The Effects of Sceletium tortuosum in the Chick Anxiety-Depression Model

by

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A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2017

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ACKNOWLEDGEMENTS

I would like to thank everyone involved in helping me with this research project. Firstly, I would like to thank Dr. Sufka for his guidance in the planning and executing of this research project, as well as his direction with the writing process of this thesis. I would also like to thank all of the Graduate and Undergraduate Research Assistants in the Psychopharmacology Lab for their support and assistance while carrying out the experiment. Thank you to the Sally McDonnell Barksdale Honors College for their funding of this research. I also thank my family and friends for their encouragement throughout the past year while I completed this research project.
ABSTRACT
The Effects of *Sceletium tortuosum* in the Chick Anxiety-Depression Model
(Under the direction of Kenneth J. Sufka)

*Sceletium tortuosum* (*S. tortuosum*), known colloquially as Kanna, is a natural botanical that is thought to reduce anxiety, elevate mood, and produce euphoria. This study explores *S. tortuosum*’s properties in the chick anxiety-depression model, a pre-clinical drug efficacy screening model that shares many features to clinical stress-related disorders and has high predictive validity. Socially-raised male Silver Laced Wyandotte chicks age 4-6 days were given intraperitoneal injections of either a vehicle, imipramine, or *S. tortuosum* fraction (10, 20, 30, 50, 75, or 100mg/kg) 15 minutes prior to being placed in a stress-inducing isolation chamber for 60 minutes and distress vocalizations were recorded. Chick distress vocalizations were quantified during anxiety (first 3 minutes) and depression-like phases (30-60 minutes, relatively). The results show that vehicle-treated chicks had an initially high DVoc rate, followed by a decline and plateau of approximately 50%; this follows the typical pattern of anxiety, followed by behavioral despair that the Chick Model of Anxiety and Depression simulates. 75 and 100 mg/kg of *S. tortuosum* decreased DVoc rates during the anxiety phase, which is indicative of anxiolytic activity. Imipramine groups increased DVoc rates in the depression phase, which is indicative of antidepressant activity; no antidepressant effect was found in *S. tortuosum*. These findings display the potential of *S. tortuosum* to have stress-relieving properties that may alleviate anxiety-like symptoms.
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INTRODUCTION

_Sceletium tortuosum_ (S. tortuosum) is a commercially available plant originating in South Africa. Leaves of the _S. tortuosum_ genus tend to be large and succulent, with characteristic veins running through them. The plant’s flowers range in color from white, yellow, and pink; seeds are encapsulated in the fruit portion of the plant and tend to be brown or black. The genus is dispersed throughout south-western parts of South Africa, particularly in dry, arid environments (for review, see Gericke and Viljoen, 2008).

The plant has been known colloquially as channa, kanna, or kougoed. Traditionally, the plant material has been chewed, but can also be ingested in the form of tea, used as snuff, or smoked. _S. tortuosum_ has been used to treat insomnia in adults and diarrhea in children; it has been thought to be a mild narcotic by indigenous people, as it relieves toothaches and abdominal pain. In the United States, _S. tortuosum_ compounds have been patented as pharmaceuticals for use in various psychological conditions, including the management of depression, anxiety, drug dependence, bulimia, and obsessive compulsive disorder (for review, see Gericke and Viljoen, 2008).

There has been extensive research on the alkaloid products of _S. tortuosum_; mesembrine and mesembrenone were first isolated in 1914 by E. Zwicky (for review, see Gericke and Viljoen, 2008). These components have been isolated and studied for their effects on the central nervous system. Harvey et al. 2011 tested purified alkaloids mesembrine and mesembrenone on a panel of receptors, enzymes, and drug targets to examine their cellular effects. These researchers found that mesembrine was active
against the 5-HT transporter by blocking binding to the receptor; mesembrenone had some action against the 5-HT transporter and PDE4 transporter. These findings may explain the clinical effects, as known 5-HT and PDE4 inhibitors reduce depression (Cashman et al. 2009).

Terburg et al. 2013 tested S. tortusoum’s inhibitory properties of serotonin and PDE4 in human subjects in relation to anxiety. Using fMRI’s, researchers scanned the brains of participants during a perceptual-load task and an emotion-matching task after receiving a single dose of 25mg dose of Zemrin, the standardized form of S. tortuosum, or a placebo. They found that amygdala reactivity was attenuated by the test compound. This provides evidence that S. tortuosum may attenuate anxiety-related neurocircuitry; this finding also supports the anxiolytic potential of PDE4 and 5-HT reuptake inhibition (Terburg et al. 2013).

