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THE ROLE OF CANNABINOIDS AND TERPENES IN CANNABIS MEDIATED ANALGESIA IN RATS

by Margaret Anne Rousseau

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford April 18 2018

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ABSTRACT

Objective: This research examined the contributions of terpenes (volatile oil) and cannabinoids in cannabis mediated analgesia in rats.

Methods: In experiment 1, separate groups of rats received IP administration of either vehicle, 5, 10, or 15 mg/kg cannabis extract, one of three concentrations of volatile oil alone in these cannabis extracts, one of three concentrations of these extracts with volatile oils removed, 2.5, 5, or 7.5 mg/kg Δ^9-THC, or 10 or 18 mg/kg morphine 30 m before hotplate and tail-flick tests of thermal nociception. One week later, separate groups of rats received a second administration of these test articles IP 30 m before testing in the acetic acid writhing test of inflammatory nociception.

Results: Both tail-flick and hotplate latencies were longer than controls for all drug groups/doses except the isolated volatile oil groups. The 15 mg/kg dose of the cannabis extract and 7.5mg/kg Δ^9 -THC group showed analgesic effects similar to the 18 mg/kg morphine group. In the inflammatory nociceptive assay, all drug groups/doses showed decreased writhes compared to the control except the isolated volatile oil groups.

Conclusions: These tests revealed that terpenes do not significantly contribute to the analgesic action of cannabis extracts. Further, these tests revealed that THC alone produced robust analgesia equivalent to the whole cannabis extract. This leads to the conclusion that THC alone mediates these analgesic actions of cannabis extracts and other components do not significantly affect the analgesic activity of the extract.

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LIST OF ABBREVIATIONS

CBD: Cannabidiol

THC: Δ^9-Tetrahydrocannabinol (also known as Δ^9-THC)

CB1: Cannabinoid receptor type 1

CB2: Cannabinoid receptor type 2

DCM: Dichloromethane

IP: Intraperitoneal

INTRODUCTION

For centuries, natural products have been used all over the world to treat various types of ailments. From as early as 2600 B.C. in Mesopotamia, people used Cypress and Myrrh naturally found in the environment to treat anything from coughs to infection (Dias et al., 2012). Though from China to Egypt these natural products have been used and documented in ancient texts, very few have been tested to determine if they are biologically active (Fabricant et al., 2001). Although some natural products are commonly used today, like salicin, which is used to synthesize aspirin (Dias et al., 2012), there is still a wide array of natural products that have not been assessed let alone considered for scientific value. This untapped potential could be the future of medicine, and in fact, has been the subject of much interest in the last few decades. As tools for chemical analysis and understanding of biological processes increases, so too does the understanding of natural products and how they can be used in medicine. With current prescription drugs becoming a cause for concern, like the opioid epidemic the United States is facing (Murthy, 2016), researchers are turning towards these natural products to find alternative treatments. One natural product that has gained huge interest in the last decade for its medicinal potential is Cannabis.

Cannabis, commonly known as marijuana, can be cultivated from different varieties of the plant, *Cannabis sativa* such as C. *sativa* varieties sativa, C. *sativa* varieties indica and C. *sativa* varieties ruderalis. The two have very different properties, the main difference being that *C. Sativa* has a higher ratio of CBD to THC, while *C. Indica* has a higher ratio of THC to CBD (Pearce et al., 2014). Throughout many

centuries, cannabis has been used by cultures for spiritual and recreational purposes. As early as 2 A.D., Chinese literature, specifically the *Book of Odes*, notes that the use of cannabis can connect one to the spirits and bring enlightenment (Warf, 2014). In Hinduism, cannabis was used in many religious ceremonies, specifically to honor the god Shiva (Warf, 2014). Hemp, non-psychoactive cannabis, was even used to create the robe of the Hebrew King Solomon (Warf, 2014). While having many spiritual applications in history, cannabis has also been used in recent decades, becoming a popular vice of choice. In the United States alone, cannabis has been legalized for recreational use, to reach feelings of "high" or euphoria, in 8 states (Gourdet et al., 2017). Although used for spiritual and recreational purposes, cannabis also has many real medicinal applications. Such uses are to relieve symptoms of epilepsy, to stimulate appetite, and to relieve nausea associated with chemotherapy treatment (Hofmann et al., 2013). One of the most promising of these applications of cannabis has been as a pain reliever and anti-inflammatory drug.

