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A Comparison of Mitragyna Speciosa and Mitragynine Against Opioids on Thermal Nociception in Rats

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A COMPARISON OF *MITRAGYNA SPECIOSA* AND MITRAGYNINE AGAINST OPIOIDS ON THERMAL NOCICEPTION IN RATS

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.

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Approved by

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Advisor: Dr. Kenneth Sufka

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Dedication

This work is dedicated to my parents, sister, and cousin, Stephen White, for all of their love and support. Without which none of this would have been possible.

Acknowledgements

I would like to thank the Sally McDonnell Barksdale Honors College for giving me the opportunity and the funding to get involved in such an interesting project. I would like to thank Dr. Khan and his team for all of their help and funding. I would like to thank my readers, Dr. Matthew Reysen and Dr. Karen Sabol. I would like to thank Dr. Kenneth Sufka and Stephen White for all of their guidance, as well as the graduate students and research assistants for all of their positive encouragement. A very special thanks to Jessica Carpenter and Daniel Marcum for all of their dedication and hard work on this project.

TABLE OF CONTENTS

LIST OF FIGURES

Abstract

CATHERINE ANNE CRIDDLE: A Comparison of *Mitragyna Speciosa* and Mitragynine against Opioids on Thermal Nociception in Rats
(Under the direction of Kenneth Sufka)

The purpose of this experiment is to compare *Mitragyna speciosa* (*M. speciosa*) and its active component, mitragynine, a known μ-opioid receptor (MOR) agonist, against well-known and commonly abused opioids, morphine and oxycodone. Another goal of this research is to determine if these compounds are also active when administered orally as is typical in human users. Male Sprague-Dawley rats were administered *M. speciosa* and mitragynine both intraperitoneally (IP) and orally and their antinociceptive effects were evaluated on the hotplate. Mitragynine exhibited antinociceptive effects similar to oxycodone when administered both IP and orally. *M. speciosa* exhibited a trend towards antinociceptive effects when administered both IP and orally. This research demonstrates that *M. speciosa* possesses properties like oxycodone and raises the possibility of an abuse liability which might warrant consideration for restrictions on the consumer marketplace.

Introduction

Many botanical products are easily accessible to consumers and are used recreationally as well as to remedy an assortment of medical conditions (Dennehy, Tsourounis & Miller, 2005). Unlike therapeutically used botanicals, botanicals used recreationally are typically under-researched (Humberston, Akhtar & Krenzelok, 2003; Teschke, Schwarzenboeck & Akinci, 2008). Often under-researched botanicals contain a myriad of components with dangerous properties such as toxicity or the potential for abuse. The importance of uncovering these dangerous properties cannot be over stressed (Humberston et al., 2003; Teschke et al., 2008). Because of the concerns outlined above, the need for a more systematic look at models that can quantify abuse liability is crucial. Our approach to this problem is discussed below using the botanical product, *Mitragyna speciosa* (*M. speciosa*).

M. speciosa, a plant native to Thailand and Southeast Asia, has traditionally been used by rural laborers in those areas as a mild narcotic in place of opium (Burkill, 1966; Leon et al., 2009). Like opium, *M. speciosa* alleviated workers' pain caused by manual labor and increased endurance by preventing fatigue (Assanangkornchai, Muekthong, Sam-Angsri & Pattanasattayawong, 2007; Jansen & Prast, 1988a, 1988b; Pichainarong, Chaveepojnkamjorn, Khobjit, Veerachai & Sujirarat, 2004; Suwanlert, 1975). Also known as kratom, krathom, ketum and biak-biak, the leaves of this plant can be smoked, chewed, or dried and steeped to make tea (Burkill, 1966; Jansen & Prast, 1988a;

Suwanlert, 1975). *M. speciosa* has also been used in bandages for wounds and served as a remedy for fever, diarrhea, and cough (Burkill & Haniff, 1930). Today, it is used in Southeast Asia primarily for recreational purposes, for pain management, and for treatment of withdrawal symptoms of opiate addiction (Assanangkornchai *et al*., 2007; Jansen & Prast, 1988a, 1988b; Suwanlert, 1975; Vicknasingam, Narayanan, Beng & Mansor, 2010).

