

Effects of *Sceletium tortuosum* in rats ☆Melissa J. Loria <sup>a,\*</sup>, Zulfiqar Ali <sup>c</sup>, Naohito Abe <sup>c</sup>, Kenneth J. Sufka <sup>a,b,c</sup>, Ikhlas A. Khan <sup>c</sup><sup>a</sup> Departments of Psychology, University of Mississippi, MS 38677, USA<sup>b</sup> Departments of Pharmacology, University of Mississippi, MS 38677, USA<sup>c</sup> National Center for Natural Products Research, University of Mississippi, MS 38677, USA

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## ABSTRACT

**Ethnopharmacological relevance:** Broad historical and current uses in addition to diverse activity on CNS targets may make *Sceletium tortuosum* a useful therapeutic in a variety of clinical settings. This study sought to more broadly characterize activity of *Sceletium tortuosum* and mesembrine in a number of common, rodent-based assays that model nociception, depression, anxiety, ataxia, and abuse liability.

**Materials and methods:** Male Sprague-Dawley were administered *Sceletium tortuosum* extract products and behavioral responses were evaluated in the conditioned place preference (CPP), hot plate, forced swim, elevated plus, and rotarod tests.

**Results and conclusions:** *Sceletium tortuosum* does not cause preference or aversion in CPP. Mesembrine appears to have analgesic properties without abuse liabilities or ataxia. The *Sceletium tortuosum* fraction has antidepressant properties but does produce ataxia. The ataxia may limit its usefulness as an antidepressant unless the antidepressant activity is associated with one constituent and the ataxia is associated with a separate constituent.

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## 1. Introduction

*Sceletium tortuosum*, also known as channa, kanna, or kougoed, is a flowering, succulent herb native to south-west South Africa. In its traditional use, the plant is most commonly chewed but can also be ingested as a tea, tincture, used as a snuff or smoked. It is used by indigenous people for toothache, abdominal pain, and for hunger relief but some reports claim the plant is primarily used by natives for pleasure (for review: Gericke and Viljoen, 2008). In the West, it has been used in multiple settings, including depression and anxiety management and was recently shown to elevate mood in a human clinical trial (zembrin.com; Nell et al., 2013). There are also anecdotal reports online to suggest that drug users are using the plant as an adjuvant to both legal and illegal substances to enhance effects.

*Sceletium tortuosum* extract, Zembrin<sup>®</sup>, shows broad activity on central nervous system (CNS) targets and has been shown to inhibit both serotonin (5-HT) uptake and phosphodiesterase-4 (PDE-4). At higher doses, this extract shows activity at GABA,  $\delta_2$ -

and  $\mu$ - opioid, cholecystokinin-1 (or -A), EP4 prostaglandin, and melatonin-1 receptors (Harvey et al., 2011). *Sceletium tortuosum*'s primary active constituent is mesembrine, which has specifically shown high selectivity for the 5-HT transporter (for review: Gericke and Viljoen, 2008; Harvey et al., 2011).

The broad historical and current uses and diverse activity on CNS targets may make *Sceletium tortuosum* a useful therapeutic in a variety of clinical settings. However, the anecdotal evidence that suggests recreational use of the plant may limit its therapeutic value. The goal of this research is to more broadly characterize activity of *Sceletium tortuosum*, and mesembrine in a number of common, rodent-based assays that model nociception, depression, anxiety, ataxia, and abuse liability.

## 2. Materials and methods

## 2.1. Subjects

Male Sprague Dawley rats (175–200 g, 6–7 weeks old; Harlan, Indianapolis, IN) were housed in pairs and maintained under a 12-h light/dark cycle in a temperature and humidity controlled vivarium. Food and water were available ad libitum. Animals were handled daily for 3–6 days prior to experimental manipulations to reduce experimenter-related stress.

Two cohorts of animals were used for these experiments. The first group was used for the Conditioned Place Preference

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paradigm. The second group was used for the Hotplate test, Elevated Plus maze, Forced Swim test, and Rotarod; tests were run in this order with at least one day off between tests to permit drug and botanical clearance.

Two cohorts of animals were used for these experiments. The first cohort was used for the Conditioned Place Preference paradigm while the second was used for four other assays that included the Hotplate, Elevated Plus Maze, Forced Swim, and Rotarod tests; These latter four tests were run in this order with 2–3 days between tests to minimize testing carry-over effects and permit compound clearance. The use of repeated testing in the second cohort was also designed to address a reduction in the number of purpose bred animals for research in accordance with the NIH policy.

