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April 12, 2018

Dr. Rainer Glaser, Professor in Chemistry
Editor, Journal of the Greatest Stories Ever Told
Department of Chemistry, University of Missouri-Columbia
Columbia, MO 65211

Re: REVISED
Delta-9-Tetrahydrocannabinol, a New Method for the Treatment of Neuropathic Pain
By: Jin Bai, Stephen J. Bogert, and Wesley Waterman

Dear Dr. Rainer Glaser:

Thank you for your communication on April 19th with the peer reviews of our original manuscript. We were pleased to see their suggestions and have taken them all into consideration during the revising of our manuscript. Based off the recommendations given to us by our peer reviewers, we have changed our manuscript accordingly. The changes are described as follows:

Major Revision

[M.1] Materials and Methods section was shortened, and the extra information was moved into the supporting information section

[M.2] All scheme numbers cited in the text have been revised to correlate with their correct schemes. Scheme 6 was omitted from the document.

[M.3] Abstract has been moved onto its own page.

[M.4] All schemes and figures in the supporting information have been relabeled as such.

[M.5] Schemes 4, 5, and 6 were referenced appropriately in the text.

[M.6] CBD is a compound that is only in its beginnings from a research perspective. Therefore, we decided it would be better for us to compare THC to a well-known NSAID, Ibuprofen, and well-known opioid, morphine.

Response to Reviewer 1

[1.1] After re-reading the manuscript, I did see the repetitions. The only reference to tetrahydrocannabinol now is in the abstract of the paper. We also elaborated on the significance of the acronyms NSAIDs and RCT.

[1.2] The abstract is now on its own page after the title page.

[1.3] After significant additional research, I was able to find more information to include into the introduction.

Response to Reviewer 2

[2.1] The abstract has now been moved to a separate page.

[2.2] Citations were and are present on page seven and the second paragraph. Only one source was used, therefore, the citation is placed at the end of the paragraph.

[2.3] The Schemes (4,5,6) are now mentioned in-text and have been modified to match the scheme intended. Scheme 6 was removed from the paper, as we believed it did not provide any purpose to the paper.

[2.4] The Supporting Information now has titles for all the schemes, figures, and tables.

[2.5] Although kappa and delta receptors were never mentioned in the paper, mu was changed to the Greek letter, μ.

[2.6] All sentence fragments and grammatical errors have been fixed.

Response to Reviewer 3

[3.1] Benefits of THC replacing opioids have been added into the conclusion to better illustrate the benefits of THC replacing opioids.

[3.2] The recommendation of the omission that THC and Mu opioid interaction and mechanism is not fully understood has been acknowledged. The information is removed from the intro; however, information regarding the recommendation of further research to be done has been added to the conclusion to generate attention towards the research of this mechanism.

[3.3] Grammatical errors in the conclusions has been fixed.

[3.4] After reviewing the NMR spectra, it was decided that no extra information was needed. The spectra serve as a standard for identification.

[3.5] Description of the Bradford Bioassay has been changed for better readability. Extra information about the bioassay has been moved to the supporting information section.

[3.6] Peer Reviewer's concern regarding acetaminophen is noted and agreed. The comparison drug will be changed to Ibuprofen, another broad-spectrum NSAID.

Sincerely,

Jin Bai
Stephen J. Bogert
Wesley Waterman

Delta-9-Tetrahydrocannabinol, a New Method for the Treatment of Neuropathic Pain

Jin Bai, Stephen Bogert, and Wesley Waterman

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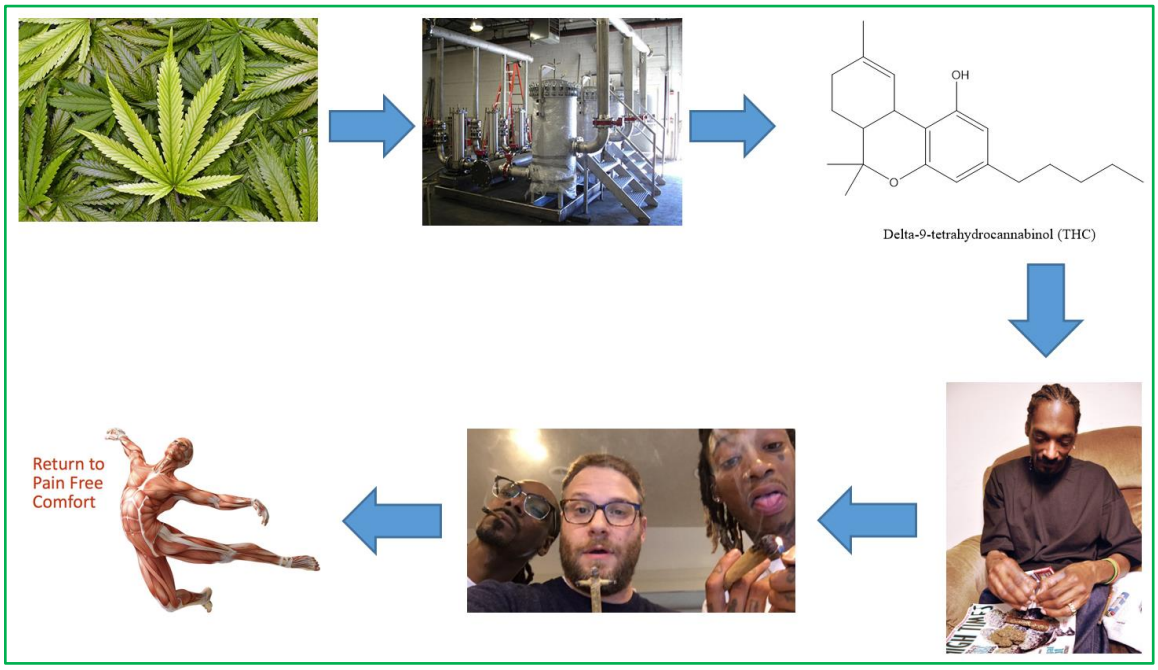


