Effects of The Administration of *Melissa officinalis* on Memory and Anxiety

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ABSTRACT

Research has shown that the development of anxiety disorders is linked to a deficiency in GABA, an inhibitory neurotransmitter, and an excess of glutamate, an excitatory neurotransmitter. Previous research has been conducted on the herbal supplement, *Melissa officinalis*, and its ability to improve cognitive functioning while increasing levels of calmness in those who had taken it. This study sought to replicate and expand on these results while incorporating the use of neuropsychological measures in addition to an anxiety measure. It was predicted that those taking *Melissa officinalis* would show decreased levels of anxiety post drug administration and improvement on various neuropsychological tasks as compared to those given a placebo. No significant difference was found between the placebo and those given *Melissa officinalis* on any measure. The discrepancy in the results of this study compared to previous literature requires further investigation and improvement upon the current study.

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LITERATURE REVIEW

Introduction

Prescribed medication is one of the many routes a person can take in treating his or her psychological ailments. Generalized anxiety disorder and other anxiety disorders such as panic disorder and social phobia are typically treated with tricyclic antidepressants, benzodiazepines or other anxiolytics (Argyropoulos, Sandford, & Nutt, 2000). These drugs produce calming effects because they often alleviate the physiological symptoms of feeling tense and fatigued that are associated with anxiety disorders. However, as with any medication, there are potential side effects that may follow which are not necessarily beneficial to the patient. For instance, Beracochea (2006) conducted a mini-review and found that benzodiazepines were related to deficits in episodic memory and difficulty with the acquisition of new information. Given these adverse side effects, many have attempted to identify an alternative pharmacological treatment for anxiety disorders. Natural supplements may represent a suitable alternative to treat mood and anxiety problems without the adverse side effects. The main goal of the present proposal was to investigate the effects that an herbal supplement, *Melissa officinalis,* had on lowering anxiety levels in addition to improving memory functioning. Previous research has demonstrated the mood altering effects of *Melissa officinalis* as well as the improvement in memory that follows use of this natural supplement (Kennedy, Scholey, Tildesley, Perry, & Wesnes, 2002). Hence, *Melissa officinalis* may be an alternative to the currently prescribed anti- anxiety medications.

The following literature review explains in further detail how anxiety impacts cognitive functioning and how *Melissa officinalis* could be used to counteract these effects. The first section will provide research explaining how anxiety alone impacts specific areas of cognitive functioning. The second section will discuss the neurotransmitters involved in anxiety and how an absence of Gamma-Aminobutyric acid (GABA) in particular can lead to higher than normal levels of anxiety in someone. The next section will describe the current medications that are used to treat anxiety and the possible side effects of these drugs. The final section will discuss the pharmacological profile of *Melissa Officinalis* with research showing its ability to improve memory and alter mood.

Anxiety Impacting Cognitive Functioning

According to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5), the main cognitive aspect associated with anxiety is persistent thoughts of worry pertaining to everyday life events, but the duration and intensity of the worry is out of proportion to the future event (American Psychiatric Association [APA], 2013). Therefore, those with Generalized Anxiety Disorder (GAD) may find it hard to control these thoughts which can impair their auditory working memory (Asmundson, 1995), executive functioning (Airaksinen, Larsson, & Forsell, 2005; Tempesta et al., 2013), immediate and delayed recall of information, (Asmundson, 1995; Airaksinen, Larsson, & Forsell, 2005; Mantella et al., 2007), visual working memory (Castaneda et al., 2011, Tempesta et al., 2013), and processing speed (Castaneda et al., 2011).

Whereas generalized anxiety has not been analyzed extensively, specific types of anxiety disorders have been researched such as panic disorder, specific phobia, social phobia and posttraumatic stress disorder. The following research will describe in further detail the exact measures used to assess various aspects of one's memory and compare the cognitive functioning between healthy controls and those with some type of anxiety disorder. Asmundson, Stein, Larsen and Walker (1995) assessed neuropsychological functioning in those with panic disorder or social phobia. All participants were free of any other comorbid disorder and were not taking medication or had discontinued medication. The Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were given to all participants followed by intellectual and neuropsychological assessments. Asmundson et al. administered the vocabulary, similarities, block design and picture completion subtests of the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) to measure intellectual ability. Assessment of neuropsychological functioning included measures of verbal learning and memory, immediate visual memory, concentration, psychomotor speed, and cognitive flexibility. Those with an anxiety disorder performed significantly worse on several of the tests compared to the healthy controls. Specifically, participants with social phobia did not immediately recall as many words after the first trial compared to healthy controls on the measure of verbal learning and memory, nor did they recall as many words after a short delay as compared to healthy controls. No significant differences were reported between the two groups on any other measures.

Airaksinen, Larsson and Forsell (2005), evaluated memory and executive functioning in individuals with different anxiety disorders. The experimental group consisted of individuals with panic disorder, social phobia, generalized anxiety disorder, obsessive-compulsive disorder, or a specific phobia. Approximately 28% of those with an anxiety disorder were taking medication for their symptoms. The control group consisted of individuals free from any presenting psychological disorder and not taking any medication. Various neuropsychological tests were used to measure executive functioning, verbal fluency, and episodic memory. Participants with an anxiety disorder recalled significantly fewer words compared to healthy controls in both cued and free recall formats. Individuals diagnosed with social phobia produced significantly fewer words when compared to healthy controls on a measure of verbal fluency. Those affected by another disorder did not significantly differ in their performance on any measures given. Participants taking medication and individuals diagnosed with panic disorder and obsessive compulsive disorder, required significantly more time to complete the measure of executive functioning.

Mantella et al. (2007) assessed cognitive functioning of not only participants with GAD, but major depressive disorder (MDD) as well. The patient groups consisted of 19 participants diagnosed with GAD and 68 diagnosed with MDD. The control group consisted of 40 participants with no history of mental illness. Benzodiazepines were currently being taken by five patients with GAD and 18 patients with MDD. Mantella et al. (2007) assessed executive functioning, verbal recall and verbal memory in all participants . The findings indicated that those with GAD and MDD recalled significantly

fewer words on the measure of verbal memory, and performed worse on the measure of executive functioning compared to the controls. No significant difference was found between those taking or not taking medication.

Casteneda et al. (2011) assessed the neuropsychological functioning of those with GAD, obsessive compulsive disorder (OCD), anxiety disorder not otherwise specified, post-traumatic stress disorder (PTSD), adjustment disorder with anxiety, and specific phobia for a total of 75 participants in the experimental group. Trait anxiety was assessed by one question on the Beck Depression Inventory, "Are you usually tense or distressed?" As in the previous study, executive functioning, verbal learning, and verbal memory were evaluated with the addition of auditory working memory. The specific subtests, visual span forward and visual span backward, of the Wechsler Memory Scale Revised (WMS-R) were also administered. The findings indicated that participants currently experiencing an anxiety disorder (not in remission) scored significantly lower on the visual span backward test. Unlike the participants in the previous study, some of the participants in this study were taking medication which may have altered their performance. Those taking medication performed worse than controls on measures of executive functioning, processing speed, and auditory working memory. Participants with GAD, PTSD, OCD, and adjustment disorder with anxiety scored significantly lower than controls on the measure of short-term verbal memory. Overall, these results show that individuals with anxiety display impaired short-term memory and that medication can induce other deficits in executive functioning and processing speed.

Tempesta et al. (2013) evaluated different aspects of neuropsychological functioning in participants with GAD currently taking escitalopram or venlafaxine and participants with GAD not taking any drug as compared to healthy individuals. Both of these drugs are commonly prescribed to treat major depressive disorder. Escitalopram is a selective serotonin reuptake inhibitor (SSRI) and venlafaxine is a serotonin and noradrenaline reuptake inhibitor (Montgomery, Huusom, & Bothmer, 2004). Working memory, visuospatial short term memory, attention, executive functioning, and non-verbal memory were assessed in all participants. The results of statistical analysis indicated that participants taking medication scored significantly lower on the measure of attention and made more errors compared to healthy controls in the measure of executive functioning. Both GAD groups, those taking medication and those not taking medication, scored significantly worse as compared to healthy controls on measures of executive functioning and non-verbal memory. No significant differences were found between those with GAD taking or not taking medication. These findings also support the research of Mantella et al. (2007) in regards to anxiety impairing executive functioning.

Although the aforementioned studies investigated the effects of anxiety disorders on memory and cognitive functioning, research has also indicated that anxiety not associated with a clinical disorder may have deleterious effects. Lavric, Rippon, and Gray (2003) evaluated the effects of anxiety on verbal and visuospatial working memory in 36 healthy volunteers. Lavirc, Rippon, and Gray induced anxiety in the participants by informing them that “Shock Block” or “Safety Block” would appear on the screen before

the tasks began. No participants received an electrical shock at any point during the experiment but had shock electrodes placed on them to make them think they would be shocked. The words "Shock Block" represented the threat condition whereas the "Safety Block" represented the safety condition. The verbal working memory task required participants to identify if letters on a computer screen were "same" or "different" as compared to the target letter presented. The visuospatial working memory task required participants to identify if letters on a screen were in the same location as compared to the target letters. All participants were fitted with a chest strap and heart rate monitor to measure heart rate. Participants performed significantly worse on the visuospatial task as compared to the verbal task when they were anticipating being shocked i.e. when the words "Shock Block" appeared. Participants also displayed significantly increased heart rate in the threat condition as compared to the safety condition regardless of the type of task being performed.

The cognitive manifestations of anxiety can have a negative effect on neuropsychological functioning in those diagnosed with a disorder and those without. The biological component of anxiety involves the neurotransmitter gamma-aminobutyric acid (GABA). Investigation of this in further detail can aid in explaining how the neurotransmitter, GABA, is incorporated into the development of anxiety.

GABA and Anxiety

The neurological and psychopharmacological mechanisms of anxiety involve several different neurotransmitters such as serotonin, dopamine, glutamate, and GABA. Several of these neurotransmitters interact with one another, but there has been a

considerable amount of research conducted specifically regarding GABA and its implication in the underlying physiological and cognitive aspects of anxiety. Research involving GABA and anxiety involve the manipulation of GABA antagonists and agonists in the brains of animals, specifically rodents. Research involving humans utilize brain imaging techniques to investigate the distribution and density of GABA receptors in the brain of those with anxiety as compared to those without.

There are two different types of GABA receptors in the human brain: $GABA_A$ and $GABA_B$. The $GABA_A$ receptors are made up of five different subunits to which different ligands can bind. One of these five subunits is able to recognize benzodiazepines. When a benzodiazepine comes into contact with the receptor, it will become active thus mimicking the effects of GABA if actual GABA came into contact with the receptor (Meyer and Quenzer, 2005). This has led to this subunit sometimes being referred as a "benzodiazepine receptor". Therefore, the possibility exists that those with anxiety disorders may be deficient in benzodiazepine receptors.

