EVALUATION OF LYTHRINE, AN ALKALOID FROM HEIMIA SALICIFOLIA, IN AN AVIAN MODEL OF ANXIETY AND DEPRESSION

_____

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

Sam Houston State University

_____

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

_____

by

Connor D. Carlton

May, 2020

EVALUATION OF LYTHRINE, AN ALKALOID FROM HEIMIA SALICIFOLIA, IN AN AVIAN MODEL OF ANXIETY AND DEPRESSION

by

Connor D. Carlton

_____

APPROVED:

Justin Williams, PhD
Thesis Co-Director

Stephen White, PhD
Thesis Co-Director

Patrick Lewis, PhD
Committee Member

James Harper, PhD
Committee Member

John Pascarella, PhD
Dean, College of Science and Engineering Technology

DEDICATION

To my family for their unending support and love throughout my life and studies.

ABSTRACT

Carlton, Connor D., *Evaluation of lythrine, an alkaloid from Heimia salicifolia, in an avian model of anxiety and depression*. Master of Science (Biology), May, 2020, Sam Houston State University, Huntsville, Texas.

The purpose of this thesis was to evaluate whether an acute exposure to lythrine, a naturally occurring alkaloid substance from *Heimia salicifolia*, has anxiolytic properties in a validated avian model of anxiety and depression. Socially raised, five-day-old chicks were exposed to an isolation stressor for five minutes after receiving one of five treatments. These were: 1) vehicle-treated, 2) clonidine (0.1 mg/kg body weight), and 3) lythrine-treated groups at each of the different dosages (0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg). All lythrine treatment doses were dissolved in 10% cremaphor, 5% ethanol, and 85% deionized water. A solution of exclusively 10% cremaphor, 5% ethanol, and 85% deionized water served as the vehicle-group. Clonidine was dissolved in saline and served as our known anxiolytic positive control comparison. During the exposure to isolation stressor, distress vocalizations (DVocs) of the chicks were recorded. Vehicle-treated animals displayed high rates of DVocs indicative of a panic state and consistent with previous findings in this model. Clonidine-treated animals displayed a reduced rate of DVocs, indicative of an anti-panic (i.e. anxiolytic) effect. All three lythrine doses failed to attenuate panic, as measured by a failure to reduce DVocs in the five-minute isolation test. The findings from this study suggest that at the doses tested lythrine does not possess anti-panic (i.e. anxiolytic) effects. However, limitations include the low dosing as well as housing maintenance issues. For this study, we chose low doses based upon lythrine's limited availability, novelty and limited *in vivo* testing of the compound, and clonidine's active dosage. Future studies should evaluate both higher and lower doses

of lythrine in this model to elucidate a dose-response curve while measuring potential anxiolytic effects. Additionally, further evaluation of lythrine's pharmacokinetics and pharmacodynamics is needed.

KEY WORDS: Heimia, Heimia salicifolia, Salicifolia, Lythraceae, Lythrine, Cryogenine, Vertine, Biphenyl quinolizidine lactone, Alkaloid, Aztecs, Anxiety, Panic disorder, Avian, Animal model, Psychopharmacology, Behavior, Sam Houston State University, Graduate School, Texas

ACKNOWLEDGEMENTS

An enormous thank you to my committee members for their guidance throughout this process.

Additionally, I want to express my deepest appreciation to the great students in the Psychopharmacology Lab led by Dr. Stephen White, without whom I could not have completed this research.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure **Page**

CHAPTER I

Background

Heimia is a genus in the Family Lythraceae that was first described by Johann Heinrich Friedrich Link and Friedrich Otto in 1822, when they erected the species *Heimia salicifolia* (Kunth). The genus name *Heimia* was assigned to honor the German physician Ernst Ludwig Heim (1747-1834) and contains four accepted species (Gledhill, 2008; Malone & Rother, 1994). The species epithet, *salicifolia*, is derived from Salix (willow genus) and folia (leaves), referencing the great resemblance the structure of the plant leaves have to the leaves of a willow tree. Common folk names of *H. salicifolia* include Abre-o-sol (Portuguese for "Sun Opener"), Herva de la vida (Portuguese for "Herb of Life"), Hierba de San Francisco (Spanish for "herb of St. Francis"), Yerba de las ánimas (Spanish for "herb of the spirits"), Shrubby yellowcrest, and Sinicuichi (alternate spelling: Sinicuiche) to name a few (Martinez, 1959; Hoehne, 1939; Dominguez, et al., 1975).

Heimia salicifolia is a deciduous shrub varying from 50 to 300 cm in height and width. The dark green leaves are decussate and are linear lanceolate to lanceolate in shape and 2 - 9.5 cm in length. *Heimia salicifolia* flowers annually between June and September as a yellow 5-7 petal bloom, about 2 cm across, emerging in solitary blooms from the leaf axils. The shape is campanulate, with 10-13 stamens and a thin style. The fruits of this shrub are dry, rounded four-celled capsules containing very small seeds (Link and Otto, 1828). *Heimia salicifolia* prefers hot, sunny, moist places along streams in the high altitudes. Reports indicate wild growth in the southwestern United States, including Texas, as well as throughout Mexico, Central and South America. In addition,

cultivation has been successful as far north as Baja, California and ranging as far south as Argentina (Graham, 1997).

Gordon Wasson first made the connection between *H. salicifolia* with Xochipilli, the Prince of Flowers (Xochi is from the Nahuatl xochitl or “flower”, while pilli means either “Prince” or “child”). This is the Aztec god of psychoactive plants, flowers, spring, fertility, maize, love, desire, games, beauty, song and dance (Wasson, 1974). He is also referred to as Macuilxochitl, which means "five flowers.” This refers to the five sacred and psychoactive entheogens carved onto the Xochipilli statue and its base, which currently resides in the Museo Nacional de Antropologia of Mexico. The five entheogens the statue depicts are mushrooms (*Psilocybe aztecorum*), tobacco (*Nicotiana tabacum*), morning glory (*Turbina corymbosa*), sinicuichi (*Heimia salicifolia*), and possibly cacahuaxochitl (*Quararibea funebris*). *Heimia salicifolia* and its medicinal use can be traced back to an Aztec herbal manuscript detailing various native plants, their medicinal properties and methods used for specific ailments. This manuscript was originally written in the Aztec native language of Nahuatl and later translated into Latin in 1552 by Martin de la Cruz and Juan Badiano titled *Libellus de Medicinalibus Indorum Herbis* (Gates, 2000).

Leaves of *H. salicifolia* have been consumed historically as a folk remedy, especially in Mexico (Martinez, 1959). Practitioners have employed this remedy for a vast majority of ailments including dysentery, inflammation of the uterus, bronchitis and other chest illnesses. It has also been used as a diuretic, laxative, antisyphilitic, emetic, and a general vulnerary (Martinez, 1959; Blomster, et al., 1964; Douglas, et al., 1964; Oliver-Bever, 1972; Lema, et al., 1986; Malone, 1986). Continued modern use seems to

be due to these reported medicinal, as well as spiritual properties. Specifically, many reports include psychoactive and hallucinogenic experiences after the consumption of a decoction of *H. salicifolia* leaves.

The traditional method of preparation the Aztecs used was for *H. salicifolia* leaves to be dried until wilted, placed into a cup or bowl of cool water, and set out in the sun for at least 24 hours. Unbeknownst to them, this allowed for fermentation to occur. Instead practitioners believed that the sun transferred its knowledge and spirit into the drink during its fermentation, which is a critical step in the preparation of the tea or "elixir of the sun". One of many reasons for *H. salicifolia*'s colloquial term "Sun-Opener" (Bechelli, n.d.). The symbolism of the sun and its energy is a historical theme surrounding this plant. Fermentation prior to consuming the tea has long been thought to reduce the side effects associated with ingesting the plant material, such as extreme muscle soreness, nausea and headache.

Consumers of the tea have reported mild states of intoxication with trance-like states (Diaz, 1979), euphoria, and auditory hallucinations (Schultes & Smith, 1976); however, in large doses, there have been reports of visual disturbances and time perception disorders (Rother, 1989). The properties of the sinicuichi tea were first published in 1897 by J.B. Calderon as possessing a unique physiological action, a pleasant drunkenness with objects appearing yellow, and the sounds of bells and human voices heard coming from a long distance (Calderon, 1897). Three decades later, Victor Reko further elaborated on the psychedelic and hallucinogenic effects of *H. salicifolia*, stating,

> *"strength, energy, and joy awaken the spirit. Objects are very clearly seen in great detail. […] Individuals feel as if walking on a soft carpet. They see a door opened but don't hear the sound. There is nothing unpleasant, except that objects have a yellow-blue or purple sheen. Users say it is the remedy to secure happiness"* (Reko, 1926).