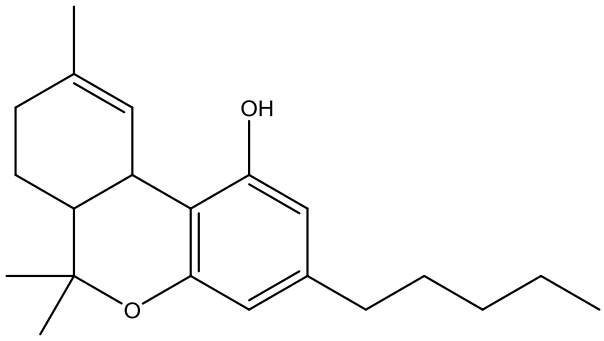
Abstract

Tetrahydrocannabinol (THC) has been used recreationally for decades but the possibility of using it for the treatment of serious and quality-of-life changing pain management has yet to be explored fully. The impact of neuropathy on the quality of life for patients suffering from HIV or cancer has yet to be alleviated. Broad-spectrum opioids and Nonsteroidal Anti-inflammatory Drug (NSAIDs) have yet to prove their efficacy in the management of neuropathic pain. THC's pharmacology and properties, when measured using a Bradford bioassay, are assessed to be on par with current day opioids and NSAIDs with similar IC_{50} values and binding affinity. When THC is implemented in double-blind randomized controlled trial (RCT), they have shown to be an effective analgesic for the decrease of neuropathic pain. Patients that supplement THC to their opioid and non-opioid painkiller in their management of neuropathy have shown an increase in pain management, decrease in opioid and non-opioid analgesic use, and prefer the side effects of THC. The functionality of THC comes from its pharmacophore and its binding to both the CB1 and CB2 receptors. THC functions effectively as an analgesic for neuropathy and is poised to replace the use of opioids and non-opioid painkillers.[1]

Introduction

Pain is encountered in many different ways and severities, such as being sore after exercise or after a surgical procedure. Depending on the severity or acuteness of the pain, it can be treated with different analgesics. The two principal classes of analgesics are opiates and non-opiates. Non-opioids are anti-inflammatories that are typically used for mild to moderate pains, while opioids are used to treat moderate to severe pain which act directly on the central nervous system. Opioid pain killers block the transmission of pain and decrease the brain's recognition of pain by acting on opioid receptors while non-opioids, such as Tylenol and Advil, work precisely on injured muscles and tissues. Opioids, however, are dangerous because they provide euphoric sensations to the user. Because of this, it has steered toward people using them recreationally, which has led to increased abuse and opioid-related deaths. Since 1999, the number of opioid related deaths has increased five-fold, which many people consider to be a major contributor to the decreased life expectancy in the United States.[2]

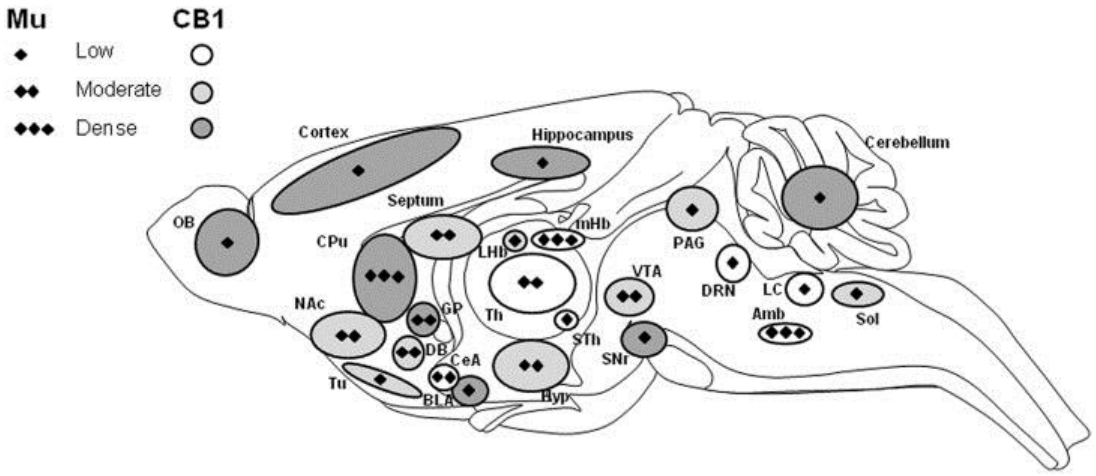
Scheme 1: Tetrahydrocannabinol (THC)