## 2.2. Drugs

The leaves of *Sceletium tortuosum* were purchased from Bouncing Bear Botanicals, Lawrence, KS, USA, respectively. The plant's material was identified by Dr. Vijayasankar Raman at The National Center for Natural Products Research, University of Mississippi (*Sceletium tortuosum* voucher no. 10851). The dried leaves powder of *Sceletium tortuosum* was extracted separately with methanol and chloroform to give the methanol extract (crude extract) and the chloroform extract on removal of solvent under reduced pressure. The chloroform extract (20 g) was applied to vacuum liquid chromatography (VLC) over reverse 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). Fraction eluted with methanol-water (8:2) was named as alkaloid enrich fraction.

Mesembrine was found to be a major compound in the crude extract (1.5%) and an alkaloid enriched fraction (11.8%) during the HPLC fingerprinting analysis (see [Supplemental materials](#)).

For the Conditioned Place Preference study, amphetamine (1 mg/kg) and haloperidol (0.8 mg/kg) served as the reference compounds that display reward and aversion, respectively. Vehicles for these drugs were physiological saline. Doses for the *Sceletium tortuosum* full alkaloid extract were 25, 50, and 100 mg/kg, for the alkaloid enriched fraction were 5, 10, and 20 mg/kg, and for mesembrine were 5, 10, and 20 mg/kg. All compounds were administered intraperitoneally (IP) in a volume of 1 mg/kg. Vehicle for *Sceletium tortuosum* conditions was 20% solution of Tween-80.

Morphine (5 mg/kg), imipramine (15 mg/kg), chlordiazepoxide (5 mg/kg), and muscimol (2 mg/kg) served as the reference compounds for the Hotplate test, Forced Swim test, Elevated Plus maze, and Rotarod, respectively. Vehicle for these four controls was physiological saline. For these four assays, the full alkaloid extract, the alkaloid enriched fraction, and mesembrine were tested at one dose each 100, 20, and 20 mg/kg, respectively. All compounds were administered intraperitoneally (IP) with a 30 min injection to test interval.

## 2.3. Conditioned place preference (CPP)

CPP was used to evaluate the rewarding and aversive properties of test compounds. Five place preference chambers (Med Associates CPP RS; Med Associates, St. Albans, VT) were used for this experiment. Each chamber has two stimulus-distinct (color and flooring) drug-conditioning chambers and a central start chamber. Guillotine doors provide confinement/access to the conditioning chambers. The CPP/CPA procedure ([Bardo and Bevins, 2000](#)) involves four phases: 1) a 15 min apparatus habituation trial, 2) a 15 min baseline preference trial, 3) eight 30 min drug conditioning trials, and 4) a final 15 min place preference trial. Animals had access to the entire place preference apparatus during the drug-free

habituation, baseline preference and final preference trials. The conditioning phase involved alternate day, counterbalanced (for drug order) pairings of test compound in one compartment (S+) and vehicle in the other (S-). Conditioning trials were counterbalanced (drug/vehicle) within treatments conditions. Test articles were administered and animals were immediately placed into test apparatus for the 30 min test. Excluding a small number of outliers, the baseline preferences for compartments were within a 60:40 split. From this, S+ chamber assignment was to the non-preferred compartment, based on baseline preference scores, except for haloperidol because it was expected to produce aversion. Sample sizes were 7–10.

## 2.4. Hotplate

The hotplate test was used to characterize analgesic properties of the test compounds against thermal nociception. Animals were injected 15 min prior to test. Rats were placed into an acrylic enclosure situated on a hotplate maintained at 52 °C (Harvard Apparatus, Model #52-8570). Latency to flutter or lick hindpaw, or to perform an escape response, was recorded (45 s cut-off score). Animals were returned to home cage upon completion of the test. Sample sizes were 10.

## 2.5. Forced swim

The forced swim test was used to characterize antidepressant properties of test compounds. Animals were injected 15 min prior to test. Animals were placed into a glass cylinder (46 cm × 20 cm) filled with water up to 11.5 cm from top, maintained at room temperature. Immobility during a 5 min test interval (i.e. not actively engaging in swimming behavior or escape behavior) served as the dependent variable. After the test, animals were towel dried and returned to their home cage. Sample sizes were 4–5.