Support for a deficiency in benzodiazepine receptors is provided by a study by Malizia et al (1998) which compared the binding of Flumazenil, a radiolabelled benzodiazepine PET ligand, in those with panic disorder to normal healthy controls. Patients with panic disorder were diagnosed via measures on the Spielberger Trait Anxiety Inventory, Spielberger State Anxiety Inventory, Agoraphobic Cognitions Questionnaire, the Marks and Sheehan Phobia Scale, and a clinical interview based on the DSM-III. Malizia et al. injected Flumazenil into the cephalic vein in the left arm of all participants followed by a PET scan for 90 minutes. The scans were divided up into

different regions of interest and then these regions were compared from those with panic disorder to see if they were significantly different from healthy controls. Patients with panic disorder displayed significantly decreased amounts of Flumenzil binding in all regions of interest with peak decreases in the middle temporal gyrus, right orbitofrontal cortex, right insula, left fusiform gyrus, and left anterior medial cortex as compared to the healthy controls. The results of this study support not only the theory that a decrease in GABA is correlated with anxiety, but that this decrease is apparent in various regions throughout the brain, rather than being restricted to a specific area.

Specific regions of the brain have also been investigated. Tiihonen et al. (1997) further added to research concerning benzodiazepine receptor binding by investigating binding affinity of the benzodiazepine radio ligand [123I]NNC 13-8241. Tiihonen et al. compared patients diagnosed with GAD, according to DSM-IV criteria, to healthy controls on the basis of benzodiazepine receptor density, specifically in the left inferior temporal lobe and right prefrontal cortex. Tihonen et al. chose these regions based upon previous literature that showed there was decreased receptor binding in the left temporal lobe in those with panic disorder. All participants were drug naïve and the Hamilton Anxiety Scale was used to evaluate their level of anxiety. The radio ligand was injected into the cephalic vein in the right arm of all participants to assess receptor density. The first SPECT scan began five minutes later, followed by a second SPECT scan that occurred 5.5 hours later. Finally, Tiihonen et al. conducted an MRI on all participants . The researchers compared the benzodiazepine receptor binding of those with GAD to the healthy controls. Tiihonen et al. found that compared to the healthy controls, those

diagnosed with GAD had significantly lower receptor binding in the left inferior temporal lobe. The MRI found no anatomical abnormalities in any of the participants.

Research involving human participants has been conducted showing the anxiolytic- like properties of GABA. The research of Abdou et al. (2006) describes the impacts that GABA has on brain waves in humans. It has been previously established that alpha waves are indicative of relaxation whereas high beta waves are produced in stressful situations (William & Harry, 1985). Abdou et al. (2006) conducted an experiment to compare the effects of orally administered L- theanine and GABA to humans on specific brain waves. For this study, Abdou et al. (2006) recorded the EEGs of 13 drug naïve volunteers with no preexisting medical conditions or history of abuse. Recordings were done before and after the administration of distilled water, distilled water containing 100mg of GABA (Pharma-GABA which is produced via natural fermentation), and distilled water containing 200 mg of L-theanine. Each administration was separated by a period of seven days. Abdou et al. (2006) analyzed the EEG recordings of each participant and found that the 100 mg of GABA produced the highest percentage of alpha waves, followed by L-theanine and distilled water alone. The 100 mg of GABA also displayed the most significant reduction in beta waves, followed by L-theanine and distilled water alone. Overall, the results show that GABA added to one's system induces relaxation and reduces brain waves associated with anxiety. However, GABA serves many purposes in the human brain other than inhibiting excitatory neurotransmissions. GABA is also utilized in different aspects of one's memory functioning.

GABA and Memory Functioning

There is also evidence to show that GABA is involved in working memory. Several different pathways containing GABA originate in the hippocampus, amygdala, and prefrontal cortex and one or more of these areas may be involved in working memory. Michels et al. (2012) found that GABA levels fluctuate in different areas of the human brain when a person is engaged in a task involving working memory. All participants were free of any presenting illness and not currently taking any type of psychotropic medication. A functional MRI was taken of each patient before beginning the task to establish baseline levels of GABA and glutamate in the left dorsolateral prefrontal cortex. Working memory of all participants was assessed by having them complete the Sternberg Memory Working Memory Task. Participants were presented with five or seven letters for approximately 2 seconds followed by a probe letter 5 seconds later. Participants then indicated whether or not the probe letter was in the set previously shown by pressing a button. An fMRI was taken every ten minutes while the participants completed this task for a total of four MRI's. Participants displayed a significant increase in GABA levels during the first time interval and then decreased levels at the following time intervals compared to baseline measures. There was no significant difference in the amount of glutamate levels present. Michel et al. (2012) attributed the significant increase in concentration of GABA in the dorsolateral prefrontal cortex, middle frontal gyrus, and insular cortex during the first time interval to the person acquiring the knowledge to complete the task and that a higher amount of GABA was

needed to retain that knowledge. As the task became easier, less and less amounts of GABA were needed resulting in the decrease in concentration across trials.

There are also certain types of drugs, specifically benzodiazepines and barbiturates, which interact with the GABAergic system that have been shown to indirectly affect memory and cognitive functioning. MacLeod, Dekaban, and Hunt (1978) assessed the short term and long term memory functioning of 19 epileptic patients that had been taking phenobarbital for grand mal seizures. All patients were men with less than 8 seizures per year. The control group consisted of 20 men with no presenting neurological abnormalities. MacLeod, Dekaban, and Hunt used the Sternberg scanning task (recalling a specific number from a sequence) to evaluate short term memory and the Posner's letter matching task (determining if two pairs of letters are identical or different) to evaluate long term memory, with reaction time being the dependent variable for both tasks. The average amount of phenobarbital given to the patients was 15.8μg during the first week and then increased to 26.2 μg for the second week of the experiment. The first week of testing established a baseline to compare to the second week of testing. The epileptic patients yielded significantly longer reaction times on the short term memory task during the second week as compared to the healthy controls and to their own baseline performance measures in the first week of testing. MacLeod, Dekaban, and Hunt found no significant differences in reaction times on the long term memory task. Overall, they attributed these results to the phenobarbital impairing the speed of access to information stored in short term memory.

The previous research focused only on the side effects produced by a barbiturate. Zimmerman-Tansella, Tansella and Lader (1979) further expanded their research to compare the side effects of a barbiturate, benzodiazepine and placebo in anxious patients. A total of 24 patients diagnosed with anxiety were randomly assigned to one of three groups: 5 mg of diazepam, 100 mg of placebo or 100 mg of amylobarbitone sodium. The dosage of the two medications fluctuated depending upon the subject's physician. The treatment lasted for a total of four weeks. The first week consisted of a "wash out period" followed by three weeks of testing and drug administration. Zimmerman-Tansella, Tansella and Lader recorded the degree of participants' anxiety at the beginning of each week via the Hamilton Anxiety Rating Scale, The Morbid Anxiety Inventory and visual analog scales. The cognitive performance of each subject was measured at the end of each week via thee different types of cancellation tasks, a card sorting task, tapping rate, a symbol copying test, Digit – Symbol Substitution test, Arithmetic and the Gibson Spiral Maze task. Patients who received the diazepam reported feeling less tired and more rested compared to those who had received the placebo and amylobarbitone sodium. However, these patients also took significantly more time to complete the cancellation tasks, Gibson Maze task, and card sorting task indicating that diazepam affected their processing speed. The following research no longer includes barbiturates as these drugs were becoming increasingly discontinued to due adverse side effects.

Lucki, Giesecke and Geller (1987) examined the short term memory and recall ability of 39 patients diagnosed with generalized anxiety disorder. However, unlike the previous studies, this experiment introduces buspirone, an anxiolytic that does not

interact with the GABAeric system. Patients were randomly assigned to be given a placebo, five mg of diazepam, five mg of buspirone or ten mg of buspirone. All participants were given the Digit Span subtest from Wechsler Intelligence Scale to assess short term memory. Long term memory was assessed by the amount of words a patient was able to recall after a 20 minute delay. All tests were administered before drug administration to establish baseline performance and 70 minutes after drug ingestion. Lucki, Giesecke and Geller found that diazepam did not hinder one's ability to immediately recall information as there was no significant difference in performance among any of the groups for the digit span test. However, those who received diazepam remembered significantly fewer words as compared to their baseline performance. No significant differences were found among the other three groups. The final study attempts to replicate the type of memory demands that one would experience in everyday situations.

Buffet-Jerrot, Stewart, and Teehan (1998) examined the effects of lorazepam and oxazepam on one's memory in 30 healthy volunteers. The volunteers were divided into three groups with ten participants each: One group received a placebo, another group received 2mg of lorazepam, and another group received 30 mg of oxazepam. Buffet-Jerrot, Stewart, and Teehandeveloped a "movie task" to make it more applicable to memory tasks that one may encounter during day to day interactions, ecological validity, as opposed to in a laboratory setting. All participants were shown a movie that none of them had previously seen before following drug administration. Buffet-Jerrot, Stewart, and Teehan paused the movie every 15 minutes and instructed participants to answer

questions about the portion of the film they just watched. The questions were then grouped into three time intervals with 15 questions each for analysis. No significant differences were reported across all three groups for the first time interval. However, differences were reported for the second and third time intervals. Those who had received lorazepam performed worse than those who received the placebo or oxazepam at the second time interval, 64-111 minutes into the film and at the third time interval, 112-160 minutes into the film. Those who had received oxazepam showed significant impairment at the third time interval. The results of this study show that benzodiazepines can impair one's attention and ability to recall information as compared to when one is not under the influence of medication. Although benzodiazepines and barbiturates may cause impairment in cognitive functioning, there is also research to show that these drugs have been proven effective in treating anxiety.

Traditional Methods to Treating Anxiety

Extensive research has been conducted to support the idea that a deficiency in GABA may be a contributing factor to the development of anxiety. This has led to a large focus on creating drugs that interact with the GABAergic system. Barbiturates and benzodiazepines are the two main classes of drugs that affect GABA and are considered to be anxiolytic because of the calming effects they produce.

Barbiturates such as pentobarbital, phenobarbital and amobarbital were the first class of drugs created to interact with the GABAergic system. These drugs were originally developed as a sedative which lead their efficacy in the treatment of sleep disorders, epileptic seizures and for use as anesthesia before surgery (López-Muñoz,

Ucha-Udabe, & Alamo, 2005). However, due to the high risk of dependence and unwanted side effects, their usage was discontinued and prompted the development of benzodiazepines. The first benzodiazepine, chlordiazepoxide, was created in 1930 but it did not come to market until 1960 (Lader, 1991). Since then other drugs such as diazepam, lorazepam and alprazolam have been introduced and are frequently prescribed to treat anxiety disorders and depression with Xanax (alprazolam) being the most prescribed (Stahl, 2002).

Barbitruates and Benzodiazepines act on the GABAergic neurons via the same mechanism. Receptors are located at the beginning and end of each neuron. GABAergic neurons have two different types of receptors, $GABA_A$ and $GABA_B$. $GABA_A$ receptors have several different sites that allow for multiple types of neurotransmitters to bind to including GABA, benzodiazepines, barbiturates and neurosteroids. When a neuron receives a neurotransmitter, this causes the generation of an action potential. The two ions that interact with each other to perpetuate the action potential are potassium (K^+) and chloride (Cl^-) (Meyer and Quenzer, 2005). The binding of a barbiturate or benzodiazepine to the $GABA_A$ receptor causes an increase in the conductance of chloride ions which, in turn, prolongs the inhibitory effect that GABA has on other neurons to create the anxiolytic effect one feels when taking that drug (Wafford, 2005; Schulz & Macdonald, 1981).