Since 2900 B.C., the Chinese have used cannabis to treat pain. This analgesic property of cannabis has been confirmed as early as 2001 in a study on capsaicin-evoked pain in rats by Johanek et al. and in 2007, a study by Wallace et al. found dose-dependent analgesia of smoked cannabis in healthy human adults. These studies, among others, have prompted 29 states in the U.S. to begin legalizing the plant for its medicinal usage. Cannabis has been tested as a method to treat pain because of the manner in which it acts in the brain. In the body, peripheral nerves detect sensations of pain (Mack et al., 2001) and in these same areas, there are also cannabinoid receptors (Lynch, 2006).

Cannabinoids bind to inherent endocannabinoid receptors in the human nervous system (Noonan, 2015). They inhibit cellular processes by altering “neurotransmission through CB1 receptors by inhibition of P/Q-type Ca2+ channels and adenyl cyclase and by activation of K+ channels and mitogen-activated protein kinase” (Walker et al., 2002). CB2 receptors also promote inhibition, but they act through adenyl cyclase and mitogen activated protein kinase alone (Walker et al., 2002). The cellular inhibition brought on by cannabinoid binding to either CB1 receptors in the central nervous system or CB2 receptors in the immune system “modulate neural conduction of pain signals by mitigating sensitization and inflammation” (Hill et al., 2017). These combined effects inhibit the sensation of pain. Along with blocking pain, cannabinoids treat pain so robustly because they produce euphoric and anti-inflammatory properties (Greydanus, 2013). In the 38 published clinical trials involving cannabinoid therapies, over 70% of patients reported positive outcomes with usage for pain treatment (Kennedy, 2014). Δ^9-Tetrahydrocannabinol alone has also been shown to have these similar analgesic effects.

Δ^9-Tetrahydrocannabinol, also known as THC or Δ^9-THC, is the main psychoactive constituent of cannabis. It is the most potent constituent in the plant material (Vale, 2007). THC has these effects on the body by enhancing “dopaminergic neurotransmission in brain regions known to be implicated in psychosis” (Vale, 2007). However, recent studies have shown THC issued as an analgesic, appetite stimulant, and anxiety reliever (Repka et al., 2006), suggesting that THC interacts in multiple pathways.

Though THC has been isolated as separate component, the whole cannabis plant, including all constituents, has been shown to be an effective treatment for neuropathic pain. One drug, Sativex®, has successfully done just so. Sativex® is an oral mucosa

spray of whole-plant cannabis extract, which contains cannabinoids, THC, and cannabidiol, CBD (Nurmikko et al., 2007). A clinical study in 2007 by Nurmikko et al. found that Sativex® was an effective treatment for peripheral neuropathic pain. Considering then that the plant as a whole can be an effective treatment, researchers are now interested to see if it is one of these cannabinoids, besides THC or CBD, which also contribute to the overall efficacy of cannabis for analgesia.

There has been extensive research to show how cannabinoids are direct agonists, which elevate the endocannabinoid function through fatty-acid amide hydrolase inhibition to produce analgesia (Suplita et al., 2005). However, the known cannabinoids, such as THC and CBD, may not be the only contributors to the overall efficacy of cannabis. There are still unknown components of the plant as well as known components whose functions and interactions are unknown. Not only are these constituents not clearly documented, but there is also uncertainty whether these have any effect on the analgesic action of the plant. As of 2005, there were already 413 of these constituents discovered, which belong to different classes of compounds (ElSohly et al., 2005). In 2017, ElSohly et al. reported 565 compounds from Cannabis (ElSohly et al, 2017). As the plant has been researched more over the last decade, more of these constituents have been found, yet the activity of the majority of these are unknown. Another effect that these constituents can have on the overall activity of the cannabis plant is the chemical interactions that they have with each other (ElSohly et al., 2005). These factors must be considered for cannabis, as a whole, to be understood.

One class of these constituents whose properties are unknown, yet the topic of current discussion, are the terpenes. Terpenes are the major components of the volatile oil

of plants and occur widely in nature (Paduch et al., 2007). They are a class of hydrocarbons and oxygenated hydrocarbons found in the resin of plants that act as natural attractants or repellents (Paduch et al., 2007), which give off the characteristic aroma. Terpenes are biosynthesized from isoprene units at their most basic form. Most terpenes in volatile oils are monoterpenes (2 isoprene units) and sesquiterpenes (3 isoprene units) but rarely diterpenes (4 isoprene units). There are many terpenes known to be useful to modern medicine, such as d-limonene which has been shown to help promote apoptosis of cancerous cells in the body (Paduch et al., 2007). However, what remains unknown is how the terpenes of cannabis can play a role in its medicinal value. There has been speculation that terpenes are responsible for some of the analgesic properties of the plant, yet this has yet to be tested. Though, there have been *in vitro* studies that found that some of the components of the cannabis volatile oils have binding affinity to CB1 and CB2 receptors (Mehmedic et al., 2014), there have been no *in vivo* studies on their specific analgesic effects. In short, just because the volatile oils can bind the CB1 and CB2 receptors, this is not direct evidence of a correlation with the inhibition of pain when these receptors are activated.

