Fitoterapia 109 (2016) 87–90

Contents lists available at ScienceDirect

Fitoterapia

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

Comparative effects of *Mitragyna speciosa* extract, mitragynine, and opioid agonists on thermal nociception in rats

Jessica M. Carpenter [a], Catherine A. Criddle [b], Helaina K. Craig [a], Zulfiqar Ali [a], Zhihao Zhang [a], Ikhlas A. Khan [a], Kenneth J. Sufka [a,b,*]

[a] National Center for Natural Products Research, University of Mississippi, MS 38677, USA
[b] Department of Psychology, University of Mississippi, MS 38677, USA

ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 2 December 2015
Accepted 9 December 2015
Available online 10 December 2015

Keywords:
Mitragyna speciosa (Korth.) Havil.
Mitragynine
Hotplate
Opioids
Oral
Intraperitoneal

ABSTRACT

This study sought to compare the effects of *Mitragyna speciosa* (Korth.) Havil. extract, alkaloids fraction, and mitragynine, a μ-opioid receptor agonist, to that of morphine and oxycodone in a test of thermal nociception. In Experiment 1, male Sprague–Dawley rats were administered test articles intraperitoneally (IP) 30 min prior to testing to compare the effects of *M. speciosa* articles to opioid reference compounds on the hotplate assay. Test articles were vehicle, 10 mg/kg morphine, 3 mg/kg oxycodone, 300 mg/kg *M. speciosa* extract, 75 mg/kg *M. speciosa* alkaloids fraction, or 30 mg/kg mitragynine. To mirror consumer usage, Experiment 2 sought to determine whether *M. speciosa* articles retained their biological activity when given orally (PO). Test articles were vehicle, 6 mg/kg oxycodone, 300 mg/kg *M. speciosa* extract, or 100 mg/kg mitragynine with hotplate tests conducted 30 and 60 min after administration. Mitragynine produced antinociceptive effects similar to the reference opioid agonists when administered IP and PO routes. These data suggest that *M. speciosa* extracts containing significant quantities of mitragynine may warrant consideration for further studies in primate self-administration models to yield insight into the abuse liability of this commercially available product.

1. Introduction

Mitragyna speciosa (Korth.) Havil., also known as kratom, ketum, or biak-biak, is a tropical tree indigenous to regions of Southeast Asia. In its traditional use, the leaves of *Mitragyna speciosa* (abbreviated to *M. speciosa*) have been chewed, smoked, or made into a tea for medicinal purposes to relieve muscle pain and fever, reduce appetite, and control diarrhea [1]. Furthermore, it has been reportedly used as a mild narcotic in place of opium [2]. Similar to opium, *M. speciosa* alleviated workers' pain caused by manual labor and increased endurance by preventing fatigue [2–4]. Today, it is consumed orally for tolerance of manual labor in extreme heat, recreational purposes among younger populations, and for treatment of withdrawal symptoms of opiate addiction [1]. Despite regulation in some regions of Southeast Asia, such as Thailand, export of the plant to internet pharmacies has made kratom powders and extracts easily accessible and unregulated around the world [1,5].

Twenty-five alkaloids have been isolated and chemically characterized from *M. speciosa* with mitragynine being the most dominant alkaloid. Mitragynine comprises 66% of the total alkaloid mixture. It has been reported to exhibit stimulant-like effects at low dosages and opiate-like effects at higher dosages [3,6]. More recent work has shown that mitragynine is a high affinity μ-opioid receptor (MOR) agonist [1,7]. MORs are found throughout the central nervous system including limbic structures that mediate reward and subserve addiction [8,9].

A recent work by Sufka et al. [10] sought to characterize the rewarding properties of *M. speciosa* extract, an alkaloids fraction, and isolated mitragynine in the Conditioned Place Preference (CPP) assay. This paradigm is based on the notion that animals prefer environments previously paired with positively reinforcing drugs. Mitragynine produced an increase in preference scores similar to the reference article, D-amphetamine. *M. speciosa* extract showed a similar but not statistically significant pattern of effects as its major active constituent. These findings suggest that *M. speciosa* containing significant amounts of mitragynine possesses an abuse liability [10].

There are two limitations of the aforementioned abuse liability study. First, *M. speciosa* products were administered intraperitoneally (IP), not orally, as CPP protocols do not lend themselves to oral route of administration due to poor absorption and bioavailability that are necessary to establish conditioning. Second, D-amphetamine was used as a positive control. Given the MOR agonist activity of mitragynine, a more appropriate positive control would be an opioid agonist such as morphine or the commonly abused prescription analgesic oxycodone.

Given that mitragynine shows MOR agonist activity, it would not be surprising to find this botanical constituent to show activity similar to

* Corresponding author at: Department of Psychology, University of Mississippi, University, MS 38677, USA.
E-mail address: pysufka@olemiss.edu (K.J. Sufka).

http://dx.doi.org/10.1016/j.fitote.2015.12.001
0367-326X/

other opioid compounds. One assay that shows robust sensitivity to opioids and easily lends itself to multiple routes of administration is the hotplate test. The opioid agonists morphine and oxycodone increase hotplate latencies that reflect the antinociceptive effects of these compounds. The goal of this research is two-fold. First, we sought to determine whether IP administration of *M. speciosa* and mitragynine mirrored hotplate activity of morphine and oxycodone. Second, we sought to determine whether mitragynine retained such effects when given via an oral route of administration.

