Journal of Ethnopharmacology 155 (2014) 731–735

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Effects of *Sceletium tortuosum* in rats ☆

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ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form
26 May 2014
Accepted 4 June 2014
Available online 12 June 2014

Keywords:
Sceletium tortuosum
Mesembrine
Conditioned place preference
Hotplate
Anxiety and Depression
Rotarod

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.

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®, 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, δ_2- and μ- 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

☆This research was conducted under the ethical standards American Psychological Association and in accordance with the principles of laboratory animal care as detailed in the National Institutes of Health publication No. 85-23. This manuscript has not been previously published nor is it currently under consideration by any other journal for publication.

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http://dx.doi.org/10.1016/j.jep.2014.06.007
0378-8741/

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 m apparatus habituation trial, 2) a 15 m baseline preference trial, 3) eight 30 m drug conditioning trials, and 4) a final 15 m 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 m 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.