Because of the historical and cultural use of S. tortuosum, a broad number of screening assays may be used to detect this botanical’s efficacy, side effect liability, and site of activity. To do this, researchers have tested varying doses of an alkaloid extract fraction, varying doses of the presumed active component (mesembrine), and varying doses of S. tortuosum extract. Loria et al. 2014 sought to characterize the effects of S. tortuosum and its extract products (alkaloid enriched extracts and mesembrine) in a variety of rodent-based assays that model nociception, depression, anxiety, ataxia, and abuse liability.
Conditioned place preference was used to measure the reward potential of the test articles (alkaloid enriched fractions, mesembrine, and *S. tortuosum* extracts) in order to gauge the abuse liability of the drug; a hot plate apparatus was used to measure analgesic properties of the test compound by measuring the time before a hind-paw flutter or escape attempt; a forced swim test was used to characterize antidepressant properties of the test compound by measuring behavioral despair (time spent not engaging in escape behavior); an elevated plus maze was used to characterize anxiolytic properties of the test compound by measuring time spent in the open portions of the maze; a rotarod apparatus was used to characterize ataxia of the test compound by measuring the time spent on the rotating trundle. These assays were used to broadly characterize the effects of *S. tortuosum* (Loria et al., 2014).

*S. tortuosum* test articles showed no evidence of preference or aversion in the conditioned place preference assay; this indicates that there is no abuse potential for the botanical. *S. tortuosum’s* active constituent, mesembrine, showed longer latency on the hotplate procedure, indicating potential analgesic activity. The alkaloid enriched fraction of *S. tortuosum* decreased behavioral despair during the forced swim test; this indicates that some constituents of the botanical may be useful for the treatment of mood disorders. *S. tortuosum* test compounds failed to show any anxiolytic properties in the elevated plus maze. Both *S. tortuosum* fraction and mesembrine tended to have a shorter latency to fall on the rotarod test, meaning that these treatment groups could not withstand the rotating apparatus as long as the vehicle-treated test subjects; this may indicate some ataxia.
caused by the botanical. These findings indicate that *S. tortuosum*’s major constituent, mesembrine, as well as fractions of the drug have psychoactive properties that should be tested for further analysis of the drug (Loria et al., 2014).

Given the potential clinical benefits of *S. tortuosum*, Murbach et al. 2014 sought to find any harm with the use of the drug during short-term use with a high dose and long-term use with a low dose. These experimenters tested SPF Wistar rats that were orally administered high doses (25, 74, 250, and 500mg/mL) and low doses (10, 30, 45, and 60mg/mL) of dissolved Zembrin, the commercially available source of *S. tortuosum*, during a 14-day study and a 90-day study, respectively. Histopathological examinations and toxicology reports were constructed after the final administration of the drug in both testing protocols; the researchers conducted examinations of hepatotoxicity to measure liver function, organ screenings to determine lesions, and blood chemistries to measure immunological functioning of the test subjects. The experimenters found no adverse symptoms or mortality due to the ingestion of the drug, and provide evidence supporting the safety and tolerability of *S. tortuosum* (Murbach et al., 2014).

Using animals as models for clinical syndromes can prove to be challenging due to the difficulties of translating human symptoms to animal behaviors. Eric Nestler and Steven Hyman recognize the innate complications of realizing how symptoms in animal models explain human disorders. Animal models are not likely to mirror the full extent of human disorders; instead, we must take individual symptoms in animals and attempt to correspond them with human symptoms. These symptoms are not universal among
humans, but we rely on observation of specific behaviors to characterize depression in animal models. Researchers often generate animal models of depression by exposing short-term stressors to normal animals; this is very different from human depression in that it does not consider genetic vulnerability and environmental factors that lead to chronic behavioral pathology. Furthermore, researchers often administer a single dose of antidepressant and record a robust response; this is not in accordance with antidepressant response in humans (Nestler et al., 2010). These issues of developing useful animal models for depression are seen repeatedly and in other neuropsychiatric conditions.

These concerns imply that there is a lack of translational relevance between animal models and human conditions. There is a need to develop and validate additional animal models to test novel compounds to provide further evidence of the effects of psychopharmacological compounds. Because of these problems with translational relevance, a number of research labs have focused on developing models that better simulate these clinical symptoms.