Tansella, Tansella and Lader (1979) examined the effects of diazepam in comparison to a placebo and amylobarbitone sodium in overall cognitive functioning and reliving the symptoms associated with anxiety in newly diagnosed patients. The course of

the experiment lasted approximately one month. Participants were given one of the three drugs per week until all participants had taken each drug and were counterbalanced across participants. The drug dosages consisted of 5 mg of diazepam, 100mg of amylobarbitone sodium or placebo. All participants completed the Hamilton-anxiety rating scale, Morbid anxiety inventory, and an Anxiety self-rating scale before at the beginning of each treatment week. Drugs were administered three times a day every day. Participants reported significantly lower anxiety levels on the Anxiety self- rating scale when taking diazepam as compared to the other drugs given which did not significantly impact their levels of anxiety. However, no significant differences were found in regards to the Hamilton-anxiety rating scale and the Morbid anxiety inventory.

Davidson, Farquharson, Khan, and Majid (1985) compared the effects of alprazolam, diazepam, and a placebo in the treatment of anxiety in those in an outpatient clinic. Participants were included in the study if they received an 18 or higher on the Hamilton Anxiety rating scale and if they were not displaying criteria of depression, schizophrenia, benzodiazepine sensitivity, alcohol or substance abuse. Participants were randomly assigned to receive one capsule, three times a day of 0.5mg of alprazolam, 5mg of diazepam or placebo over a period of 28 days. Participants completed the Hamilton Anxiety Rating Scale (HARS) after the first, second, and fourth week of treatment. Participants taking alprazolam or diazepam showed significant improvement over the placebo in their ratings on the anxiety scale. Those taking alprazolam displayed significant improvement in depression component of the HARS as compared to diazepam

and placebo. Overall, those taking alprazolam displayed significant improvement over those taking diazepam and placebo.

The previous research has demonstrated the relationship between GABA levels and the development of anxiety. GABA added to one's system induces a calming effect whereas a lack of GABA and benzodiazepine receptors can result in higher levels of anxiety. Barbiturates and benzodiazepines are the two the main classes of drugs used to treat anxiety because of how they interact with the GABAergic system. Although these drugs have been proven to be useful in treating those with anxiety, the side effects of these drugs can lead to impairment in cognitive functioning which has led to research involving the use of a naturally derived herbal supplement, *Melissa officinalis*.

Pharmacological Profile of *Melissa officinalis*

Examination of the pharmacological components that make up *Melissa officinalis* will provide a better understanding and explanation of how it could be used to treat anxiety and improve memory functioning. Previous research has established that GABA is an important inhibitory neurotransmitter required for normal brain functioning and that a deficiency in GABA may be an underlying biological factor in the development of anxiety disorders. Excess GABA remaining after an action potential has occurred is inactivated via synaptic reuptake or metabolic breakdown (Meyer & Quenzer, 2005). The enzyme responsible for the metabolic breakdown of GABA is GABA aminotransferase (GABA-T). Preventing GABA-T from metabolizing GABA is one way to allow GABA to remain in one's system and there is evidence to support that *Melissa officinalis* has GABA-T inhibiting properties.

Awad, Muhammed, Durst, Trudeau, and Arnason (2009) isolated the active component in *Melissa officinalis* via bioassay guided fractionation of 11 different samples of the plant. The two primary compounds identified were rosmarinic acid and ursolic acid. The rosmarinic acid displayed a 40% inhibition of GABA-T in rat brain tissue whereas the ursolic acid displayed only a 19% inhibition. The sample labeled "F" displayed significantly higher inhibition rates of GABA-T compared to the other samples and also had the highest amount of rosmarinic acid. The sub fractionation of sample "F", "FG4" consisted of 95% of rosmarinic acid. It can be said that the inhibitory properties of *Melissa officinalis* are due to the rosmarinic acid. This compound in *Melissa officinalis* can be what is contributing to the alteration in mood that participant experience when taking the drug. There has been only one study to date investigating the effects that *Melissa officinalis* has in a clinical sample of those with a diagnosis of an anxiety disorder.

Cases, Ibarra, Feuillére, Roller and Sukkar (2011) evaluated the efficacy of Cyracos on its ability to reduce the negative side effects produced by anxiety. Rosmarinic acid makes up approximately 7% of Cyracos. Participants consisted of 20 volunteers who met the DSM-IV-TR criteria for an anxiety disorder and sleep disturbance. The Free Rating Scale for Anxiety was used to assess the symptoms of anxiety and the Hamilton Rating Scale for Depression to assess insomnia. All participants received 600 mg of the drug for 15 consecutive days and completed the Clinical Global Impression-Improvement Scale on the final day of the trial. Participants

reported significantly reduced agitation and tension and significantly reduced initial, middle and delayed insomnia.

Melissa officinalis and Cognitive Functioning

GABA is the main inhibitory neurotransmitter thought to be involved in anxiety. As previously mentioned, many synthetic drugs such as benzodiazapines, target the GABAergic system and produce anxiolytic or calming effects. However, there has also been research to show that herbal supplements may not only help reduce anxiety but improve memory as well. One of these in particular is *Melissa officinalis*. Kennedy et al. (2002, 2003, & 2004) has been studying this herbal supplement in regards to its effect on memory and anxiety in humans.

Kennedy, Scholey, Tildesley, Perry and Wesnes (2002) assessed the cognitive functioning of 20 healthy adult volunteers not currently taking any medication. Cognitive functioning was assessed via the Cognitive Drug Research (CDR) computerized assessment battery. The battery included measures of working memory, short-term memory, long-term memory and attention. Participants received a placebo on the first session, 300 mg at the second session, 600 mg at the third session and 900 mg at the fourth testing session of dried leaf extract of *Melissa Officinalis*. Testing sessions were separated by one week to establish an adequate wash out period between dosages. The CDR was given to all participants before drug administration and 1 hour, 2.5 hours, 4 hours and 6 hours after administration. Kennedy et al. (2002) found that the 600 mg dose improved attention at all time points but found that all three dosages resulted in impairments on the spatial memory tasks at the 4 hour time point. Kennedy et al. (2002)

found decrements in performance in those who took 600 and 900 mg on tests of immediate and delayed recall of verbal information. All three dosages also impaired working memory at all time points. However, all participants recorded increased levels of "calmness" via the Bond –Lader Visual Analog Scales when taking 300 mg and 900 mg.

Kennedy, Scholey, Tildesley, Perry and Wesnes (2003) conducted another study using the same methodology as in their previous experiment but chose a specific sample of *Melissa officinalis* via fractionation, administered different dosages, and established different time intervals. The specific sample of *Melissa officinalis* was chosen out of eight different samples due to its high displacement of scopolamine, an anticholinergic which can impair memory functioning. Cognitive functioning was assessed in 20 healthy adult volunteers not taking medication. Participants received a placebo on the first session, 600 mg at the second session, 1000 mg at the third session, and 1600 mg at the fourth testing session of dried leaf extract of *Melissa officinalis*. Testing sessions were separated by one week to ensure an adequate wash out period between dosages. The CDR and the rapid visual information processing task was given to all participants before drug administration, 1 hour, 3 hours, and 6 hours after administration. Kennedy, (2003) found that the 1600 mg dosage improved performance on the immediate and delayed recall word task at the 3 hr. and 6 hr. time interval. Participants also showed improved performance on the spatial memory task with the 1600 dosage at all time intervals. Participants given 1000 mg displayed improved performance on the picture-recognition task 1 hour later. All participants reported significantly increased ratings of calmness

when administered the 1000mg and 1600 mg dosage as measured via the Bond-Lader Visual Analog Scales.

The final study conducted by Kennedy, Little, and Scholey (2004) focused just on the mood altering aspects of *Melissa officinalis*. The Defined Intensity Stressor Simulation (DISS) computerized battery was used to induce stress in the participants. Completion of the DISS requires the participant to attend to and solve four different tasks on a screen simultaneously. Participants consisted of 18 healthy adult volunteers free from medication and were divided into three different groups: placebo, 300 mg and 600 mg of dried leaf extract. Participants completed the DISS and the Bond-Lader Visual Analog Mood Scales to establish baseline measures before drug administration. Feelings of "calmness","alertness", and "contentedness"was assessed again one hour after the completion of the DISS. Participants given the 600 mg dosage reported significantly increase ratings of calmness upon completion of the DISS compared to placebo. Significant improvement on the memory search task of the DISS battery was found for the 300 mg dosage.

Summary

The constant presence physical and cognitive manifestations of anxiety such as: persistent worrying, feeling tense, and having excessive fear about the anticipation of future events and can lead to debilitating effects on a person's cognitive functioning. Review of the literature on anxiety and cognitive functioning has yielded similar results as to the extent cognitive functioning can become impaired. Specific areas of functioning

that are affected is one's processing speed, executive functioning, and short-term and long term memory.

A deficiency in the inhibitory neurotransmitter, GABA, may be one of the underlying biological causes of developing an anxiety disorder. Research regarding this theory has focused on the amount of $GABA_A$ receptors present in one's brain and compared to healthy individuals, those with an anxiety disorder have significantly decreased amounts of $GABA_A$ receptor binding. Since GABA receptors have an important role in understanding how anxiety develops, several types of drugs have been developed to alleviate the symptoms associated with anxiety that interact with these receptors. Barbiturates and benzodiazepines interact with $GABA_A$ receptors to produce anxiolytic effects thus reducing anxiety levels. Benzodiazepines and barbiturates are able to significantly improve self-ratings of anxiety levels and even reduce some of the symptoms associated with depression. However, as with any type of drug, there are unwanted side effects that some individuals experience.

GABA is also involved in memory functioning, which could aid in the explanation of the memory deficits as previously mentioned. When compared to patients not taking a benzodiazepine, those who took the benzodiazepine, diazepam, experienced slowing of processing speed and impairment in executive functioning. Comparable results have been reported for those taking the barbiturates as well in which patients displayed longer reaction times when completing a short-term memory task. These side effects have prompted research investigating alternative treatments to benzodiazepines and barbiturates. *Melissa officinalis*, the Lemon Balm plant, has been shown to produce

the same type of anxiolytic effects as those reported in the benzodiazepines studies but without any adverse side effects to date.

Melissa officinalis consists of a large amount of rosmarinic acid which is known for its GABA-transaminase, GABA-T, inhibitory properties. The purpose of GABA-T is to break down excess GABA in one's brain. By inhibiting GABA-T, GABA can continue to inhibit neuron's from becoming activated in the brain which results in the calming effects the person reports feeling. Patients with an anxiety disorder taking Cyracos, which contains rosmarinic acid, reported decreased levels of agitation and muscle tension. Three separate studies have been conducted to investigate cognitive functioning and alterations in mood after administration of *Melissa officinalis*. These are the only studies to date that involve *Melissa officinalis* without the simultaneous comparison of other botanical extracts. There is consistency among the studies regarding how *Melissa officinalis* was beneficial in lowering stress levels and increasing feelings of calmness in participants. However, future research still needs to address memory functioning since the 2002 study found decrements in memory whereas the 2003 study found improvements. Continuing research would clarify the inconsistency of the previous results and add the existing research since no further research has been conducted since 2004 exclusively examining *Melissa officinalis*.

Another limitation of the three Kennedy et al. (2002, 2003 & 2004) studies is the low amount of participant response. Each study had a total of only 20 participants or less (one study had 18 participants) which can be considered a small sample size. Future research should include a larger sample size to help attain more data on how people of

different genders, ethnicities and ages react to *Melissa officinalis.* This would also allow for replication of the mood altering effects on a more varied population.

