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Sceletium tortuosum and Mesembrine: A Potential Alternative Treatment for Depression

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Sceletium tortuosum and Mesembrine: A Potential Alternative Treatment for Depression

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Abstract

Major depressive disorder affects people's productivity and ability to function in everyday life. The disorder can be attributed to neurochemical imbalances of various neurotransmitters including but not limited to serotonin, dopamine, and norepinephrine. Conventional pharmacological treatments have focused primarily on these three neurotransmitters, and have been shown to be effective in alleviating most of the major symptoms of depression. Although these treatments are effective with most patients, they are known to have adverse side effects, causing patients to seek alternative treatments. *Sceletium tortuosum*, a succulent plant found in the Cape region of South Africa, has been shown to have anxiolytic effects when used recreationally. Studies have confirmed the presence of a family of alkaloids mesembrines that are present within the plant and believed to be responsible for the calming effects. Pharmacological analyses have revealed that individual members of the alkaloid family act as either serotonin reuptake inhibitors (SRI) or phosphodiesterase-4 (PDE4) inhibitors. The current study seeks to elucidate the antidepressant properties of the mesembrine alkaloids in a mouse model of depression. Isolated alkaloids were administered at a low dose (10 mg/kg) and a high dose (80 mg/kg) to BALB/c mice in the forced swim test a rodent model of behavioral despair. This was compared with paroxetine (Paxil) (1 mg/kg), a selective serotonin reuptake inhibitor with proven antidepressant efficacy, and 0.9% saline. Each trial of the forced swim test was administered for six minutes and the duration of swimming and immobility was measured. In order to assess any locomotor effects of the drug treatments, an open field exploration test was also employed one week following the forced swim task. Results from the forced swim test revealed a statistically significant reduction in the duration of immobility (behavioral despair) between the low dose of alkaloids and saline. No significant effects in immobility were found across the other drug treatment conditions (high dose mesembrine, paroxetine, and saline). Further, none of the treatment groups showed statistically significant locomotor interference effects in the open field exploration test. We conclude that the mesembrine alkaloids present in *Sceletium tortuosum* have antidepressant properties and may represent a suitable alternative for the treatment of major depressive disorder.

Introduction

Sceletium tortuosum is one of many plants within the *Sceletium* species that have been present in the historical writings from the Cape region of South Africa. The genus name comes from "sceletus" meaning skeleton, pointing to the characteristic skeleton-like leaf venation pattern that is most visible when the leaves are dried. *Sceletium* plants show climbing behaviors in growth and have the characteristic idioblasts or "bladder cells" found in most succulents. The plant flowers, and the flowers are normally pale pink, yellow, or white. The seeds found in the fruit capsule have brown or black kidney-shaped seeds. The plant fairs best in arid or dry regions, explaining its prominence in the Cape region. *Sceletium tortuosum* has had many documented recreational uses over the years, and its psychotropic effects are of profound interest to researchers. Specifically, its known ability to increase a sense of wellbeing has been an area in which researchers have begun to turn their attention (Gericke and Viljoen, 2008).

Historical Background

Many plants of the genus *Sceletium* have been used for the relief of thirst and hunger, as medicines, to increase alertness, and as a social and spiritual herb for the Bushmen and Hottentots in the Cape region of South Africa, long before the earliest written reports of usage among European explorers and settlers in the area. However, the oral tradition that served to teach others about the plant has been worn away over time as a product of the conflicts between these settlers and the indigenous people who used the plant for its many uses. Concurrently, the plant itself has diminished in population due to over-harvesting, poor veld-management, and potentially plant

disease too. A lot of attention has been brought to the plant to try to save it, which has caused many scientists to begin assessing its chemical properties as well as its potential as a dietary supplement, phytomedicine, or new drug application (Gericke and Viljoen, 2008).

Its first illustration and recording by Europeans was in the journal of the Cape of Good Hope by Governor Simon van der Stal when he took an expedition to Namaqualand in 1685. Van der Stal wrote that the Namaqua people called the plant *Canna*, later spelled *kanna* by other writers. *Sceletium* may have been mixed with 'dagga,' or *Cannabis sativa L*. as it supposedly "induced Bushmen users to dance." (Laidler, 1928) The area where these people lived, called Little Karoo, was given the name 'Cannaland' by the white settlers for its abundance of the plant. These writings made it clear that the plant had known intoxicating properties, noting that the Hottentots would ferment the root, and if they chewed it immediately afterwards, would be intoxicated. If not used immediately afterwards, it would relieve thirst. The Hottentots knew it was of great value, and it was used as a form of currency to exchange for cattle and other items of value. The Bushmen made it the center of their trade, and there were numerous accounts of it being used to treat insomnia in adults, diarrhea in children, and as a mild narcotic or general intoxicant (Gericke and Viljoen, 2008).

The fermented version of *Sceletium tortuosum* is given the name 'kougoed' in Afrikaans, which is derived from "*kou*," meaning "to chew," and "*goed*," meaning "stuff." This name was first recorded in 1830 (Smith, 1966). The fermentation process involves crushing the plant material between two stones and placing it in a sealed container for many days. The container used took the form of a skin or canvas bag, but in modern day

preparations, plastic bags are used (Jacobson, 1960; Smith et al. 1996). However, it was not always fermented, and writings indicated the use of tinctures, as cited by Pappe (1868) and chewing of the raw plant material.

Meiring wrote that *Sceletium tortuosum* was used for its soporific effect on young children, silencing them when they were suffering from what he called "acidity." Parents would use a few drops of fresh juice from the plants on their children, inducing a very deep rest for many hours (as cited in Meiring, 1898). Hartwich and Zwicky concluded their scientific reports on *Sceletium tortuosum* by saying that the indigenous people almost certainly used the plant recreationally the majority of the time as opposed to medicinally (Hartwick and Zwicky, 1914).