The chick anxiety-depression model is a simulation of behavioral despair. This assay has proven to be a well-validated screening assay for known anxiolytics and antidepressants, (Sufka et al., 2009; Warnick et al., 2006; Warnick et al., 2009) as well as novel compounds from natural products that contain anxiolytic/antidepressant properties (Feltenstein et al., 2003; Kochanowska et al., 2008; Lewellyn et al., 2013; Smith et al., 2001; Sufka et al., 2001). This model uses socially raised chicks at 4-6 days old that are subjected to a 1-2 hour isolation period. Isolated chicks display an initially high rate of
distress vocalizations (DVocs) that decline approximately 50% within 25-30 minutes. This pattern replicates an anxiety phase that is followed by a depression phase. Anxiolytics attenuate distress vocalizations in the anxiety-like phase; antidepressants attenuate behavioral distress, seen by an increase in distress vocalizations during the depression-like phase. This models the hypothesis that anxiety and depression are a part of a “temporal continuum” (Sufka et al. 2006). Thus, this assay serves as a simultaneous screening tool for both anxiety and depression.

Warnick et al. 2006 sought to distinguish the specific anxiety state in the chick model by screening the efficacy of anxiolytics. Researchers explored both low-stress and high-stress responses by incorporating mirrors into some groups’ isolation chambers; the mirrors simulated a group setting (low-stress) rather than complete isolation (high-stress). The findings indicate that a low-stress environment led to significantly lower DVoc rates than those in the high-stress environment. Furthermore, results showed that all drugs that are clinically effective the treatment of Panic Disorder significantly attenuated DVocs. This experiment adds to the validity of the chick model as an in-vivo screening assay for anxiety, specifically for drugs that attenuate anxiety seen in Panic Disorder (Warnick et al., 2006).

In a follow-up study, Warnick et al. 2009 sought to further validate the temporal continuum hypothesis of anxiety and depression by utilizing the chick model of anxiety and depression to test the efficacy of clinically validated anxiolytics and antidepressants. In addition, researchers tested for biomarkers of stress; the change in cytokine levels were
collected and analyzed in the chick subjects. Researchers found the DVoc pattern to be consistent with previous studies; anxiolytic activity was seen by chlorodiazepoxide, clonidine, imipramine, and maprotoline in the anxiety-like phase, and antidepressant activity was seen by imipramine, maprotoline, and fluoxetine in the depression-like phase. Elevation in interleukin-6, a biomarker of depression, was found in response to social isolation during the late-depression phase of the experiment. This study offers further validation of the chick model as a screening assay of clinically relevant drugs and as a simulator of the anxiety-depression continuum (Warnick et al., 2009).

Sufka et al. 2009 constructed a set of experiments that utilizes the chick model to identify novel antidepressant compounds and identify false positives in antidepressant compounds that have failed in clinical trials. In this study, all drugs had tested positive for antidepressant properties in rodent models and were being used test if the chick model was as sensitive as rodent models. Two of the test compounds showed negative effects; these two compounds failed in human clinical trials, indicating that the rodent model gave false positive results of antidepressant properties. Thus, the chick model avoided two false positives by identifying compounds that didn’t work in human clinical trials. This set of experiments validated the effects of anxiolytics by measuring the attenuation of DVocs during the anxiety phase, as well as validated clinically used antidepressants by evaluating the attenuation of behavioral despair. In addition, this study correctly identified drugs that failed in clinical trials; this assay can predict false positives that are not identified in rodent models. This set of experiments provides evidence for this assay’s
high utilization for in-vivo screening (Willner, 1991); compared to rodent models of behavioral distress, the chick model uses a more economical approach to screen for drug properties by utilizing a lower cost animal, tests at a young age, and screens for two drug properties in a single test (Willner, 1991). This study provides evidence for a replacement assay for the traditionally used rodent model of anxiety and depression (Sufka et al., 2009).

This screening assay has been validated many times for its usefulness in testing the efficacy of known anxiolytics and antidepressants; it has also been successful at screening novel compounds from natural products that possess anxiolytic/antidepressant properties. Feltenstein et al. verified anxiolytic properties of *Piper methysticum* by utilizing the chick model of anxiety; researchers showed *P. methysticum* to attenuate D Voc equivalent to that of chlordiazepoxide, a clinically used anxiolytic (Feltenstein et al., 2003). Other studies have since replicated the findings of novel anxiolytic and antidepressant properties in natural products (see Kochanowska et al., 2008; Lewellyn et al., 2013; Smith et al., 2001; Sufka et al., 2001).

