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The effects of *Sceletium tortuosum* (L.) N.E. Br. extract fraction in the chick anxiety-depression model

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ABSTRACT

Ethnopharmacological relevance: Sceletium tortuosum (L.) N.E. Br. has been reported to elevate mood, reduce anxiety and stress and alleviate pain.
Aim of study: This study sought to examine the effects of an *S. tortuosum* alkaloid enriched fraction in the chick anxiety-depression model, a model that shows high predictive validity as a pharmacological screening assay.
Material and methods: Socially-raised male Silver Laced Wyandotte chicks (4–6 days old) were given IP vehicle, imipramine (10 mg/kg), or *S. tortuosum* fraction (10, 20, 30 mg/kg in Exp. 1 or 50, 75, 100 mg/kg in Exp. 2) 15 min prior to a 60 min isolation test period in which distress vocalizations (DVoc) were continuously recorded.
Results: Vehicle chicks displayed high DVoc rates in the anxiety phase (first 3 min). DVoc rates declined about 50% (i.e., behavioral despair) in the depression phase (30–60 min). *S. tortuosum* fraction at 75 and 100 mg/kg decreased DVoc rates during the anxiety phase indicative of an anxiolytic effect. Imipramine, but not *S. tortuosum* groups, increased DVoc rates in the depression phase indicative of an antidepressant effect.
Conclusions: The findings suggest that an alkaloid enriched *S. tortuosum* fraction may benefit some forms of stress-related disorders.

1. Introduction

Sceletium tortuosum (L.) N.E. Br., colloquially known as *kougoed* or *kanna*, is a flowering, succulent plant indigenous to South Africa. The plant is traditionally chewed, smoked, or used as a tea or snuff predominantly for pleasure, but also for ailments such as toothache and abdominal pain (Gericke and Viljoen (2008) for review). *S. tortuosum* has been reported to elevate mood and reduce anxiety, stress and tension (Gericke and Viljoen, 2008). The antidepressant and anxiolytic clinical effects of *S. tortuosum* have been shown both in case reports (Gericke, 2001) and more recently, double-blind studies (Nell et al., 2013).

S. tortuosum has a rich alkaloid profile that contains mesembrine, mesembrenone, mesembrenol, alkaloid A4, tortuosamine, and chennaine (Gericke and Viljoen (2008) for review; Smith et al., 1996). These alkaloids have been shown to affect a number of central nervous system (CNS) targets. For example, Zembrin®, an ethanolic extract of *S. tortuosum* with the purified alkaloids mesembrine, mesembrenone and mesembrenol, shows inhibitory effects on serotonin (5HT) reuptake and phosphodiesterase 4 (PDE4) activity (Harvey et al., 2011). At higher concentrations, this extract also binds to gamma butyric acid (GABA), μ-opioid, δ_2-opioid, cholecystokinin-1, EP4 prostaglandin, and melatonin-1 receptors (Harvey et al., 2011; Gericke and Viljoen (2008) for review). Mesembrine was found to be the most abundant alkaloid constituent of *S. tortuosum* yielding 0.7% m/m (total alkaloid constituent extracted was 1.0–1.5% m/m) (Smith et al., 1996).

Previous work sought to broadly characterize activity of *S. tortuosum* extract, an alkaloid enriched fraction and the isolated constituent mesembrine respectively on measures of addiction, nociception, motor coordination, depression and anxiety (Loria et al., 2014). Mesembrine produced an antinociceptive effect similar to that of morphine on the hotplate. In line with previous anecdotal evidence of mood elevating properties, the *S. tortuosum* enriched fraction produced a modest antidepressant effect similar to that of imipramine in the forced swim test. However, this

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enriched fraction also produced ataxia similar to its positive control muscimol on the rotor-rod. Given the emerging evidence that *S. tortuosum* may mitigate stress-related disorders, it would be useful to demonstrate whether such properties generalize to other efficacy screening models.

The chick anxiety-depression model is a well-validated simulation and pharmacological screening assay (Hymel et al., 2010; Sufka and White, 2013). In this model, socially raised chicks are separated from conspecifics at 4–6 days old for a 1–2 h test session. Isolated chicks display high rates of distress vocalizations during the initial 3 min period (i.e., anxiety-like phase); distress vocalizations then decline by about 50% of the initial rate over the next 25–30 min period to enter into a stable rate for the remainder of the test session (i.e., depression phase) and is typical of behavioral despair models (Sufka et al., 2006). Anxiolytics attenuate distress vocalizations during the anxiety-like phase whereas antidepressants attenuate behavioral despair as evidenced by an increase in distress vocalizations during the depression-like phase (Sufka et al., 2006; Warnick et al., 2009). This assay has proven efficacious in screening clinically established anxiolytics and antidepressants (Sufka et al., 2009; Sufka and White, 2013; Warnick et al., 2006; Warnick et al., 2009) as well as novel compounds from natural products that possess anxiolytic/antidepressant properties (Feltenstein et al., 2003; Kochanowska et al., 2008; Lewellyn et al., 2013; Smith et al., 2001; Sufka et al., 2001).