Clinical Depression

Clinical depression is an increasingly prevalent problem for people in the United States with 1 in 10 US adults having some form of depressive disorder (Centers for Disease Control and Prevention, 2008). Major depressive disorder, also called unipolar affective disorder, refers to a form of depression that recurs for many months and sometimes years. This depression often keeps the individual from leading a normal life, including keeping a job as well as maintaining healthy relationships. According to a study done at Harvard University, the number of patients in the U.S. diagnosed with clinical depression increases by about 20% per year, with the total number of adults affected estimated to be approximately 14.8 million (National Institutes of Mental Health, 2011). Within the US, different regions appear to have varying occurrences of depression with the highest prevalence of depression found in the southeast. It has

been speculated that this could be related to high rates of obesity, heart disease, stroke, and sleep disorders in the same region (Census Bureau, 2011). It has also been shown that women are twice as likely as men of the same age to have depression or symptoms of depression with 12% of women in the United States experiencing symptoms of clinical depression at some point in their lives. The diagnosis is most notably common among women between the ages of 45-64, often associated with a lack of feeling physically beautiful or feeling that one's physical beauty is past its peak, stressful life events, and poverty. Hispanics (4.0%), Blacks (4.0%), and Others (4.3%) tend to have slightly higher rates of depression than non-Hispanic whites (3.1%) (Faris and Krucik, 2012).

Clinical depression is also a large and growing problem around the world. It is estimated that 121 million people around the world have some form of depression (Science Daily, 2011), fewer than 25% of whom have access to adequate healthcare to address their needs. The World Health Organization called depression the fourth leading cause of disability worldwide, predicting that it would become the second leading cause of disability in older adults by 2020. It is currently the leading cause of disability in young people (15-24 years) (World Health Organization, 2012).

Clinical depression is characterized by a few necessary criteria and a variety of possible symptoms. The National Institute of Mental Health explained the variance of symptoms by three dimensions: (1) anxiety-physical agitation-somatization; (2) depressed mood-motor retardation; and (3) hostility-interpersonal sensitivity (Katz and Maas, 1994). According to this characterization, these symptoms coexist in varying proportions among severely depressed patients. The Diagnostic and Statistical Manual

of Mental Disorders 4th Edition (DSM-IV) characterizes major depressive disorder or a major depressive episode as having a depressed mood for at least two weeks, often having the worst of the depressed mood occurring in the morning, and five of a list of criteria, including feelings of worthlessness or excessive guilt, decreases in interest in pleasurable activities or relationships (also called anhedonia), fatigue, impaired concentration, insomnia or hypersomnia, significant weight loss or gain, aches and pains in various parts of the body, restlessness, and thoughts of death or suicide (American Psychiatric Association, 2000). Men often experience more outward signs such as irritability and drug or alcohol abuse. Many anxiety disorders, such as post-traumatic stress disorder (PTSD), panic disorder, social phobia, and generalized anxiety disorder, tend to coexist with depression (National Institutes of Mental Health, 2011).

Treatment of depression varies depending on the severity of the disorder. Many patients enter psychotherapy, attending sessions with a psychologist. In conjunction with this, people may take medications such as antidepressants. In situations where medication and psychotherapy do not help or if the symptoms are particularly severe, some patients undergo electroconvulsive shock therapy (ECT) as a last resort. However, newer medications are being developed at a rapid pace, creating hope for many that are afflicted with depression (National Institutes of Mental Health, 2011).

Neurochemistry of Depression

The neurochemistry of depression is a complex interaction of many different systems of neurotransmitters within the brain. One of the theories behind the alterations of neurochemistry implicated in depression involves the corticotropin-

releasing factor (CRF) system and its role in stress. CRF is a peptide found in the hypothalamic region of the brain, and it interacts heavily with what is called the hypothalamic-pituitary-adrenal (HPA) axis. CRF is released by the paraventricular nucleus (PVN) of the hypothalamus. It travels from here to the anterior pituitary, and this stimulates the release of adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal cortex to secrete glucocorticoids, primarily cortisol. Cortisol acts as negative feedback, which allows the maintenance of normal cortisol levels. When this system is overused, it can begin to malfunction, and CRH is produced in non-stressful as well as stressful situations. For this reason, increased CRH production has been associated with an increased risk of developing major depression. However, there is evidence of a similar nature that cortisol can be implicated in depression, and that it is the causal component in the relationship between cortisol, CRH, and depression.

The hippocampus, located in the medial temporal lobe, is a part of the limbic system whose change in function has been connected with various symptoms of depression such as the cognitive deficits. It is postulated that a neurotoxicity takes hold, in the form of excess glucocorticoids, in the hippocampi when experimental subjects are raised in "stressful" environments. In humans, "stressful" conditions are things such as childhood abuse or trauma (Medscape Faculty and Disclosures, 2011).

There are studies that have shown norepinephrine (NE) to be potentially important. The CRF system in the hypothalamus projects to the locus coeruleus, which is where many NE-containing cell bodies reside. Therefore, the NE system has become hypersensitive to things such as early-life trauma that promote stress. This supports the "Stress-Diathesis Model" of depression. This model states that early trauma can have

neurotoxic effects on the hippocampus, causing sensitization of the CRF system, which can cause a hyperactive stress response in adults. When an adult is then exposed to consistent stress, the CRF system can be taxed even more, leading to very high levels of cortisol release. This is one theoretical cause of major mood disorders such as clinical depression (Medscape Faculty and Disclosures, 2011).

Many studies focus on the monoamine hypothesis of depression, relating to the reuptake of monoamines or inhibition of the breakdown of monoamines. Many researchers tried to measure the levels of various neurotransmitters and their metabolites in body fluids such as cerebrospinal fluid (CSF), but the data are difficult to interpret. There have also been many studies using post-mortem brain tissue to study the neurochemical effects of depression. Researchers have taken post-mortem tissues from many different suicide victims, and used it to study various noradrenergic and serotonergic receptors, uptake sites, and metabolites. They also looked at GABA, acetylcholine, and corticotropin releasing factor (CRF). For the most part, these studies weren't very consistent across patients, with the various neurotransmitter markers being studied not producing anything significant when compared with the post mortem tissue of normal brain. There are also too few studies with too few subjects, which also makes consistency an issue. The areas of examination within the brain are increasing beyond the study of just the frontal cortex, and there is increasing hope for this method to provide more information about those with depression that commit suicide by a certain method (Horton, 1992).

Lastly, the neurotrophic model of depression focuses on neurotrophic factors such as nerve growth factor, brain-derived neurotrophic factor (BDNF), and a few

others that are expressed in the central nervous system and have many varying effects on neurons. It has been shown that stress can down-regulate BDNF in the hippocampus as well as cause neuronal atrophy of hippocampal neurons, resulting in a decrease in volume of the entire structure. All of this forms the basis of this theory for depression, especially stress-associated cases. Further supporting this theory is the evidence that BDNF is up-regulated in the hippocampus by antidepressant treatment, such as serotonin and norepinephrine reuptake inhibitors. BDNF has been shown to increase synaptic strength between hippocampal neurons as well as to promote the growth and vitality of cortical neurons, norepinephrine neurons, and serotonin neurons. Finally, BDNF has shown antidepressant-like effects in behavioral models of depression such as the forced swim test and other learned helplessness models. This was shown through either BDNF knockout mice or infusion of BDNF into certain brain regions such as the hippocampus and frontal cortex (Duman, 1999).