These descriptions remain very similar to modern personal accounts on the effects of sinicuichi tea based upon multiple online resources for psychoactive botanicals, such as *The Vaults of Erowid* (http://www.erowid.org/) and *The Lycaeum* (http://www.lycaeum.org/); two of the most comprehensive and up-to-date psychoactive plant databases online today. Although anecdotal evidence, these unusual effects described by Calderon, Reko and online forum users are the reasons into the rigor of scientific testing and discovery associated with *H. salicifolia.* These described effects are an important aspect in the evaluation of this plant considering its history and common cultural practices. However, little scientific research has been conducted on these perceived behavioral effects, even though it remains lawful.

Currently, *H. salicifolia* nor any of its constituents fall under DEA scheduling for controlled substances in the United States. This means all parts of the plant and its constituents are legal to cultivate, buy, possess, and distribute (sell, trade or give) without a license or prescription.

Heimia salicifolia products that are available include viable seeds, live plants, harvested plant material, and tincture extracts labeled "herbal dietary supplements" (http://www.amazon.com/), which do not necessarily comply with Food and Drug Administration's (FDA) safety standards for other medications. Safety testing including

contamination prevention measures to ensure harmful pesticides, insecticides, herbicides, heavy metals, or residual solvents are not required. No systems, governmental or otherwise, are currently in place to confirm any *H. salicifolia* source's safety, validity or known quantity. This all poses serious public health concerns for those seeking to medicate with this substance.

Numerous alkaloids have been identified in the *H. salicifolia* plant with the three most abundant (in order) being cryogenine, lyfoline, and lythrine (Rother, 1990). They all belong to a class of alkaloids known as biphenyl quinolizidine lactones, which are the primary biologically active compounds in the *Heimia* genus. Also included in this class are minor alkaloids such as nesodine, heimidine, sinicuichine, dehydrodecodine, lythridine, and more, totaling 24 known alkaloids, including six newly isolated ones (Kitajima et al, 2018; Kitajima et al, 2019). Interestingly, this group of compounds form in the plant at a much larger amount than the biosynthetically simpler group of phenylquinolizidinyl esters. The cause of this is unknown.

The most abundant alkaloid in *H. salicifolia*, cryogenine ($C_{26}H_{29}NO_5$), has long thought to be responsible for the reported psychoactive and hallucinogenic experiences associated with this plant (Calderon, 1896; Schultes, 1969; Rother, 1989). Although the debate is ongoing, as none of the individual alkaloids of the genus *Heimia* have been classified as a psychotomimetic or psychodysleptic (Malone & Rother, 1994; Rumalla et al., 2008). A psychotomimetic or psychodysleptic is a drug or substance that produces psychological and behavioral changes resembling those of psychosis; a hallucinogen. Cryogenine treatment in animal studies resulted in decrease spontaneous motor activity, muscle relaxation, hypothermia, blepharoptosis and ataxia, in addition to being a diuretic

(Singh, 2011). Additionally, in rats, cryogenine had anticholinergic, anti-inflammatory, antispasmodic, hyperglycemic, hypotensive, sedative, tranquilizer, and vasodilatory effects (Malone and Rother, 1994). Cryogenine is also shown to be 2.48 times more active than aspirin as a prostaglandin synthetase inhibitor (Lema et al., 1986), which may explain much of its anti-inflammatory activity.

Lythrine ($C_{26}H_{29}NO_5$), the third most abundant alkaloid within the plant (Rother 1989), is a diastereomer of the purported psychoactive alkaloid cryogenine. Lythrine has a (*trans*)-Z-configuration at C-10 of the quinolizidine ring, while cryogenine has a (*cis*)-E-configuration (Figure 1).

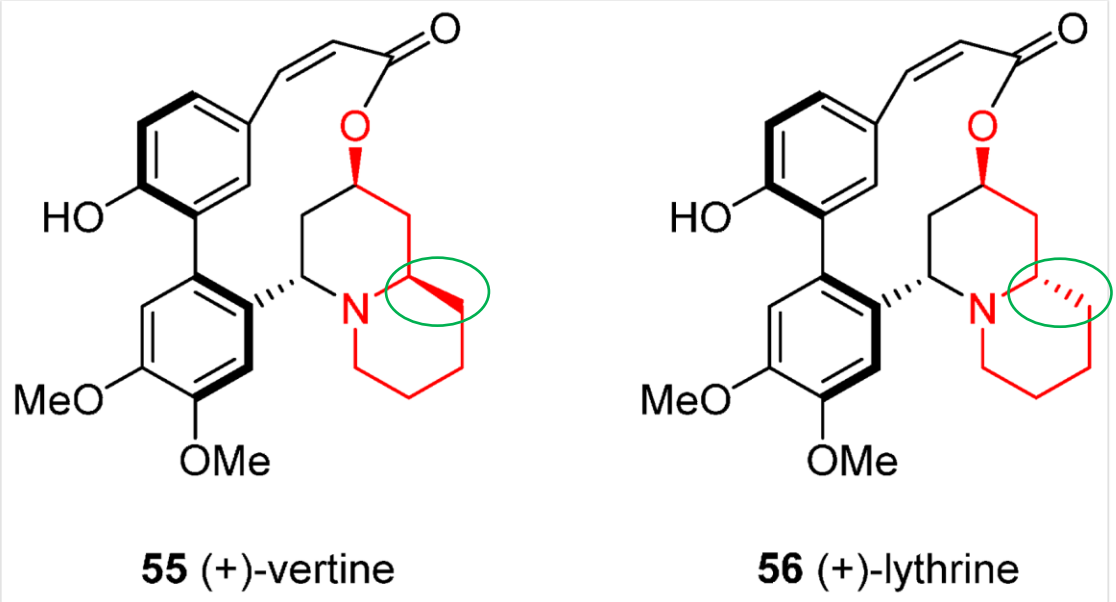

Figure 1. Lythrine and cryogenine (vertine) are diastereomers.

Lythrine is the *trans*-isomer, which is lower in enthalpy, and thus is a more stable isomer (Bendz, 2013). Lythrine is reported to be a diuretic and to have anti-inflammatory effects (United States Patent No. 3,184,446; Weisbach, 1965; Byrne and Malone, 1981), and the most potent vasorelaxant alkaloid of *H. salicifolia* (Guzmán-Hernández et al.,

2018). As demonstrated in a phenylephrine (Phen) induced model of vasoconstriction using isolated and perfused rat mesenteric vascular bed (MVB) preparations with intact endothelium, lythrine produced a concentration-dependent vasorelaxation effect. Action mechanisms were elucidated via the successful reduction of lythrine-induced vasodilation when individually perfusing the *in vitro* MVB preparations with N^{ω}-nitro-L-arginine-methyl ester (L-NAME) (an unspecific nitric oxide synthase inhibitor), methylene blue (a guanylate cyclase inhibitor), Wortmannin (a phosphatidylinositol 3-kinase inhibitor), and atropine (a muscarinic cholinergic receptor antagonist). Additionally, the MVB preparations with eliminated endothelial cells significantly reduced the lythrine-induced vasodilation. These results suggest that lythrine's vasorelaxant effect is dependent on the release of endothelial nitric oxide synthase (eNOS). The activation of eNOS depends on the formation of calcium-calmodulin complex (Su et al., 2014). However, eNOS can also be activated by phosphorylation with phosphatidylinositol 3-kinase (PI3K), a calcium-independent mechanism (Zhang et al., 2014). The PI3K-dependent activation of eNOS is also an important mechanism in the vasodilation effect of lythrine, as demonstrated by Wortmannin's strong reduction of lythrine-induced vasodilation. The involvement of cyclic guanosine monophosphate (cGMP) in the relaxant and hypotensive effects of lythrine was also verified by the complete elimination of lythrine's vasodilation effects as the guanylate cyclase (GC) inhibitor, methylene blue, was introduced. Evidence lythrine is mediated through the interaction with cholinergic muscarinic receptors was verified through the reduction of lythrine-induced vasodilation with the introduction of the muscarinic cholinergic receptor antagonist atropine. In every test, lythrine's effects were reported as similar to the effects of the study's control drug acetylcholine (ACh)

(Guzmán-Hernández et al., 2018). ACh serves as the main neurotransmitter of the parasympathetic nervous system with functions of dilating blood vessels, reducing blood pressure, and slowing heart rate. These findings demonstrate the close similarity of lythrine's potent vasodilation and hypotensive effects to the established vasodilation and hypotensive effects of ACh, which was a critical factor in determining the basis for performing this study and formulation of its hypothesis. Additionally, the researchers of this study found it logical to evaluate lythrine's potential anxiolytic effects due to all the findings observed in the Guzmán-Hernández study, especially lythrine's reported potent vasodilation properties via the activation of the nitric oxide (NO)/GC pathway. The NO/GC pathway initiates and maintains vasodilation through a series of biological events resulting in the relaxation of smooth muscle cells lining arteries, veins, and lymphatics thus causing a reduction in blood pressure and heart rate. This pathway works when NO, formed by vascular eNOS, binds to the heme group of hemoglobin in red blood cells (RBC) and to the heme group of the enzyme GC, which is found in vascular smooth muscle cells. Once it diffuses into the vascular smooth muscle cells adjacent to the endothelium and binds to the GC enzyme, NO activates GC. The GC enzyme then catalyzes the dephosphorylation of GTP to cGMP. The cGMP serves as an important secondary messenger for signaling smooth muscle relaxation via the phosphorylation, or addition of a phosphate group, to the smooth muscle contractile protein of myosin. Once myosin is phosphorylated, it relaxes. This relaxation results in the dilation of blood vessels originally exposed to NO, and a decrease in blood pressure and heart rate occurs. With this information, the scientific backing for the evaluation of lythrine to treat anxiety via two common physiological symptoms of anxiety, high blood pressure and increased

heart rate, was determined. At 87 percent, accelerated heart rate is the most common symptom patients suffering from anxiety and panic attacks self-report (Rapee, Craske, & Barlow, 1990). Additionally, according to a systematic review and meta-analysis of epidemiological studies, there is a strong association between anxiety and hypertension (Yan, et al., 2015).