The ingestion of *M. spe*ciosa produces a stimulant effect at low dosages and an opioid-like effect at medium to high dosages. This dose-dependent effect has been reported by consumers and exhibited in animal models (Assanangkornchai *et al*., 2007; Grewal, 1932; Jansen & Prast, 1988a, 1988b; Macko, Weisbach & Douglas, 1972; Pichainarong *et al*., 2004; Suwanlert, 1975; Watanabe, Yano, Horie & Yamamoto, 1997; Yamamoto *et al*., 1999). In low to medium doses, some individuals regard the effects of *M. speciosa* as unpleasant, experiencing anxiety and agitation ("Kratom Dosage," 2015; Siebert, 2006; "Kratom Addiction," n.d.). Similarly, in high doses, whether the effects of *M. speciosa* are euphoric or dysphoric is highly dependent on the individual. Some individuals experience nausea, sedation, constipation, and itching after consuming high doses of *M. speciosa* (Assanangkornchai *et al*., 2007; Jansen & Prast, 1988a, 1988b; Suwanlert, 1975).

Mitragynine, an indole alkaloid and the primary active component of *M. speciosa*, is responsible for the opioid-like activity of the plant (Adkins, Boyer & McCurdy, 2011; Matsumoto, Horie, Takayama, *et al*., 2005; Takayama, 2004). Mitragynine has been found to exhibit antinociceptive and analgesic effects in animal models (Matsumoto *et*

al., 2005; Takayama *et al*., 2002; Takayama, 2004; Watanbe *et al*., 1997; Yamamoto *et al*., 1997). Additionally, long-term exposure to mitragynine has been shown to produce dependence and withdrawal symptoms similar to those of opioid withdrawal upon cessation, in animal models (Babu, McCurdy & Boyer, 2008; Matsumoto *et al*., 2005; Thongpradichote, Matsumoto, Tohda *et al*., 1998). Mitragynine also exhibits a high affinity for μ-opioid receptors (MORs), classifying it as a MOR agonist (Watanabe *et al*., 1997; Yamamoto *et al*., 1999).

MORs are highly involved in analgesic and euphoric pathways as well as play a significant role in addiction (Contet *et al*., 2004; McDonald & Lambert, 2005; Watanabe *et al*., 1997; Yamamoto *et al*., 1999). Considering their role in addiction, it is not surprising that MORs are largely located along reward pathways in the brain. MORs mediate the reinforcing activity of morphine and other opioids and are also thought to play a role in the continuance of drug use, cravings, and relapse (Gerrits, Lesscher & van Ree, 2003). Commonly used opioids that act on MORs include morphine, codeine, hydrocodone, heroine, and cocaine (European College of Neuropsychopharmacology, 2007; Contet, Kieffer & Befort, 2004; Trescot, Datta, Lee & Hansen, 2008).

Although *M. speciosa* has been outlawed in Thailand since 1943, and several other East Asian countries, it has not yet been banned in the United States (Siebert, 2006; Vitayanartpaisarn *et al*., 2005). However, according to the U.S. Drug Enforcement Administration (2006) the increasing use and availability of *M. speciosa* is concerning. Over the past 15 years, *M. speciosa* has become increasingly used and widely available in "smoke" or "head" shops as well as over the Internet (US Drug Enforcement

Administration [DEA], 2006; Krauth, 2011; Osterhaus, 2008; Schmidt, Sharma, Schifano & Feinmann, 2011; Adkins *et al*., 2011) In fact, *M. speciosa* products in the form of tablets, capsules, concentrated extracts, and dried leaves are easily obtained from suppliers via the Internet (Krauth, 2011; Osterhaus, 2008).