Forced Swim Test as a Behavioral Model of Depression

Although we have more informed theories about the mechanisms behind antidepressant activity, we are still not entirely sure how they exert their effects in alleviating depression. Many animal behavioral tests are used on specific compounds being developed as antidepressants. Tests such as the tail suspension test and olfactory bulbectomies have been used in many cases in the testing component of animal models of depression. Tests vary as to their reliability, specificity, and ease of use. The forced swim test, which is very widely used, is known for its high reliability and specificity, as its incredible ease in administration. It is also sensitive to acute administrations of

antidepressants. It's most commonly cited downside is that it does not reliably differentiate SSRIs from other types of antidepressants (Cryan, Markou & Lucki, 2002).

The forced swim test, also called the 'behavioral despair' test (Porsolt et al., 1977, 1978), is a popular behavioral test used to observe potential antidepressant activity, especially as a test one can use early on to determine if an antidepressant has any kind of efficacy. It is fairly easy to conduct and it is sensitive to all major classes of antidepressants (Borsini and Meli, 1988). Rodents are placed in a glass cylinder half-filled with water, and they will swim and attempt to escape in the beginning. Eventually, they will become much more immobile, and immobility is thought to represent a failure of persistence in escape-directed behavior or passivity in response to stress, also called learned helplessness. This is thought to come from the rodent's acceptable that escape is not possible. Learned helplessness is believed to be an analog of a diagnosis of clinical depression in humans. In the specific case of the SSRIs fluoxetine, sertraline, and paroxetine, immobility was reduced but swimming was increased without affecting climbing behaviors (Detke et al., 1995), and this was replicated in other studies (Hemby et al., 1997) It would appear that climbing behaviors are increased by norepinephrine and dopamine reuptake inhibitors (Reneric and Lucki, 1997; Hemby et al., 1997).

The popularity of the forced swim test has allowed for large amounts of data to be published and compared among many different research groups. This has made it very important in the drug discovery process of antidepressants. The forced swim test has also been used in neurogenetic research looking at the genetic basis for certain depression-related behaviors. Mouse strains that are genetically modified or selectively bred are compared with wild type mice in the forced swim test, with or without

administration of antidepressants (Lucki, Dalvi, and Mayorga, 2001). The forced swim test has high predictive validity, as it is insensitive to compounds that are not effective antidepressants in humans, while being sensitive to those that are (Willner, 1984).

Antidepressants

Conventional antidepressants primarily act on the neurotransmitter systems of the brain, such as serotonin, dopamine, and norepinephrine. In the early 1950s, iproniazid, an antimycobacterial agent, used as a potential treatment for tuberculosis, was seen to have psychoactive properties; patients became noticeably happier and more active after ingesting the drug. It was subsequently shown that it slowed the enzymatic breakdown of a class of neurotransmitters called monoamines. This was the beginning of a class of antidepressants called monoamine oxidase inhibitors, or MAOIs (Lieberman, 2003). The monoamine oxidase family of mitochondrial enzymes is important in the breakdown of monoamine neurotransmitters, which include dopamine, norepinephrine, and serotonin (Lieberman, 2003). Some examples of FDA approved MAOIs are isocarboxazid (Marplan) and phenelzine (Nardil). These worked well with the specific symptoms, of atypical depression, a type of depression sharing symptoms with major depressive disorder but characterized by occasional improved mood due to positive events, but they were riddled with problems. People who take these medications have to avoid eating foods that contain a substance called tyramine, which is found in fermented foods. Other things to avoid are certain types of birth control pills, cold and allergy medications, certain herbal supplements, and prescription painkillers. Some of these cause drastic increases in blood pressure when they interact

with MAOIs. To alleviate these symptoms, a transdermal patch was developed, marketed under the name Emsam (selegiline), to administer the drug. This route of administration causes fewer side effects, and with low enough doses, dietary restrictions may not be necessary since the patch not only circumvents the gastrointestinal tract, but low doses also only affect MAO-B, which is found predominantly in the brain. These medications also interact significantly with SSRIs, putting a patient at risk for developing serotonin syndrome. Symptoms of this syndrome include hallucinations, seizures, increased sweating, confusion, muscle stiffness, and potentially dangerous changes in blood pressure or heart arrhythmia (Mayo Clinic, 2013).

Another older, once popular class of antidepressants are called tricyclics. These were developed when researchers made molecular modifications to antipsychotic drugs called phenothiazines, leading to the synthesis of a drug called imipramine (Kuhn, 1958). Their name is derived from their chemical structure, which contains three benzene rings. These drugs work by inhibiting the reuptake of serontonin and norepinephrine by blocking both transporter systems. It has little efficacy on the transporter system of dopamine, so its effects on this system are negligible. These drugs proved to be effective, but there were also significant side effects. They cause dizziness, weight gain, dry mouth, drowsiness, and possibly cardiac problems in people with pre-existing heart conditions. Further modification of the phenothiazine molecule made slightly safer and better-tolerated tricyclics (Lieberman, 2003). Changing the dosage or changing medications could improve most of these side effects, but physicians have to be cautious since these drugs are very dangerous when taken in high doses. Low doses

of these drugs are sometimes used in conjunction with an SSRI to increase the positive effects, while decreasing the negative side effects. Some examples of tricyclics include imipramine (Tofranil) and notriptyline (Pamelor) (National Institutes of Health, 2013).

The newest and most popular class of antidepressants are the SSRIs; and serotonin-norepinpehrine reuptake inhibitors (SNRIs) have also gained popularity. These drugs were developed when the serotonin hypothesis of depression was being popularized, causing researchers to search for structural analogs of diphenhydramine, a compound that had been shown to be active against serotonin but inactive against norepinephrine (Fuller et al., 1974). They have very few serious side effects, but they can cause nausea, insomnia, headaches, or jitters at the beginning of administration. There are, however, sexual side effects including decreases in libido, but these can be remedied by adjusting dosage or switching medications. Examples of popular SSRIs include fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), and escitalopram (Lexapro). Two of the most common SNRIs are venlafaxine (Effexor) and duloxetine (Cymbalta). Bupropion (Wellbutrin) is also a popular antidepressant that acts primarily on dopamine, it tends to have similar side effects as SSRIs and SNRIs, but it is less likely to cause sexual side effects. However, it has been known to increase a person's risk for having seizures. SNRIs and bupropion are often classified as atypical antidepressants because they do not fit well into any other category (Mayo Clinic, 2011).