This study aims to determine the contribution of terpenes in mediated cannabis extract by evaluating the plant extract with and without terpenes, isolated terpenes, and isolated THC across a battery of nociceptive assays, including two thermal pain assays and one inflammatory pain assay.

METHODS

Chemical Preparations

Four products were prepared from high potency *Cannabis* sativa (THC rich plant) for this study. These four drugs were the cannabis extract heated under reflux, volatile oil, extract without volatile oil, and the major cannabinoid, Δ^9 -THC. To make the total extract, the dried powdered plant material was extracted by maceration in hexanes for 17 hours. The hexanes solution was drained and evaporated under vacuum to give dried extract. In order to prepare the first group (total extract under reflux), a portion of the extract was heated with 500 mL of distilled water under reflux for 3 hours then dichloromethane (DCM), a heavy solvent was added to a separating funnel where the extract separated from the water. Anhydrous sodium sulfate was used to dry the DCM extract. The solvent was then filtered out, and the solution was evaporated under vacuum using a rotatory evaporator to get rid of DCM. After this process, the extract was further dried via a pressure vacuum for 15 minutes to make sure that all solvent was out of the product and we were left with extract under reflux (**group 1**). This reflux step was done to decarboxylate the extract on hand (in order to convert all the cannabinoid acids into natural ones) and to subject the extract to the same conditions to be used to remove the volatile oil (terpenes) from the extract for pharmacological activity comparison between extract with terpenes versus extract without terpenes.

The next drug group was the extract without volatile oil. The volatile oil isolated from the extract will be administered to group 3. To do this, the extract is hydrodistilled where the volatile oil, or terpenes, evaporate at a low boiling point and are carried by the

steam under distillation conditions. The steam (carrying the terpenes) is allowed to condense and the volatile oil distilled from the extract is collected using a volatile oil preparation apparatus. Fifteen grams of the total cannabis extract and 500 mL of water were hydrodistilled for three hours. Just like the extract under reflux, after the hydrodistillation, the extract without volatile oil was separated from water using methylene chloride via a separatory funnel. Anhydrous sodium sulfate was used to dry the extract, the sodium sulfate was then filtered out and washed again with methylene chloride. The final extract without volatile oil evaporated till dryness under reduced pressure. The DCM extract was further placed under vacuum to ensure complete evaporation of any solvent. The remaining product was the extract without the volatile oil (**group 2**).

The steam condensed product collected from the hydrodistillation process was a mixture of the volatile oil and water. In order to separate the water out from the volatile oil, the mixture was frozen. Since water has a higher freezing point than the volatile oil, the oil was drained off once the water froze. The drained product was the volatile oil (**group 3**). We tested the volatile oil via gas chromatography to make sure than there is no THC left in the solution after this process.

The final drug group (Δ^9 -THC) was provide by Coy Waller lab. (Dr. ElSohly). The preparation of Δ^9 -THC in brief:

The high potency extract (total extract) was subjected to thin film distillation using POPE® 2`` still under specified rotation, flow rate and temperature. Then the distillate was chemically derivatized and purified by chromatography to obtain pure Δ^9 – THC (the method of distillation and purification has been filed as a patent).

In Vivo Screening

i. *Apparatus*

For the hot plate assay, Harvard Apparatus, Model #52-8570 measured thermal nociception. Animals were confined to the heated plate with an open top acrylic enclosure. A foot switch was used to start and stop a timer, which measured the time (seconds) that the animal remained on the plate. The hotplate was set at 53.9 degrees Celsius.

For the tail flick assay, Columbus Instruments, Model #0104-300M measured thermal nociception. The animals were inserted into a small chamber to confine them and their tails were placed in the apparatus' groove, below which there was a heat lamp that was turned on by a button on the front of the apparatus. When the animal flicked its tail away from the light stimulus, the apparatus automatically turns off, and the latency is displayed on the apparatus' timer. The intensity of the light was adjusted to yield a baseline latency around 5 seconds in control animals.

For the acetic acid writhing assay, 4 chambers with open tops were placed on a solid counter to confine animals for the duration of the observation. The boxes measured 42 cm x 20 cm x 21 cm, allowing enough room in the chambers for animals to move freely. The sides of the chambers were solid so animals could not see each other during the observation period.

ii. *Procedure*

Male Sprague Dawley rats of 200 grams were selected for this study. The rats were housed two to a cage. They were fed a diet of pellet food and water. Their cages were cleaned twice a week. The rats were randomly assigned to drug

groups using a random number generator. There were on average 12 animals in each drug group. The rats were broken down into cohorts and tested subsequent weeks in the same pattern to eliminate potential confounding variables. A cohort of rats arrived on day one and were handled twice a day for five days. On the sixth day, rats were weighed and then injected intraperitoneally (IP), with the assigned drug group delivered in a volume of 1 mL/kg. The injectors nor testers knew which drug was being injected as a coded system was used to eliminate potential experimental bias. Thirty minutes later the rats were tested with a hotplate and then immediately with the tail flick apparatus.