Sufka *et al*. (2014) sought to determine if *M. speciosa* or its major constituents have an abuse liability. Rats were exposed to conditioned place preference, a behavioral assay, in which either test compounds or saline were administered via intraperitoneal (IP) injections on alternating days in two distinctly different chambers (Sufka *et al*., 2014). These researchers found mitragynine and *M. speciosa* extract, to a lesser extent, produce a place preference comparable to amphetamine. These findings suggest that *M. speciosa* has a high abuse potential (Sufka *et al*., 2014).

Given that mitragynine exhibits a high affinity for MORs, it seems necessary to compare *M. speciosa* to other known and abused MOR agonists, morphine and oxycodone (Koob, Sanna & Bloom, 1998; Lemberg *et al*., 2006; Matsumoto *et al*., 1996; Watanabe *et al*., 1997; Wood and Iyengar, 1988; Yamamoto *et al*., 1999). A considerable amount of literature is available on the abuse liabilities of morphine and oxycodone (Bannon & Malmberg, 2007; Contet *et al*., 2004). The standard assay for measuring MOR agonist activity on antinociception is the hotplate test (Bannon & Malmberg, 2007). Similar hotplate response latencies between *M. speciosa* and the two well-known opioids, morphine and oxycodone, will suggest a high abuse potential for the under-researched botanical. The purpose of this research is 1) to investigate *M. speciosa* and its components for activity similar to other well-known and commonly abused opioids,

morphine and oxycodone 2) to see if this activity is still exhibited when the botanical compound or its components are administered orally and 3) after oral administration at what time points (30 or 60 minutes) during testing are drug effects apparent. These objectives will be addressed in two separate experiments.

Materials and methods

Subjects

Male Sprague Dawley rats (175-200 g, 6-7 weeks old; Harlan, Indianapolis, IN) were housed in pairs and maintained under a 12-hour light/dark cycle in a temperature and humidity controlled vivarium. Food and water were available ad libitum. In experiment 1, 60 animals were handled daily 5 days prior to testing in order to minimize any experimenter-related stress. In experiment 2, 40 naive animals were handled and trained to drink a small amount of sucrose water (.2 ml) via a 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.

Drugs

In 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* fraction, or 30 mg/kg mitragynine IP 30 minutes prior to hotplate testing. In experiment 2, rats were given, via oral gavage, vehicle, 6 mg/kg oxycodone, 300 mg/kg *M. speciosa* extract, or 100 mg/kg mitragynine. During gavage administration, the rat was restrained in the upright position while a stainless steel feeding tube was inserted into the stomach to deliver the test compounds. Hotplate tests were conducted at 30 and 60 minutes post drug administration.

Hotplate

The hotplate test was utilized to characterize analgesic properties of test compounds against thermal nociception. For testing, animals were placed into an acrylic enclosure positioned on top of a hotplate maintained at 52 °C (Harvard Apparatus, Model # 52-8570). Latency to flutter or lick a hindpaw or perform an escape response (i.e. jumping out of the apparatus) was recorded. A 45 second cut-off score was employed for testing. All animals were returned to their home cages upon completion of testing (and in between tests in Experiment 2).

Results

Experiment 1

The effects of test articles on hotplate response latencies are presented in Fig. 1. Compared to the vehicle group, the reference groups, morphine and oxycodone, had longer hotplate response latencies. The *M. speciosa* extract and fraction failed to show any antinociceptive effect, but mitragynine exhibited a longer hotplate response latency compared to the vehicle group. A one-way ANOVA revealed a significant main effect for treatment condition $F(5,53)=6.734$, $p < 0.0001$. Post-hoc analyses revealed that the mean hotplate response latency for morphine ($p=0.0001$) and oxycodone ($p=0.039$) were significantly longer than the vehicle. Mitragynine also showed a mean hotplate latency ($p=0.022$) significantly longer than the vehicle. All other relevant comparisons were not statistically significant.