The SSRI paroxetine, marketed as Paxil, is the most potent SSRI that is clinically available, but it has a lower selectivity to serotonin compared with fluvoxamine (Luvox) and sertraline (Zoloft). It has also been shown to block musacrinic acetylcholine receptors very effectively, but anticholingeric side effects seem to be rare except at very

high doses (Owens et el., 1997). It's a chiral molecule, marketed as a pure enantiomer (Dechant 1991). It is mostly absorbed in the gastrointestinal tract, and a large amount of it is metabolized during its first pass through the liver. It forms more hydrophilic excretable compounds, and none of these metabolites are though to contribute to the pharmacological effects of the drug (Kaye et al., 1989).

Regarding efficacy, most antidepressant treatments that have been successful at some point in their history are very similar. Studies comparing tricyclic antidepressants and SSRIs have found very little difference in efficacy and most differences in tolerability of side effects (Anderson, 2000). Similar results have been found in comparing SSRIs and SNRIs (Sir et al., 2005). Marketed antidepressants tend to have similar effectiveness in treating major depressive disorder, anxiety disorders, and other mood disorders, but their tolerability related to side effects can be very different and influence their popularity in being prescribed.

Mouse Strain Differences

The use of mice in laboratory research is common in the testing of neurological and neuropsychiatric agents. Mice are very similar to humans in the genetics and physiology of their brains. They are also small, making them easy to handle as well as house. They have a short generation time, and are therefore quick to reproduce. This makes them ideal candidates for research.

Among the various strains of mice, they are defined among the large categories of inbred and outbred strains. Inbred strains are strains that reproduce through the mating of siblings in order to keep certain characteristics within the populations. In

order to be considered inbred, mice but have be the product of twenty generations of brother x sister matings (Les, 1990).

BALB/cJ mice are a commonly used inbred strain, “popular for their resistance to experimental autoimmune encephalomyelitis (EAE) as well as a susceptibility to developing demyelinating disease upon infection with Theiler’s murine encephalitis.” (Jackson Labs, actual citation). Three major substrains can be traced as far back as 1940 and potentially back further. There has been little genetic contamination in the strains. They tend to have greater immobility times in the forced swim test, making the reductions in this time very apparent when tested for regarding the efficacy of antidepressants (Festing, 1998).

Not all mouse strains respond in the same way to validated behavioral testing or specific models of a given psychiatric or physical phenomenon. Sugimoto et al (2011) compared strains of mice within the forced swim test when administered with paroxetine, a very successful SSRI. They compared BALB/c, C57BL/6, DBA/2, ICR, and ddY strains, and they found that only DBA/2 and BALB/c mice were significantly different from the negative control at all three doses of paroxetine. C57BL/6 and ddY mice were significantly different at the two higher doses, and ICR mice were significantly different at the highest dose only. This comparison shows a clear difference in the reactions of different strains to certain behavioral tests, and researchers need to take this into account when choosing specific strains to experiment with using a behavioral model.

Herbal Treatments

The most popular natural alternative to the many antidepressants prescribed is St. John's wort, or *Hypericum perforatum* L., an herbal supplement that can be purchased over the counter. It is often used to avoid the potentially negative side effects associated with prescribed antidepressants, but it is not as effective in treating severe cases of depression. The Food and Drug Administration has not approved it for the treatment of depression, but it is used commonly in Europe. Studies have shown that St. John's wort is equally as effective for the short-term treatment of mild to moderate depression (lasting 1-3 months) as the tricyclics. It is not very clear if it is as effective as the SSRIs (Hypericum Depression Trial Study Group, 2002). Studies have also shown that it can have strong interactions with prescribed antidepressants and other supplements, so caution should be taken by those who already take other drugs to treat their depression (National Center for Complementary and Alternative Medicine, 2012).

Another alternative treatment takes the form of a dietary supplement is called *S*-Adenosyl-L-methionine or SAMe. It has been available in the US since 1999, but it has been used as a prescription drug in parts of Europe since the 1970s and 1980s. It's made naturally in the body by the combination of adenosine triphosphate (ATP) and methionine via methionine adenosyltransferase. It is involved in many bodily functions, and is found in high concentrations in the brain, liver, and adrenal gland. One controlled clinical trial showed that, after two weeks of administration, 66% of clinically depressed patients given the drug had significant improvement in their symptoms, compared to 22% of patients given the tricyclic imipramine (Bell et al., 1988). Other clinical trials have shown that it helps with the treatment of liver disease and the pain

of osteoarthritis (Berger and Nowack, 1987). Its mechanism of action is not known, but there are many hypotheses. It is known, through preclinical trials, that SAMe has an effect on monoamine metabolism, increasing norepinephrine and serotonin levels. Vitamin B12 deficiency is known to decrease endogenous levels of SAMe, which is linked to depression (Mischoulon, 2002). It is cautioned that those with bipolar disorder should not take these supplements as the pills could worsen symptoms of mania. This could be a potentially helpful solution, although insurance companies do not cover any of the cost because it is not approved as a treatment for depression by the FDA , and a month's worth of supplements would cost roughly $143. This is similar to what some pay for very effective antidepressants without any insurance coverage (Parker-Pope, 2010), so for patient's with any kind of insurance, it is in their best interest to purchase antidepressants that are approved as treatments for depression by the FDA.

Alkaloid Chemistry: The Mesembrine family

Sceletium was first shown to contain an alkaloid by Meiring in 1898, who isolated it and injected a small amount into frogs subcutaneously, causing a quick physiological response such as uneasiness and loss of appetite, the first *in vivo* experiment with mesembrine. It was not until it was isolated again in 1914 by Zwicky that the group of alkaloids was given the name mesembrin, now called mesembrine. Although Zwicky ran alkaloid tests to show the presence of mesembrine, it was not confirmed as these tests were non-specific. The extracted alkaloids were members of

the mesembrine family that are present in *Sceletium tortuosum*, predominantly mesembrine and mesembrinine (mesembrenone) (Meiring, 1898).