## 2.6. Elevated plus

The elevated plus maze was used to characterize anxiolytic effects of the test compounds. The apparatus is a + shaped maze with four 56 cm long arms. Two arms have side walls (15.25 cm) (closed arms) and two arms are without walls (open arms) and is elevated 76 cm above the ground. Animals were injected 15 min prior to test. Rats were placed into the center (hub) facing an open arm. Time spent on open arms was recorded. After the 5 min test, animals were returned to home cage. Sample sizes were 4–5.

## 2.7. Rotarod

The rotarod (San Diego Instruments, ROTOR-ROD™) was used to characterize ataxia of the test compounds. One day prior to test session, three training sessions, separated by 10 min intervals, were conducted under drug-free states. On test day, animals were tested twice with a 15 min interval between. Animals were injected 15 min prior to the first run which served as further training and were tested 30 min after injection. Dependent measure included latency to fall from the rotating drum on the second trial. Animals were returned to homecage after completion of each trial. Sample sizes were 7–10.

## 2.8. Statistical analyses

Data analyses were conducted using SPSS® software. Group differences in all tests were analyzed using one-way ANOVAs. Post-hoc analyses were performed using Fisher's LSD. A statistically significant finding was determined by a group having a  $p < 0.05$  relative to vehicle treated groups.

### 3. Results

#### 3.1. Conditioned place preference

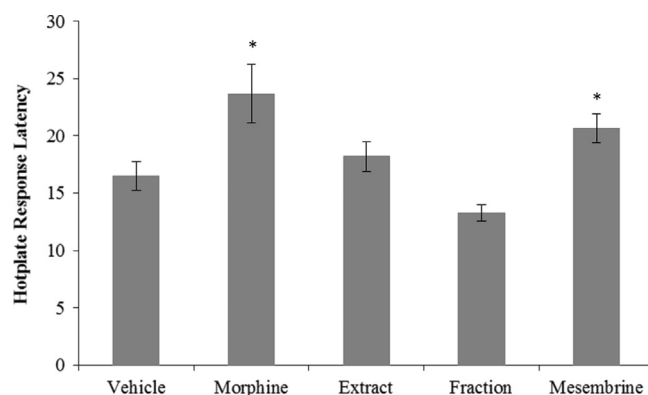
The effects of test articles on preference scores are summarized in Fig. 1. Amphetamine and haloperidol, displayed higher (i.e. reward) and lower (i.e. aversion) preference scores when compared to the vehicle group, respectively. In contrast, the test articles showed no change in preference scores compared to the vehicle group. Consistent with these findings, a one-way ANOVA was performed on these data and revealed a significant main effect for treatment condition,  $F(11,106)=3.811$ ,  $p<0.001$ . Post-hoc analyses revealed that preference scores for d-amphetamine were significantly higher than vehicle ( $p=0.043$ ). Preference scores for haloperidol were significantly lower than the vehicle ( $p<0.001$ ). All other relevant comparisons were not statistically significant.

#### 3.2. Hotplate

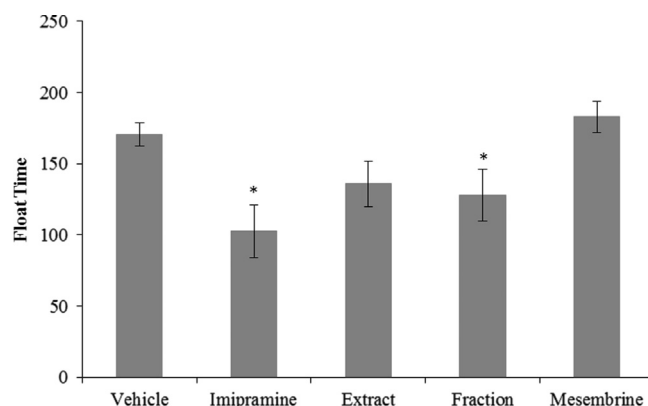
The effects of test articles on hotplate response latencies are summarized in Fig. 2. The reference group, morphine, had an increased hotplate response latency compared to the vehicle group. Mesembrine, but not *Sceletium tortuosum* or its selected fraction, showed an increased response latency. Consistent with these observations, a one-way ANOVA revealed a significant main effect for treatment condition,  $F(4,55)=5.873$ ,  $p=0.001$ . Post-hoc analyses revealed that the mean hotplate response latency for morphine was significantly higher than the vehicle, ( $p=0.001$ ). The mean hotplate response latency for mesembrine was also significantly higher than the vehicle, ( $p=0.047$ ). All other relevant comparisons were not statistically significant.