The hotplate assay was a measure of analgesia. When a rat motioned to lick its paw, it was removed from the hot plate. If no response was monitored after 35 seconds, the rat was removed from the hotplate and a latency of 35 seconds was recorded as full analgesia. The more robust analgesia the given drug produced, the longer the rat remained on the hotplate, or a greater latency was measured.

The tail flick assay was another measure of analgesia. The rat was placed in a restraint chamber and its tail was laid flat on the device. When the machine was turned on, a light projected onto the rats' tail. When the rat made a tail flick, or distinct movement of the tail away from the stimulus, the test ended and the latency was measured. If no tail flick was produced after 20 seconds, the light was turned off and a latency of 20 seconds was recorded as full analgesia. Like the hot plate test, the more robust analgesia a drug produced, the longer the latency.

After the rats were tested on day 6, they were brought back to their housing. They were handled once a day for six more days. On the 14th day, the rats were weighed and injected IP again with the drug group they had been assigned, delivered in a volume of 1 mL/kg. Thirty minutes later, the rats were injected IP with a 0.4% acetic acid solution delivered in a volume of 10 mL/kg and placed in the observation chambers. The number of times the rats writhed, or stretched their bodies out forcibly, were tallied. This test measured how well the drug groups produced anti-inflammatory properties. Unlike the other tests, the more the drug produced effects, the less the animals writhed.

iii. *Treatment Groups*

The four drug groups (extract under reflux, extract without volatile oil, volatile oil, and THC) were each tested in three dosages. The extract under reflux was tested at 5.0 mg/kg, 10.0 mg/kg, and 15.0 mg/kg. The extract without volatile oil and the volatile oil were tested at the amount of each component that would be found in 5.0 mg/kg of total extract, 10 mg/kg of total extract, and 15.0 mg/kg of total extract. The THC was tested at 2.5 mg/kg, 5.0 mg/kg, and 7.5 mg/kg. These groups were also compared to the vehicle in which they were dissolved, which was 10 % cremaphor, 10% ethanol, and 80% water.

For the tail flick and hotplate tests, these experimental drugs were also compared to the "gold standard" for analgesia. This positive control was morphine, tested at 10 mg/kg and 18 mg/kg dosages. Since the morphine was dissolved in saline, we also tested saline as a vehicle in these two assays.

The day after the abdominal writhing tests were completed, the animals were euthanized. This was done a day after testing so that the acetic acid would not interfere with the Euthasol to ensure that the animals were handled as humanly as possible.

Statistical Analysis

Data were analyzed using SPSS software. For each assay, one-way ANOVAs were used to identify treatment effects followed by SNK procedures to identify group differences. Saline and vehicle treatment groups for both tail flick and hotplate data were condensed as there was no significant difference in latencies between the two vehicles. For the acetic acid writhing data, only the vehicle was used as a negative control. In this assay, it is not uncommon for 15-20 % of animals to be non-responders (see Collier, 1998 for further details). Indeed, 2 rats in the vehicle group did not respond to the acetic acid administration. These observations prompted us to remove 2 non-responders each treatment group that contained non-responders. This amounted to 9 out of the 13 conditions tested.

RESULTS

Hotplate Latency

Latencies from the hotplate assay are summarized in Figure 1. For the vehicle group, average hotplate latencies were around 10 seconds. As before, the 5.0, 10.0, and 15.0 mg/kg doses of volatile oil were not statistically different from the control. The hotplate assay revealed increased latency than control for all treatments besides the volatile oil groups. Consistent with these findings, an one-way ANOVA of hotplate data revealed a significant main effect for treatment groups, $F(14,161) = 10.54$, $p < 0.0001$. SNK procedures identified several homogenous subsets. It revealed increased latency parallel to increased doses of cannabinoid extracts, both with and without the volatile oil, and pure THC. The latency of these groups increased as the dosage of drug increased, and the responses became significantly more different from the vehicle.