The structure of mesembrine was found to be N-methyl-3a-(3',4'-di-methoxyphenyl)-6-oxo-*cis*-octahydroindole through synthetic and degradative studies (Popelak and Lettenbauer, 1968). This structure was confirmed later by a total synthesis of (+/-)-mesembrine by Shamma and Rodriguez (Jeffs, 1969).

Sceletium tortuosum's mechanism of action was recently identified as an inhibitor of the serotonin (5-HT) transporter system, which is responsible for the reuptake of serotonin from the synaptic cleft (Harvey, 2008) and inhibition of the phosphodiesterase-4 (PDE4) enzyme (Napoletano et al., 2001). 5-HT reuptake inhibitors, also called SSRIs, are often used in the treatment of depression as was stated earlier. It has been argued that a combination of this property with the inhibition of PDE4 will have greater therapeutic value than either of the properties on their own because PDE4 has been shown to inhibit second messenger systems regulated by cyclic AMP (cAMP) by promoting the metabolism of cAMP. It has been previously proposed that antidepressant effects may also result from upregulating cAMP, which PDE4 accomplishes via this mechanism (Zhang, 2009) SSRIs have been shown to upregulate cAMP in many cells, providing another potential explanation for the antidepressant actions of SSRIs if the PDE4 hypothesis of antidepressant effects holds true. Although it takes time for SSRIs to upregulate cAMP, mesembrine appears to have a similar or faster effect on the up-regulation of cAMP.

It is uncertain as to which of the compounds within the plant, mesembrine or mesembrinone, produce anxiolytic effects, mood enhancement, and generally more

positive well-being. Although the synthetic mesembrine has been shown to act as an SRI *in vitro*, a recent study explored the phytochemical content of *Sceletium tortuosum*. It was reported that fermentation alters the alkaloid content, transforming mesembrine into mesembrenone (Patnala and Kanfer, 2009). Therefore, it is uncertain as to which of these compounds is responsible for the effects, or if some combination of the alkaloids produce the desired effect.

Pharmacological activity of Mesembrine

Through the use of *in vitro* studies, mesembrine and some of the related compounds in the family were shown to be serotonin-uptake inhibitors, as well as standardized amounts of these various compounds have been used for the potential management of psychiatric and psychological disorders, such as depression, anxiety, OCD, bulimia, and drug dependence (Gericke and Van Wyk, 2001). Synthetic formulations of (-)-mesembrine confirmed inhibitory activity on the serotonin transporter, with limited inhibition of norepinephrine uptake and dopamine uptake at very high concentrations (Harvey, 2008). As stated earlier, mesembrine has also been shown to inhibit phosphodiesterase-4 (PDE4) at an IC_{50} of 29 μM (Napoletano et al., 2001). PDE4 inhibitors are currently a new area of research for the treatment of asthma, chronic pulmonary diseases, psoriasis, and depression. Cyclic AMP (cAMP), a prominent second messenger, is influenced by many subtypes of the PDE4 family, and it has been found that selective inhibition of these enzymes can have significant functional effects that may contribute to antidepressant activity (Houslay et al., 2005).

Another study involved observing the effects of *Sceletium tortuosum* in an *in vivo* model of psychological stress. This was performed on male Wistar rats, which were given an extract of the plant and exposed to repeated restraint stress. The rats' behavior was assessed using the elevated plus maze on their last day of restraint, and were sacrificed 24 hours after the last restraint exposure. Their observed behavior showed little difference between the experimental and control groups when *Sceletium tortuosum* was administered at a low dose (Smith, 2011).

In a more recent study, the safety of administration of *Sceletium tortuosum* was confirmed when it was given to seven healthy beagles and one dog with dementia at a dose of 10 mg/kg twice per day for six days. The researchers did blood tests to check red cell count, blood urea nitrogen, glucose, white cell and platelet count, total cholesterol, and many other measures to see how the plant affected these. It was reported that there were no significant changes in the tests, and observations of behavior showed that all of the dogs behaved as before. There were no cardiac issues, changes in lipid or glucose metabolism, and no problems in liver or kidney functioning (Hirabayashi et al., 2002).

A pharmaco-fMRI study with humans ingesting acute doses of Zembrin, the marketed pill that consists of a proprietary extract of *Sceletium tortuosum* on rice powder, was conducted to observe anxiety-related activities in areas such as the amygdala and its associated structures. The amygdala's reaction to fearful facial expressions was reduced when participants took one 25 mg dose of Zembrin, accompanied by an attenuation of the amygdala-hypothalamus coupling. This was evidence for the belief that the SRI capabilities combined with the PDE4 action of

Sceletium may have potential not just in reducing depression, but also can help anxiety, a condition often associated with depression (Terburg & Syal, 2013).

Current Study

The purpose of this experiment was to compare mesembrine with an effective antidepressant, paroxetine (Paxil) serving as the positive control in an *in vivo* model of behavioral despair using BALB/c male mice. All drugs will be dissolved in 0.9% saline, and 0.9% saline alone will serve as the negative control. Administration of mesembrine, a potential, natural alternative treatment for depression, should produce calming effects as witnessed in behavioral tests such as the forced swim test in the mouse model of depression. Mice will also be tested with the open field exploration test to account for any changes in locomotor activity that may occur as a result of drug administration. Blood analysis will be performed to check for the presence of certain metabolites and how these might influence any noticeable behavioral changes. We hypothesize that the high dose of mesembrine will produce an equivalent antidepressant effect to that of paroxetine, and the low dose of mesembrine will produce some antidepressant effect although less noticeable. All three experimental groups will show greater antidepressant activity, via the forced swim test, than the saline group. Also, we predict there will be no significant locomotor differences between the four groups, indicating no locomotor interference by mesembrine.

Materials and Methods

Subjects

Twenty male BALC/c mice (Simonsen Labs, Gilroy CA), weighing approximately 19 grams at the beginning of the experiment, were housed in groups of four in a temperature controlled colony room (22 ^{o}C) with a 12-hour light/dark cycle (lights on 0800 Hrs). All testing was done during the same time each day, and food and water were freely available throughout the duration of the study. Mice were randomly assigned to four treatment groups prior to behavioral testing; saline, low-mesembrine alkaloids (10 mg/kg), high-mesembrine alkaloids (80 mg/kg), paroxetine (1 mg/kg). All drug injections were intraperitoneal (i.p.). Testing procedures and animal care were done in compliance to guidelines indicated by the Keck Science Center Institutional Animal Care and Use Committee.