Another direction that would enhance the research regarding *Melissa officinalis* and the ecological validity of the drug's impact on memory functioning is incorporating a different test battery. Kennedy et al. (2002, 2003, & 2004) used the computerized version of the Cognitive Drug Research battery to assess neuropsychological functioning of the participants. This battery was developed by Keith Wesnes and consists of six different subtests that are designed to assess different areas of cognitive functioning such as short-term memory, processing speed, and recognition. The test is designed to be used for those roughly between the ages of 62 and 85 (Wesnes, 1987). However, the studies conducted by Kennedy et al. (2002, 2003, & 2004) involved participants between the ages of 18 and 30 years old. Using the battery for an age range that it was not designed for could lead to flaws in the validity and accuracy of the results attained from the tests. Furthermore, the reliability and validity of this test battery is not stated in any of the Kennedy studies which can be another cause for concern. Future research should incorporate the use of tests that are appropriate for the age ranges being studied and have research regarding the reliability and validity of the tests.

Another possible confound which future research should achieve to reduce and or eliminate is practice effects and participant fatigue. While the Kennedy et al. (2002 & 2003) studies assess changes in neuropsychological functioning over time, the time span in between each time interval may have been too short. Each participant was assessed using the exact same tests before drug administration and 1hour, 2 hours, 4 hours and 6

hours or 1 hour, 3hours, and 6hours after drug administration. It is possible that participants may have found different subtests of the battery to be easier to complete due to the frequency of being tested and familiarity with the materials which is known as practice effects. Both of these studies found decrements in memory functioning on the tasks that measured working memory, visuospatial memory, and processing speed. Instead of the drug causing these deficits, it may be that the participants became fatigued due to being tested for an extensive period of time. Decreasing the amount of time the participants are being tested in addition to using different forms or versions of the same subtest as opposed to identical forms may prove useful in future research.

A final area of improvement which needs to be addressed in future research is the tools that will be used to measure the levels of anxiety of the participants. Kennedy et al. (2002, 2003, & 2004) used the Bond-Lader Visual Analog Scales to assess the mood of participants before and after administration of *Melissa officinalis*. However, these scales were not designed to measure anxiety specifically. Therefore, future research should consider using scales specifically constructed to measure more thoroughly one type of emotion or state.

Purpose of Study

The purpose of this study was to add to the literature on the cognitive and mood altering effects of *Melissa officinalis* using improved methodology, specifically using a within-subjects design using alternate forms. A larger sample size increases population variance and allows for the results to be more generalizable. The current study also reduced the amount of time the participants were tested, approximately two hours total

instead of 6 hours, which reduces fatigue and practice effects. The overall goal of the current study was to increase existing literature on the use, possible benefits, and the cognitive side effects of *Melissa officinalis* while improving upon the methodology used to study this drug.

Hypotheses

The following hypotheses were proposed:

1. Participants were predicted to decreased levels of anxiety as indicated by lower raw scores on the SSAI post administration of the *Melissa officinalis*. No change in levels of anxiety was expected in those given the placebo. This is supported by the results of previous studies conducted by Kennedy et al. (2002, 2003 & 2004).

2. Participants given 1500 mg were predicted to perform better as compared to those given 500 mg of *Melissa officinalis* on the Digit Span forward and backward subtests. Those given 500 mg were predicted to perform better than those given the placebo. No change in scores was expected between pre and post drug administration for the placebo group.

3. Participants given 1500 mg were predicted to perform better as compared to those given 500 mg on the Hopkins Verbal Learning Test-Revised. Both of these groups wereexpected to perform better as compared to those given the placebo.

4. Participants given 1500 mg *Melissa officinalis* were predicted to perform better than those given 500 mg and the placebo on the Logical Memory I and II subtests of the WMS-III post drug administration as compared to pre drug administration. Those given the 500mg of *Melissa officinalis* were predicted to perform worse than those given the

placebo post drug administration. No change in scores wasexpected between pre and post drug administration for those given the placebo.

5. Participants given 1500 mg were predicted to perform worse as compared to those given the 500 mg of *Melissa officinalis* on the Symbol Digit Modalities test post drug administration. However, both groups given *Melissa officinalis* were expected to be more accurate as compared to the placebo. No change is expected in scores between pre and post drug administration to those given the placebo.

6. Participants given the 500 and 1500mg dosages of *Melissa officinalis* were predicted to improve on the Stroop task and Verbal Fluency post drug administration. Participants were expected to produce more words for the letters given post drug administration compared the amount of words given pre drug administration. Participants also were predicted to produce more correct responses on the third portion of the Stroop task. Those given the placebo would display no difference in performance on either task.

CHAPTER II

METHOD

Participants

Participants were recruited from the Middle Tennessee State research pool. Participants consisted of 54 undergraduate women between the ages of 18 and 26 years old (M = 19.69, SD = 1.59). Twenty-two of whom identified as Caucasian, 28 as African American, three as Hispanic, and one as Asian. Participants were excluded if they were currently taking psychotropic medication (to prevent any interactions with the *Melissa officinalis*). All participants were instructed to abstain from products containing caffeine during the day of the experiment and to abstain from alcohol 12 hours prior to the day of the experiment. Any participants with a current anxiety disorder, depressive disorder, had been diagnosed with ADHD, and or who had suffered a major head injury or other neurological illness were excluded from the study.

Measures

Spielberger State-Trait Anxiety Scale (State Anxiety only) (Speilberger et al., 1983)**.** The Spielberger State-Trait Anxiety Inventory (STAI) was designed to measure the state anxiety and trait anxiety of someone through self-report with a total 40 items where 20 items assess state anxiety and 20 items assess trait anxiety. The first 20 items assess one's state anxiety (their level of anxiety at that particular moment). The items are rated using a 4 point scale ranging from 1 = "*Not at all*" to 4 = "*Very Much So*". The dependent variable will be the participant's score where higher scores indicate greater anxiety (Spielberger, et al., 1983). The average internal consistency of the State Anxiety scale was .91 and the average internal consistency of the Trait Anxiety scale was .89.

Barnes, Harp and Jung (2002) concluded that the STAI is a reliable measure and applicable for use in several different populations.

Wechsler Test of Adult Reading (Wechsler, 2001). The WTAR is a brief measure designed to assess premorbid intellectual functioning. The examiner places a card with a word printed on it in front of the examinee and instructs him or her to pronounce the word aloud. The examiner continues to administer more cards until the test is complete or the discontinuation criterion has been met. The discontinuation criterion is fulfilled when the examinee mispronounces 12 words consecutively. The dependent variable was the number of words pronounced correctly. The WTAR has been shown to have good internal consistency reliability with coefficients as high as .90. The test also displays adequate test-retest reliability with correlations greater than .90 (Wechsler, 2001).

Digit Span Forward and Backward subtests of the WAIS-IV (Wechsler, 2008). The forward and backward trials of the Digit Span subtest of the WAIS-IV are designed to measure the working and short-term memory of an individual. It can be said that this test also measures verbal and auditory memory as these numbers are read aloud. The examiner reads of a string of numbers at the rate of one number per second and once they are finished, the participant is required to repeat as many as they can remember and in the correct sequence back to the examiner. In the forward trials, participants repeated the numbers verbatim and in the backwards trials, participants repeated the numbers in the reverse order in which they were given. The first trial of both formats begins with a sequence of two numbers and increases each time with one more number until the longest trial which has nine numbers. The dependent variable for the current study was the

number of trials the participant recalled correctly. The more trials the examinee can remember correctly, the higher the score. There are two trials for each string of digits. These trials were divided to create two different versions of the Digit Span Forward and Backward subtest. These two different versions were counterbalanced into pre and post drug administration tests. The WAIS-IV has excellent reliability with coefficients of .92 for the Working Memory index of the entire battery and more specifically, the Digit Span subtest has an average internal consistency reliability coefficient of .93 (Sattler & Ryan, 2009). The WAIS-IV has been shown to have good criterion validity when the Working Memory index is correlated with the WAIS-III Working Memory Index (r =.86) (Sattler & Ryan, 2009).

Hopkins Verbal Learning Test-Revised (Forms 1 and 3) (Brandt & Benedict, 2001). The Hopkins Verbal Learning Test – Revised is a measure of short-term and long-term memory. The test consists of a list of 12 words and participants are given three trials to remember as many words as they can. The last trial is followed by a delay of 20 -25 minutes after which participants are asked to recall as many words as they can remember. The last portion of the test requires participants to identify the 12 words they learned from a list of 24 words. The dependent variables were the number of words the participant remembers immediately across the three trials, the total amount of words recalled after the delay and the number of words correctly identified during the recognition task. Benedict et al. (1998) administered the six different forms to college students over a period of 6 weeks and found that the forms were equivalent in regards to the learning trials. The test also has been proven to have good convergent validity with

other measures of verbal short-term and long-term memory. Lacritz et al. (2001) found a correlation of .62 between the HVLT-R and the California Verbal Learning Test on the delayed recall trial. This indicates that the HVLT-R is valid in measuring short-term and long-term memory.

Logical Memory I and II Subtests of the Wechsler Memory Scale-III (Wechsler, 1997). The Logical Memory subtests of the WMS-IV are a measure of verbal long-term and short term-memory. For this subtest, the examiner reads two short stories to the examinee and then the examinee is required to immediately recall as much information as they can remember about the two stories and then again after a 20 minute delay. The dependent variables will be the amount of information they recall immediately and after the delay. This specific subtest has been shown to have good reliability with coefficients ranging from .86 to .88 for Logical Memory I and coefficients of .73 to .76 for Logical Memory II (The Psychological Corporation, 1997).

Symbol Digit Modalities (Form B and Form D). (Smith, 1991). The Symbol Digit modalities test is a measure of attention and processing speed. The participant is given a piece of paper with a coding key located at the top. The top row of the coding key has symbols and the bottom row has a number that corresponds to each symbol. The rest of the page contains rows with only the symbols given. The participant is required to fill in as many spaces as possible in the bottom rows with the correct number in 90 seconds. The dependent variable in this study was the number of boxes filled in correctly within the given time limit. The test has been shown to have high test-retest reliability in regards to short time intervals of 29 days (.80) and longer time intervals of 6 months (.79)

(Uchiyama et al., 1994). Forms B and D were used in the current study for pre administration and post administration. Hinton Bayre and Geffen (2005) found the four different forms to be highly correlated with each other (.91, .73, .68, .71) when used to assess cognitive functioning in those who had experienced a concussion.

*Verbal Fluency (FAS, PRW, animals, and fruits and vegetables) (*Benton, Hasher & Sivan, 1994) (Gladsjo, Schuman, Miller, & Heaton, 1999). Verbal fluency is also used to measure executive functioning. The researcher provides the participant with a letter of the alphabet and then the person says aloud as many words as they can think of in one minute that begin with that letter. Participants may also be given a specific category such as animals in which the participant says aloud as many animals as they can think of in one minute. Participants will be given the letters "F", "A", "S" or "P", "R", "W" depending upon the order in which they are counterbalanced. Participants will be given the semantic category of animals or fruits and vegetables depending upon the order in which they are counterbalanced. Rosen and Engle (1997) suggest that a measure of verbal fluency reflects a person's inhibition and self-monitoring ability because they must restrict their responses based on rules. This test also can measure processing speed as this can be evaluated based upon the number of words produced. The dependent variable in the current study was the number of words produced during the given time periods. There has been much research regarding the reliability and validity of the different versions of this test. Tombaugh et al. (1999) found the letters "F", "A", and "S" to have a high internal consistency reliability coefficient of .83 and "animal naming" to have a significant correlation of .52 with the letters "F", A", and "S". Dikmen et al. (1999)

found there to be adequate alternate forms reliability between the FAS and BDT versions with a reliability coefficient of .72. Cohen and Stanczak (2000) found high validity between the FAS and PRW version of the test with a correlation of .81.