Delta-9-tetrahydrocannabinol (THC)

One drug of significant importance today, is THC as shown in Scheme 1, also known as THC. THC is derived from *Cannabis sativa*, which contains about 110 cannabinoids that all produce psychotropic effects when ingested. THC, however, is the primary psychoactive constituent of *c. sativa*. Because of this, THC is commonly regarded as an analgesic, which is why medicinal use is allowed in many states, while recreational practices are more widely rejected in comparison. THC's main sites of action are located in the brain and spinal column. It binds to two types of G-protein-coupled receptors, known as cannabinoid type 1 receptor (CB1) and cannabinoid type 2 receptor (CB2). The increased activation of the cannabinoid receptors causes inhibition of neurotransmitters, acetylcholine and glutamate. The cannabinoid receptors are largely found in the presynaptic position rather than postsynaptic neuron.[3] However, there is a strong correlation in the density and location of cannabinoid receptors and μ receptors as depicted in Scheme 2.[4]

Scheme 2: Density of CB1 Receptor vs. μ Receptor[4]



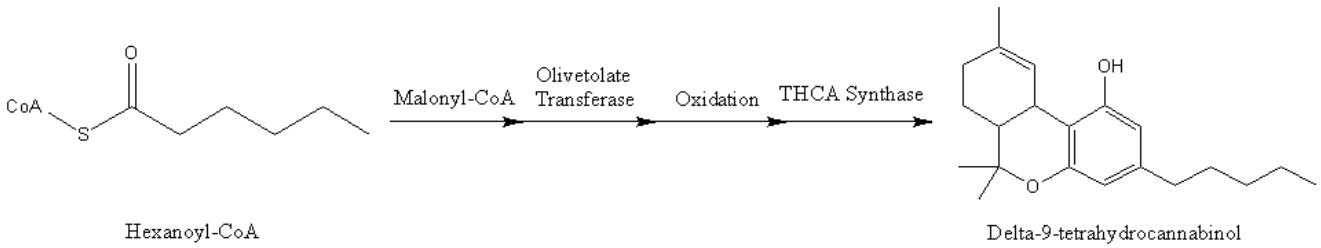
Here we report the results of THC. It is an effective analgesic and can serve as an acceptable substitute to opioids. Marijuana is a much safer analgesic compared to opioids because it would take about 1260mg/kg of pure THC to overdose. Additionally, THC does not act on any control centers of the brain that are involved with the respiratory system, so decreased respirations does not occur as it does when taking opiates. THC is very efficient in decreasing neuropathic pain sensation from treatment of cancer and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).

Materials and Methods

Preparation of Materials

THC is bio-synthesized in *c. sativa*, using the starting substrate, Hexanoyl-CoA as shown in Scheme 3. Hexanoyl-CoA is then reacted with 3 parts Malonyl-CoA to produce Olivetolic acid, a benzylic ring system. The resulting acid is then enzymatically catalyzed in a bisubstrate reaction, by a transferase, with Geranyl phosphate to yield Cannabigerolic acid. Through oxidation, followed by a self-nucleophilic ring closing reaction, the intermediate acid is then catalyzed by THCA synthase to produce the product, THC.[5]

Scheme 3: Biosynthesis of THC[5]



THC is extracted from *c. sativa* by gathering the plant material and removing the buds and leaves from the stem. The material is then air-dried at room temperature (~23°C) to reduce moisture content until a 70% loss of mass occurs. The dried material is then heated at a high temperature (105°C) in an oven to remove the remaining water before being crushed into a fine powder. The powder is then extracted with a solvent, either petroleum ether or chloroform. Both solvents yield nearly 100% of the THC stored in the plant. The solvent-THC mixture is then boiled off leaving just the THC.[5]

Cells derived from human embryonic cells (HEK293) were transfected via electroporation with recombinant human cDNA that contains CB1 and CB2 receptors. The transfected cells are maintained at 37°C and 5% CO_2 in a medium. The cells were given nutrients and supplements. A single cell was placed in a fresh plate with the appropriate medium and allowed to proliferate. The grown-in cells were collected in a HCl buffer and centrifuged. The resulting protein concentrate is then used in a Bradford assay. Each assay plate was washed seven times with ice-cold wash buffer. The filtered material is allowed to dry, then radioactive counts were measured using a scintillation cocktail. The results were calculated to obtain binding affinity and IC_{50} values.[6]