Gas Chromatography/Mass Spectroscopy (GC/MS) of Plant Material

A GC/MS analysis was performed on the ether soluble alkaloids used in the animal experiments using a HP-6890 of the Pomona College Chemistry Department. The procedure was a modified version of a previous method conducted by Candice Delphine Gaffney (2006). Two concentrations of the alkaloids, 0.94 mg/mL and 4.23 mg/mL were prepared from the 20mg/mL stock solution used for portioning out the doses used for the mouse injections. Three injections were done for each into the instrument. Method: injector 250oC; 2.5 min solvent delay; 50^{o}C hole 2 min; 10^{o}C/min to 190^{o}C, 5^{o}C/min for 190-240^{o}C, 10^{o}C/min for 240-290^{o}C then hold 1 min; 1 μL splitless injection; HP-5 5% phenylmethylsiloxane capillary column (30.0m x 250μm x

0.25 μm); helium flow 1.3 mL/min. The percentages of each alkaloid present in the material were based on the averaged relative peak areas of the total ion current for the four main alkaloids, mesembranol, memembrenol, mesembrine and mesembrenone. Automatic peak identification and integration of the ChemStation software was used for the peak integration data. The respective alkaloids were identified by both their retention on the GC column and their mass spectrum.

Drug Preparation

The alkaloid HCl mixture was taken up into anhydrous EtOH, making a 20.06 mg/mL stock solution. Amounts needed for individual alkaloid vials for drug administration were taken from this stock solution and evaporated *in vacuo* into the individual vials. Deionized water was added to each vial to bring them to either 1 or 8 mg/mL concentration, corresponding to the low and high alkaloid doses used. The vials were capped with a Teflon faced silicone septa and stored in a refrigerator until use. Paroxetine HCl hemihydrate was dissolved in filtered deionized water to yield a concentration of 0.10 mg/mL and a dose of 1.0 mg/kg. An effective paroxetine dose was determined from the reference, Sugimoto et al. 2011. Control mice received saline, which was prepared by dissolving sodium chloride in deionized water, making 0.9% w/v saline. All drug administrations were performed using sterile injection procedures.

Behavioral Tests

Forced Swim Test

The forced swim test was done according to the methods described by Porsolt et al. 1977. The apparatus consisted of two 2-liter Pyrex beakers (height= 193 mm) that were filled to the 1600 mL mark with water. Each mouse was injected with either low dose mesembrine alkaloids, high dose mesembrine alkaloids, paroxetine, or saline, and were put in a holding cage for 30 minutes prior to testing. They were then placed in the testing apparatus for six minutes (water temperature ~25-26° Celsius). Their behavior was monitored, and each mouse was scored on their swimming, immobility, and jumping behaviors. For the purposes of this experiment, immobility was defined as a mouse ceasing to struggle and remaining floating motionless in the water, only making movements necessary to keep their head above the water. All tests were videotaped, and videos were analyzed afterwards to quantify the data. The process of quantifying the data involved watching the videos, and counting seconds of immobility duration.

Open Field Exploration Test

A clear, plastic enclosure (60x45x10 cm) was used, with the floor divided into a series of lines comprising nine squares of equal area. Mice were injected with their assigned substance, and placed in a holding cage for 25 minutes prior to testing. They were then placed in the enclosure for five minutes to acclimate to the apparatus before open field assessment. Open field exploration was determined by counting the number of line crossings each animal made over three consecutive 10-minute test intervals. A line was considered crossed if both sets of limbs moved across a line. The total number

of line crossings for a given interval constituted that mouse's locomotor activity score for that interval. Test bins were cleaned with ethanol following each mouse to neutralize olfactory cues. Each mouse's locomotor behavior was video recorded.

Blood collection

After performing the Open Field Exploration test, mice were euthanized using isoflourane followed by decapitation. Immediately after cervical dislocation, trunk blood was collected using a pipettemen and were spotted on blood collection cards. These blood samples were analyzed for any metabolites or compounds of interest, and the data collected from this analysis will be included in another publication.

Neurochemical Methods

Extraction of Mesembrine Alkaloids

The dried, milled *Sceletium tortuosum* was obtained from the website http://www.sceletium.com/. The material came from South Africa and was labeled with the logo: Big Tree, Lot Number: AHSC011, Manufacture Date: March 2013. Three possible extraction methods were tested on~10 g of the plant material: Ethanol Soxhlet extraction, cold acetone extraction, and direct acid extraction using aqueous sulfuric acid. Of these three methods, the direct acid extraction was chosen for the bulk of the plant material because it had the largest yield.

This method was performed according to Shikanga et al 2012 with a few modifications. Dried milled *Sceletium tortuosum*, 24.2631 g, was stirred for 1 hour with 250 mL 0.25M aqueous sulfuric acid (pH < 1 as measured by indicating paper). The

solution was screened through a paint filter bag and then through Celite. The aqueous filtrate was brought to pH 10-11 with 10 mL conc. ammonium hydroxide and extracted three times 200 mL dichloromethane (DCM). The combined DCM extracts were dried over anhydrous sodium sulfate and evaporated *in vacuo* in a tared round bottom flask. This left 0.6670 g of crude alkaloid. The ether insoluble alkaloids were removed following part of the mesembrine isolation from Archiv der Pharmazie 1957, 290:441. The crude alkaloid (0.6670 g) was dissolved in 2 mL ethyl acetate (EtOAc) and then added to 50 mL diethyl ether and allowed to stand covered overnight (24 hours). The etheric solution was gravity filtered through glass wool and Celite. The filtrate was evaporated *in vacuo* to leave a yellow liquid 1.1202 g, indicating that it may contain some residual EtOAc. These crude ether soluble alkaloids were converted to their respective hydrochloride salts by dissolving in 2 mL ether. To this solution 2.0 mL 2M HCL in ether (Sigma-Aldrich) was added leading to a cream colored precipitate. The precipitate was triturated with 2 x 20 mL ether with the supernatant being pipetted off the top of the precipitate settled at the bottom. Finally, the mixture was evaporated in vacuo on a rotovap and then kept under vacuum from 13:00 in a vacuum dessicator. The vacuum chamber was filled with argon before opening and closing the vial with the solid. This left 103.5 mg of ether soluble alkaloid hydrochloride salts. Yield: 0.379% alkaloid/g plant material.