There is emerging evidence that *S. tortuosum* may alleviate stress-related disorders, as seen in rodent models (Loria et al. 2014); it is therefore useful to demonstrate these properties in other screening assays. The chick model of anxiety and depression is a well-validated simulator of anxiety and depression, and has proven to provide predictive validity of pharmacological products. Because no single assay can perfectly predict the properties of a pharmaceutical, it is worthwhile to test *S. tortuosum’s* on other types of
animal models. Therefore, this study seeks to find the potential stress-relieving properties of *S. tortuosum* in the chick model of anxiety and depression.
MATERIALS AND METHODS

Subjects and Housing

Male Silver Laced Wynadotte chicks were received two-days post-hatch (Gallus gallus, Ideal Poultry, Cameron, TX, USA) and kept in 34 x 57 x 40-cm stainless steel cages with approximately 12 chicks per cage. The chicks had free access to food (Purina Start and Grow, St. Louis, Missouri, USA) and water via one-quart gravity feeders and waterers. The room temperature was maintained at approximately 30-32°C with a 12 hour light cycle (7am-7pm) via overhead fluorescent lighting. Daily maintenance included replacing paper in the chicks’ cages, and replacing food and water in the feeders and waterers.

Apparatus

The testing apparatus is a six unit testing apparatus containing Plexiglas chambers (25cm x 25cm x 22 cm) that are equipped for collection of behavioral data. The individual chambers have 25 watt light bulbs for the illumination; ventilation is achieved by a rotary fan within each chamber (Model FP- 108AXS1; Rodale, Great River, New York, USA). Video cameras (Model PC60XP; SuperCircuit, Liberty Hill, Texas, USA) were placed in each testing unit that allowed for visual observation via a live stream to an onscreen display. Microphones (Model 3-675-001 [modified for AC current]; Lafayette Instruments, Lafayette, Indiana, USA) were situated at the top of each testing unit and
were connected to a digital software system that continuously recorded distress vocalizations.

**Procedure**

Experiment 1 tested chicks at 4-6 days post-hatch, and chicks were only tested one time throughout the experiment. Imipramine-treated chicks (10mg/kg) were tested with chicks treated with various fractions of *S. tortuosum* (10mg/kg, 20mg/kg, and 30mg/kg) and chicks treated with vehicles of the drug. Deionized water served as the vehicle for imipramine; deionized water and 20% tween served as the vehicle for *S. tortuosum*.

Sample sizes for Imipramine and *S. tortuosum* groups were n=18; the vehicle groups had sample sizes of n=12. Intraperitoneal injections (1ml/kg) of the vehicles and drugs were given 15 minutes prior to testing and behavioral observations were made over a 60-minute time period. Distress vocalizations were continuously collected throughout the experiment. Once testing was complete, animals were returned to their home cage until the completion of the experiment and were euthanized upon completion. These testing procedures were approved by the University of Mississippi’s Institutional Animal Care and Use Committee (protocol # 16-015).

Experiment 2 incorporated the exact same procedure with the exception of drug dosages; *S. tortuosum* was increased to 50mg/kg, 75mg/kg, and 100mg/kg.
Data Analyses

Distress vocalizations were converted to a rate of DVocs/minute function. Independent t-tests revealed no statistical difference between the two vehicle groups, thus they were collapsed into a single control group for the remainder of data analyses. The DVoc rates for the anxiety phase, and the early and late depression phases (0-3m, 31-45m, and 46-60m) were analyzed using a one-way analysis of variance (ANOVA); post-hoc tests were conducted using Fisher’s LSD using SPSS software. Significance was set at p < 0.05.
RESULTS

Experiment 1

Figure 1 summarizes the effects of imipramine and *S. tortuosum* on mean DVoc rates during the 60-minute isolation testing period. The vehicle-treated chicks displayed an initially high DVoc rate in the anxiety phase; this rate steadily decreased over the next 30 minutes as they entered the early-depression phase (30-45 minutes), and remained relatively stable as the chicks entered the late-depression phase (46-60 minutes). This pattern typifies the standard of stress-related DVocs in behavioral despair models of depression.