To showcase the efficacy and fast onset of THC, a double-blinded medical trial was performed for patients who suffered from chronic neuropathic pain. Adult patients suffering from painful HIV-associated neuropathy were chosen to participate in the randomized control trial (RTC) for 22 days. The study is divided into four phases: 7 day out-patient intervention phase, 2-day in-patient lead-in phase, 5-day inpatient intervention phase, and a 7-day outpatient post-intervention phase. The patient is required to rate their pain for recording every day. The pain levels for each patient was measured on a pain scale ranging from 1 to 100. The median pain level for the cannabis group was 52 while the median pain level for the placebo group was 57 at the beginning of the trial. The patients that were admitted into the trial were all in stable health and without substance abuse. Out of 56 total participants chosen, 28 individuals were randomly placed in the placebo group and 28 individuals were randomly placed in the cannabis group. Three individuals dropped out of the study from the cannabis group resulting in a 25:28 cannabis/placebo ratio. The patients were assigned to smoke a THC cigarette (20mg) or a placebo tobacco-THC (2mg) mixed cigarette.[7]

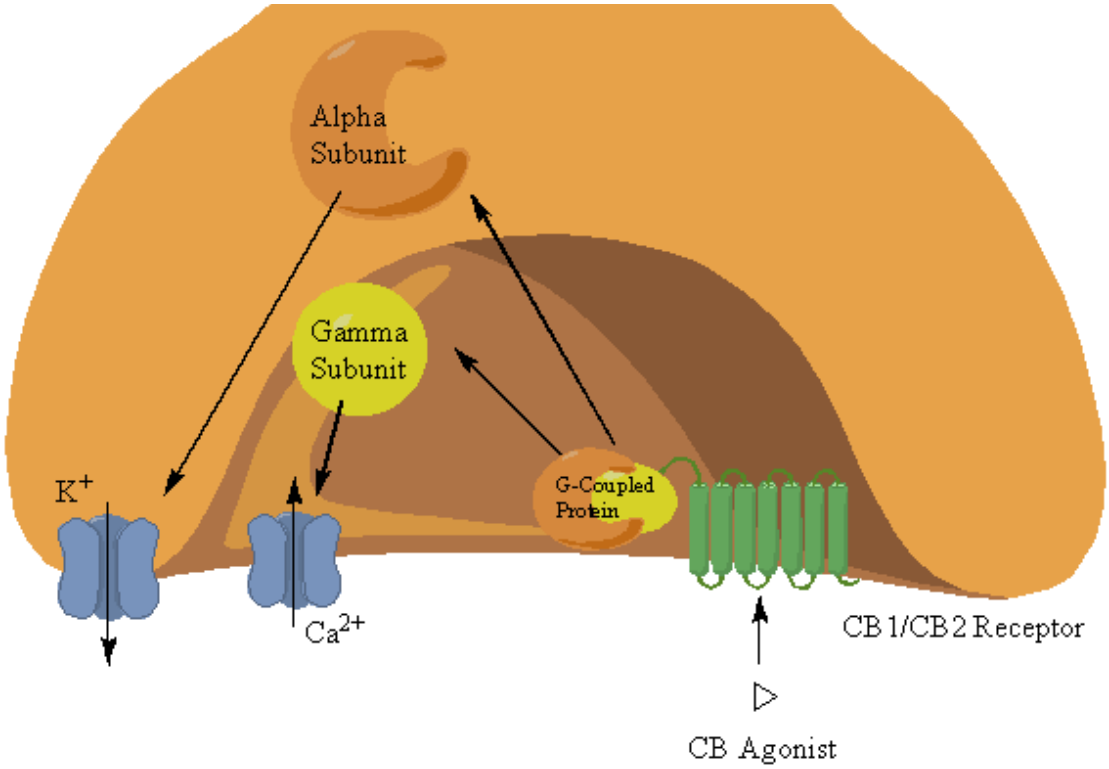
The first 9 days of the trial consist of taking a background measurement of each patient's pain intensity. After the initial threshold had been established, each patient was then admitted into the hospital as an outpatient. Each patient was allowed to smoke twice each day during the five-day outpatient procedure: 10 a.m. and 4 p.m. At the end of the five-day outpatient procedure, each patient rated their pain on the previous pain scale again and continues to do so for the next seven days.[7]

In a third study that comprises of questionnaires sent out to medical cannabis patients in the state of California using the HelloMD patient database. The questionnaire is answered by patients online. Only patients with existing neuropathic pain that has been given supplemental THC for the management of pain are included in the study. The study involves two groups: a group that suffers from neuropathy and is currently using opioids for analgesia and a group that suffers from neuropathy that is using non-opioid painkiller for analgesia. The opioid using group comprised of 881 individual using differing degree of cannabis for the supplemental management of pain. The non-opioid group consist of 1715 individuals using cannabis as a supplemental management of pain. The questionnaire comprised of questions asking the patient regarding their preference for analgesic, their feelings about the side effects of THC, whether a decrease in opioid and non-opioid painkiller use was seen, etc.[8]