Experiment 2

The effects of orally administered test compounds on hotplate response latencies are presented in Fig. 2. At 30 minutes post-administration, data trended towards but failed to reach significance. At 60 minutes post-administration, oxycodone, the reference group, had a longer hotplate response latency compared to the vehicle group. Mitragynine, but not *M. speciosa*, also exhibited a longer response latency as compared to the vehicle group. A one-way ANOVA revealed a significant main effect for treatment condition $F(3,30)=2.093$, $p < 0.122$. Post-hoc analyses revealed that the mean hotplate latency for

oxycodone (p=0.048) were significantly longer than the vehicle. Mitragynine also showed a mean hotplate latency (p=0.037) significantly longer than the vehicle. All other relevant comparisons were not statistically significant.

Discussion

The goal of this research was to compare the effects of *M. speciosa* and mitragynine against the effects of known, classic opioids, morphine and oxycodone. Our secondary goal was to determine if mitragynine and its parent compound produced similar effects when administered orally as when administered IP. Typically, the temporal dynamics of orally administered compounds differ from those administered IP, and consequently we sought to determine their time point of action (30 or 60 minutes). In our assays, the reference compounds, morphine and oxycodone, illustrated antinociceptive effects on the hotplate test regardless of administration route. These results are consistent with the extensive research exhibited in numerous models of the role of morphine and oxycodone, MOR agonists, in analgesia (Koob, Sanna & Bloom, 1998; Lemberg *et al*., 2006; Wood and Iyengar, 1988).

As expected, mitragynine showed analgesic effects similar to oxycodone when administered IP. *M. speciosa* trended towards but failed to reach significance. This pattern of effect was also exhibited by mitragynine when administered orally. Again, *M. speciosa* trended towards but failed to reach significance when orally administered. Given that mitragynine is a MOR agonist it is not surprising that it exhibits analgesic effects on the hotplate (Watanabe *et al*., 1997; Yamamoto *et al*., 1999). These results are consistent with Macko *et al*. (1972), Sabetghadam, Ramanathan, and Mansor (2010), and Takayama, Ishikawa, Kurihara (2002) who found analgesic effects on the hotplate in rodent models.

There are many major concerns with the botanical *M. speciosa*. First, *M. speciosa* has become increasingly used by and widely available to consumers in the United States (DEA, 2006; Krauth, 2011; Schmidt *et al*., 2011; Adkins *et al*., 2011). Additionally, mitragynine, the active component in *M. speciosa*, is a MOR agonist like other known and commonly abused opioids, morphine and oxycodone (Adkins *et al*., 2011; Matsumoto *et al*., 2005; Takayama, 2004; Watanabe *et al*., 1997; Yamamoto *et al*., 1999). MORs play a substantial role in addiction as well as analgesic and euphoric pathways (Contet *et al*., 2004; McDonald & Lambert, 2005; Watanbe *et al*., 1997; Yamamoto *et al*., 1999). Furthermore, Sufka *et al*., (2014) found that mitragynine and its parent compound, *M. speciosa*, exhibit an abuse liability comparable to that of amphetamine. In fact, mitragynine and its parent compound exhibit analgesic effects similar to oxycodone on the hotplate. Finally, mitragynine illustrates these effects even when orally consumed. The concerns outlined above illustrate the danger in consuming *M. speciosa*, albeit the need for higher doses and the longer period of activation. Further restrictions need to be placed on this dangerous botanical in the consumer marketplace.