Tail Flick Latency

Latencies from the tail flick assay are summarized in Figure 2. In the vehicle group, latencies averaged around 5 seconds. The 5 mg/kg and 15 mg/kg doses of the volatile oil group did not statistically differ from the vehicle group. Consistent with these findings, an one-way ANOVA revealed a significant main effect for treatment groups, $F(14,165) = 9.77$, $p < 0.0001$. SNK procedures identified several homogenous subsets. As with the hotplate assay, the test revealed increased tail flick latency than control for all drug groups other than the volatile oil groups. It also revealed increased tail flick

latencies with 15 mg/kg extract without volatile oil, 15 mg/kg full extract, and the 7.5 mg/kg THC. The latency of all treatments besides volatile oil increased as the dosage of drug increased.

Acidic Acid Writhing

Writhes from the acetic acid assay are summarized in Figure 3. For the vehicle group, average writhes were around 15.75 writhes per 30 minute observation. Every treatment group, besides volatile oil, showed decreased writhes from the vehicle group. Consistent with these findings, an one-way ANOVA of acetic acid writhing data revealed a significant main effect for treatment groups $F(15,102) = 6.133$, $p < 0.001$. SNK procedures revealed significant decreased average writhes from the vehicle group for each treatment group besides 2.5 mg/kg THC and 5.0, 10.0, and 15.0 mg/kg doses of volatile oil. Further, for the 10 mg/kg dosage of volatile oil, there was a significant increase in average writhes from the vehicle group.

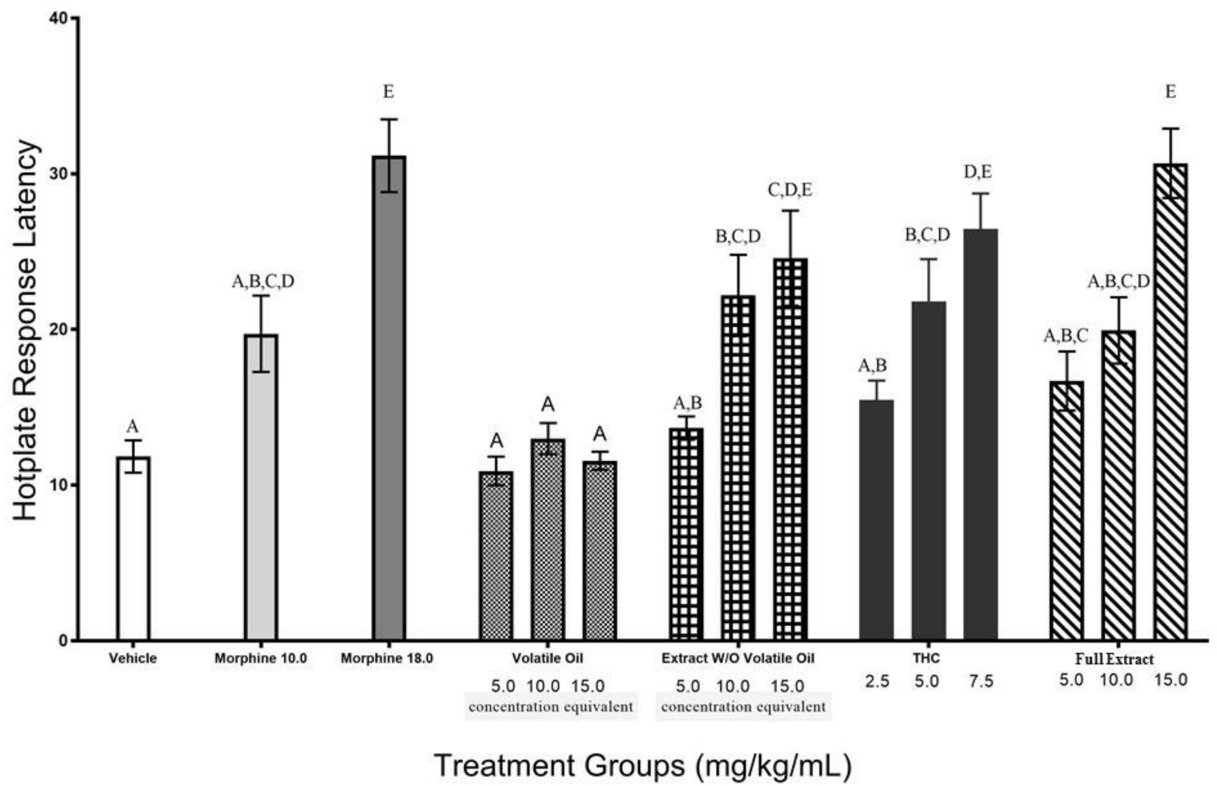

Figure 1: Hotplate latency: This is a representation of average hotplate latencies (in seconds) as a function of drug groups. The treatment groups are compared to the vehicle and the similarities between groups are denoted by the letters above the data bars. Concentration equivalent is the concentration of oils or extract without oils equivalent to that found in the full extract.