Figure 2a shows that DVoc rates in the anxiety phase were not affected by either *S. tortuosum* or imipramine. A one-way ANOVA of these data failed to reveal a significant treatment effect, $F(4,86) = 1.14, p = 0.34$. No further analyses were performed on this data set.

Figures 2b and c show that, relative to the vehicle-treated group, imipramine increased the DVoc rates during both the early (see Figure 2b) and late (see Figure 2c) depression phases; *S. tortuosum* had no effect on the early or late depression phases (see Figures 2b and c). A one-way ANOVA of the DVOC data in the early-depression phase revealed a significant treatment effect, $F(4,86) = 6.06, p < 0.001$. Planned comparisons with Fishers demonstrated that the mean DVoc rate for the imipramine group was significantly higher than the vehicle group, ($p < 0.005$) with a Cohen’s D effect size of
0.90. All other planned comparisons (i.e., *S. tortuosum* doses against vehicle group) were not statistically significant.

**Figure 1:** DVoc Pattern During 60 Minute Isolation Period

*Figure 1*

Drug effects on the mean DVoc rate (counts per minute) as a function of isolation phase (anxiety 0-3 minutes; depression 30-60 minutes) with error bars that show standard error.
Figure 2: Mean DVocs as a Function of Treatment Group

A

![Bar chart showing mean DVocs for different treatment groups.]

```
Mean DVoc Rate

0.00  20.00  40.00  60.00  80.00  100.00  120.00

Vehicles  Imprimine  S. tortuosum 10 mg/kg  S. tortuosum 20 mg/kg  S. tortuosum 30 mg/kg

Treatment
```

B

![Bar chart showing mean DVocs for different treatment groups.]

```
Mean DVoc Rate

0.00  20.00  40.00  60.00  80.00  100.00

Vehicles  Imprimine  S. tortuosum 10 mg/kg  S. tortuosum 20 mg/kg  S. tortuosum 30 mg/kg

Treatment
```
Figure 2
Drug effects on mean DVoc rate as a function of isolation phase. Figure A represents the anxiety phase; figure B, and C represent the early-depression phase and late-depression phase, respectively. Asterisks denote a significant increase of DVoc relative to the vehicle group (p < 0.05).

Finally, a one-way ANOVA of the DVoc data in the late-depression phase revealed a significant treatment effect, $F(4,86) = 7.18$, $p < 0.001$. Planned comparisons with Fishers demonstrated that the mean DVoc rate for the imipramine group was significantly higher than the vehicle group, $(p < 0.001)$ with a Cohen’s D effect size of 1.29. All other planned comparisons were not statistically significant.
Experiment 2

Figure 3 summarizes the effects of imipramine and *S. tortuosum* on mean DVoc rates during the 60-minute testing period. As in Experiment 1, the vehicle-treated chicks displayed an initially high DVoc rate in the anxiety phase, and decreased in the early-depression phase, then plateaued late depression-phase. This pattern mirrors Experiment 1 and is typical of the standard of stress-related DVocs in behavioral despair models of depression.

Figure 4a shows that groups who received high doses of *S. tortuosum* (75 and 100mg/kg) displayed decreased DVoc rates during the anxiety phase. A one-way ANOVA of the mean DVoc rate revealed significance for the treatment group, $F(4,80) = 3.2, p = 0.017$. Planned comparisons with Fishers demonstrated that the mean DVoc rate for *S. tortuosum* (75mg/kg and 100mg/kg) was significantly lower than the vehicle group, ($p < 0.05$) with a Cohen’s D effect size of 0.844 and 0.82, respectively. All other planned comparisons (i.e., imipramine and *S. tortuosum* 50mg/kg against vehicle group) were not statistically significant.

Figures 4b and c show that neither imipramine or *S. tortuosum*-treated groups displayed increased DVoc rates during either half of the depression phase. A one-way ANOVA of the DVoc rate failed to reveal significant treatment effect for either early or late-depression phases $F(4,80) = 1.72, p = 0.154$ and $F(4,80) = 1.87, p = 0.123$, respectively. Planned comparisons with Fishers revealed that imipramine approached significance at the late-depression phase ($p = 0.059$) with a Cohen’s D effect size of
0.761. All other planned comparisons (i.e., *S. tortuosum* doses against vehicle group) were not statistically significant.