The second most abundant alkaloid, lyfoline has no reported effects.

Important to note is the fact that isolated *Heimia* alkaloids have been experimentally shown to mimic qualitatively and semi-qualitatively the action of the total alkaloid extracts of *H. salicifolia*, especially vasodilation (Schultes, 1969; DeKorne, J. 2011; Guzmán-Hernández et al., 2018). Therefore, emphasizing further therapeutic and medicinal research of isolated lythrine is necessary, as its effects may resemble closely the effects of the whole-plant folk decoction of sinicuichi, as well as provide therapeutic benefits reported for centuries.

Additionally, with further testing and understanding of lythrine and the therapeutic alkaloids and constituents of *H. salicifolia*, targeted application of useful compounds and respective constituents can be performed. Given this, along with lythrine's diuretic, anti-inflammatory, sedative, anti-hypertensive, and vasorelaxant effects, an investigation of this individual compound's potential psychological benefits, especially on anxiety, would be valuable.

CHAPTER II

Anxiety

According to the American Psychiatric Association's (APA) Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), anxiety disorders include conditions that share features of excessive fear and anxiety and related behavioral disturbances. Anxiety is described as the anticipation of future threat. More relatedly to this project's model, panic attacks are an abrupt surge of intense fear or intense discomfort that reaches a peak within minutes, and during which time four or more of a list of 13 physical and cognitive symptoms occur. These symptoms include palpations, pounding heart or accelerated heart rate; sweating; trembling or shaking; sensations of shortness of breath or smothering; feelings of choking; chest pain or discomfort; nausea or abdominal distress; feeling dizzy, unsteady, light-headed, or faint; chills or heat sensations; paresthesia (numbness or tingling sensations); derealization (feelings of unreality) or depersonalization (being detached from one-self); fear of losing control or "going crazy"; and fear of dying (APA, 2013, pp. 189, 208-217).

According to facts and statistics presented by the Anxiety and Depression Association of America (ADAA), anxiety disorders are the most common mental illness in the U.S., affecting 40 million adults in the United States age 18 and older, or 18.1% of the population every year. Additionally, anxiety disorders affect 25.1% of children between 13 and 18 years old. Untreated children with anxiety disorders are at a higher risk of performing poorly in school, absence at important social experiences, and engaging in substance abuse. Anxiety disorders are highly treatable, yet only 36.9% of those suffering receive treatment. Many people with an anxiety disorder have

comorbidity, commonly depression or physical illness, which can make their symptoms worse and recovery more difficult. People with an anxiety disorder are three to five times more likely to go to the doctor and six times more likely to be hospitalized for psychological disorders than those who do not suffer from anxiety disorders.

Generalized Anxiety Disorder (GAD) affects 6.8 million adults, or 3.1 percent of the United States' population, yet only 43.2 percent are receiving treatment. Panic Disorder (PD) affects 6 million adults, or 2.7 percent of the United States' population (ADAA, 2018). This affects women twice as often as men on paper, although these numbers are based on reported individuals. The number for men may be higher as they are less likely to report and seeking treatment is less prevalent in this group. Additionally, confusing panic attacks with heart attacks are a concern, as they manifest similarly (i.e. chest pains).

In 2013, the annual cost of anxiety disorders in the United States was $48.72 billion, which is a significant portion of the United States healthcare resources (Shirneshan, 2013). However, these costs can be split between the psychological treatment of anxiety and the physiological or somaticized symptoms resulting from anxiety. According to The Economic Burden of Anxiety Disorders, a study commissioned by the ADAA and based on data gathered by the association and published in the Journal of Clinical Psychiatry, anxiety treatments cost almost one third of the $148 billion total mental health bill per year for the U.S. and more than $22.84 billion of those costs are associated with the repeated use of healthcare services due to the somatization of anxiety, which is the conversion of the anxious mental state into physical symptoms, such as severe stomach aches, chest pains, and migraines.

There is a bifurcation of the common treatments for anxiety disorders. One treatment option is non-pharmacological, which includes therapies such as Cognitive Behavioral Therapy (CBT), cognitive restructuring, interoceptive and structured exposure, Ost's applied relaxation, and breathing retraining. The goals of these techniques include gaining skills to control physiological activity. They seek to restructure one's thoughts via change in behavior – calming the mind through physiological actions. In some cases, such as Ost's applied relaxation and breathing retraining, this includes reducing heart rate by controlling respiration rate.

The second treatment option is the use of pharmaceutical drugs, such as barbiturates and benzodiazepines. Barbiturates were the original class of anxiolytic drugs used largely in the 1960s and 1970s as a treatment for anxiety, insomnia, and seizure disorders. Barbiturates are known as central nervous system (CNS) depressants. They enhance the action of gamma-aminobutyric acid (GABA), a neurotransmitter that inhibits the activity of nerve cells in the brain. Apart from a few specific indications, they are rarely prescribed for anxiety anymore, having been superseded by benzodiazepines. In the 1970s, these barbiturates were given the nickname "downers" due to their strong suppressive effect on the CNS, causing many people to die after taking high doses. Barbiturates are also extremely addictive. Together, these factors are why they are rarely prescribed today.

Benzodiazepines, such as alprazolam, clonazepam, diazepam, and lorazepam, reduce cellular activity in the amygdala – a small, paired structure of the brain located in the medial temporal lobe, just anterior to the hippocampus and is primarily associated with autonomic responses of emotional processes, especially fear. It has been

demonstrated to be hyperactive in patients experiencing anxiety symptomology (Bremner, 2004; Damsa, Kosel, & Moussally, 2009; Kent & Rauch, 2003; Rauch, Shin, & Wright, 2003). Benzodiazepines are agonists of the gamma-aminobutyric acid-alpha (GABA-A) receptor, and when bound, causes an allosteric modulation to occur in the receptor resulting in the opening of chloride-ion channels. This allosteric modulation is a structural change of the GABA-A receptor, thus allowing increased chloride-ion migration into the motor neurons and an increase in GABA-A receptor activity. Ultimately, this results in cellular hyperpolarization, or the flooding of negative ions into the neuron, causing the inhibition of the ability of an action potential to fire, as an action potential must reach a firing rate at a positively charged threshold. The amygdala is densely packed with GABA-A receptors and their sustained activation by benzodiazepines decreases amygdala activity via the mechanisms described above and provides anxiety symptom relief (Shin & Liberzon, 2009). The primary shortcoming of benzodiazepines is their high risk for abuse and dependence. Long-term use is not advised or recommended, as withdrawal reactions will occur when discontinuing use. Common side effects for benzodiazepines are drowsiness, sleepiness, or dizziness. Additionally, unusual sleep behaviors and even anterograde amnesia may occur.

Alternatively, clonidine, an FDA-approved anxiolytic, produces its anxiolytic effects through a different mechanism. Clonidine belongs to a drug class called alpha-agonist hypotensive agents. Clonidine is an agonist at the presynaptic noradrenaline alpha-2 autoreceptor. When introduced to the system, it binds to the alpha-2 autoreceptor, which is coupled to an inhibitory G-protein and located in the noradrenaline pre-synaptic neuron. When bound, the inhibitory G-protein is activated, thus inactivating the enzyme

adenylate cyclase. Consequently, this inhibits the production of the enzymes cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA, also known as cAMP-dependent protein kinase). A derivative of adenosine triphosphate (ATP), cAMP is used for intracellular signal transduction, such as transferring into cells the effects of hormones like adrenaline, which cannot pass through the plasma membrane. The reduction of cAMP and PKA production leads to the reduction of calcium current influxes into the neuron. Ultimately, causing the neuron to restore its polarization state and stop the release of noradrenaline to the synaptic cleft. The result is the reduction of sympathetic outflow from the CNS and decreases of noradrenaline activity in the brainstem and on cardiac muscle, thus decreasing heart rate and blood pressure. It is this reduction in these physiological measures, blood pressure and heart rate, that result in clonidine's anxiolytic effect. Side effects of clonidine are also numerous and undesirable and include, for example, hypotension and tachycardia (Drugs.com, 2019).