#### 3.3. Forced swim

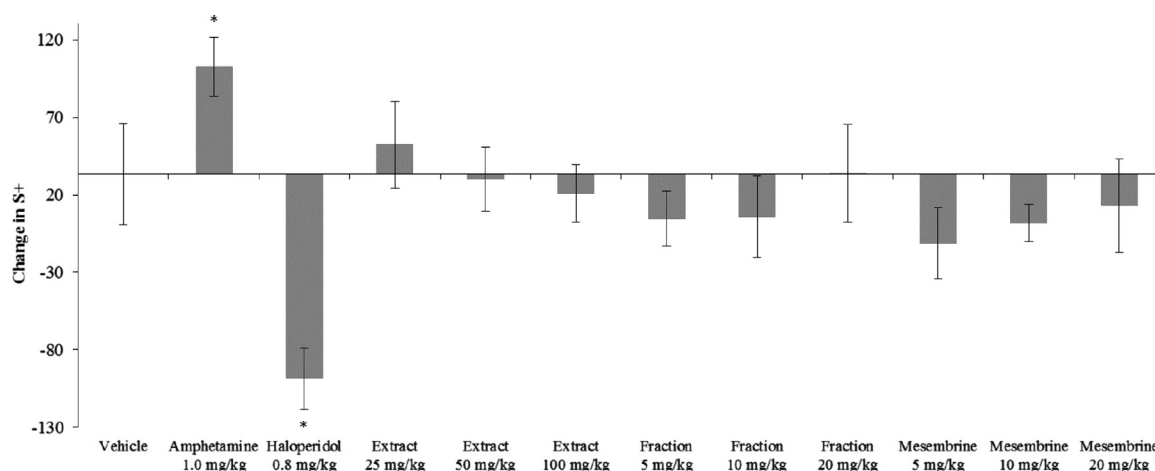
The effects of test articles on float time are summarized in Fig. 3. Imipramine decreased float time in the test compared to the vehicle group. *Sceletium tortuosum* and the fraction groups also spent less time floating. Mesembrine did not alter float time. Consistent with these observations, a one-way ANOVA revealed a significant main effect for treatment condition,  $F(4,22)=5.998$ ,  $p=0.002$ . Post-hoc analyses revealed that the mean float time for



**Fig. 2.** The effects of *Sceletium tortuosum* extract products and morphine on the hotplate test. Morphine served as the control and was tested at 10 mg/kg. The full alkaloid extract, the alkaloid enriched fraction, and mesembrine were tested at one dose each 100, 20, and 20 mg/kg, respectively. Values represent mean latency to flutter or lick hindpaw. \* indicates a significant difference from the vehicle group. Sample sizes were  $n=10$  except for vehicle ( $n=20$ ), that collapsed saline and *Sceletium tortuosum* vehicle groups.



**Fig. 3.** The effects of *Sceletium tortuosum* extract products and imipramine on the forced swim test. Imipramine served as the control and was tested at 15 mg/kg. The full alkaloid extract, the alkaloid enriched fraction, and mesembrine were tested at one dose each 100, 20, and 20 mg/kg, respectively. Values represent mean float time (i.e. not struggling or engaging in escape behavior). \* indicates a significant difference from the vehicle group. Sample sizes were 4–5 except for vehicle ( $n=9$ ), that collapsed saline and *Sceletium tortuosum* vehicle groups.



**Fig. 1.** The effects of *Sceletium tortuosum* extract products and reference compounds on place preference. Doses for the control drugs, amphetamine and haloperidol, were 1.0 and 0.8 mg/kg, respectively. Doses for the *Sceletium tortuosum* full alkaloid extract were 25, 50, and 100 mg/kg, for the alkaloid enriched fraction were 5, 10, and 20 mg/kg, and for mesembrine were 5, 10, and 20 mg/kg. Values represent mean change in time (seconds) spent in S+ (drug-paired) chamber between baseline and preference trials. Solid horizontal line reflects the mean preference score for the vehicle group and is provided for comparative purposes. Scores significantly above and below baseline reflect CPP and CPA, respectively. \* indicates a significant difference from the vehicle group. Sample sizes were 9–10.

imipramine was significantly lower than the vehicle group, ( $p=0.001$ ). Mean float time for the fraction was also significantly lower than the vehicle group, ( $p=0.032$ ). Mean float time for the full alkaloid extract when compared to the vehicle group approached significance, ( $p=0.076$ ). No another comparison was statistically significant.