Results and Discussion

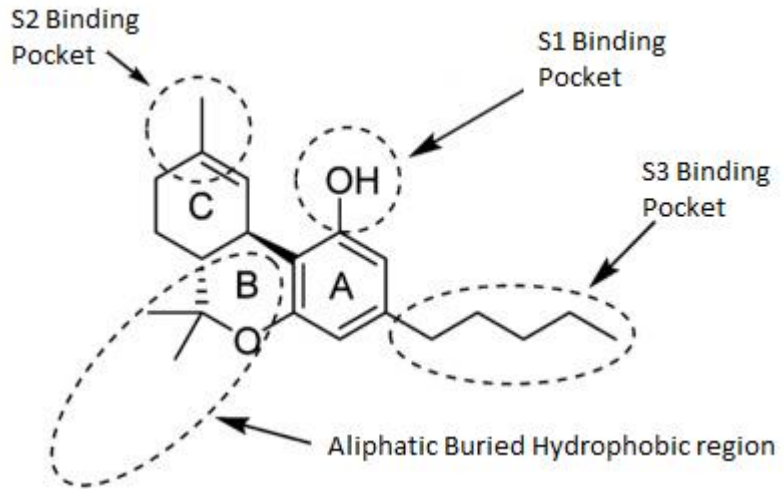
THC is able to mimic the functions of endogenous ligands that binds to cannabinoid receptors. THC is an agonist to the CB1 receptor, which is part of the central nervous system (CNS) and is associated with pain.[1] The CB1 receptor is located in the pre-synaptic clefts and acts as a retrograde neurotransmitter. The activation of the CB1 receptor sends a signal backwards and inhibits the signaling of the pre-synaptic neuron, which prevents pain signals from traveling up the nerve pathway.[9]

Scheme 4: Neuronal CB Signaling



Scheme 4 shows the pathway of cannabinoid signaling. The CB1/CB2 receptors are activated with the interaction of an agonist such as THC resulting in the dissociation of the G-coupled protein into its subunit: Gα and Gγ. The Gα subunit interacts with the potassium channel deactivation in nerve cells which prevents the depolarization of nerve cells. The Gγ subunit binds directly to the calcium channel and inhibit channel function. The result is a nerve cell that is unable to depolarize and unable to repolarize; therefore, the cell is unable to maintain tonic or phasic function, stopping the transfer of electrical signal from the limbs and spine to the brain.

Scheme 5: THC Binding Interactions



The molecular structure of THC has a lot of buried hydrophobic groups that are capable of interactions with the receptor active sites. A picture of these binding pockets is displayed above in Scheme 5. The S1 binding pocket is able to participate in hydrogen bonding with the amino acid residues of the active site and provides 2-5 kcal/mol of binding energy. The S2 and S3 binding pockets both feature hydrophobic interaction with the pentane group and the methyl group. The aliphatic properties of ring B also participates in binding to the active site of CB1 receptor. Hydrophobic interactions are weaker than hydrogen bonding interactions at only 0.5 kcal/mol, but consists significantly due to the number of buried hydrophobic surfaces capable of interaction.

Table 1: THC Pharmacology versus NSAID and Opioid[1,9,10]

Properties	THC	Ibuprofen	Morphine
logP	6.97	3.5	1.10
Bio-Availability	4-12% (Oral) 2-56% (Inhalation) 50-75%(Sublingual)	63-89% (Oral)	30-40% (Oral)
IC_{50}	5.69 mM	2.6 μM (COX-1) 1.5 μM (COX-2)	6 nm
Binding Affinity	18 nM (CB1) 42 nM (CB2)	13 μM (COX-1) 370 μM (COX-2)	29 nM (μ)

In Table 1, the pharmacology of three different compounds with analgesic effects is compared. THC is shown to have a binding affinity for the CB1/CB2 receptor that rivals morphine. THC has a very high logP value indicative of its ability to penetrate into cells because of its buried non-polar surfaces.[9] This should theoretically increase THC bioavailability; however, ingestion yields a much lower bioavailability because THC availability suffers due to the gut environment, digesting processes, and rate of metabolism.[10] The human gut is also very polar because it contains large amounts of water which means THC, a very hydrophobic molecule, will dissolve poorly into it.

While bio-availability is poor for ingested THC, its effects last longer than the other methods of consumption due to the slower breakdown and release associated with digestion.[1] The bioavailability of THC when using a sublingual method or when inhaled is much higher than the ingestion method due to the very thin mucus membranes lining your lungs and mouth. Compared to Ibuprofen and Morphine with a logP value that coincides with Lipinski's rule, they are somewhat non-polar enough to penetrate through the cell's lipid bilayer but too hydrophobic that they fail to effectively dissolve into the blood stream.[10]

Table 1 also addresses the efficacy of THC against a broad spectrum NSAID and a broad-spectrum opioid. Both the Ibuprofen and morphine have a much lower IC_{50} value compared to THC which means they have higher efficacy because it takes a smaller amount to illicit a 50% activity.