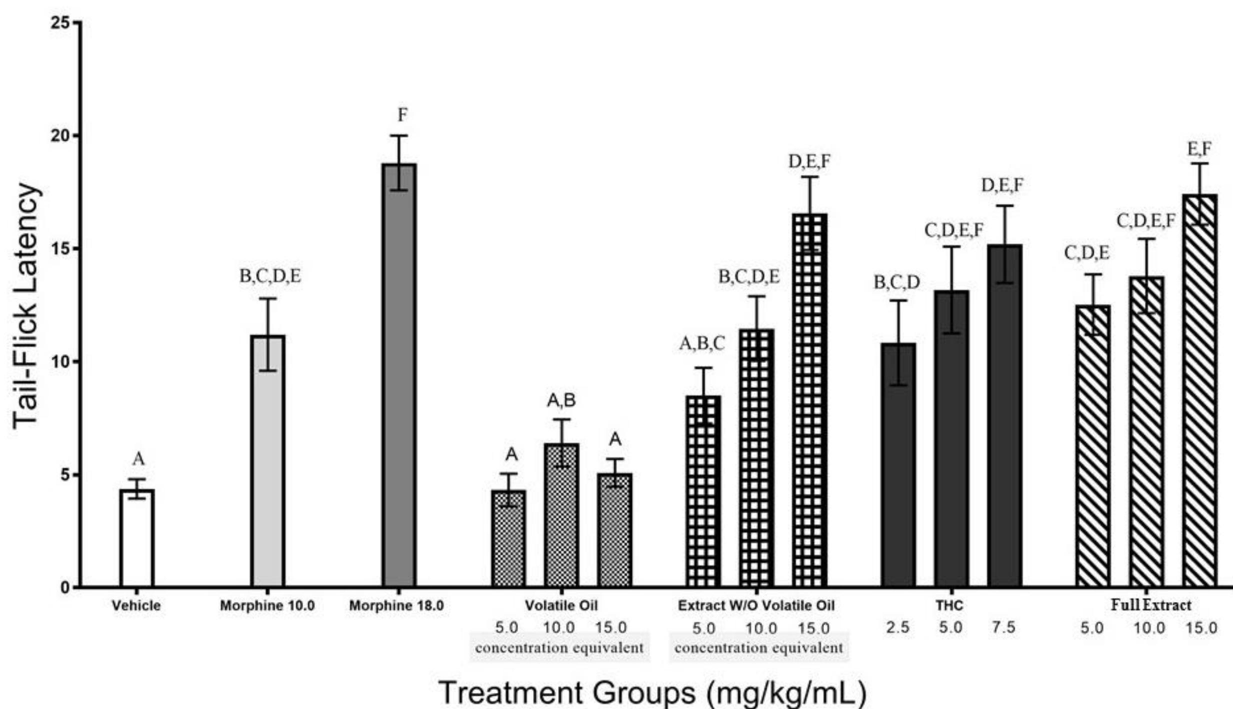

Figure 2: Tail Flick Latency: This is a representation of average tail flick latencies (in seconds) as a function of drug groups. The treatment groups are compared to the vehicle and the similarities between groups are denoted by the letters above the data bars. Concentration equivalent is the concentration of oils or extract without oils equivalent to that found in the full extract.