### 3.4. Elevated plus

The effects of test articles on time spent on the open arms of the Elevated Plus Maze are summarized in Fig. 4. Chlordiazepoxide animals spent more time on the open arms compared to the vehicle group. *Scelletium tortuosum*, its selected fraction, and mesembrine did not alter time spent on the open arms. A one-way ANOVA did not reveal a significant main effect for treatment condition,  $F(4,25)=2.196$ ,  $p=0.099$ . However, planned post-hoc analyses revealed that the chlordiazepoxide group spent significantly more time on the open arms than the vehicle group ( $p=0.019$ ). All other relevant comparisons were not statistically significant.

### 3.5. Rotarod

The effects of test articles on latencies to fall from the rotating drum are summarized in Fig. 5. Muscimol animals fell from the drum more quickly than vehicle animals. *Scelletium tortuosum*, the fraction, and mesembrine tended to have a shorter latency to fall. Consistent with these observations, a one-way ANOVA revealed a significant main effect for treatment condition,  $F(5,44)=9.576$ ,  $p<0.001$ . Post-hoc analyses revealed that the mean latency to fall for muscimol was significantly lower than for saline, ( $p=0.016$ ). The mean latency to fall for the fraction was significantly lower than that of the *Scelletium tortuosum* vehicle group, ( $p=0.001$ ). All other relevant comparisons were not statistically significant.

## 4. Discussion

The goal of this research was to broadly characterize the effects of *Scelletium tortuosum* extract products in models of abuse liability, nociception, depression, anxiety, and ataxia. Assays included CPP, hotplate, forced swim, elevated plus maze, and rotarod procedures.

Post-conditioning preference scores for vehicle-treated animals did not alter from their pre-conditioning baseline scores. The reference

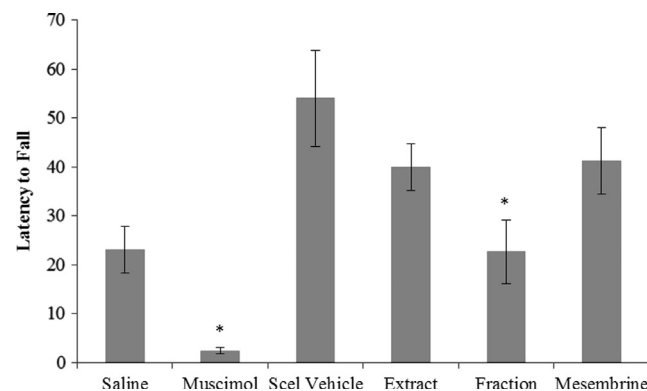


Fig. 5. The effects of *Scelletium tortuosum* extract products and muscimol on the rotarod. Muscimol served as the control and was tested at 2 mg/kg. The full alkaloid extract, the alkaloid enriched fraction, and mesembrine were tested at one dose each 100, 20, and 20 mg/kg, respectively. Values represent latency to fall off of the rotating drum. \* indicates a significant difference from the vehicle group. Sample sizes were 7–9.

compounds, amphetamine and haloperidol, produced place preference and place aversion, respectively. This is consistent with rodent and human literature that amphetamine possesses powerful rewarding properties while haloperidol produces unpleasant side effects (for review: Bardo et al., 1995; for review: Tzschentke, 1998; Schatzberg and Nemeroff, 2004). Despite limited anecdotal evidence of the recreational use of *Scelletium tortuosum* extract products, the present study showed no evidence of preference or aversion. Thus, this botanical does not appear to possess psychoactive properties (i.e. positive reinforcement or positive punishment) that would limit its use in consumers. Although one could argue that the absence of activity on CPP and CPA may be due to the lack of the compound's bioavailability, evidence revealed below for activity on hotplate and forced swim test will suggest that this is not the case and that the animals showed no evidence of preference or aversion to the botanical.