Results

GC/MS Analysis

Relative percentages of individual members of the mesembrine alkaloid family present in the dried, milled plant material that was used for drug preparation were found using Gas Chromatograpy/Mass Spectrometry (GC/MS) analysis. Mesembranol was the most abundant alkaloid at 67.0%, and the namesake mesembrine made up 9.2% of the sample. Mesembrenol made up 15.8% of the sample, and mesembrenone made up the smallest percentage of the sample at 8.0%.

Forced Swim Test

A separate one-way analysis of variance (ANOVA) was conducted on the duration of immobility and swimming behaviors in the forced swim test as a function of drug group. Statistically significant effects were subsequently analyzed using the Tukey HSD test (alpha= 0.05). One-way ANOVA revealed significant effects of drug on immobility $F(3,19) = 4.0774$, $p = 0.025$ with non-significant effects for swimming $F(3, 19) = 1.222$, $p > 0.05$.

Comparisons using the Tukey HSD test indicated that the mean immobility score for the low dose mesembrine alkaloid group was significantly lower than the saline group ($p<0.05$). There were no significant differences in the duration of immobility between low dose mesembrine alkaloid, high dose mesembrine alkaloid, and paroxetine ($p > 0.05$).

As indicated in Figure 1, all conditions showed far greater swimming duration than immobility duration over the course of the six-minute test. The low dose mesembrine group exhibited the shortest duration of immobility and the longest duration of swimming. The paroxetine and the high dose mesembrine groups had very similar immobility and swimming durations. The saline group had the longest duration of immobility and the shortest duration of swimming. All began the tests with erratic or quick swimming, only to result in greater immobility towards the end of the test.

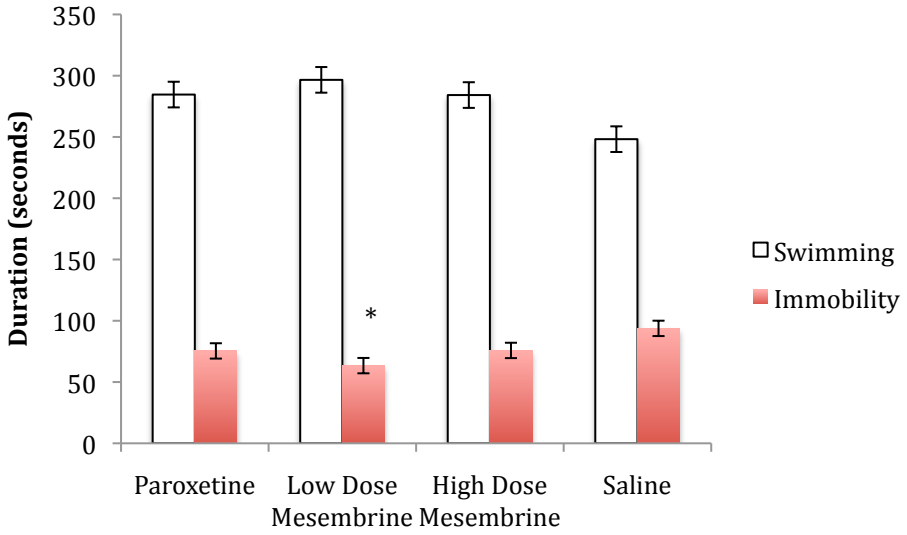

Figure 1. Swimming and immobility duration for four different groups of mice (n=5) as measured by the forced swim test (red=immobility, blue=swimming).
*$p < 0.05$ relative to saline

Open Field Exploration Test

A 4x3 ANOVA with repeated measures was conducted on the open field exploration data. The between groups factor was Drug, and the within groups factor was Time. The line crossings for each mouse were totaled for every ten-minute period. No statistical significance was found on the Time x Drug interaction $F(6, 32) = 0.481$, $p > 0.05$, nor was there a main effect for Drug. However, statistical significance was found for Time $F(2, 32) = 11.081$, $p < 0.0001$. As seen in Figure 2, locomotor behavior decreased across all Drug conditions irrespective of drug treatment.

Mice in the paroxetine group were the most active at each time point, crossing the most lines in each ten-minute period. The low dose mesembrine alkaloid group had the second-highest level of activity, followed by the saline group and finally the high dose mesembrine alkaloid group which experienced the lowest levels of activity at each time point. All groups had their highest locomotor score at the first time point. The paroxetine and low dose mesembrine alkaloid groups had their lowest locomotor scores at the second time point while the high dose mesembrine alkaloid and saline groups both had their lowest locomotor score at the third time point (see Figure 2).

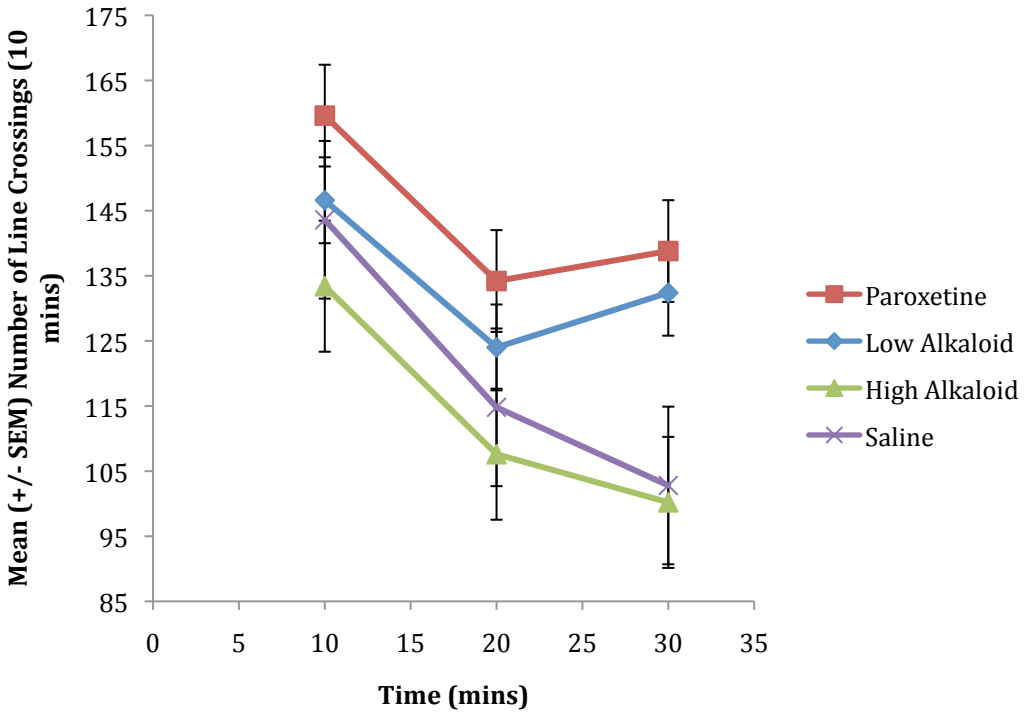

Figure 2. Number of line crossings for four different groups of mice (n=5) as measured by the open field exploration test

Discussion

Forced Swim Test

In the current study, we were looking at the effect of antidepressant activity on the swimming behavior and locomotor activity of mice.