Because of the societal impact of anxiety disorders combined with the side effects or consequences of current anxiolytic pharmacotherapies, the need to identify novel therapeutics for these disorders is vital. Exploring natural products may be a promising avenue.

Natural products are beginning to gain more recognition in mainstream science as possible remedies for psychological ailments such as anxiety, post-traumatic stress disorder (PTSD) and various addictions. For example, the Multidisciplinary Association for Psychedelic Studies (MAPS) has been testing entheogens marijuana, ayahuasca, ibogaine, peyote, kratom, mescaline, and psilocybin to treat a variety of mental disorders, and various behavioral and substance addictions. Entheogens are psychoactive plants or

chemical substances that induce alterations in perception, mood, consciousness, cognition, or behavior, especially in a spiritual context. In a similar vein of focusing on natural products as potential psychological therapeutics, including those derived from a psychedelic source, this study attempted to add to that body of research. To date, no study has examined *H. salicifolia*, and specifically lythrine's potential therapeutic value for psychological disorders. Thus, the current study aims to evaluate the potential anxiolytic (i.e. anti-panic) effects of lythrine in a well validated avian model of anxiety and depression.

As mentioned previously, Guzmán-Hernández and colleagues reported lythrine's potent vasodilation properties with physiological effects similar to ACh, such as reduction in blood pressure and heart rate. These are similar physiological responses to the anti-panic drug, clonidine. Therefore, it is logical to evaluate lythrine for potential anxiolytic effects.

CHAPTER III

Objective

The objective of this project was to evaluate lythrine for potential anxiolytic effects. Given lythrine's vasodilation and resulting blood pressure and heart rate reduction, it was reasonable to assume that it may have anxiolytic effects similar to clonidine.

The researcher hypothesized (H_1) one, or more, of the doses of lythrine would produce anxiolytic effects as measured by a reduction in DVoc rates during the five-minute isolation period, similar to that of clonidine.

The null hypothesis (H_0) is accepted if no statistically significant differences (i.e. reduction in DVoc rates) are observed between lythrine-treated groups and vehicle-treated group. Additionally, in order to compare lythrine to a known anxiolytic, a clonidine-treated group was included as a positive control.

CHAPTER IV

Materials and Methods

Chick Anxiety-Depression Model

Although the aim of this study is focused on exclusively identifying potential anxiolytic effects of lythrine, a description of the entire model and its utility follows.

The chick anxiety-depression model is a dual pharmacological screening assay in which both anxiety and depression present sequentially over a 90-minute isolation period. This paradigm utilizes socially raised domestic fowl chicks (*gallus gallus domesticus*), aged 4-6 days post hatch. Exposure to isolation stress produces the behavioral measure known as distress vocalizations (DVocs), which are recorded via custom designed software. The DVoc rate during the first five minutes of isolation are high and models a panic state (i.e. anxiety), then declines over the next 20-30 minutes to about half of their initial rate and remain stable for the remainder of isolation. The last 60 minutes of isolation models behavioral despair (i.e. depression). These two phases are pharmacologically dissociable in that anxiolytics reduce DVoc rates during the initial anxiety phase and antidepressants increase DVoc rates in the depression phase (i.e. attenuation of behavioral despair).

Even though rodent-based models seemingly remain the preferred models for pharmacological screening of anxiolytic compounds, with the open-field and elevated plus maze tests being the most commonly used to screen anxiolytics, the chick model has accurately screened all current FDA-approved drugs for the treatment of anxiety, specifically panic disorder, and depression (Sufka et al., 2009; Warnick, Wicks, and Sufka, 2006). It has-accurately screened several clinically efficacious anxiolytics, such as

the benzodiazepine chlordiazepoxide, and noradrenaline alpha-2 agonist clonidine. Additionally, the model has accurately screened clinically relevant antidepressants for anxiolytic effects. For example, high doses of antidepressants, such as the tricyclic antidepressant imipramine and the tetracyclic antidepressant maprotiline, produce anxiolytic effects in the clinical setting, as well as in the model (Sufka et al., 2006; Warnick et al., 2008). Combined, these findings support evidence for the high predictive validity for this avian model. Meaning the model can detect the anxiolytic effects of a wide range of compounds. The high predictive validity of the model reduces the vulnerability to false positives, where a drug shows efficacy in the animal model but fails in clinical trials, and/or false negatives, where a drug screens ineffective in the model but would have benefited individuals with ailment. Models with less predictive validity are more prone to false positives, which push ineffective drugs into Phase I and II of clinical trials exposing human subjects to potentially harmful compounds and using valuable investment resources by costing millions of dollars. Additionally, models prone to false negatives prevent effective compounds from clinical use, and thus fail to provide effective treatment for those suffering. This also effects corporate shareholder profitability.

A major concern regarding the rodent models listed above is their ability to meet the National Institute of Health's (NIH) and United Kingdom's policy of Reduction, Refinement, and Replacement. Compared to rodent models, this avian model reduces the number of purpose-bred research animals as male chicks are a by-product of the commercial egg-laying industry, which are otherwise discarded at hatch. The male chicks used in drug screening adds no purpose-bred animals, and even decreases the necessity of

purpose-bred animals for research. The methodology of this model also meets the second "R" of refinement by minimizing the stress-provoking stimuli to a single 90-minute test session. The current study further minimized the stress-provoking stimuli to a 5-minute test session. Finally, the model also replaces the standard rodent-based models of anxiety and depression with a lower phylogenetic, and perhaps, less sentient species according to the NIH hierarchy of sentient species (National Institute of Health, 2020). Through the development and validation of the avian model described above, many of these concerns have been addressed.

This chick paradigm provides a more clinically relevant, non-rodent-based model of psychological syndromes and is useful as a dual drug screening for both anxiolytic and antidepressant compounds. As mentioned above, evidence of the model's strong predictive validity has been demonstrated by its ability to accurately screen all current FDA-approved drugs for the treatment of anxiety (i.e. panic disorder) and depression (Sufka et al., 2009; Warnick, Wicks, and Sufka, 2006). Furthermore, given the high comorbidity of anxiety and depressive disorders, this chick anxiety-depression model more closely resembles the clinical presentation of these disorders, as it can simulate both an anxiety phase and depression phase over a 90-minute test period.

Methods

Cockerels are received into the laboratory on hatch day and housed in 34 x 57 x 40 cm cages with 12 chicks per cage. Food and water are available *ad libitium* via gravity feeders (Figure 2). Daily maintenance that entails the replacement of tray liners and filling food and water gravity feeders is conducted during the hour that precedes the

animal's dark cycle. Lights are operated on a 12:12 light-dark cycle. Supplemental heating sources are provided to maintain appropriate housing temperatures in the range of 34-35ºC.

Figure 2. Chick housing. 34 x 57 x 40 cm cages. Food and water are available *ad libitium* via gravity feeders.

A six-unit testing apparatus containing Plexiglas chambers (25 x 25 x 22 cm) surrounded by sound attenuating media is used to record separation-induced vocalizations aimed at modeling anxiety (0-5 minutes of social separation) and depression (30-90 minutes of social separation) patterns of responses (Figures 3 & 4). Each unit is lined with acoustical fiber media, illuminated by a 25-Watt light bulb, and ventilated by an 8-cm-diameter rotary fan (Model FP-108AX S1, Commonwealth Industrial Corp., Taipei, Taiwan). Miniature video cameras (Model PC60XP, SuperCircuits, Inc., Liberty Hill, Texas, USA) mounted in the sound-attenuating enclosures at floor level and routed

through a multiplexor (Model PC47MC, SuperCircuits, Inc.) provided televised display of the chicks for behavioral observation (Figure 5). To record DVocs, microphones [Radio Shack Omnidirectional Model 33-3013 (modified for AC current)] are mounted at the top of the Plexiglas chamber. These vocalizations are routed to a computer equipped with custom designed software for data collection.