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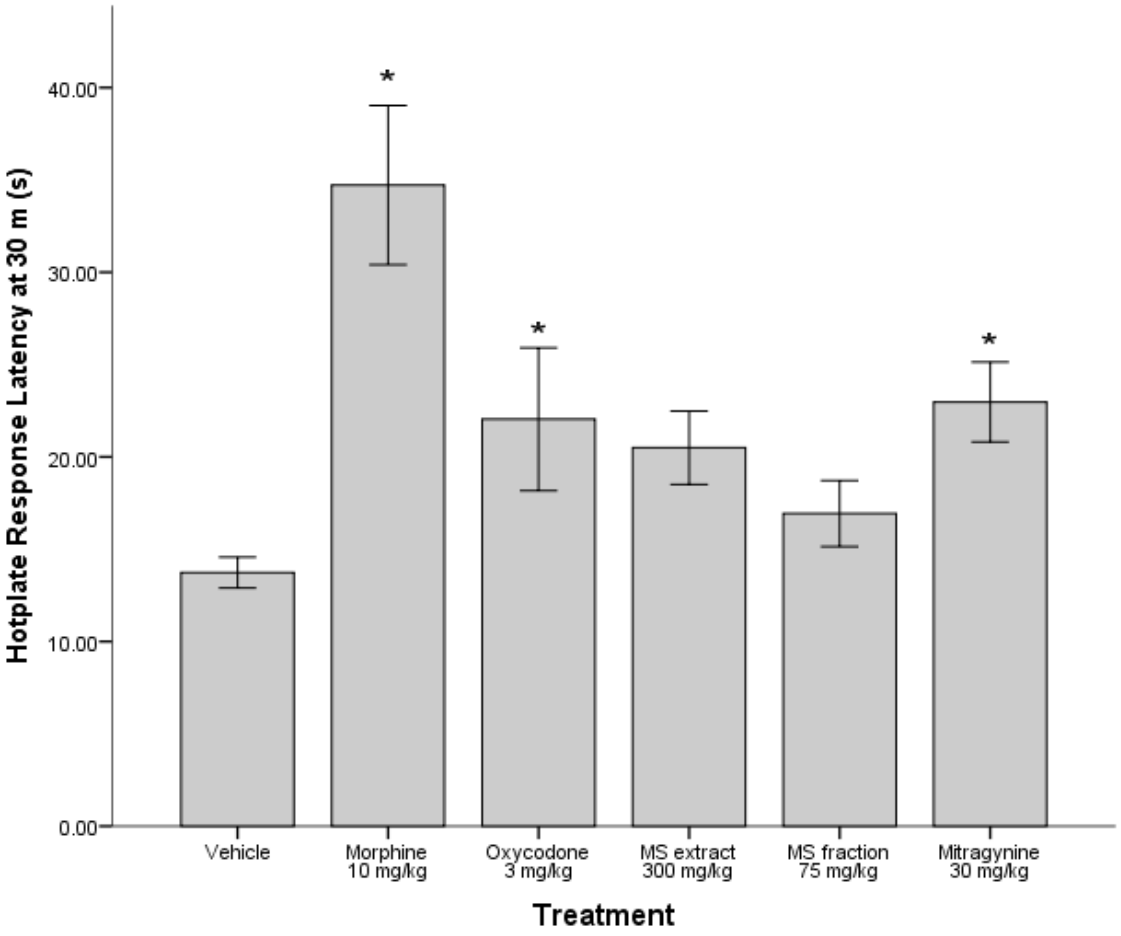

Fig. 1. Hotplate Response Latencies at 30 Min Post IP Administration. The effects of *M. speciosa* extract, fraction, and active component, mitragynine, compared against morphine and oxycodone on the hotplate test. All drugs were administered IP. Morphine and oxycodone served as the control and were tested at 10 mg/kg and 3 mg/kg, respectively. The extract, fraction, and active component, mitragynine, were tested at 300 mg/kg, 75 mg/kg, and 30 mg/kg, respectively. Values represent mean latency to flutter, lick hindpaw, or attempt escape from the apparatus. * indicates a significant difference from the vehicle group. Sample sizes were n=10.