It is known that paroxetine exhibits its antidepressant effects in the forced swim test by reducing immobility duration and thereby increasing swimming duration as compared to the saline group. This is through the phenomenon known as "behavioral despair," which is exhibited by rodents in the forced swim test and is an analog to depression in humans (Porsolt, 1977). Paroxetine is an antidepressant of proven efficacy, so its effects on forced swim behavior was comparable to the different dosages of mesembrine alkaloids. Sugimoto (2011) showed that paroxetine was effective at producing changes in immobility in BALB/c mice, so this served as the positive control.

The high dose mesembrine alkaloid group had an almost identical immobility score to the paroxetine group. The saline group had the longest duration of immobility among the four conditions. However, since the only significant difference was found between the low dose mesembrine alkaloid group and the saline group, we concluded that the high dose mesembrine alkaloid group was only marginally effective at producing antidepressant effects as measured in the forced swim test.

The low dose mesembrine alkaloid group had a lower immobility score compared to both the high dose mesembrine alkaloid group and the paroxetine group. It was concluded that the low dose of mesembrine alkaloids is an effective antidepressant, and this suggests that the alkaloids and paroxetine could be working through a similar mechanism of action to achieve the antidepressant effects as seen

through the forced swim test. Paroxetine works as a selective serotonin reuptake inhibitor (Mellerup and Plenge, 1986), and some of the mesembrine alkaloids are thought to act through this mechanism as well. The alkaloids are also thought to act as phosphodiesterase-4 inhibitors, a mechanism of action that has previously been shown to have antidepressant effects (Zeller, 1984). It is also possible that the low dose of mesembrine alkaloids produces a similar effect to that seen in paroxetine and the high dose of mesembrine alkaloids, but due to the small sample size, there is a greater chance for random error to be introduced.

Open Field Exploration Test

Locomotor activity was assessed using the open field exploration test to see if the drugs being administered had any effect on the mice's movement. The open field exploration test uses the natural exploratory behaviors of rodents to assess their baseline locomotor ability and to determine if the possibility that motoric effects may have influenced the behavioral despair results. It was used to show if any of the drugs administered interfered with the locomotor ability of the mice.

All of the conditions had their largest locomotor score during the first time point, which is a hallmark of the open field exploration test. It was found that there was a significant effect for the main effect of time, which makes sense as the locomotor scores at each time point decrease naturally as the mice become more accustomed to their surroundings, and therefore less exploratory.

The paroxetine and low dose mesembrine alkaloid groups had very similar shapes in their data curves, showing similar decreases from the first to second time

point, followed by a slight increase from the second to third time point. This is not a hallmark of paroxetine in the forced swim test, but the similarity in the curves of the paroxetine group and the low dose mesembrine alkaloid group could suggest a similar mechanism of action between these two groups in their effects. The high dose mesembrine alkaloid and saline groups were similar in the shape of their graph, consistently decreasing along each successive time point. Both groups also had lower average locomotor scores than the low dose mesembrine alkaloids and paroxetine groups. This data indicates that paroxetine and the low dose mesembrine alkaloid groups increase the locomotor activity compared to the saline group, but none of these differences were statistically significant. It was concluded from this that none of the drugs produced any kind of locomotor interference, and this can be ruled out when analyzing the data of the forced swim test.

Conclusions and Future Directions

The data suggests that all groups improved immobility scores significantly compared with the negative control group (saline) (See Figure 1). Therefore, it can be concluded that all drug conditions had an antidepressant effect as measured through the forced swim test. The low dose mesembrine alkaloid group appeared to be the most effective in its antidepressant effects as seen by its low immobility score. However, it is possible that, in a larger sample size, it would have similar antidepressant effects to the positive control group (paroxetine). A test with a larger sample size would need to be conducted for stronger evidence supporting the idea that the low dose of mesembrine alkaloids has greater efficacy as an antidepressant than paroxetine.

The high dose mesembrine alkaloid group seems to work as well as paroxetine, and coupled with the locomotor data, it was concluded that the high dose of mesembrine alkaloids might cause some small amount of locomotor interference. However, this is speculation since none of the locomotor data were significantly different between groups (see Figure 2). With all of this in mind, I speculate that the high dose of mesembrine alkaloids is just as effective as an antidepressant as compared with the low dose of mesembrine alkaloids, but the locomotor interference makes a small difference in the results of the forced swim test.

The GC/MS data indicated there is a very high concentration of mesembranol as compared to all of the other mesembrine alkaloids. It is possible that this specific alkaloid causes some kind of subtle locomotor interference at higher doses, which could lend some reason as to why differences between the high and low doses existed. The percentage of alkaloids varies by individual plant, and the exact mechanism of action for each individual member is not yet understood. There have not been many studies on mesembranol alone, and an area of further study would be to isolate mesembranol to see if it exhibits antidepressant effects on its own when administered at the lower dose. I would conclude, based on previous studies that have looked at mesembrine or mesembrenone as individual alkaloids, that the antidepressant effects seen through the ingestion of *Sceletium tortusoum* are a product of all alkaloids present and their interactions with one another.

All in all, it was concluded from the data that *Sceletium tortuosum*, and specifically the mesembrine alkaloids, would serve as an effective natural alternative for the treatment of major depressive disorder as well as many depressions disorders

across the spectrum. However, to reach further and sounder conclusions, the same test could be done with a much larger sample size such as 48 mice (n=12). The open field exploration test could also be run for 60 minutes as opposed to 30 to see if the increase in locomotion at the third time point seen in the paroxetine and low dose mesembrine alkaloid groups would continue, stay at the same level of locomotion, or drop back down at a similar rate as seen in the high dose mesembrine alkaloid and saline groups. Since the literature does not indicate that paroxetine should increase locomotion at any point during the open field exploration test, I believe this is another issue with the small sample size, or it would have been corrected with a 60-minute test as opposed to the 30-minute test used here.

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