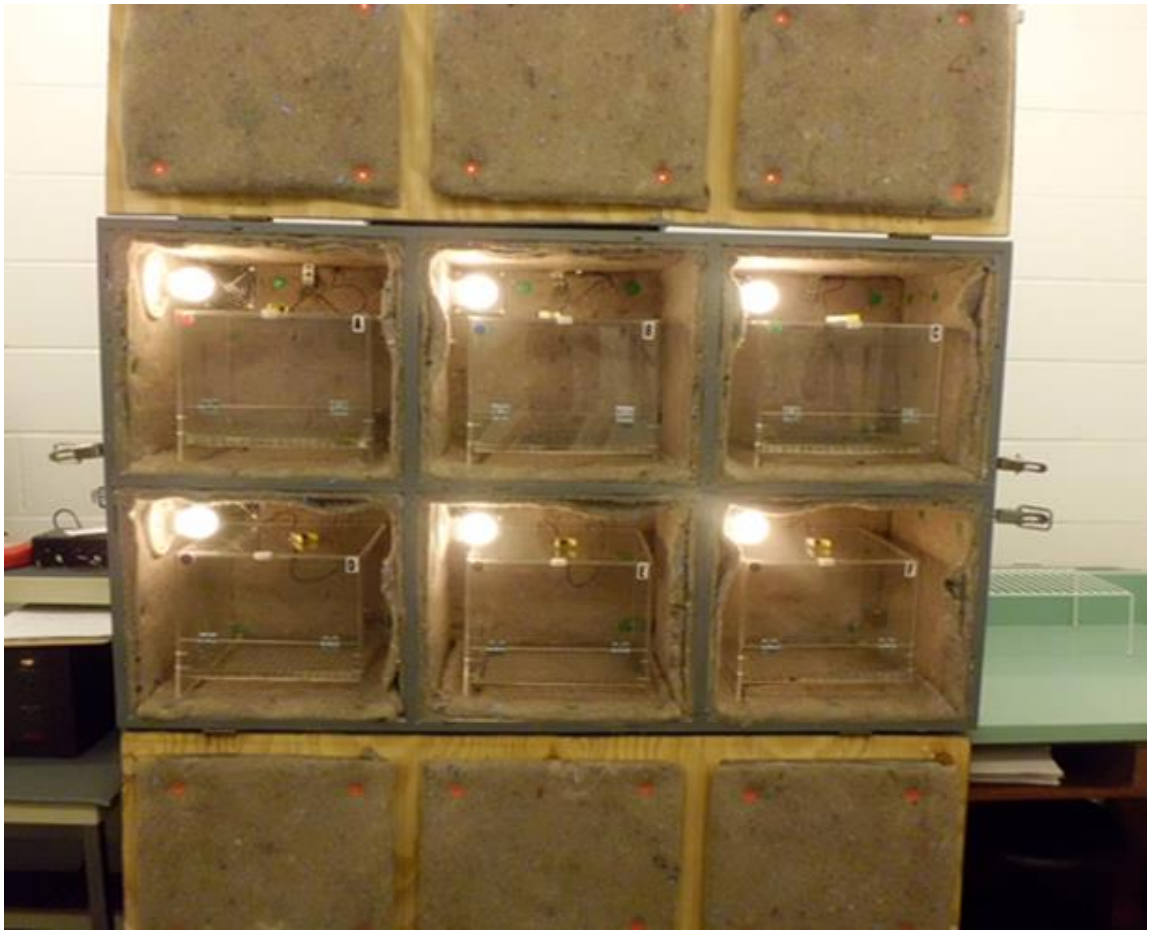

Figure 3. Six-unit testing apparatus. Used to record separation-induced vocalizations aimed at modeling anxiety (0-5 minutes of social separation) and depression (30-90 minutes of social separation) patterns of responses.

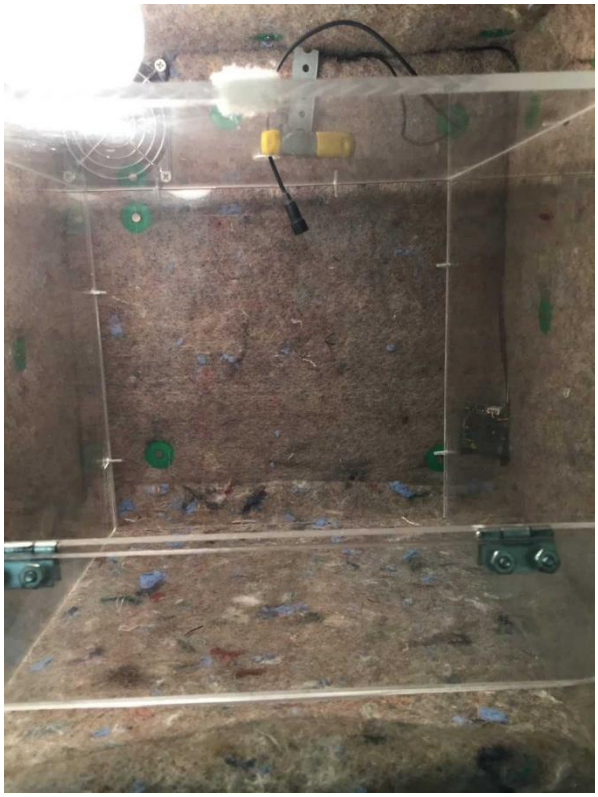

Figure 4. Plexiglas chamber. Surrounded by sound attenuating media with a microphone inserted inside to record separation-induced vocalizations aimed at modeling anxiety and depression.

Figure 5. Televised display. Provides for visual behavioral observation of chicks.

Procedure

Test compounds were dissolved in 10% cremaphor, 5% ethanol, and 85% deionized water. Isolated conditions included a vehicle-treated group, a clonidine-treated group (0.10 mg/kg) and 0.1, 0.3, and 1.0 mg/kg lythrine-treated groups. Lythrine was sourced from Cayman Chemical at 95+% purity (CAS 5386-10-2).

Squads of six chicks were taken from their home cage (labeled A-I) and placed within a lidded plastic transport container. To track subject assignment to various treatment conditions, chicks were marked using colored felt pens. Body weight was recorded for each chick to determine volume of drug administration and to identify outliers (i.e. low body weight (<30g), which may signify unhealthy development). Drugs were administered via intraperitoneal (IP) injection. Injection-to-test interval was 15 minutes. This is the time from which the chick was injected to the time the chick was placed into the testing apparatus, beginning the evaluation. This time was based on the standard practice of the earlier research in this lab, and the limited body of previous studies on lythrine and its pharmacokinetics. Chicks were group transported inside the lidded container to an adjacent testing room. Each chick was placed into an individual testing unit. Doors for the testing chamber were then closed and secured. The program for recording vocalizations was started and allowed to run for 6 minutes to record behavior in the anxiety-phase only. The extra minute was run for any possibility of computer-automated timing errors. The original 90-minute test was abbreviated to minimize the exposure of the subjects to excess stress, as there is no reasonable expectation of antidepressant effects by lythrine according to previous literature. Following the completion of the test session, chicks were removed from the testing apparatus and

returned to their home cage. Records of the electronic files from the data collection program recording vocalizations were stored on the hard drive and backed up on a flash drive for data analysis. The dependent measure of distress vocalizations was recorded as a rate function (i.e. DVoc/min). For statistical analysis, data was converted into a DVoc/5min rate function. Data was analyzed via IBM SPSS Statistics v22 software package performing a univariate analysis using treatment factor one-way ANOVA, and Dunnett's test for post-hoc analysis. Each treatment group included 18 chicks, totaling 90. This sample size was determined based on prior research in this chick model to yield an adequate statistical power of 0.80 or above. An anxiolytic effect is defined as a significant reduction in average DVoc rates over the five-minute isolation period, compared to the vehicle-treated animals. The miniature cameras located within each isolation unit allowed for the observation of any sedative (i.e. lethargy) effects produced by test articles.

Animals were observed for potential sedative effects which would have confounded DVoc rates. Data sheets were kept next to the laptop. Mild sedative effects (i.e. lethargy) were observed in approximately 25% of clonidine animals, however, no sedation was observed in any of the lythrine-treated groups at any of the doses test, nor the vehicle-treated groups.

CHAPTER V

Results

No significant effect of lythrine on DVocs was observed (Figure 6). The pattern of DVocs in vehicle-treated isolated chicks is consistent with previous findings from this laboratory. Vehicle-treated isolated birds displayed high rates of distress calls during the five minutes of isolation (i.e. anxiety phase). A one-way ANOVA of the isolated groups during anxiety phase of the model (0-5 min) showed a significant main effect for treatment ($F_{4,89} = 10.111$, $p = 0.000$), $\eta_p^2 = 0.322$, observed power = 1.000. Dunnett's post-hoc analysis revealed a significant effect for clonidine (i.e. anxiolytic effect) and failed to reveal significant differences between the vehicle group and any of the three lythrine groups (i.e. lythrine failed to produce anxiolytic effects).