THC pharmacology is compared to a larger cast of opioids. In Table 2, the oral bio-availability of THC is much lower than opioids because of its inefficiency to be ingested. THC has a faster activation onset time than other opioids and a higher half-life, so it won't be metabolized quickly; however, THC does have a shorter typical duration than other opioids resulting in more frequent dosage of THC. The oral availability of THC is much lower than opioid; ingestion of THC is not an effective way to incorporate THC as a way to manage neuropathy.[11]

Table 2: THC vs. Common Opioids[11]

Compound	Oral Bio-availability	Onset Effect	Avg. Half-life	Typical Duration
THC	**4-12%**	**5-30 min**	**24 hr**	**2-3 hr**
Codeine	70-90%	45-60 min	Prodrug	4-6 hr
Pethidine	40-60%	20-40 min	4 hr	2-4 hr
Morphine	30-40%	30-45 min	3 hr	3-4 hr
Oxycodone	60-80%	45-60 min	3.5 hr	4-6 hr
Hydrocodone	60-80%	45-60 min	3.5 hr	4-6 hr
Hydromorphone	24%	30 min	2.6 hr	2-3 hr
Oxymorphone	10%	20-40 min	1.3 hr	3-4 hr
Levorphanol	50%	20-40 min	11-16 hr	4-8 hr
Methadone	80%	60-90 min	22 hr	6-12 hr
Fentanyl	10-15%	10-20 min	3.5 hr	1-2 hr

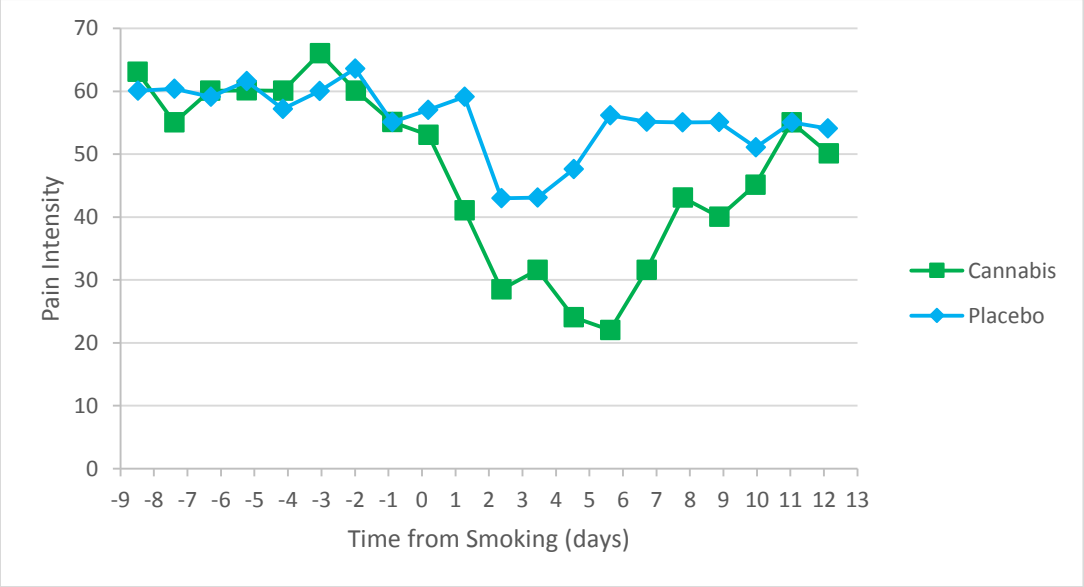


Figure 1: Primary outcome of THC vs. Placebo in chronic pain management.[7]

From the medical RCT, the raw data and pain rating for both cannabis and placebo groups were plotted as a graph to better show relationship in Figure 1. An average of 34% decrease in pain for the THC group is recorded compared to an average of 17% pain decrease in the placebo group. Both sets of patients experienced a decrease in chronic pain levels after smoking their given cigarette, but the high dose THC cigarette yielded a statically significant decrease in neuropathic pain (100% greater than the placebo group). Figure 1 shows the data from the clinical trial comprised into a graph. The pain levels of both groups are very similar and constant all nine days prior to the start of the study. From Day 0, there is a clear divergence in pain level between the THC group and the placebo group. From Day 0 to Day 5, a constant drop in pain is elicited. After the trial has ended after Day 5, it can be seen that both group's pain intensities began to rise and converge to their pre-trial levels.[7]

