes in cognitive performance measures: CDR (for determination of progression to dementia), MMSE (general cognition), IADL (functional ability), HVLT (memory), BNT (language), Rey CFT (spatial function), anagrams (verbal cognitive flexibility), and VPS (spatial cognitive flexibility).

4.2 A description of the type/design of trial:

Double-blinded placebo-controlled pilot trial

4.3 A description of the measures taken to minimize/avoid bias:

Twenty of the subjects will be randomly assigned to receive elderberry juice and twenty control subjects will be randomly assigned to receive an equal amount of a flavored liquid with no nutritional content as a placebo control group. The clinicians will be blinded as to group, as will be the assessor. Neetu Nair, a graduate student in Dr. Beversdorf's lab who is otherwise uninvolved in the study, will keep the unblinding code in case a need arises to break the code for clinical purposes or preliminary data analyses.

4.4 A description of the trial treatment(s) and the dosage and dosage regimen of the investigational product(s):

Subjects will drink 5 ml juice by mouth, diluted in 8oz of water, three times daily. Subjects will be given sufficient juice at each visit to last until the next visit. Juice containers will be clearly labeled indicating the contents as a research supplement with directions advising subjects of dose schedule. Subjects and caregivers will also be asked to check each dose on a dose log (indicating day of trial, date, time, and dose), and will bring the juice containers to the next visit to confirm that a proper amount has been administered.

4.5 Subject Involvement:

Prior to initiation of elderberry juice or placebo, baseline visit 1, subjects will be assessed with the CDR (for determination of progression to dementia), MMSE (general cognition), IADL (functional ability), HVLT (memory), BNT (language), Rey CFT (spatial function), anagrams (verbal cognitive flexibility), and VPS (spatial cognitive flexibility). They will also be administered a Food Frequency Questionnaire to determine any individual differences in diet that might be relevant to the current study's findings. After initiation of elderberry juice or control, subjects will be retested at 12 weeks, visit 2, (the timeframe that revealed significant effects with other anthocyanin-rich fruit preparations (33,34), 6 months, visit 3, and (pending resubmission for second year of support) 12 months, visit 4. At each visit following baseline the Clinical Global Impression of Change (CGIC) will also be administered. Blood samples (30mL) will be obtained at baseline and each time point in the trial to determine immunological markers, 4 times total if subject progresses through all time points.

4.6 Maintenance of trial treatment randomization codes:

The clinicians will be blinded as to group, as will be the assessor. Neetu Nair, a graduate student in Dr. Beversdorf's lab who is otherwise uninvolved in the study, will keep the unblinding code in case a need arises to break the code for clinical purposes or preliminary data analyses. This will be securely stored in her lab space at 181 Galena Hall. All subject data will be coded to a study specific code and all identifying information will be stored on a separate secure server.

6.2 The number of subjects planned to be enrolled and reason for choice of sample size:

In other research examining the effects of other anthocyane-rich fruit extracts on MCI, a significant effect was revealed on word list memory with 12 subjects (33). In this study, the subjects in the treatment group improved their word list memory scores from 35.2 to 38.6, with a

standard deviation of less than 2. Based upon this data, the power of a study at the level of α=0.008 (adjusted for multiple measures for the various cognitive tasks) with a sample of 13 would be 0.95 for detecting an effect of elderberry juice if it has similar effects as the grape juice on memory, and a sample of 18 would yield a power of 0.99. Therefore, we expect that the sample size of 20 per group (total 40) would be sufficient to reveal effects on word list memory (HVLT), as well as our other cognitive and behavioral outcome measures (VPS, MMSE, Rey CFT, BNT, and IADL), if their performance follows the same response as observed with word list memory. This pilot study will also yield results for the purpose of deriving power calculations for determination of appropriate sample sizes for larger, subsequent studies.

Dr. Beversdorf and Dr. Shenker see at least 80 patients with MCI each year in their clinics. Most of these patients have expressed a willingness to participate in research. We may need to enroll up to 60 subjects to obtain a sufficient set of 40.

6.3 The selection of subjects to be included in the analyses:

Subjects taking less than 75% of the scheduled dose or failing to complete at least one follow-up session will be excluded from analysis.

7. Potential Risks Related to Drug

The elderberry can cause problems for people with a known allergy in the *Caprifoliaceae* family (honeysuckle). The related side effects are given below.

- There are reports of gastrointestinal distress, diarrhea, vomiting, abdominal cramps, and weakness after drinking elderberry juice made from crushed leaves, stems, and uncooked elderberries. **Notably, toxicity typically arises from uncooked berries and preparations for this study will be carried out to minimize such reactions.**
- In theory, high doses or long-term use of elder flowers may have diuretic (urine-producing) effects. People taking diuretics or drugs that interact with diuretics should use caution when taking products containing elder.
- Elder may also lower blood sugar levels. Dizziness, headache, convulsions, and rapid heart rate have also been reported.

8. Data Handling and Recordkeeping

Information produced by this study will be stored in the study PIs files located in M741 in the Center for Translational Neuroscience in the University of Missouri School of Medicine and on a secure server identified by a code number only. The code key will be kept on a separate secure server. Identifiable information contained in the records will not be given to anyone not associated with the study without written consent except as required by law.