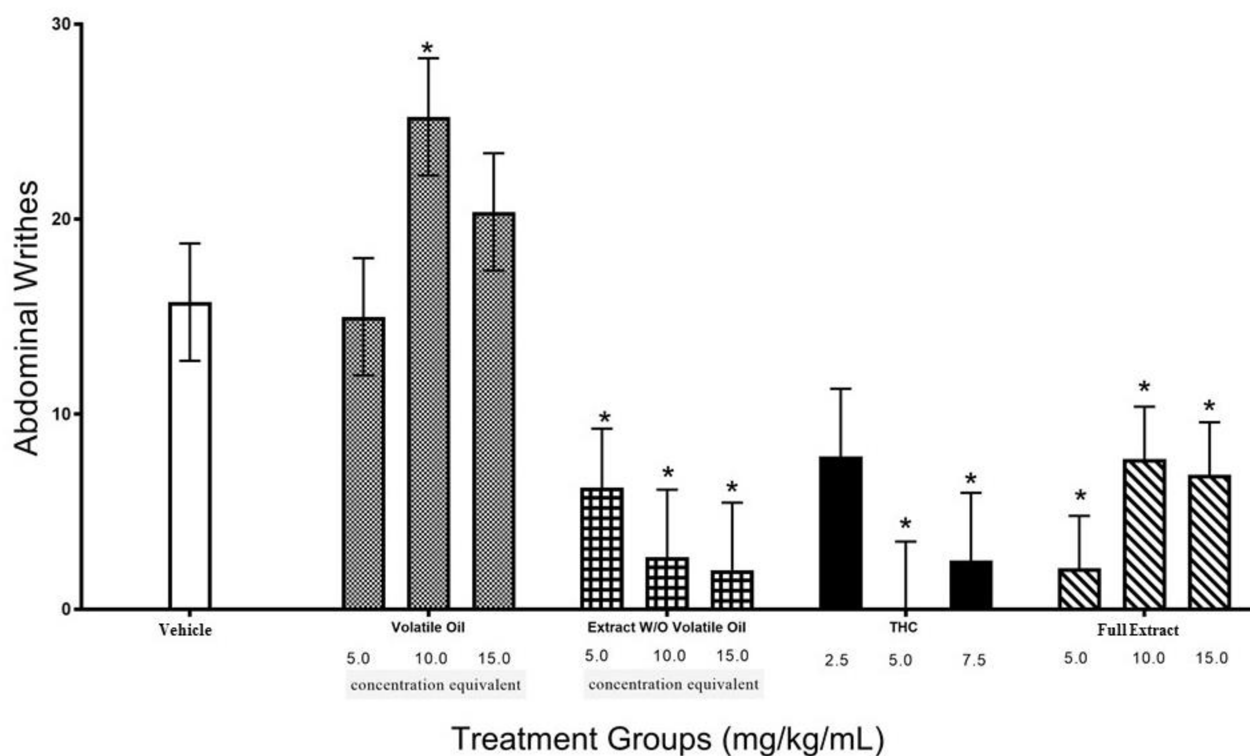

Figure 3. Acetic Acid Writhing: This is a representation of average abdominal writhes as a function of drug groups. A star above the data bar represents significant difference from the reference group, in this case cremaphor. Concentration equivalent is the concentration of oils or extract without oils equivalent to that found in the full extract.

DISCUSSION

This study sought to determine whether volatile oils (terpenes) and other cannabis constituents contribute to cannabis mediated analgesia. To answer this question, we prepared four cannabinoid products that included the full extract, isolated THC, extract without volatile oils, and pure volatile oils and tested these products at three different doses for each. We evaluated these products on three nociceptive assays: tail flick apparatus and hotplate apparatus, both thermal nociception screens, as well as acetic acid writhing, which measured inflammatory nociception.

On both tests of thermal nociception, the vehicle animals should produce the shortest latencies (time the animal remained on the hotplate or with their tail under the light), since there is no drug mediating the animal's response to pain. In contrast, animals from a treatment group with a robust analgesic effect will produce longer latency. In the tail flick test of thermal nociception, the isolated THC treatment groups showed the longest latencies suggesting these treatments produced robust analgesia. The extract without volatile oil and the full extract produced long latencies as well, suggesting these treatments also produced robust analgesia. Since the isolated THC produced equivalent analgesia to the full extract, it can be suggested that THC alone is responsible for the analgesic actions of cannabis or that other constituents including the terpenes have no significant effect on the analgesic activity of cannabis. Furthermore, these tests revealed a dose-dependent response in these three groups. The highest dosage in all three of these groups produced robust analgesia equivalent to the 18.0 mg/kg dosage of morphine, the gold standard for pain treatment. Meanwhile, the latency of the volatile oil treatment

groups did not differ statistically from the latency of the vehicle treatment group. This suggests that volatile oils do not contribute to the analgesic action of cannabis. Since the extract without volatile oil treatment group did not differ statistically from the full extract treatment, it is further suggested that volatile oils do not play a role in the analgesic action of cannabis. The hotplate test of thermal nociception also produced robust analgesia in isolated THC, full extract, and extract without volatile oil in a dose-dependent response. This assay also showed no effect in the volatile oil group.

On the test of inflammatory nociception, the control animals are expected to show increased writhes, as there is no drug-induced analgesia to mediate the pain response. Animals that produce less writhes show more robust analgesic action of the treatment group that they received. In this assay, a similar pattern of analgesia against chemo-inflammatory pain was observed. The isolated THC treatment group showed robust analgesia, with the 5.0 mg/kg treatment group being the only compound to average 0 writhes for the testing period. The extract without volatile oil and the full extract both produced significantly less writhes than the vehicle. On the other hand, the volatile oils showed equivalent average writhes to the vehicle group, suggesting that volatile oils have no effect on inflammatory nociception. The attenuation of writhing behavior suggests that the isolated THC, the extract without volatile oils, and the full extract produced robust analgesia against chemo-inflammatory pain, while the volatile oils (terpenes) did not.