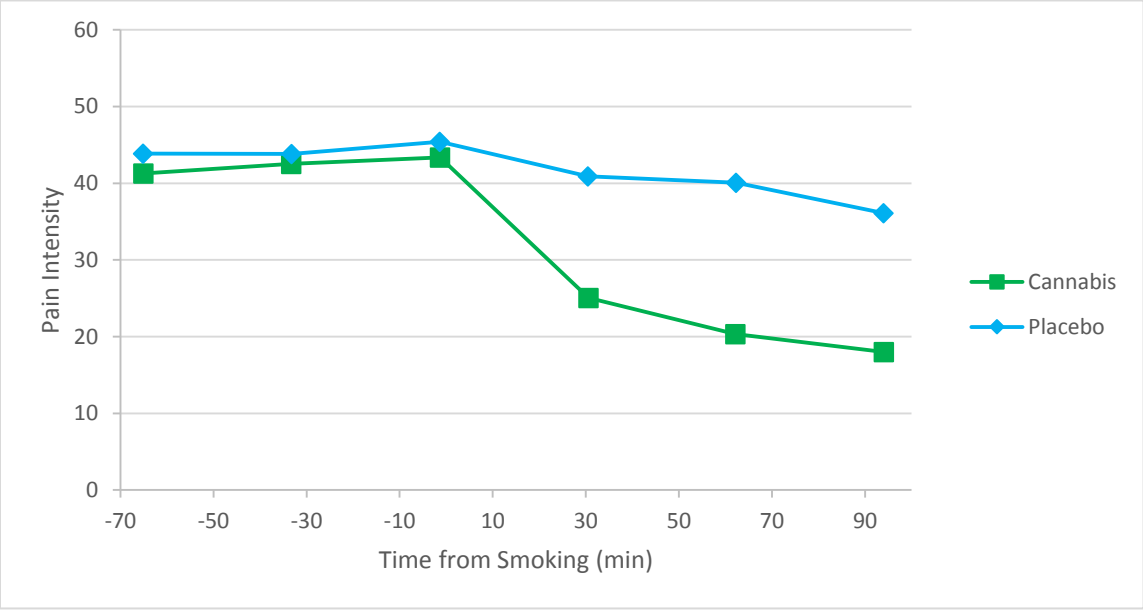


Figure 2: Onset speed of THC in chronic pain management.[7]

In Figure 2, the same RCT data was used to plot the onset activation of THC. THC elicited analgesia very quickly. Thirty minutes after smoking the THC cigarette, the patients experienced a dramatic decrease in chronic pain. It can be concluded that THC is a fast agonist.[7]

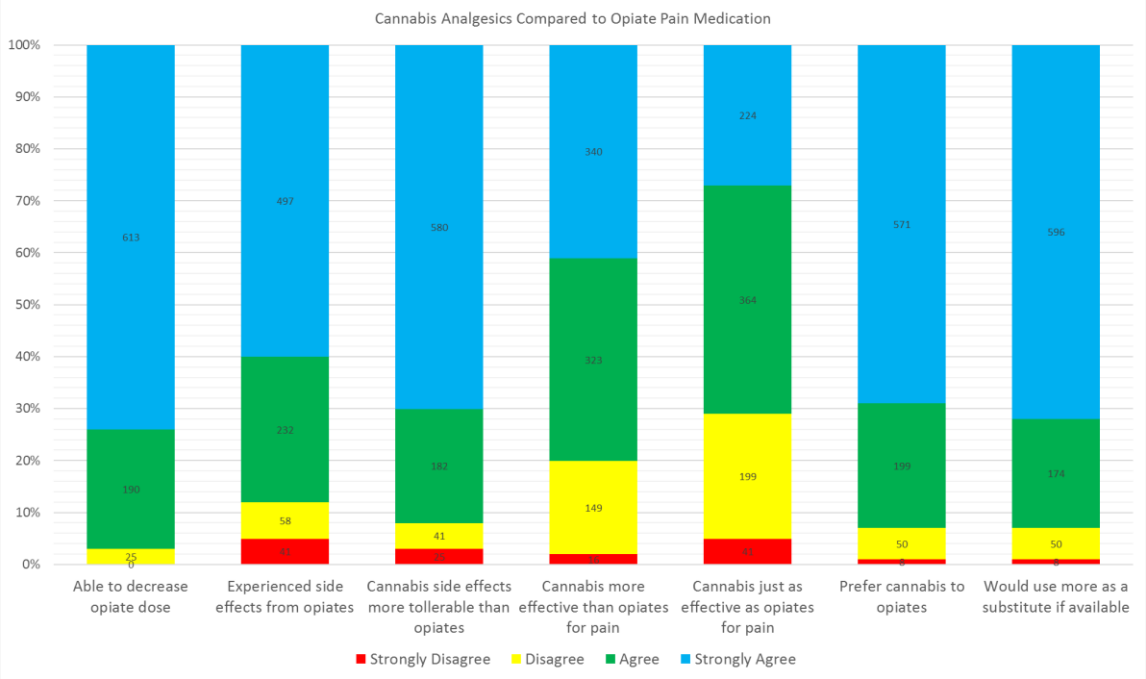


Figure 3: THC vs. Opioid painkiller questionnaire for neuropathy patients.[8]

Figure 3 is the aggregation of all the questionnaire data formulated into a chart. An overwhelming majority of participants (97%) agreed that using THC has helped reduce their opioid usage in the management of pain and the patient's experience on THC has been a positive one. A majority of patients also agree that cannabis is just as effective as or more effective than opioids for analgesia. An overwhelming majority prefers cannabis to opioids and would use more cannabis in their regulation of pain if available.[8]