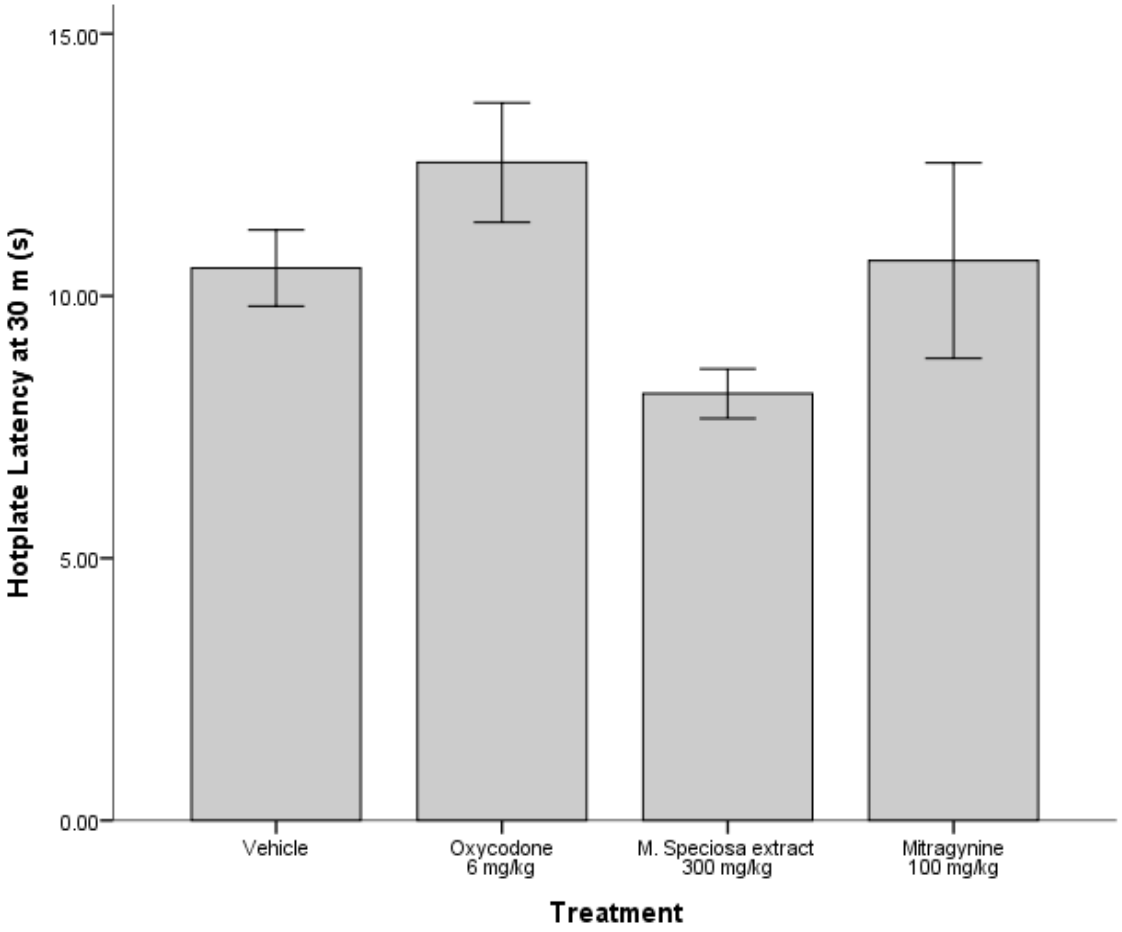

Fig. 2. Hotplate Response Latencies at 30 Min Post Oral Administration. The effects of *M. speciosa* extract and active component, mitragynine, compared against oxycodone on the hotplate test at 30 minutes. All drugs were administered orally. Oxycodone served as the control and was tested at 6 mg/kg. The extract and active component, mitragynine, were tested at 300 mg/kg and 100 mg/kg, respectively. Values represent mean latency to flutter, lick hindpaw, or attempt escape from the apparatus. * indicates a significant difference from the vehicle group. Sample sizes were n=(8-10).