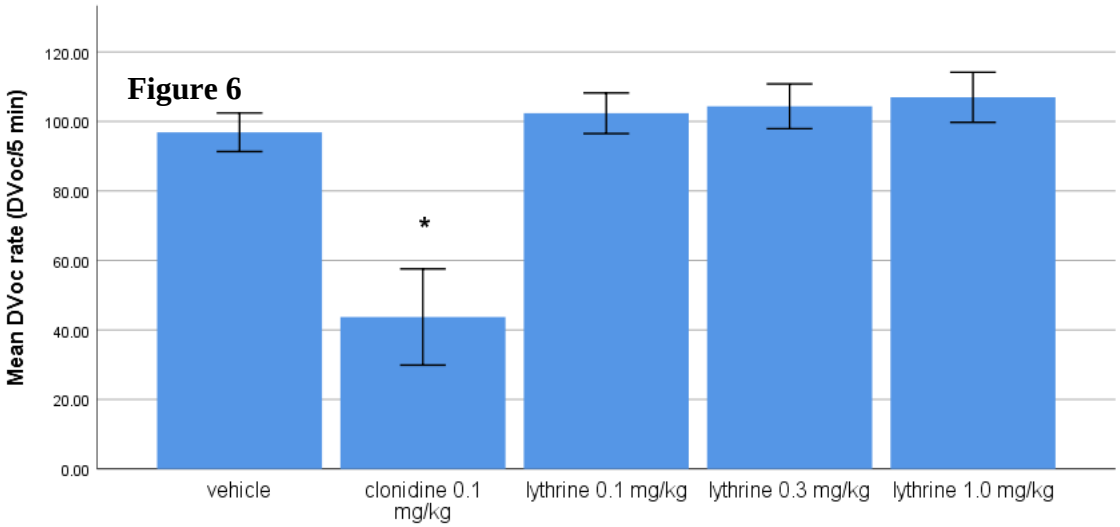

Figure 6. The effects of clonidine and lythrine on isolated-induced distress vocalizations (DVocs). Values represent mean (±SEM) DVoc for a 5-min isolation period as a function of the five treatment groups. *Indicates significant decrease in DVoc compared with vehicle condition ($F_{4,89} = 10.111$, $p = 0.000$).

CHAPTER VI

Discussion

The goal of this project was to evaluate the naturally occurring alkaloid of *H. salicifolia*, lythrine, for potential anxiolytic effects in the chick anxiety-depression model. This model is well-validated and possesses high predictive validity as all FDA-approved anti-panic medications have been positively screened in the model (Warnick, Wicks and Sufka, 2006).

Due to the high predictive validity of this model, lythrine's vasorelaxant properties and, more specifically, its resulting reduction of heart rate and blood pressure (Guzmán-Hernández et al., 2018), it was hypothesized that one or more of the lythrine doses would demonstrate anti-panic effects as measured by a reduction in distress vocalizations. Lythrine's effect on heart rate and blood pressure reduction is similar to that of the known anxiolytic clonidine. As mentioned previously, clonidine reduces heart rate and blood pressure by its reduction of noradrenergic activity on heart muscle, and therefore, used specifically in the treatment of panic disorders. As such, clonidine was chosen as the positive control compound.

In congruence with previous findings of this lab, the vehicle-treated animals displayed high rates of distress calls during the five-minute isolation period (i.e. anxiety phase), indicative of the animals in a panic state. As expected, clonidine-treated animals attenuated DVoc rates, indicative of an anti-panic (i.e. anxiolytic) effect. However, none of the three doses of lythrine (0.1, 0.3, 1.0 mg/kg) produced a significant attenuation of panic (i.e. did not produce anxiolytic effects), as measured by change in DVocs compared to vehicle treated (Figure 6). The findings from this study suggest, at the doses tested,

lythrine does not produce anti-panic (i.e. anxiolytic) effects. Therefore, the hypothesis is rejected, and the null must not be rejected.

The lack of statistically significant results for lythrine may be due to the poor absorption of lythrine when injected IP. The study referenced earlier by Guzmán-Hernández et al. produced significant results indicating lythrine's potent vasodilation effects via intravenous (IV) administration. Although a very common route of administration for this model and standard in pre-clinical trials, IP injection depends on the absorption of numerous cell layers, thus slowing the substance's absorption rate compared to IV. Given the factors of age and size of the subjects, IV was not considered in this study, as the difficulty of locating and correctly injecting blood vessels of 4-day-old chicks may have been extremely challenging and an additional stressor on the animal, thus confounding stress manipulation. Intramuscular (IM) injection could be an effective alternative for future studies, as this route of administration is also common and may allow for better absorption into the blood stream compared to IP injection.

Limitations of the current study include dosage potency, housing maintenance issues, and the lack of research involving lythrine's pharmacokinetics, especially in avian models. Originally, 25 mg of lythrine were ordered and drug was diluted according to company specifications to test 10 mg/kg, 3 mg/kg, and 1 mg/kg. These doses were chosen based on previous research in rodents with lythrine and its diastereomer cryogenine wherein higher doses of these compounds (100 mg/kg for lythrine; 68 mg/kg for cryogenine) with IP administration resulted in death (Kaplan & Malone, 1966; Robichaud et al., 1964), and 5-15 mg/kg of lythrine administered orally to rats was active as a diuretic (Weisbach, 1965). The vendor of lythrine recommended to dissolve with

DMSO at a ratio of 20mg lythrine:1mL DMSO. In order to perform serial dilution for purpose of injection, saline was added to attain test doses. However, data included with the drug failed to describe lythrine's hydrophobic properties. Thus, after adding saline to the DMSO/lythrine solution to perform injections, a precipitate formed, rendering the solution unusable. After consultation with the vendor, a significantly reduced volume of lythrine was acquired (6.0 mg). The highest dosages available were the ones used in this study (1 mg/kg, 0.3 mg/kg, and 0.1 mg/kg). The resulting reduction in doses is potentially an additional factor contributing to the null results.

Additionally, maintenance of the animals on the final day of housing in their vivarium housing units was absent. While unconfirmed, the lack of resources (i.e. food and water) may have influenced the bioavailability (i.e. absorption) of the drug, and behavioral stress response (i.e. DVocs) once isolated. Evidence for this possibility can be seen in clonidine's effects. Compared to previous studies in this model, clonidine showed exaggerated anxiolytic effects and atypical sedative effects at the dose tested. The lack of previous research regarding lythrine's pharmacokinetic properties in any model is a limitation, especially in conjunction with the accidental lack of resources provided to the chicks on the final day of housing. Without having a better understanding of lythrine's pharmacokinetics, we are unable to speculate whether lythrine was metabolized at an altered rate, thus affecting the inject-to-test interval and, more importantly, the DVoc rate of each treatment group. However, data suggests the lack of resources had an effect, as the clonidine group showed more sedation and fewer DVocs compared to previous studies in the lab. In clinical settings, clonidine is recommended to take on a full stomach.

Lythrine-treated groups at all doses tested increased in DVocs compared to the vehicle-treated group. Although not statistically significant, lythrine did produce a dose response, thus these increase in DVocs may suggest that the doses tested were too high. However, this increase could also be caused by the stress factor of experiencing dehydration and/or empty stomach due to the lack of housing resources. Alternatively, this may indicate an angiogenic property of lythrine, however, more testing is necessary to make that claim.

Regarding these findings the researcher suggests multiple future studies are necessary. Firstly, testing both higher and lower dosages of lythrine concentrations to elucidate a dose-response curve for potential anxiolytic effects. This could further explore if lythrine and biphenyl quinolizidine lactones as a group are potentially a new class of anxiolytics – a drug class at an impasse for new discoveries for quite some time (Griebel and Holmes, 2013).

Additionally, the further evaluation of the pharmacodynamic and pharmacokinetic properties of lythrine, both *in vivo* and *in vitro* settings are necessary in order to more comprehensively understand how this compound may work in clinical settings. These studies are needed to further elucidate the mechanisms and pathways by which lythrine works in the body, including the affinity, efficacy, and potency, as well as the time course of lythrine's absorption, distribution, metabolism and extraction. A method beneficial for further understanding the compound's pharmacokinetics of the circulatory system, and help injection-to-test interval timing, would be to test the blood concentration of lythrine at various time points from 0 to 60 minutes after various methods of administration. The objective being the determination of how quickly the compound circulates throughout the

subject's bloodstream, when concentration peaks, and how long complete metabolization occurs.

Finally, the researcher suggests the evaluation of a traditionally made whole plant extract of *H. salicifolia* using this chick model to test anxiety effects via gavage administration of the sinicuichi tea decoction. The substance should be chemically analyzed to ensure the inclusion of all major alkaloids mentioned. This evaluation would be valuable to determine if the alkaloids possess anxiolytic properties synergistically.

REFERENCES

Abiatha, S., & Jaehn, M. (2014). *U.S. Patent No. 20160174603*. Washington, DC: U.S. Patent and Trademark Office.

Bechelli, J. (n.d.). *Via Oneira: Three Dreaming Herbs of Mexico.* Unpublished manuscript.

Bendz, G. (2013). *Chemistry in Botanical Classification Medicine and Natural Sciences*. Burlington: Elsevier Science.

Blomster, R. N., Schwarting A. E., Bobbitt J. M. (1964). Alkaloids of *Heimia salicifolia*. I. A preliminary report. Lloydia 27:15-24.