**Figure 3: DVoc Pattern During 60 Minute Isolation Period**

Drug effects on the mean DVoc rate (counts per minute) as a function of isolation phase (anxiety 0-3 minutes; depression 30-60 minutes) with error bars that show standard error.
Figure 4: Mean DVocs as a Function of Treatment Group

A

![Bar chart showing mean DVocs as a function of treatment group.](image)

B

![Bar chart showing another set of mean DVocs as a function of treatment group.](image)
Figure 4
Drug effects on mean DVoc rate as a function of isolation phase. Figure A represents the anxiety phase; figures B, and C represent the early-depression phase, and late-depression phase, respectively. French Cross denotes a significant decrease of DVocs relative to the vehicle group (p < 0.05).
DISCUSSION

This study sought to determine whether the antidepressant properties of *S. tortuosum* that are seen in rodent models can be generalized in another non-rodent, well validated screening assay. The chick anxiety-depression model is a dual screening assay that induces both acute and chronic stress that triggers anxiety and depression in the test subject; this assay can therefore test the efficacy of anxiolytics and antidepressants. This animal model has repeatedly shown high predictive validity when screening the efficacy of FDA-approved pharmaceuticals (Feltenstein et al., 2004; Sufka et al., 2009; Warnick et al., 2006, 2009) and also novel compounds that mitigate stress-related disorders (Feltenstein et al., 2003; Sufka et al. 2006). This assay screened *S. tortuosum* for its ability to alleviate stress-induced behaviors that reflect anxiety and depression phases.

The model shows a pattern of an initial high DVoc rate that is indicative of an anxiety phase, followed by a decline and a plateau of DVocs as the chicks enter into a period of behavioral despair (i.e. the depression phase). In both experiments, the vehicle-treated chicks’ DVoc rates showed the characteristic pattern of an initially high rate during the anxiety phase (first 3 minutes), followed by a gradual decline of approximately 50% as the chicks entered into the depression phase (next 30 minutes). This pattern is consistent with existing literature of the chick anxiety-depression model (Sufka et al., 2006) and illustrates transition from a panic state into behavioral despair.

Imipramine served as an antidepressant control and its effects in both of these experiments mirror the results found in previous studies (Sufka et al. 2009; Warnick et al. 2006, 2009) and also novel compounds that mitigate stress-related disorders (Feltenstein et al., 2003; Sufka et al. 2006). This assay screened *S. tortuosum* for its ability to alleviate stress-induced behaviors that reflect anxiety and depression phases.
In Experiment 1, imipramine attenuated behavioral despair; this is identified higher DVoc rates in both the early and late depression phase than the vehicle-treated chicks. The effect of imipramine in Experiment 2 trended towards significance and had a very large effect size; this difference may be due to the high variance with a smaller sample size.

Two *S. totuosum* doses (75 and 100mg/kg) reduced DVocs in the anxiety phase; this mirrors results of anxiolytic compounds that have previously been tested in this assay (Feltenstein et al., 2004; Sufka et al., 2006; Warnick et al., 2006, 2009). *S. tortuosum* did not affect DVoc rates in either the early depression phase or late depression phase; there is no indication of antidepressant properties in any of the tested doses of *S. tortuosum*.

The anxiolytic findings of *S. tortuosum* is unsurprising due to the known effects of this botanical on the central nervous system; lower doses show preferential binding to serotonin transporters, and higher doses affect GABA receptors (Harvey et al., 2011). GABA receptor agonists are known to possess anxiolytic effects (Feltenstein et al., 2003), thus it is unsurprising that the two highest doses of *S. tortuosum* showed the attenuation of DVocs in the anxiety phase, which is an indication of anxiolytic activity.

The fact that *S. tortuosum* fractions that were tested in this study failed to show anti-depressant effects is a surprising finding due to a previous study’s findings that an alkaloid-enriched fraction showed activity in the rat Forced Swim Test (Loria et al., 2014). Rodent models of depression are known to show false positives (Hyman, Nestler et al., 2010). One possibility is that our current findings in the chick model represent a
true negative of antidepressant effects of *S. tortuosum*. It may also be the case that other concentrations of the active constituents of *S. tortuosum* were not tested in this study show antidepressant properties; the antidepressant properties may be caused by a constituent of *S. tortuosum* that was active in the alkaloid enriched fraction that did not show up in the fraction that we tested in this study.

Collectively, these findings are indicative of the following: this botanical can alleviate behaviors of acute stress rather than chronic stress behavior. This can be seen by the attenuation of DVocs in the anxiety phase, but the absence of attenuation of behavioral despair in the depression phase.
BIBLIOGRAPHY