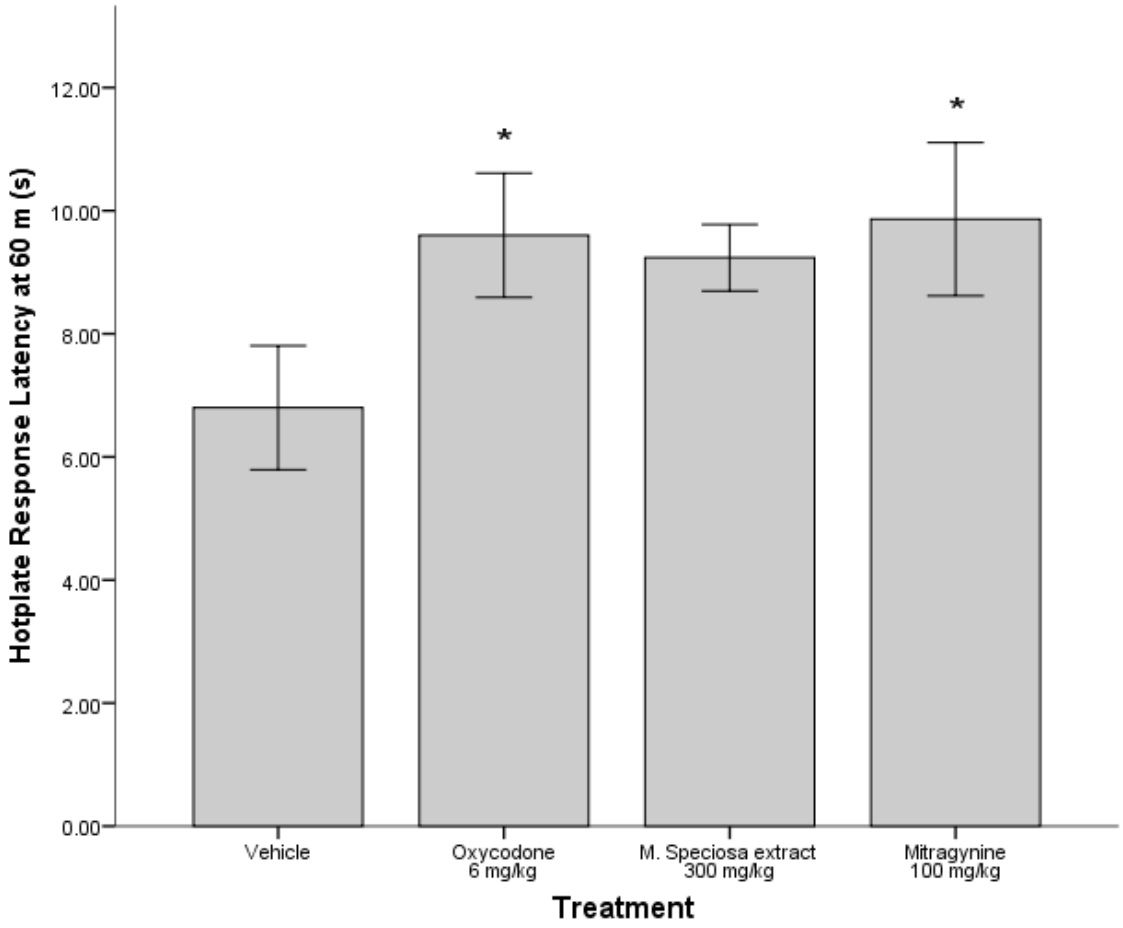

Fig. 3. Hotplate Response Latencies at 60 Min Post Oral Administration. The effects of *M. speciosa* extract and active component, mitragynine, compared against oxycodone on the hotplate test at 60 minutes. All drugs were administered orally. Oxycodone served as the control and was tested at 6 mg/kg. The extract and active component, mitragynine, were tested at 300 mg/kg and 100 mg/kg, respectively. Values represent mean latency to flutter, lick hindpaw, or attempt escape from the apparatus. * indicates a significant difference from the vehicle group. Sample sizes were n=(8-10).