Bremner, J. D. (2004). Brain imaging in anxiety disorders. *Expert Review of Neurotherapeutics*, 4(2), 275–284. doi: 10.1586/14737175.4.2.275

Byrne, J. A., & Malone, M. H. (1981). Protective effects of cryogenine, lythrine, and certain derivatives in croton oil induced inflammation. *Proceedings of the Western Pharmacology Society, 24*, 183-187.

Calderon, J.B. (1897). Estudio sobre el arbusto llamado sinicuiche. *Instituto Mediconacional Anales. (Mexico)* 2: 36-42.

Clonidine Side Effects: Common, Severe, Long Term. (2019, January 2). Retrieved from https://www.drugs.com/sfx/clonidine-side-effects.html

Cruz, M. D., & Badiano, J. (1991). *Libellus de medicinalibus indorum herbis*. México: Fondo de Cultura Económica.

Damsa, C., Kosel, M., & Moussally, J. (2009). Current status of brain imaging in anxiety disorders. *Current Opinion in Psychiatry*, 22(1), 96–110. doi: 10.1097/yco.0b013e328319bd10

DeKorne, J. (2011). *Psychedelic shamanism: The cultivation, preparation, and shamanic use of psychotropic plants*. Berkeley, Calif: North Atlantic Books.

Diagnostic and Statistical Manual of Mental Disorders: DSM-5. (2013). Arlington, VA: American Psychiatric Association.

Diaz, J. L. (1979). Ethnopharmacology and Taxonomy of Mexican Psychodysleptic Plants. *Journal of Psychedelic Drugs* 11

Dominguez, X. A., Marroquín, J., & Quintero, B. S., Vargas, S. B. (1975). Two new quinolizidine alkaloids from Heimia salicifolia. *Phytochemistry,14*(8), 1883-1884. doi:10.1016/0031-9422(75)85325-8

Douglas B., Kirkpatrick J. L., Raffauf R. F., Ribeiro O., Weisbach J. A., (1964). Problems in chemotaxonomy. II. The major alkaloids of the genus *Heimia*. Lloydia 27:25-31.

Facts & Statistics | Anxiety and Depression Association of America, ADAA. (2019). Retrieved 11 December 2019, from https://adaa.org/about-adaa/press-room/facts statistics

Gates, W. (2000). *An Aztec herbal: The classic codex of 1552*. Mineola, NY: Dover Publications.

Gledhill, D. (2008). *The Names of Plants*. Cambridge: Cambridge University Press.

Graham, S. “Type Species: Heimia Salicifolia.” Archive. Kent University, 1997. http://web.archive.org/web/19970624061507/http://simon.kent.edu/Biology/Research/Shirley_Graham/Genera/heimia.html

Griebel, G. and Holmes, A. (2013). 50 years of hurdles and hope in anxiolytic drug discovery. *Nature Reviews Drug Discovery*, 12(9), pp.667-687.

Guzmán-Hernández, E. A., Segura-Cobos, D., Amato, D., Avila-Acevedo, J. G., & Vazquez-Cruz, B. (2018). Evaluation of antihypertensive and vasorelaxant effects of Heimia salicifolia (family: Lythraceae). *African Journal of Pharmacy and Pharmacology,12*(3), 41-51. doi:10.5897/ajpp2017.4860

Hoehne, F. C. (1978). *Plantas e substâncias vegetais tóxicas e medicinais*. São Paulo: Graphicars.

Kaplan, H. R., & Malone, M. H. (1966) A pharmacologic study of nesodine, cryogenine and other alkaloids of *Heimia salicifolia*. *Lloydia* 29. 348-359.

Kent, J. M., & Rauch, S. L. (2003). Neurocircuitry of anxiety disorders. *Current Psychiatry Reports*, 5(4), 266–273. doi: 10.1007/s11920-003-0055-8

Kitajima, M., Yanagisawa, T., Tsukahara, M., Yamaguchi, Y., Kogure, N., Kikura-Hanajiri, R., . . . Takayama, H. (2018). Biphenyl ether and biphenyl quinolizidine lactone alkaloids from Heimia salicifolia. *Tetrahedron,74*(4), 441-452. doi:10.1016/j.tet.2017.12.012

Kitajima, M., Yamaguchi, Y., Yanagisawa, T., Kogure, N., Ogata, J., Kikura-Hanajiri, R., & Takayama, H. (2019). Biphenyl quinolizidine lactone alkaloids from “sinicuichi” (Heimia salicifolia). *Tetrahedron,75*(27), 3733-3739. doi:10.1016/j.tet.2019.05.045

Link, H. F., & Otto, F. S. (1828). *Icones plantarum selectarum horti regii botanici Berolinensis cum descriptionibus et colendi ratione*.

Lema W. J., Blakenship J. W., Malone M. H. (1986). Prostaglandin synthetase inhibition by alkaloids of *Heimia salicifolia*. J Ethnopharmacol 15:161-167

Malone M. H. (1986). Cryogenine. Drugs of the Future 11:95-96

Malone, M. H., & Rother, A. (1994). Heimia salicifolia: A phytochemical and phytopharmacologic review. *Journal of Ethnopharmacology*,*42*(3), 135-159. doi:10.1016/0378-8741(94)90080-9

Martínez, M. (1959). *Las plantas medicinales de Mexico*(4th ed.). Mexico: Ediciones Botas, 293-295.

National Institute of Health. (2020, March 11). Research Using Vertebrate Animals. Retrieved from https://www.niaid.nih.gov/grants-contracts/research-vertebrate-animals#A13

Nucifora, T.L. and Malone, M.H. (1970). A psychopharmacological evaluation of certain nonsteroidal antiinflammatory compounds. *Federation Proceedings* 29, 620.

Nucifora, T.L. and Malone, M.H. (1971). Comparative psychopharmacological investigation of cryogenine, certain nonsteroid antiinflammatory compounds, lupine alkaloids and cyptoheptadine. *Archives internationales de Pharmacodynamie et de Therapie* 191, 345-356.

Oliver-Bever B. (1972). Drug plants in ancient and modern Mexico. Q J Crude Drug Res 12:1957-1972.

Rapee, R. M., Craske, M. G., & Barlow, D. H. (1990). Subject-described features of panic attacks using self-monitoring. *Journal of Anxiety Disorders*, *4*(2), 171–181. doi: 10.1016/0887-6185(90)90009-x

Rauch, S. L., Shin, L. M., & Wright, C. I. (2003). Neuroimaging Studies of Amygdala Function in Anxiety Disorders. *Annals of the New York Academy of Sciences*, 985(1), 389–410. doi: 10.1111/j.1749-6632.2003.tb07096.x

Reko, V. (1926). Sinicuichi. La Revista Médica De Yucatán. Vol. 14, 22-27.

Robichaud, R.C., Malone, M.H. and Schwarting, A.E. (1964). Pharmacodynamics of cryogenine, an alkaloid from *Heimia salicifolia* Link and Otto. Part 1. *Archives internationales de Pharmacodynamie et de Therapie* 150, 220-232.

Robichaud, R.C., Malone, M.H. and Kosersky, D.S. (1965). Pharmacodynamics of cryogenine, an alkaloid from *Heimia salicifolia* Link and Otto. Part 2. *Archives internationales de Pharmacodynamie et de Therapie* 157, 43-52.

Roth, B. (2004). Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: A novel approach for CNS drug discovery. *Pharmacology & Therapeutics,102*(2), 99-110. doi:10.1016/s0163-7258(04)00048-8

Rother A. (1989) *Heimia salicifolia*: In Vitro Culture and the Production of Phenyl- and Biphenylquinolizidines. In: Bajaj Y.P.S. (eds) Medicinal and Aromatic Plants II. Biotechnology in Agriculture and Forestry, vol 7. Springer, Berlin, Heidelberg

Rother, A. (1990). Alkaloids of Heimia montana. *Phytochemistry, 29*(5), 1683-1686. doi:10.1016/0031-9422(90)80146-8

Rother, A., & Schwarting, A. (1972). Phenylalanine as a precursor for cryogenine biosynthesis in Heimia salicifolia. Phytochemistry, 11(8), 2475-2480. doi:10.1016/s0031-9422(00)88520-9

Schultes, R. E. (1969). “The New World Indians and Their Hallucinogenic Plants.” *7th Annual Lecture Honoring Dr. Laura L. Barnes.*

Schultes, R. E., & Smith, E. W. (1976). *Hallucinogenic plants*. Retrieved from https://issuu.com/marthapina/docs/richard_evans_schultes_-_hallucinog.

Schultes, R. E., Hofmann, A., & Rätsch, C. (2001). *Plants of the gods: Their sacred, healing, and hallucinogenic powers*. Rochester, VT: Healing Arts Press.