2. Materials and methods

2.1. Plant materials and extraction

The leaves of *S. tortuosum* (L.) N.E. Br. (www.theplantlist.org, accessed May 2016) were purchased from Bouncing Bear Botanicals, Lawrence, KS, USA. The plant species was identified by Dr. Vijayasankar Raman at the National Center for Natural Products Research, University of Mississippi (voucher no. 10851). The plant extraction followed procedures detailed elsewhere (Loria et al., 2014). Briefly, the dried leaf powder of *S. tortuosum* was extracted with chloroform and the solvent was removed under reduced pressure to give the chloroform extract. The chloroform extract (20 g) was applied to vacuum liquid chromatography (VLC) over reversed phase silica (RP-18) and eluted with methanol-water (7:3, 1 L), (8:2, 1 L), (9:1, 1 L) and (1:0, 1 L). The fraction eluted with methanol-water (8:2) was defined as the alkaloid enriched fraction while the other three fractions did not contain alkaloids. Mesembrine was found to be a major compound in the alkaloid enriched fraction (11.8%) during HPLC fingerprinting analysis.

2.2. Subjects

Male Silver Laced Wyandotte chicks (Ideal Poultry, Cameron, TX, USA) were obtained two days post-hatch and housed in stainless steel cages ($44 \times 61 \times 40$ cm) with 12 chicks per cage. Food (Purina 5065, Lab Diet, Chick Chow S-G) and water were available ad libitum. Room temperature was maintained at approximately 30–32 °C and overhead illumination was maintained on a 12:12 h light-dark cycle.

2.3. Drugs

Imipramine (Sigma-Aldrich, St. Louis, MO) dissolved in deionized water served as the reference control for both experiments and was tested at a dose of 10 mg/kg. This dose was selected from pilot data showing 10 and 15 mg/kg imipramine had significant dose-dependent antidepressant activity in this genetic line. Deionized water served as the vehicle control for the imipramine group. The *S. tortuosum* enriched fraction was dissolved in a solution of 20% Tween 80 and deionized water and tested at doses of 10, 20 and 30 mg/kg in Experiment 1 and 50, 75 and 100 mg/kg in Experiment 2. As in previous work, a solution of 20% Tween 80 and deionized water served as the vehicle control for the *S. tortuosum* groups (Loria et al., 2014). All compounds were administered IP in a volume of 1 ml/kg.

2.4. Apparatus

A six-unit testing apparatus containing Plexiglas chambers ($25 \times 25 \times 22$ cm) surrounded by sound attenuating media was used to record separation-induced vocalizations. Each unit was illuminated by a 25-W light bulb, and ventilated by an 8-cm-diameter rotary fan. Miniature video cameras mounted outside the observation chambers at floor level and routed through a multiplexor provided televised display of chicks for observation. Distress vocalizations (DVocs) were detected via microphones [Radio Shack Omnidirectional Model 33-3013 (modified for AC current)] mounted at the top of the Plexiglas chamber and routed to a computer equipped with software that continuously counted distress vocalizations at a sample rate > 10 events/s.

2.5. Procedure

Two separate dose response experiments were conducted at 4–6 days post-hatch and chicks were tested only once. Time constraints prevented testing a greater number of doses within a test session. In experiment 1, sample sizes were $n=22$ for both vehicle groups combined and $n=15$–18 for imipramine and *S. tortuosum* enriched fraction groups. In experiment 2, sample sizes were $n=21$ for both vehicle groups combined and $n=16$ for imipramine and *S. tortuosum* enriched fraction groups. Chicks received vehicle or pharmacological substances 15 min prior to a social-separation stressor. This injection to test interval is based on extensive validation studies of this procedure as a drug efficacy screening assay (Sufka et al., 2006; Warnick et al., 2009; Sufka et al., 2009). Chicks were placed into individual isolation chambers for a 60 m test session and distress vocalizations were continuously recorded. Upon completion of experimental testing, chicks were returned to their home cage. These procedures were approved by the University of Mississippi's Institutional Animal Care and Use Committee (protocol no. 16-015).

2.6. Data analyses

Distress vocalizations were converted to a rate/minute function (DVoc rate). From these data, DVoc rates were derived for the anxiety-like phase (0–3 min), and the first and second halves of the depression-like phases (31–45 min and 46–60 min). Independent *t*-tests revealed that DVoc rates of the two vehicle groups from these three test phases were not significantly different from one another and were collapsed to form a single control group. A two way ANOVA (between-within) was used to determine main effects for both treatment and test phase. One-way ANOVAs were used to reveal group differences at each test phase. Fisher's LSD was used to determine specific group differences and Cohen's *d* was calculated to determine effect size.

3. Theory/calculation

Given the historic cultural use of *S. tortuosum* as an anxiolytic and antidepressant and that its extract fraction shows antidepressant-like activity in a rodent model of depression, it would be of value to determine whether these effects can be observed in