Stroop (Golden Version) (Golden & Freshwater, 2002). The Stroop test is a measure of executive functioning and cognitive flexibility. The test consists of three parts: In the first part of the test, participants read the words "Blue", "Green", or "Red" printed in black ink aloud while the researcher records how many words are said correctly within 45 seconds. Participants thenare presented with "X"s in red, green, or blue ink and say the color of the ink as the researcher records the number of correct responses within 45 seconds. In the final part of the test, participants are presented with the words "Blue", "Green", or "Red" but the word is printed in a different color of ink than what the word actually is. Participants say aloud the color of ink or the word, depending upon what the researcher chooses, as the researcher records correct responses for 45 seconds. The person's score on the third portion of the test represents their ability to inhibit their response which is an indication of one's executive functioning ability. The dependent variable in this study was the number of words or colors said correctly, depending on the portion of the test being administered, within the given time limits. Higher scores indicate strong executive functioning whereas lower scores indicate impairment. The Golden Version of the Stroop test has been evaluated to be a reliable measure with test-retest reliability coefficients of .89 (Word), .84 (Color), and .73 (Color-Word) (Golden, 1975).

Procedure

Approval was obtained from the Institutional Review board at Middle Tennessee State University prior to recruiting participants (see Appendix A). Consent was obtained from each participant prior to neuropsychological testing (see Appendix B). The participants were also given a brief demographics questionnaire that included questions about the participant's psychiatric/mental history. (See Appendices C and D). Any person with a disorder or taking any medications were excluded if they answered "yes". Each participant was tested for approximately 2.25 hours and all neuropsychological tests were administered according to standardized procedures in counter balanced order to obtain a baseline measurement of the person's functioning. Alternate forms of each neuropsychological test were given in counterbalanced order to reduce potential order effects as a possible confound. Once pre-assessment was complete, the research assistant randomly assigned participants to one of three groups: Placebo, 500 mg or 1500 mg of *Melissa officinalis*. The primary researcher and the participant were blind to the medication. Once the *Melissa officinalis* or placebo was administered, participants then read "Crow Killer: The Saga of Liver-Eating Johnson" for 45 minutes. Then all post assessments were administered in counterbalanced order. Finally, participants took a brief quiz over the material they read from "Crow Killer: The Saga of Liver-Eating Johnson." Participants then signed debriefing statement and were awarded the appropriate amount of class credit for participation.

CHAPTER III

RESULTS

Initial Analyses

Initial analyses were conducted to determine if there were any differences in age, years of education, body mass index, and scores on the Wechsler Test of Adult Reading between the placebo group, those given 500 mg, and those given 1500mg. Descriptive Statistics for demographic variables of each group are shown in Table 1. (See Appendix E). All analyses were conducted with alpha = .05. The results of a series of one way ANOVAs on each of these variables indicated no significant differences between the groups in age $F(2,51) = 1.23$, $p = 0.28$, years of education $F(2,51) = .57$, $p = 0.57$, body mass index $F(2,51) = 0.57$, $p = 0.77$, or their scores on the Wechsler Test of Adult Reading $F(2, 51) = .05$, $p = 0.95$.

Primary Analyses

Because the initial analyses indicated no significant group differences in age, years of education, body mass index, or WTAR scores, these variables were not included as covariates in subsequent analyses. To analyze each of the neuropsychological variables of interest a series of 2 x 3 ANOVAs were conducted, with a between factor of Group (Placebo, 500mg, 1500mg) and a within factor of Time (Pre and Post). All analyses were conducted with alpha = .05.

Speilberger State Anxiety Scale. There was no significant interaction between the Time and Group for the scores on the SSAI, $F(2,51) = 1.97$, $p = 0.15$. The results indicated a significant main effect for Time, $F(1, 51) = 13.97$, $p < .001$, but no significant

main effect for Group, $F(2, 51) = 0.15$, $p = 0.87$. Descriptive statistics for this test can be found in Table 2 (See Appendix A).

Digit Span Forwards and Backwards. There was no significant interaction between Time and Group for the total score on the Digit Span Forward task, $F(2,51) = 0.07$, $p = 0.93$. The results indicated a significant main effect for Time, $F(1, 51) = 6.29$, $p = .015$, but no significant main effect for Group, $F(2, 51) = 0.71$, $p = 0.50$. There was no significant interaction between Time and Group for the total score on the Digit Span Backwards Task, $F(2,51) = 0.22$, $p = 0.80$. The results also indicated no significant main effect for Time, $F(1,51) = 0.38$, $p = 0.54$, and no significant main effect for Group, $F(2,51) = 0.99$, $p = 0.38$. Finally, there was no significant interaction between Time and Group for the combined score on the Digit Span Forward and Backward Tasks, $F(2,51) = 0.30$, $p = 0.74$. There was a significant main effect for Time, $F(1,51) = 4.41$, $p = .04$, but no significant main effect for Group, $F(2,51) = 0.68$, $p = 0.51$. Descriptive statistics for this measure can be found in Table 3 (See Appendix A).

Hopkins Verbal Learning Test – Revised. There was no significant interaction between Time and Group for the amount of words recalled across the first three trials, $F(2, 51) = 0.34$, $p = 0.72$. The results also indicated no significant main effect for Time, $F(1,51) = 3.25$, $p = 0.08$, and no significant main effect for Group, $F(2,51) = 0.18$, $p = 0.84$. There was no significant interaction between Time and Group for the amount of words recalled after a 20 minute delay, $F(2, 51) = 0.54$, $p = 0.59$. The results also indicated no significant main effect for Time, $F(1,51) = 1.61$, $p = 0.29$, and no significant main effect for Group, $F(2,51) = 0.22$, $p = 0.80$. There was no significant interaction

between Time and Group for the amount of information retained across the three trials, $F(2,51) = 1.65$, $p = 0.20$. The results indicated a significant main effect for Time, $F(1,51) = 4.55$, $p = .04$, but no main effect for Group, $F(2,51) = 1.08$, $p = 0.35$. There was no significant interaction between Time and Group for the amount of words recalled during the forced choice recognition task, $F(2,51) = 1.45$, $p = 0.24$. The results also indicated no significant main effect for Time, $F(1,51) = 3.05$, $p = 0.08$, or Group $F(2,51) = 0.47$, $p = 0.63$. Descriptive statistics for this measure can be found in Table 3 (See Appendix A).

Logical Memory I and II. There was no significant interaction between Time and Group for the amount of information recalled on the Logical Memory I task, $F(2,51) = 0.13$, $p = 0.88$. The results indicated a significant main effect for Time, $F(1,51) = 9.53$, $p = .003$, but no significant main effect for Group, $F(2,51) = 0.15$, $p = 0.86$. There was no significant interaction between Time and Group for the amount of information recalled on the Logical Memory II task, $F(2,51) = 0.51$, $p = 0.61$. The results also indicated no significant main effect for Time, $F(1,51) = 3.60$, $p = 0.06$, or Group, $F(2,51) = 0.06$, $p = 0.95$. Descriptive statistics for this measure can be found in Table 3 (See Appendix A).

Symbol Digit Modalities. There was no significant interaction between Time and Group for the total score, $F(2,51) = 0.02$, $p = 0.98$. The results also indicated no significant main effect for Time, $F(1,51) = 4.20$, $p = 0.05$, or Group, $F(2,51) = 1.64$, $p = 0.21$. Descriptive statistics for this measure can be found in Table 4 (See Appendix A).

Verbal Fluency. There was no significant interaction between Time and Group for the amount of words given per letter, $F(2,51) = 0.80$, $p = 0.45$. The results indicated a significant main effect for Time, $F(1,51) = 18.28$, $p < .001$, but no significant main effect

for Group, $F(2,51) = 2.07$, $p = 0.14$. There was no significant interaction between Time and Group for the amount of words given per category, $F(2,51) = 2.42$, $p = 0.10$. The results also indicated no significant main effect for Time, $F(1,51) = 0.10$, $p = 0.77$ or Group, $F(2,51) = 0.58$, $p = 0.56$. Descriptive statistics for this measure can be found in Table 4 (See Appendix A).

Stroop (Golden Version). There was no significant interaction between Time and Group for the amount of colors correctly named during the "Color-Word" portion of the Stroop task, $F(2, 51) = 0.32$, $p = 0.73$. The results indicated a significant main effect for Time, $F(1,51) = 13.30$, $p = .001$, but no significant main effect for Group, $F(2,51) = 0.29$, $p = 0.75$. Descriptive statistics for this measure can be found in Table 4 (See Appendix A).

Crow Killer Quiz. The results of a one way ANOVA indicated no significant differences between the groups on the percentage of questions answered correctly post drug administration, $F(2,51) = 0.90$, $p = 0.41$.

CHAPTER IV

DISCUSSION

The data from the current study were used to assess several hypotheses. It was predicted that participants given *Melissa officinalis* would show decreased levels of anxiety on the SSAI post drug administration as compared to those given the placebo. There would also be no difference in anxiety levels pre and post drug administration for those in the placebo group. However, the results of this study indicated that *Melissa officinalis* had no effect on anxiety.

Several hypotheses also were formulated regarding the effect of *Melissa officinalis* on memory functioning. It was first predicted that those given 1500 mg of *Melissa officinalis* were to perform better than those given 500mg on the Digit Span Forward and Backwards subtests followed by 500 mg group in performance. No change in scores was expected for those given the placebo. It also was predicted that participants given 1500 mg would perform better than those given 500 mg and that both of these groups would perform better than those given the placebo pre and post drug administration on the Hopkins Verbal Learning Test – Revised. Finally, it was predicted that participants given 1500 mg were to show the greatest improvement on their scores for the Logical Memory I and II subtests and those given 500mg were expected to perform worse than those given the placebo. However, the results of the analyses did not support any of these hypotheses, indicating that *Melissa officinalis* has no effect on memory functioning.

There were also a number of hypotheses regarding the effects of *Melissa officinalis* on tests of executive functioning, processing speed and verbal fluency were evaluated. Specifically, it was predicted that participants given 1500 mg would perform worse as compared to those given 500 mg on the Symbol Digit Modalities task. It was predicted that participants given 1500mg or 500 mg would produce a greater amount of words on the Verbal Fluency tests as compared to the placebo group. The last hypothesis evaluated predicted that those given 500mg or 1500 mg would show improved performance on the "Color-Word" portion of the Stroop task. The results produced from the repeated measures ANOVAs did not support the hypotheses related to these tests.