2. Materials and methods

2.1. Plant material

M. speciosa leaves were purchased from Bouncing Bear Botanicals (Lawrence, KS, USA). The plant material was identified by Dr. Vijayasankar Raman at The National Center for Natural Products Research at the University of Mississippi (voucher no. 12433).

2.2. Extraction

M. speciosa leaves were extracted with methanol (3 l × 4× for 24 h) at room temperature and the solvent was removed using rotavap to yield dried extract. The extract was mixed with 5% HCl in water and extracted with ethyl acetate. The water soluble part was made basic (pH 9–10) with liquid ammonia and extracted with ethyl acetate. The ethyl acetate soluble part from basic media was dried in vacuum to obtain an alkaloid enrich fraction. Repeated column chromatography of alkaloid enrich fraction over silica gel, using chloroform/methanol (9:1) and hexanes/acetone/liquid ammonia (210:90:1) solvent systems, mitragynine (97% pure) was purified. The structure of mitragynine was confirmed by mass and NMR data analyses including 1D NMR and 2D NMR [11], as well as by comparing NMR data with reported values [12].

2.3. Animals

Male Sprague Dawley rats (175–200 g 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 up to the day before testing. In Experiment 1, animals were handled daily 5 days prior to testing in order to minimize any experimenter-related stress. In Experiment 2, naive animals were handled and trained to drink a small amount of sucrose water (.2 ml) via gavage-type feeding tube twice daily for a period of 5 days. Such training was performed in order to acclimate animals to the gavage procedure for oral route of administration and reduce experimenter-related stress. Food was restricted 12-h and 24-h prior to drug administration and testing in Experiments 1 and 2, respectively. Food was available upon completion of tests.

The present research was conducted under the ethical standards of the American Psychological Association and in accordance with the principles of laboratory animal care as detailed in the National Institutes of Health publication # 85-23. Research protocol # 12-020 was approved by the University of Mississippi's Institutional Animal Care and Use Committee.

2.4. Drugs and chemicals

A solution of 20% Tween 80 served as vehicle for these experiments. In Experiment 1, morphine (Research Biochemicals International, Natick, MA) and oxycodone (Sigma-Aldrich, St. Louis, MO) served as controls and were delivered in a volume of 1 ml/kg. In Experiment 2, oxycodone served as control and was administered orally in a volume of 2 ml/kg. Dosages of test articles were derived from Sufka et al. [10], which showed activity of *M. speciosa* extract, alkaloids fraction, and mitragynine in the CPP paradigm. Drug dosages were increased by 2–3× to accommodate pharmacodynamic differences of absorption and first pass metabolism when using oral gavage route over IP route of administration [13]. Therefore, dosages in Experiment 2 were increased accordingly for both reference compound and mitragynine [14,15]. However, we found that dosage of *M. speciosa* was at the limit for solubility and was unable to increase further.

2.4.1. Experiment 1

Rats were given vehicle, 10 mg/kg morphine, 3 mg/kg oxycodone, 300 mg/kg *M. speciosa* extract, 75 mg/kg *M. speciosa* alkaloids fraction, or 30 mg/kg mitragynine IP 30 min prior to hotplate testing. Sample sizes were n = 9–10.

2.4.2. Experiment 2

Rats were given vehicle, 6 mg/kg oxycodone, 300 mg/kg *M. speciosa* extract, or 100 mg/kg mitragynine orally. During drug administration, the rat was gently restrained in an upright position while a stainless steel gavage feeding tube was inserted into the stomach to deliver compounds. Hotplate tests were conducted at 30 and 60 min post drug administration. Sample sizes were n = 8–9.

2.5. Hotplate test

The antinociceptive effects of test compounds against thermal nociception were quantified using the hotplate test. Rats were gently placed into an acrylic enclosure positioned on top of a hotplate (model # 52-8570, Harvard Apparatus) maintained at 52 °C. The latency to flutter or lick a hindpaw or perform an escape response (i.e. jumping out of the apparatus) was recorded. A 45 s cut-off score was employed to avoid tissue damage. All animals were returned to their home cages upon completion of testing (and in between tests in Experiment 2).

2.6. Data analysis

Data analyses were conducted using SPSS software. Data was analyzed using one-way ANOVAs with Fisher's planned post-hoc analyses.

3. Results

3.1. Experiment 1 — IP administration of test articles

The effects of intraperitoneal (IP) administration of *M. speciosa* products and the opioid reference compounds on hotplate response latencies are summarized in Fig. 1. Morphine, oxycodone, and mitragynine produced antinociceptive effects in this assay. A one-way ANOVA of these data revealed a significant main effect for treatment, $F(5,53) = 6.734$, $p < 0.0001$. Post-hoc analyses revealed that the mean hotplate response latency for morphine ($p = 0.0001$), oxycodone ($p = 0.039$) and mitragynine ($p = 0.022$) groups was significantly longer than the vehicle group. All other relevant comparisons were not statistically significant.

3.2. Experiment 2 — PO administration of test articles

The effects of oral (PO) administration of *M. speciosa* products and the opioid reference compound on hotplate response latencies are summarized in Fig. 2. Oral administration of oxycodone and mitragynine produced antinociceptive effects in this assay at 60 min but not 30 min post administration (data for 30 min not shown). A one-way ANOVA on data at 60 min revealed a main effect for treatment that approached significance, $F(3,30) = 2.093$, $p < 0.122$. Post-hoc analyses revealed that the mean hotplate latency for oxycodone ($p = 0.048$) and mitragynine ($p = 0.037$) was significantly longer than the vehicle group. All other relevant comparisons were not statistically significant.