The results of the two thermal nociception assays and the inflammatory nociception assay revealed that isolated THC produced robust analgesia equivalent to the whole extract. This observation suggests that THC alone is the responsible constituent for mediating analgesia and it does not necessitate any other constituent. Furthermore, since

analgesia was produced by the cannabis products in both thermal and inflammatory nociception assays, it can be concluded that these products induce analgesia in a spectrum of pain models and thus, have a broad range of clinical applications.

The use of the hotplate and tail flick test of nociception, specifically, are aimed at understanding the location where the treatments produce analgesic action. Tail flick test is a nociceptive assay that is mediated entirely by spinal reflexes. Analgesia produced on the tail flick apparatus yields evidence of spinal-mediated analgesia (Deuis et al.). However, the hotplate nociceptive assay engages more organized nociceptive behavior such as paw flutter, licking, and lifting driven by supraspinal mechanisms. If analgesia is produced in the hotplate assay, then the pain pathway is supraspinal (Deuis et al.). Analgesic actions that are produced in one assay, yet not the other help researchers determine where these drugs have analgesic effects. If there is analgesia on the tail flick assay, but not the hotplate assay, then pain is mediated on the spinal pathway. If analgesia is on the hotplate assay, but not the tail flick, then pain is mediated through the supraspinal pathway (South et al., 1998). In this study, we found that the isolated THC, extract without volatile oil, and full extract all showed analgesia on both assays. Since there were robust effects in both tail flick and hotplate assays, it is suggested that these compounds mediate analgesia through both spinal and supraspinal pathways.

This study is not the first to reveal that cannabinoid extracts produce analgesia. A study conducted in 1997 documented the role of cannabinoids in mediating analgesia (Bloom et al. 1999). In this study, albino mice placed equivalent latencies on tail flick test when injected with (±)-9-nor-9β-hydroxyhexahydrocannabinol (β-HHC) as they did when treated with morphine (Bloom et al., 1977). Furthermore, this study is not the first

to suggest that THC alone produces analgesia. Buxbaum et al. showed in 1972 that isolated THC was comparable in mediating analgesia equivalent to morphine in both rats and mice using both the hot plate and tail flick tests of thermal nociception (Buxbaum et al., 1972). More recently, a study by Varvel et al. in 2005 found that in mice, tests of tail flick latencies, as well as catalepsy and hypothermia revealed Δ^9-THC acting via CB_1 receptors was the sole constituent responsible for cannabis-mediated analgesia and that no other known cannabis constituent produced analgesia to this effect (Varvel et al., 2005). These findings, as well as the findings of this study, lead to the suggestion that THC should be further explored as a means of treating clinical pain.

Although it is not new evidence that THC alone can mediate analgesia, this study also found that cannabinoid constituents do not add to the analgesic actions of cannabis beyond the analgesic actions of isolated THC. While there is evidence that cannabidiol can have analgesic actions of its own (Notcutt et al., 2004), the extracts used in this study are from a high THC variety that has only a small amount of CBD. A study to ascertain the contribution of CBD to the analgesic activity of cannabis needs to be carried out using extracts of the mixed cannabis variety which contains significant amounts of both cannabinoids. Also, this study is the first to find that the volatile oils of cannabis produced no analgesic effect in any of the three assays. This suggested that volatile oils do not cause alterations in cannabis medicated analgesia. The necessity of this information is that treatments that complex botanicals with a multitude of chemicals that are not well understand could have many potential interactions with organ systems that may lead to an array of side effects. The work herein displays how cannabis extract can produce robust analgesia without the volatile oils. Indeed, isolated THC by itself may

prove to be all that is required for producing significant analgesia, thus removing the potential side effects associated with other constituents in the plant material.

Although THC alone produces robust analgesic action, this study does not necessarily suggest that other constituents should be completely disregarded. In 2004, Notcutt et al. found that in a double-blind study of patients with chronic pain from multiple sclerosis, THC alleviated chronic pain, but a mixture of THC and CBD also alleviated this pain to the same extent. Further, this study found that patients with chronic pain placed preference for the THC and CBD mixture (Notcutt et al., 2004). In 1982, a double-blind study was conducted on healthy subjects who were given either THC, THC and CBD, diazepam, or placebo. The subjects reported less anxiety, as well as less side effects, with the THC and CBD treatment than with THC alone (Zuardi et al., 1982). The study "verified that CBD blocks the anxiety provoked by Δ^9-THC, however this effect also extended to marijuana- like effects and to other subjective alterations induced by Δ^9-THC" (Zuardi et al., 1982). This finding indicates a possible importance of CBD, and potentially other constituents, on mediating potential side effects that may occur from using THC alone for treatment of pain. Even if CBD does not add to the overall analgesic action, it could mitigate the potential for abuse or use of these products for reward, or recreational, purposes. Further research should be conducted to answer this question.

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