Overall the results from this study did not support the previous research conducted by Kennedy et al. (2002, 2003, & 2004). Rather, the present results are contradictory. The inconsistency and difference in the findings of the current study could be due to several factors. One of these factors could be due to the dosage levels that were used. All three studies conducted by Kennedy et al. (2002, 2003, & 2004) used dosage levels of 300 mg, 600 mg, 900 mg, 1000 mg, or 1600 mg of *Melissa officinalis*. The current study only used 500 and 1500mg. Therefore, it is possible that the use of different dosages caused discrepant findings between the present study and those reported by Kennedy et al. However, the differences in dosages was rather negligible and it is difficult to believe that this caused the variability in findings across investigations.

Another factor to consider is the state of the participants at the beginning of the experiment. Kennedy et al. (2004) created a state of stress in their participants before beginning any type of cognitive task via the DISS (Defined Intensity Stressor Simulation)

Computerized Battery. The current study did not employ any method to create a sense of tension or stressfulness in the participants before neuropsychological testing began. There were also two participants that reached their basal level on the SSAI (i.e., obtained the lowest score possible) before drug administration had begun. In fact, the average score on the SSAI pre drug administration was 32.81 which is not considered in the clinical range (Julian, 2001).Therefore, it is possible that *Melissa officinalis* may only be beneficial to those with high levels of anxiety or those in an anxious state as Kennedy et al. (2004) obtained significant results when this method was used.

Another factor to consider is the amount of time that it takes for *Melissa officinalis* to take effect in one's system. Kennedy et al. (2002 & 2003) found significant differences in performance on certain cognitive tasks 2.5, 4, and 6 hours post drug administration. Significance for changes in mood were found 6 hours post drug administration. The current study included a period of 45 minutes where no neuropsychological measures were administered to allow for the drug to become effective which may not have been enough time for the drug to fully take effect.

Another factor that could have contributed to the discrepancy between the previous research and the current study is the motivation behind the participants' performance on the cognitive tasks that were given to them. The current study collected data from undergraduate college students in exchange for extra credit or to earn research credit for their General Psychology class. Further examination of participant's raw scores indicated that nine participants in the current study performed in the first percentile compared to other people their age on the HVLT-R and another eight

participants scored in the 5th percentile. Therefore, it is possible that the differences in motivation in one third of the sample could have introduced a large amount of variance in the sample and be a contributing factor to the discrepancy in findings between this study and the Kennedy et al (2002, 2003, & 2004) studies.

Although the aforementioned differences may explain the discrepant findings between the present study and those of Kennedy et al. (2002, 2003, & 2004), the possibility also must be considered that *Melissa officinalis* actually has no effect on memory and cognitive functioning. It is important to note that there was a significant main effect for Time across all groups on their scores on the SSAI and every neuropsychological test administered, with the exception of the Symbol Digit Modalities task. This means that each group experienced a reduction in anxiety regardless of the type of drug given. The present study was interested in examining the effect of reduced anxiety across groups, however the results do not allow this for everyone experienced a reduction in anxiety.

The present investigation represents an improvement in methodology as compared to the previous investigation. For instance, whereas the Kennedy et al. (2002, 2003, & 2004) collected data from 20 participants, the current study nearly tripled the amount of data collected, with a total of 54 participants. The measures used in the current study also had different forms which reduced the possibility of practice effects. Previous research does not state if different forms were utilized between pre and post drug administration and each testing session following post drug administration.

Additionally, the current study is also unique compared to the previous research due to the type of measures that were used. The current study is the only study to date to use actual neuropsychological measures that were specifically designed to assess memory, executive functioning, processing speed, and verbal fluency. All measures used had well established reliability and validity values whereas the previous research utilized measures that were tailored for that specific study, such as in the Kennedy et al. (2003) study. The current study also used a measure specifically designed to assess levels of anxiety in participants as compared to previous research that used measures that assessed a person's level of "calmness", and "alertness" which are not specifically related to those suffering from medically defined anxiety. These strengths of the present study buttress the possibility that *Melissa officinalis* actually has no effect on memory and cognitive functioning.

There are also several limitations that exist in the study that should be mentioned. The greatest limitation in this study pertains to the methodology. Among the most common mental disorders that is frequently co-morbid with anxiety is depression. The current study only assessed anxiety in participants. Although the current study was assessing specifically anxiety and memory functioning, it is possible that one group may have been more significantly depressed than another group. High levels of depression can also cause impairment in cognitive functioning which could have caused the inconsistency in findings and a lack of significant difference between the groups. Measuring the participants' level of depression also could have improved upon the

previous research as well because no research has been conducted on *Melissa officinalis* effecting depression.

Another concern regarding the characteristics of the sample is that data from only women were collected. Previous research has shown that women are more prone to developing an anxiety disorder than men (McLean & Anderson, 2009). Although the sample sizes were small, previous research found significant differences between those given the placebo and those given the drug when both men and women were assessed (Kennedy et al.,2002; 2003; & 2004). It is possible that *Melissa officinalis* affects men and women differently which could have led to significant results in the previous research.

It also should be mentioned that the level of anxiety in participants pre-drug administration may have not been adequate for the *Melissa officinalis* to be beneficial. Previous research has shown *Melissa officinalis* to lower levels of alertness when participants were induced into an anxious state. The current study did not create an anxious sate in participants but just measured their current anxiety levels before the testing session began. Further examination of the participants' scores on the SSAI showed that only 13 participants in the current study obtained a score high enough for clinical levels of anxiety. It is possible that not enough participants with clinical levels of anxiety were assessed. To date, only a pilot study has been conducted on *Melissa officinalis* and anxiety using a clinical population. It is unclear if *Melissa officinalis* impacts those with high levels of anxiety or those only in an anxious sate.

Implications and Future Directions

The results of the current study suggest that *Melissa officinalis* does not impact levels of anxiety or improve cognitive functioning. They also suggest that there may not be as many beneficial properties of this drug as once previously postulated. However, due to the limitations previously discussed, the results of the current study are inconclusive which requires further investigation. Future studies should seek to improve upon the characteristics of the sample. Ideally, the sample size should consist of equal amounts of men and women for each group, and participants should be diagnosed with an anxiety disorder or experience high levels of state anxiety in the situations evaluated. This would allow for further insights as to how *Melissa officinalis* impacts both sexes and also in those in a clinical population. Future researchers also should incorporate the use of other mood measures such as the Beck Depression Inventory to eliminate the possibility of other factors impacting the results. However, given that it is quite difficult to achieve a sample size in a clinical population where only one mental disorder is present, any other clinical factors present could later be used as a covariate in analyses.

In conclusion, the current study expanded upon the previous research while helping gain insight to the direction of future research on the use of herbal supplements as opposed to synthetic drugs. Although the findings of the current study were not consistent with the previous research, it does help provide a better understanding of how herbal supplements impact cognitive functioning, if any at all. Inconsistencies in the measures and neuropsychological tools used to assess anxiety and cognitive functioning need to be addressed to establish more conclusive findings. Further investigation of how

this supplement impacts those with clinical anxiety can help develop a better understanding of the role GABA plays in anxiety and cognitive functioning while providing more possible treatment avenues for those with an anxiety disorder.

REFERENCES

Abdou, A. M., Higashiguchi, S., Horie, K., Kim, M., Hatta, A., & Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of γ-amino-butyric acid (GABA) administration in humans. *BioFactors, 26*, 201-208. doi: 10.1002/biof.5520260305

Airaksinen, E., Maria Larsson, M., & Forsell, Y. (2005). Neuropsychological functions in anxiety disorders in population-based samples: evidence of episodic memory dysfunction. *Journal of Psychiatric Research, 39,* 207-214. doi: 10.1016/j.jpsychires.2004.06.001

Allison, C. & Pratt, J. A. (2003). Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. *Pharmacology and Therapeutics, 98,* 171-195. doi:10.1016/S0163-7258(03)00029-9

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders: DSM-5.* (5th ed.). Washington, D.C: American Psychiatric Publishing.

Argyropoulos, S. V., Sandford, J. J., & Nutt, D. J. (2000). The psychobiology of anxiolytic drugs Part 2: pharmacological treatments of anxiety. *Pharmacology and Therapeutics, 88,* 213-277. doi.org/10.1016/S0163-7258(00)00083-8

Asmundson, G., Stein, M. B., Larsen, D. K., & Walker, J. R. (1995). Neurocognitive function in panic disorder and social phobia patients. *Anxiety, 1,* 201-207. doi: 10.1002/anxi.3070010503

Awad, R., Muhammed, A., Durst, T., Trudeau, V. L., & Arnason, J. T. (2009). Bioassay-guided fractionation of lemon balm (*melissa officinalis* l.) using an *in vitro* measure of GABA transaminase activity. *Phytotherapy Research, 23,* 1075-1081. doi: 10.1002/ptr.2712

Barnes, L.B., Harp, D. & Jung, W. S. (2002). Reliability generalization of scores on the speilberger state-trait anxiety inventory. *Educational and Psychological Measurement, 62,* 603-618. doi:10.1177/0013164402062004005

Benedict, R. H. B., Schretlen, D., Groninger, L., & Brandt, J. (1998). The hopkins verbal learning test-revised: normative data and analysis of interform and test-retest reliability. *The Clinical Neuropsychologist, 12,* 43-55. doi: abs/10.1076/clin.12.1.43.1726

Benton, A. L., Hamsher, K. deS., & Sivan, A. B. (1994). *Multilingual Aphasia Examination* (3rd ed.). San Antonio, TX: Psychological Corporation.

Beracochea, D. (2006). Anterograde and retrograde effects of benzodiazepines on memory. *The Scientific World Journal, 6,* 1460 – 1465. doi: 10.1100/tsw.2006.243

Boudaba, C., Schrader, L. & Tasker, J. G. (1997). Physiological evidence for local excitatory synaptic circuits in the rat hypothalamus. *Journal of Neurophysiology, 77,* 3396-3400. Retrieved from http://jn.physiology.org/content/jn/77/6/3396.full.pdf

Brandt, J., & Benedict, R. H. B. (2001). *Hopkins Verbal Learning Test-Revised.* Odessa, Fla.: PAR.

Buffet-Jerrott, S. E., Stewart, S. H., & Teehan, M. D. (1998). A further examination on the time- dependent effects of oxazepam and lorazepam on implicit and explicit memory. *Psychopharmacology, 138,* 344-353. doi: 10.1007/s002130050680

Cases, J., Ibarra, A., Fueillére, N., Roller, M., & Sukkar, S. G. (2011). Pilot trial of Melissa officinalis L. leaf extract in the treatment of volunteers suffering from mild-to-moderate anxiety disorders and sleep disturbances. *Mediterranean Journal of Nutrition Metabolism, 4,* 211–218. doi: 10.1007/s12349-010-0045-4

Castaneda, A.E., Suvisaari, J., Marttunen, M., Perälä, J., Saarni, S. I., Aalto-Setälä, T., Lönnqvist, J., & Tuulio-Henriksson, A. (2011). Cognitive functioning in a population-based sample of young adults with anxiety disorders. *European Psychiatry, 26,* 346-353. doi: 10.1016/j.eurpsy.2009.11.006

Charter, R. A., Adkins, T. G., Alekoumbides, A., & Seacat, G. F. (1987). Reliability of the WAIS, WMS, and Reitan Battery: Raw scores and standardized scores corrected for age and education. *International Journal of Clinical Neuropsychology, 9,* 28-32.