In the hotplate, the reference compound morphine increased hotplate latency. This analgesic effect is consistent with both rodent studies and clinical uses. Mesembrine, but not the extract or fraction, altered nociceptive responses approximate to morphine. That mesembrine has analgesic activity is not surprising, given this botanical has shown activity at  $\delta_2$ - and  $\mu$ - opioid receptors (Harvey et al., 2011); agonists at these sites show analgesic effects (Rev, 1996). These findings suggest that mesembrine may be a useful analgesic.

In the forced swim test, the reference compound imipramine decreased float time. This antidepressant effect is consistent with both rodent studies and its use as an antidepressant in clinical settings (for review: Petit-Demouliere et al., 2005; Schatzberg and Nemeroff, 2004). The fraction, but not the full extract or mesembrine, altered float time approximate to imipramine. That the fraction has antidepressant properties is not surprising given that this botanical acts as a 5HT reuptake inhibitor (Harvey et al., 2011); 5HT reuptake inhibitors show antidepressant effects (Schatzberg and Nemeroff, 2004). These findings suggest that some component(s) in *Scelletium tortuosum* may be useful in treatment of depressive disorders.

In the elevated plus maze, the reference compound chlordiazepoxide increased time spent in open arms. This anxiolytic effect is consistent with both rodent studies and its use as an anxiolytic in clinical settings (Rodgers and Dalvi, 1997; Schatzberg and Nemeroff, 2004). *Scelletium tortuosum* extract products showed no evidence of anxiolytic properties. Thus, this botanical does not appear to possess psychoactive properties that would be useful in treating anxiety disorders in humans.

In the rotarod, the reference compound muscimol decreased latency to fall from the rotating drum. This motor coordination

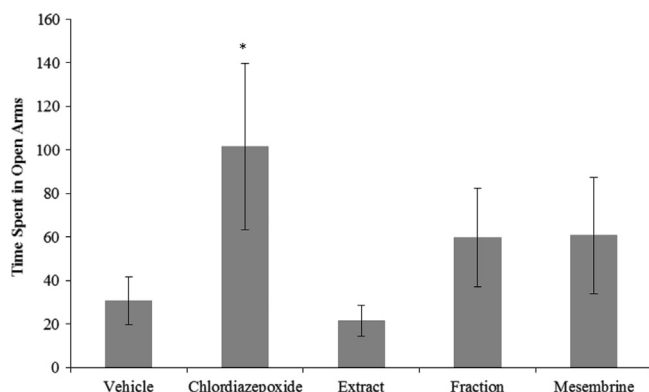


Fig. 4. The effects of *Scelletium tortuosum* extract products and chlordiazepoxide on the elevated plus maze. Chlordiazepoxide served as the control and was tested at 5 mg/kg. The full alkaloid extract, the alkaloid enriched fraction, and mesembrine were tested at one dose each 100, 20, and 20 mg/kg, respectively. Values represent mean time spent in open arms. \* indicates a significant difference from the vehicle group. Sample sizes were 4–5 except for vehicle ( $n=9$ ), that collapsed saline and *Scelletium tortuosum* vehicle groups.

impairment is consistent with both rodent studies and side effects of the drug in clinical settings. These findings suggest that some component(s) in *Sceletium tortuosum* may cause ataxia.

In summary, mesembrine appears to have analgesic properties without abuse liabilities or ataxia. The *Sceletium tortuosum* fraction has antidepressant properties but does produce ataxia. The ataxia may limit its usefulness as an antidepressant unless the antidepressant activity is associated with one constituent and the ataxia is associated with a separate constituent. The finding of differential patterns of activity across assays, between these compounds highlights the possibility of *Sceletium tortuosum* constituents that possess agonist and antagonist properties to one another. This would suggest isolation of additional constituents and evaluation of activity and interaction with other major constituents would be a worthwhile endeavor.

A recent double-blind, parallel-group, placebo-controlled trial study sought to characterize long-term use of *Sceletium tortuosum* in a clinical population. Safety and tolerability of the botanical were monitored throughout the three-month study. Results showed no long-term toxicity of *Sceletium tortuosum* and positive emotional changes in the participants taking the botanical. It was concluded that *Sceletium tortuosum* is well tolerated in healthy adults when taken consistently for 3 months (HG&H Pharmaceuticals, 2013). These findings, along the present research, suggest that *Sceletium tortuosum* looks to be a botanical that could potentially have a number of uses in clinical settings and be void of an abuse liability.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jep.2014.06.007>.

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