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of visualized experiments: JoVE*, (96), e52434. doi:10.3791/52434

Shin, L. M., & Liberzon, I. (2009). The Neurocircuitry of Fear, Stress, and Anxiety Disorders. Neuropsychopharmacology, 35(1), 169–191. doi: 10.1038/npp.2009.83

Shirneshan, Elaheh, "Cost of Illness Study of Anxiety Disorders for the Ambulatory Adult Population of the United States" (2013). Theses and Dissertations (ETD). Paper 370. http://dx.doi.org/10.21007/etd.cghs.2013.0289.

Singh, A. (2011). *Herbalism, phytochemistry and ethnopharmacology*. Retrieved April 3, 2019, from https://www.academia.edu/17381789/Herbalism_Phytochemistry_and_Ethnopharmacology

Su, K. H., Lin, S. J., Wei, J., Lee, K. I., Zhao, J. F., Shyue, S. K., & Lee, T. S. (2014). The essential role of transient receptor potential vanilloid 1 in simvastatin-induced activation of endothelial nitric oxide synthase and angiogenesis. *Acta Physiologica, 212*(3), 191–204. doi: 10.1111/apha.12378

Sufka, K. J., Warnick, J. E., Pulaski, C. N., Slauson, S. R., Kim, Y. B., & Rimoldi, J. M. (2009). Antidepressant efficacy screening of novel targets in the chick anxiety-depression model. *Behavioural Pharmacology, 20*(2), 146-154. doi:10.1097/fbp.0b013e32832a8082

Sufka, K. J. (2015). *U.S. Patent No. 8999293 B2*. Washington, DC: U.S. Patent and Trademark Office.

Warnick, J. E., Wicks, R. T., & Sufka, K. J. (2006). Modeling anxiety-like states: Pharmacological characterization of the chick separation stress paradigm. *Behavioural Pharmacology, 17*(7), 581-587. doi:10.1097/01.fbp.0000236269.87547.9d

Warnick, J. E., Huang, C. J., Acevedo, E. O., & Sufka, K. J. (2008). Modelling the anxiety-depression continuum in chicks. *Journal of Psychopharmacology*, 23(2), 143–156. doi: 10.1177/0269881108089805

Wasson, R. G. (1974). The Role of 'Flowers' in Nahuatl Culture: A Suggested Interpretation. *Journal of Psychedelic Drugs, 6*(3), 351-360. doi:10.1080/02791072.1974.10471987

Weisbach, J. A. (1965). *U.S. Patent No. 3184446*. Washington, DC: U.S. Patent and Trademark Office. Heimia Alkaloids.

Wiebelhaus, V. D. (1973). *U.S. Patent No. 3843795.* Washington, DC: U.S. Patent and Trademark Office. Methods for Producing Glucocorticoid-like Water Diuretic and Anti-Inflammatory Activity with Decinine.

Yan, J., Pan, Y., Cai, W., Cheng, Q., Dong, W., & An, T. (2015). Association between anxiety and hypertension: a systematic review and meta-analysis of epidemiological studies. *Neuropsychiatric Disease and Treatment*, 1121–1130. doi: 10.2147/ndt.s77710

Zhang, W., Han, Y., Meng, G., Bai, W., Xie, L., Lu, H., … Ji, Y. (2014). Direct Renin Inhibition with Aliskiren Protects Against Myocardial Ischemia/Reperfusion

Injury by Activating Nitric Oxide Synthase Signaling in Spontaneously Hypertensive Rats. *Journal of the American Heart Association*, *3*(1). doi: 10.1161/jaha.113.000606

VITA

CONNOR D. CARLTON

EDUCATION

Sam Houston State University, Huntsville, Texas
Committee Co-Chairs: Justin Williams, Stephen White
Master of Science, May 2020 (Department of Biological Sciences)

University of Florida, Gainesville, Florida
Bachelor of Science, May 2016 (Department of Biological Sciences)

RESEARCH INTERESTS

Former interests
Post-mortem Interval Methodology
3D Photogrammetry Modeling of Wound Trauma
Micro-CT Application of Wound Trauma

Current interests
Neuropsychopharmacology
Behavioral Pharmacology
Pharmacodynamics and Pharmacokinetics of psychotropics
Cannabis cultivation efficiency techniques
Medicinal Agriculture
Ethnobotanical Pharmacology

FIELD WORK

Texas Research Institute for Environmental Studies (TRIES), Huntsville, Texas
Directors: Justin Williams, Ph.D., and William Godwin, Ph.D.
Survey of the Long Leaf Pine in the Big Thicket National Preserve
June 2019 – September 2019

Southeast Texas Applied Forensics Science Facility, Huntsville, Texas
Directors: Dr. Joan Bytheway and Dr. Patrick Lewis
Bone marrow biopsies and 3D Photogrammetry of cadavers
May 2016

RESEARCH EXPERIENCE

Sam Houston State University, Department of Biological Sciences, Huntsville, Texas
Committee: Justin Williams, Patrick Lewis, James Harper, Stephen White
M.S. Thesis: Evaluation of lythrine, an alkaloid from Heimia salicifolia, in the

chick model of anxiety and depression
May 2018 - Current

Sam Houston State University, Department of Biological Sciences, Huntsville, Texas
Principal Investigator: Dr. Patrick Lewis
Evaluating the decomposition process of machete wounds on human cadavers using 3D Photogrammetry technology
May 2016 – April 2017

Sam Houston State University, Department of Biological Sciences, Huntsville, Texas
Principal Investigator: Dr. Patrick Lewis
Understanding the microbiome of the bone marrow in decomposing human cadavers
May 2016 – October 2016

University of Florida, Department of Food Science and Human Nutrition, Gainesville, FL
Principal Investigator: Dr. James Collins
Determining if iron transporter DMT1 can transport copper through intestines
January – May 2016

University of Florida, College of Veterinary Medicine, Small Animal Hospital, Gainesville, FL
Principal Investigator: Dr. Stanley Kim
Evaluating knee kinematics in dogs using 3D bone modeling and X-ray imaging for shape-matching using JointTrack
January – April 2014

POSTER PRESENTATIONS

American Association of Physical Anthropologist (AAPA)
April 2017

Texas Association of Biological Anthropologists (TABA)
November 2016

PUBLICATIONS

Carlton, C. D., Mitchell, S., & Lewis, P. (2018). Preliminary application of Structure from Motion and GIS to document decomposition and taphonomic processes. *Forensic Science International*, 282, 41-45. doi:10.1016/j.forsciint.2017.10.023

Bailey, C. A., **Carlton, C. D.**, White, S. W. (2019). *A Call to Policy Change: Cannabis in the NFL.* Manuscript submitted for publication.

GRANT APPLICATIONS – NOT FUNDED

Sigma Xi - *How distance affects the microanatomy of gunshot wounds to the skull*
September 2016

PROFESSIONAL EXPERIENCE

Sam Houston State University, Texas Research Institute for Environmental Studies
Student Assistant to Museum Curator, Dr. William Godwin
June 2019 – September 2019

Sam Houston State University, Department of Biological Sciences
Secretary to the Department Chair, Dr. Tamara Cook
September 2018 – December 2018

Sam Houston State University, Department of Biological Sciences
Lab Instructor – Botany
August 2017 – May 2018

Sam Houston State University, Department of Biological Sciences
Lab Instructor – Foundations of Sciences
August 2016 – May 2018

COURSES TAUGHT

BIOL 1411 – General Botany Lab, August 2017 - May 2018

BIOL 1436 – Foundations of Science Lab, August 2016 - May 2018

CLINICAL EXPERIENCE

Global Medical Training, Arraijan District, Panama
Doctor's Assistant
June 2015

Shands Hospital, University of Florida, Gainesville, Florida
Physician Shadowed: Vincent Bird, MD
May – August 2014

COMMUNITY OUTREACH

President, Biological Sciences Graduate Student Organization, SHSU
Huntsville, TX
Leader and head representative for all graduate students in the Department of Biological Sciences
May 2018 - Present

Webmaster, Biological Sciences Graduate Student Organization, SHSU

Huntsville, TX
Maintained clear communication between members, officers, faculty and the community
January - May 2018

Adaptive Golf Program Founder, Shriners Hospital for Children
Tampa, Florida
Organized and designed various teaching activities for three annual events
May 2009 – May 2016

Internet and Social Media Relations, Community Health Service Corp.
Gainesville, FL
Managed all community outreach via social media for organization operations
August – December 2015

Dream Team Monthly Volunteer, Shands Hospital
Gainesville, Florida
Volunteered to spend time with children in the Cardiology department
January 2014 – April 2014

AWARDS & AFFILIATIONS

Deliberative Dialogue Competition – 2nd Place
Sam Houston State University, Fall 2019

College of Science and Engineering Technology (COSET) Graduate Achievement Scholarship
Sam Houston State University, Fall 2019

College of Science and Engineering Technology (COSET) Graduate Achievement Scholarship
Sam Houston State University, Spring 2019

Deliberative Dialogue Competition – 1st Place
Sam Houston State University, Fall 2018