Cohen, M. J. & Stanczak, D. E. (2000). On the reliability, validity, and cognitive structure of the Thurstone Word Fluency Test. *Archives of Clinical Neuropsychology, 15,* 267-279.doi: S0887617799000177.

Davidson, K., Farquharson, R. G., Khan, M. C., & Majid, A. (1985). A double-blind comparison of alprazolam, diazepam and placebo in the treatment of anxious out-

patients. *British Journal of Clinical Pharmacology, 19,* 37S-43S. doi: 10.1111/j.1365-2125.1985.tb02741.x

Dikmen, S.S., Heaton, R. K., Grant, I., & Temkin, N. R. (1999). Test retest reliability and practice effects of expanded Halstead-Reitan neuropsychological test battery. *Journal of the International Neuropsychological Society, 5,* 346-356. doi: 10.1017/S1355617799544056

Golden, C. J. (1975). A group version of the Stroop Color and Word Test. *Journal of Personality Assessment, 39,* 502-506.

Golden, C. J., & Freshwater, S. M. (2002). *Stroop Color and Word Test: Revised examiner's manual.* Wood Dale, IL: Stoelting Co.

Gladsjo, J. A., Schuman, C. C. Miller, S. W., & Heaton, R. K. (1999). *Norms for letter and category fluency: Demographic corrections for age, education, and ethnicity.* Odessa, FL.: PAR.

Hinton-Bayre, A. & Geffen, G. (2005). Comparability, reliability, and practice effects on alternate forms of the digit symbol substitution and symbol digit modalities tests. *Psychological Assessment, 2,* 237-241. doi: 10.1037/1040-3590.17.2.237.

Julian, L. J. (2011). Measures of anxiety. *Arthritis Care and Research, 63,* S467-S472. doi: 10.1002/acr.20561.

Kennedy, D. O., Scholey, A. B., Tildesley, N. T. J., Perry, E. K., & Wesnes, K. A. (2002). Modulation of mood and cognitive performance following acute administration of Melissa Officinalis (lemon balm). *Pharmacology, Biology and Behavior, 72,* 953-964. doi: S0091305702007773.

Kennedy, D. O., Wake, G., Savelev, S., Tildesley, N. T. J., Perry, E. K., Wesnes, K. A., & Scholey, A. B. (2003). Modulation of mood and cognitive performance following acute administration of single doses of melissa officinalis (lemon balm) with human cns nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology, 28,* 1871-1881. doi: 10.1038/sj.npp.1300230.

Kennedy, D. O., Little, W., & Scholey, A. B. (2004). Attenuation of laboratory-induced stress in humans after acute administration of melissa officinalis (lemon balm). *Psychosomatic Medicine, 66,* 607–613. doi: 10.1097/01.psy.0000132877.72833.71.

Lacritz, L. H., Cullum, M. C., & Weiner, M. F. (2001). Comparison of the hopkins verbal learning test-revised to the california verbal learning test in alzheimer's disease. *Applied Neuropsychology, 8,* 180-184. doi: 10.1207/S15324826AN0803 8

Lader, M. (1991). History of benzodiazepine dependence. *Journal of Substance Abuse Treatment, 8,* 53-59. Retrieved from http://www.benzo.org.uk/amisc/lader.pdf

Lavric, A., Rippon, G., & Gray, J. R. (2003). Threat-evoked anxiety disrupts spatial working memory performance: an attentional account. *Cognitive Therapy and Research, 27,* 489-504. doi: 10.1023/A:1026300619569

López-Muñoz, F., Ucha-Udabe, R., & Alamo, C. (2005). The history of barbiturates a century after their clinical introduction. *Neuropsychiatric Disease and Treatment, 4,* 329-343. doi: PMC2424120

Lucki, I., Rickels, K., Giesecke, M. A., & Geller, A. (1987). Differential effects of the anxiolytic drugs, diazepam and buspirone, on memory function. *British Journal of Clinical Pharmacology, 23,* 207-211. doi:10.1111/j.1365-2125.1987.tb03031.x

MacLeod, C. M., Dekaban, A., S., & Hunt, E. (1978). Memory impairment in epileptic patients: Selective effects of phenobarbital concentration. *Science, 202,* 1102-1104. doi: 10.1126/science.715461

Malizia, A. L., Cunningham, V. J., Bell, C. J., Liddle, P. F., Jones, T. & Nutt, J. (1998). Decreased brain $GABA_A$-Benzodiazepine receptor binding in panic disorder. *Arh Gen Psychiatry, 55,* 715720. doi:10.1001/archpsyc.55.8.715.

Manetlla, R. C., Butters, M. A., Dew, M. A., Mulsant, B. H., Begley, A. E., Tracey, B., Shear, M. K., Reynolds C. F., Lenze, E. J. (2007). Cognitive impairment in late-life generalized anxiety disorder. *American Journal of Geriatric Psychiatry, 15,* 673-679. doi: http://dx.doi.org/10.1097/JGP.0b013e31803111f2.

McLean, C. P., & Anderson, E. R. (2009). Brave men and timid women? A review of the gender differences in fear and anxiety. *Clinical Psychology Review, 29, 496-505.* doi:10.1016/j.cpr.2009.05.003.

Michels, L., Martin, E., Klaver, P., Edden, R., Zelaya, F., Lythgoe, D. J., Lu¨ chinger, R., Brandeis, D. & O'Gorman, R. L. (2012). Frontal GABA levels change during working memory. *Public Library of Science, 7,* 1-8.
doi: 10.1371/journal.pone.0031933

Montgomery, S. A., Huusom, A. T., & Bothmer, J. (2004). A randomised study comparing escitalopram with venlafaxine xr in primary care patients with major depressive disorder. *Neuropsychobiology, 50,* 57-64. doi: 10.1159/000078225.

Meyer, J. S. & Quenzer, L. F. (2005). *Psychopharmacology: Drugs, the brain and behavior.* Sunderland, Massachusetts: Sinauer Associates, Inc.

Pineda, D. A., & Merchan, V. (2003). Executive function in young Colombian adults. *International Journal of Neuroscience, 113,* 397-410. doi: 10.1080/00207450390162164

Rosen, V. M., & Engle, R. W. (1997). The role of working memory capacity in retrieval. *Journal of Experimental Psychology General:, 126,* 211-227. doi: 10.1037/0096-3445.126.3.211

Sander, S. K., Morzorati, S. L., Shekar, A. (1995). Priming of experimental anxiety by repeated subthreshold GABA blockade in the rat amygdala. *Brain Research, 699,* 250-259. doi: 10.1016/0006-8993(95)00915-D

Sattler, J. M. & Ryan, J. J. (2009). Assessment with the WAIS-IV. *Wechsler Adult Intelligence Scale – Fourth Addition (WAIS-IV): Description* (pp. 31-80). La Mesa, California: Jerome M. Sattler, Publishing, Inc.

Schulz, D. W. & Macdonald, R. L. (1981). Barbiturate enhancement of gaba-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Research, 209,* 177-188. doi: 10.1016/0006-8993(81)91179-3

Sherif, F. M. & Ahmed, S. S. (1995). Basic aspects of gaba transaminase in neuropsychiatric disorders. *Clinical Biochemistry, 28,* 145-154. doi: 10.1016/0009-9120(94)00074-6

Smith, A. (1991). *Symbol Digit Modalities Test.* Los Angeles, CA: Western Psychological Services.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press. Retrieved from http://www.apa.org/pi/about/publications/caregivers/practice-settings/assessment/tools/trait-state.aspx

Stahl, S. M. (2002). Don't ask, don't tell, but benzodiazepines are still the leading treatments for anxiety disorder. *Journal of Clinical Psychiatry, 63,* 756-757. Retrieved from http://www.psychiatrist.com/jcp/article/Pages/2002/v63n09/v63n0901.aspx

Strauss, E., Sherman, E. M. S., & Spreen, O. (2006). *A compendium of neuropsychological tests: Administration, norms and commentary.* New York, New York: Oxford University Press.

Tansella, Z. C., Tansella, M., & Lader, M. (1979). A comparison of the clinical and psychological effects of diazepam and amylobarbitone in anxious patients. *British Journal of Clinical Pharmacology, 7,* 605-611. doi: /10.1111/j.1365-2125.1979.tb04650.x/pdf.

Tempesta, D., Mazza, M., Serroni, N., Moschetta, F.S., Giannantonio. M. D., Ferrara, M., & De Berardis, D. (2103). Neuropsychological functioning in young subjects with generalized anxiety disorder with and without pharmacotherapy. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 45,* 236–241. doi: 10.1016/j.pnpbp.2013.06.006.

Tombaugh, T. N., Kozak, J., & Rees, L. (1999). Normative data stratified by age and education for two measures of verbal fluency: FAS and animal naming. *Archives of Clinical Neuropsychology, 14,* 167-177. doi:10.1016/S0887-6177(97)00095-4

Tiihonen, J., Kuikka, J., Ra¨sa¨nen, P., Lepola, U., Koponen, H., Liuska, A., Lehmusvaara, A., Vainio, P., Ko¨no¨ nen, M., Bergstro¨m, K., Yu, M., Kinnunen, I., Åkerman, K., & Karhu, J. (1997). Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: a fractal analysis. *Molecular Psychiatry, 2,* 463-471. Retrieved from https://www.nature.com/mp/journal/v2/n6/pdf/4000329a.pdf.

Uchiyama, C. L., D'Elia, L. F., Delinger, A. M., Selnes, 0. A., Becker, J. T., Wesch, J. E., Chen, B. B., Satz, P., Van Gorp, W., & Miller, E. N. (1994). Longitudinal comparison of alternate versions of the symbol digit modalities test: Issues of form comparability and moderating demographic variables. *The Clinical Neuropsychologist, 8,* 209-218. doi: 10.1080/13854049408401558

Wafford, K. A. (2005). $GABA_A$ receptor subtypes: any clues to the mechanism of benzodiazepine dependence? *Current Opinion in Pharmacology, 5,* 47-52. doi: 10.1016/j.coph.2004.08.006.

Wechsler, D. (1997). *Wechsler memory scale–third edition*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. (1997). *WMS-III administration and scoring manual.* San Antonio, TX: The Psychological Corporation.

Wechsler, D. (2001). *Wechsler test of adult reading*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. (2008). *Wechsler adult intelligence scale- fourth edition.* San Antonio , TX: The Psychological Corporation.

Wesnes, K., Simmons, D., Rook, M., & Simpson, P. (1987). A double-blind placebo controlled trial of tanakan in the treatment of idiopathic cognitive impairment in the elderly. *Human Psychopharmacology, 2,* 159-169. doi: 10.1002/hup.470020305

Zimmerman-Tansella, C., Tansella, M., & Lader, M. (1979). Psychological performance in anxious patients treated with diazepam. *Neuro-Psychopharmacology, 3,* 361-368. doi:10.1016/0364-7722(79)90047-X

APPENDICES

APPENDIX A

IRB Approval Letter

Monday, June 06, 2016

Investigator(s): Cara Otte; Paul Foster
Investigator(s') Email(s): clo3m@mtmail.mtsu.edu; Paul.Foster@mtsu.edu
Department: Psychology
Study Title: Effects of the Administration of Melissa officinalis on memory and Anxiety
Protocol ID: 16-2258

Dear Investigator(s),

The above identified research proposal has been reviewed by the MTSU Institutional Review Board (IRB) through the EXPEDITED mechanism under 45 CFR 46.110 and 21 CFR 56.110 within the category (1) Clinical studies of drugs and medical devices A summary of the IRB action and other particulars in regard to this protocol application is tabulated as shown below:

IRB Action APPROVED for one year from the date this notification

Date of expiration 6/6/2017

Participant Size 54

Exceptions

Restrictions :Participants will be female between the ages of 18 and 26 years old. Inclusion criteria include no history of head injury, no neurological illness, and not taking any psychotropic medications. Additional exclusions: Anyone pregnant or nursing, taking sedatives, taking medication to regulate thyroid, or taking medications for HIV.

Comments

This protocol can be continued for up to THREE years (6/6/2019) by obtaining a continuation approval prior to 6/6/2017. Failure in obtaining an approval for continuation will automatically result in cancellation of this protocol. Moreover, the completion of this study MUST be notified to the Office of Compliance in order to close-out the protocol.

The investigator(s) indicated in this notification should read and abide by all of the post-approval conditions imposed with this approval. Refer to the post-approval guidelines posted in the MTSU IRB's website. Any unanticipated harms to participants or adverse events must be reported to the Office of Compliance at (615) 494-8918 within 48 hours of the incident. Amendments to this

protocol must be approved by the IRB. Inclusion of new researchers must also be approved by the Office of Compliance before they begin to work on the project.

All of the research-related records, which include signed consent forms, investigator information and other documents related to the study, must be retained by the PI or the faculty advisor (if the PI is a student) at the secure location mentioned in the protocol application. The data storage must be maintained for at least three (3) years after study completion. Subsequently, the researcher may destroy the data in a manner that maintains confidentiality and anonymity. IRB reserves the right to modify, change or cancel the terms of this letter without prior notice. Be advised that IRB also reserves the right to inspect or audit your records if needed.

Sincerely,

Institutional Review Board

Middle Tennessee State University

Quick Links:

Click here for a detailed list of the post-approval responsibilities. More information on expedited procedures can be found here.

APPENDIX B

Informed Consent

Principal Investigator: Cara L. Otte
Study Title: The Effect of Melissa officinalis on Memory and Anxiety.
Institution: Middle Tennessee State University

Name of participant: _____
Age: _____

The following information is provided to inform you about the research project and your participation in it. Please read this form carefully and feel free to ask any questions you may have about this study and the information given below. You will be given an opportunity to ask questions, and your questions will be answered. Also, you will be given a copy of this consent form.

Your participation in this research study is voluntary. You are also free to withdraw from this study at any time. In the event new information becomes available that may affect the risks or benefits associated with this research study or your willingness to participate in it, you will be notified so that you can make an informed decision whether or not to continue your participation in this study.

For additional information about giving consent or your rights as a participant in this study, please feel free to contact the MTSU Office of Compliance at (615) 494-8918.

1. **Purpose of the study:**
   You are being asked to participate in a research study because we are interested in investigating how taking Melissa officinalis affects memory and cognitive functioning.

2. **Description of procedures to be followed and approximate duration of the study:**
   The study should take approximately 2.0 hours to complete. You will first be asked to complete a demographics questionnaire where you will indicate current height and weight and complete a questionnaire regarding your medical history. Immediately following, you will be given an anxiety measure followed by a series of tests of memory and cognitive functioning, after which you will be given either 500mg or 1500mg of Melissa officinalis, or a placebo. After taking one of these you will be asked to read a book for 50 minutes. Following this, you will be asked to once again complete the tests of memory and cognitive functioning.

   Melissa officinalis is typically taken by individuals to increase mental functions and mood.
   You must not participate if you meet any of the items listed on the Melissa officinalis Checklist that was handed to you. If you meet any of the items on the Checklist and still take Melissa officinalis then you might experience problems with sleepiness and drowsiness and possibly other medical problems. There are no data regarding the safety or the effects of taking Melissa officinalis for women who are pregnant or nursing.

3. **Expected costs:**
   none

4. **Description of the discomforts, inconveniences, and/or risks that can be reasonably expected as a result of participation in this study:**
   Studies have been conducted with Melissa officinalis that have reported no adverse effects of this supplement, which is available over-the-counter in many

pharmacies and vitamin shops. However, some have reported side effects of upset stomach, nausea, vomiting, wheezing, and dizziness.
These side effects, if experienced, are also temporary.

The product label for Melissa offcinalis states the following: Melissa (*Melissa officinalis*) is a member of the mint family. It is commonly referred to as Lemon Balm because of its lemon-like flavor and fragrance.

1. **Compensation in case of study-related injury:**
   MTSU will not provide compensation in the case of study related injury.

2. **Anticipated benefits from this study:**
   a) The potential benefits to science and humankind that may result from this study are that we may gain an increased understanding of the effects of taking Melissa officinalis on memory and cognitive functioning. This may eventually help researchers to devise new treatments for anxiety disorders, such as Generalized Anxiety Disorder.
   b) The potential benefits to you from this study are that you will gain a better understanding of how research is conducted and you will earn extra-credit points for your course.

3. **Alternative treatments available:**
   Not applicable.

4. **Compensation for participation:**
   None.

5. **Circumstances under which the Principal Investigator may withdraw you from study participation:**
   Non compliance with the study procedures and failure to comply with instructions. Also, you may be withdrawn if you have any history of significant head injury, neurological illness, or are taking a psychotropic medication.

6. **What happens if you choose to withdraw from study participation:**
   Participation in this study is strictly voluntary and there are no penalties for refusing or participate and there are no consequences from withdrawing from the study. The participants may choose to withdraw from the study at any point.

7. **Contact Information. If you should have any questions about this research study or possible injury, please feel free to contact Paul S. Foster at 898-2007.**

8. **Confidentiality. All efforts, within reason, will be made to keep the personal information in your research record private but total privacy cannot be promised. Your information may be shared with MTSU or the government, such as the Middle Tennessee State University Institutional Review Board, Federal Government Office for Human Research Protections, if you or someone else is in danger or if we are required to do so by law.**

9. **<u>STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY</u>**
   I have read this informed consent document and the material contained in it has been explained to me verbally. I understand each part of the document, all

my questions have been answered, and I freely and voluntarily choose to participate in this study.

Date	**Signature of patient/volunteer**
Consent obtained by:	
Date	**Signature**
	Printed Name and Title

APPENDIX C

Subject History and Demographic

Subject Number:

Date of Birth:

Date of Study:

Age:

Height:

Weight:

Handedness:

Education:

History of significant head injury (meaning loss of consciousness)? Y / N

If yes then explain. How long was the loss of consciousness?

History of neurological or psychological/psychiatric illness? Y/ N

If yes then explain.

Currently taking psychotropic medications? Such as meds for depression or anxiety?

APPENDIX D

Checklist

Please read the following very carefully and indicate if you:

☐ Are pregnant, attempting to become pregnant, or are nursing.

☐ Taking sedatives (for insomnia or anxiety) that include clonazepam, (Klonopin, lorazepam (Ativan), phenobarbital (Donnatal), zolpidem (Ambien), and others.

☐ Taking medications to regulate your thyroid.

☐ Taking medication for HIV.

☐ **Eligible to participate.**

☐ **Not eligible to participate.**

_____ _____

Signature Date

APPENDIX E

Tables

Table 1

Descriptive statistics of demographics and group differences (placebo, 500mg and 1500mg).

	Placebo (n = 18)	500mg (n = 18)	1500mg (n = 18)
	M (SD)	*M (SD)*	*M (SD)*
Age	19.38 (1.24)	19.5 (1.38)	20.17 (2.01)
Years Education	14.83 (0.86)	14.89 (1.18)	15.22 (1.44)
BMI	26.36 (4.85)	26.38 (6.23)	25.12 (6.50)
WTAR IQ	99.56 (8.79)	100.11 (10.70)	100.56 (8.62)

*$p < .05$.

Table 2

Descriptive statistics and group differences for the Speilberger State Anxiety Scale (SSAI).

		Placebo (n = 18)	500mg (n = 18)	1500mg (n = 18)
Measure		*M (SD)*	*M (SD)*	*M (SD)*
SSAI	Pre	31.78 (9.93)	32.89 (1.53)	33.78 (10.57)
	Post	30.72 (12.20)	27.17 (5.75)	29.56 (10.74)

*$p < .05$.

Table 3

Descriptive statistics and group differences for memory measures

Measure		Placebo (n = 18) *M (SD)*	500mg (n = 18) *M (SD)*	1500mg (n = 18) *M (SD)*
Digit Span Forwards	Pre	5.11 (1.08)	4.78 (1.26)	4.94 (0.94)
	Post	5.50 (0.99)	5.06 (1.30)	5.33 (1.03)
Digit Span Backwards	Pre	4.44 (0.92)	4.50 (0.92)	4.78 (1.06)
	Post	4.61 (1.14)	4.44 (1.54)	5.00 (1.28)
Digit Span Total	Pre	9.56 (1.76)	9.28 (1.84)	9.72 (1.67)
	Post	10.11 (1.60)	9.50 (2.43)	10.33 (1.81)
HVLT-R (Total Recall)	Pre	25.61 (3.85)	25.61 (4.78)	25.61 (3.70)
	Post	28.78 (13.50)	27.17 (4.03)	26.72 (4.36)
HVLT-R (Delayed Recall)	Pre	9.06 (1.63)	8.78 (3.00)	9.56 (1.62)
	Post	8.33 (3.25)	8.94 (2.15)	8.67 (2.89)
HVLT-R (Retention)	Pre	90.89 (13.24)	80.50 (26.13)	93.89 (18.07)
	Post	72.11 (30.90)	82.00 (15.73)	81.28 (25.80)
HVLT-R (Forced Choice)	Pre	11.06 (1.21)	11.67 (0.59)	11.50 (0.79)
	Post	11.89 (2.25)	11.83 (0.38)	11.56 (0.78)
Logical Memory I	Pre	12.61 (3.65)	12.83 (4.00)	13.61 (3.52)
	Post	14.50 (4.96)	14.83 (5.17)	14.94 (5.47)
Logical Memory II	Pre	12.39 (3.62)	11.78 (4.19)	12.28 (4.10)
	Post	12.72 (4.40)	13.61 (5.51)	13.67 (4.43)

*$p < .05$.

Table 4

Descriptive statistics and group differences for measures of processing speed, executive functioning, and verbal fluency.

Measure		Placebo (n = 18) *M (SD)*	500mg (n = 18) *M (SD)*	1500mg (n = 18) *M (SD)*
Symbol Digit Modalities	Pre	55.61 (8.25)	56.50 (8.81)	60.11 (9.55)
	Post	53.89 (6.08)	54.50 (6.49)	57.89 (9.96)
COWAT	Pre	30.61 (7.25)	33.56 (7.70)	36.89 (8.92)
	Post	35.78 (8.82)	39.33 (10.85)	39.56 (6.54)
Semantic Fluency	Pre	20.17 (5.61)	23.06 (7.87)	19.39 (3.82)
	Post	20.56 (4.25)	20.00 (2.68)	21.22 (5.04)
Stroop (Color-Word)	Pre	42.72 (6.63)	45.89 (12.68)	44.61 (7.80)
	Post	46.50 (6.26)	48.06 (14.34)	47.78 (8.84)

*$p < .05$.