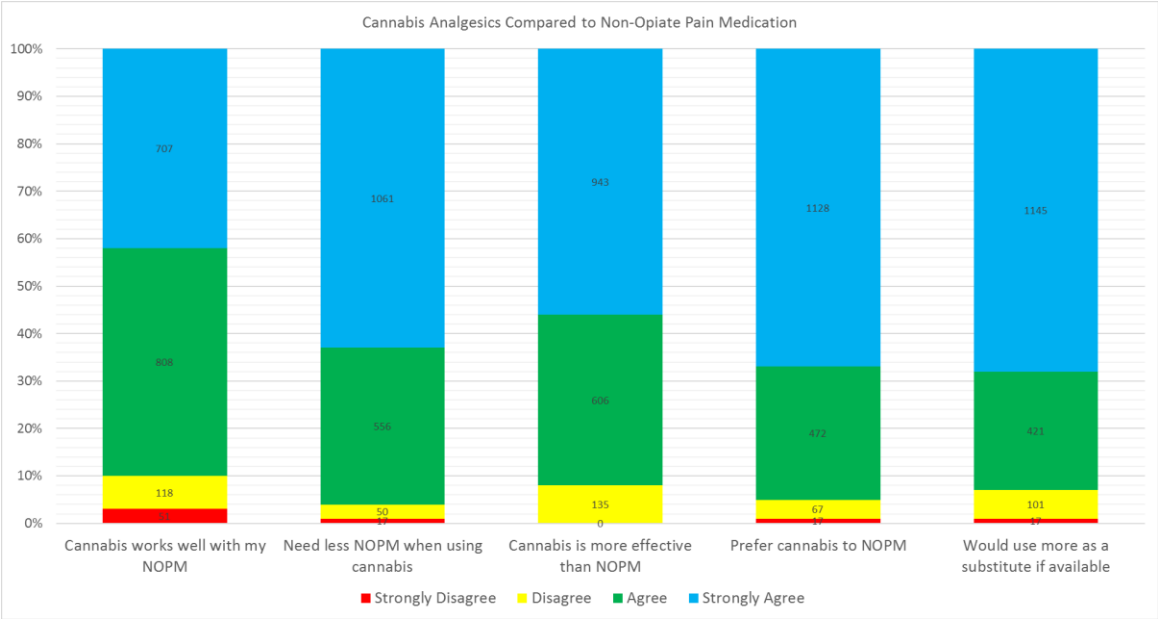


Figure 4: THC vs. Non-opioid painkiller questionnaire for neuropathic patients.[8]

In Figure 4 illustrates the second group of polled patients who uses non-opioid painkiller in their management of pain. The results from the questionnaire shows that a majority of patients agree that cannabis works well with their existing non-opioid painkiller and that their dosage decreases when using cannabis. An overwhelming majority prefers cannabis to opioids and would use more cannabis in their regulation of pain if available.[8]

Conclusion

The completion of the bioassay involving THC has showcased its pharmacology and properties that allows it to be as effective as opioids and NSAIDs. When compared to broad-spectrum opioids and NSAIDs, THC has comparable properties. When showcased in FDA approved clinical trial involving patients suffering from neuropathy, THC proves

to be statistically significant in their analgesia and depicts a faster onset time compared to other opioids. THC is not only effective as the sole analgesic in the management of neuropathy but is also effective as a supplement to opioids and non-opioid painkiller. Patients that uses THC as a supplement to their original pain medication has shown an improvement in pain management, decrease in opioid and non-opioid painkiller dosage, and prefers THC to their current regiment of analgesic.

The analgesic effects of THC can be extended to include the treatment of patients suffering from HIV. Advances in the treatment of HIV through antiretroviral medication has increased the life expectancy of HIV patients, but neuropathic pain still remains as a persistent problem. In RCT, smoked cannabis has shown to significantly decrease neuropathic pain in HIV associated neuropathy. This significance pain decrease can be considered a clinically meaningful pain relief. Previous treatment for HIV neuropathy includes the use of anti-seizure medication, antidepressants, and opioids. THC functions as a better analgesic for neuropathy than the previously mentioned drugs because of its low interaction rate with other drugs and its low dependency rate, and minimal side effects. In 2016, there has been more than 64,000 deaths due to opioid overdose and the death toll for opioids has been doubled in the past decade.[12] There have been no reports of death caused by THC overdose and THC has not shown the abuse and addiction rates of opioids. Roughly 21-29% of patients misuse prescription opioids and 8-12% of all opioids users develop an opioid use disorder.[13] THC can help curb the spread and the misuse of opioids in addition to its many uses including the application to manage cancer induced neuropathy. THC has also shown an anti-proliferative effect on tumor cell

growth. THC can also modulate metabolism, inflammatory response, and mood. The multitude of application that THC has to offer for the medicinal field has yet to be fully realized. Further research in determining the mechanism between THC and μ-receptor should be conducted.

Supplementary Material Available

The appendix contains a more detailed description of the processes and preparations of the substrates and intermediates, as well as their characterization spectra

References

(1) Husni, A.; McCurdy, C.; Radwan, M.; Ahmed, Safwat.; Slade, D.; Ross, S.; ElSohly, M.; Cutler, S. Evaluation of Phytocannabinoids from High Potency *Cannabis sativa* using *In Vitro* Bioassays to Determine Structure-Activity Relationships for Cannabinoid Receptor 1 and Cannabinoid Receptor 2. *NCBI,* **2015**. *23*(9), 4295-4300.

(2) Waterman, W; Frappier, A. Opioid Epidemic: Uses, Abuses and Innovation. **2018**. 1-2

(3) Oberbarnscheidt, T; Miller, N.S. Pharmacology of Marijuana: Marijuana: Clinical, Research, Policy. *J Addict Res Ther*. **2016.**

(4) Befort, K. Interactions of the opioid and cannabinoid systems in reward: Insights from knockout studies. *Front Pharmacol*. **2015**, *6*, 1-10

(5) Fairbairn, J.; Liebmann, J. The extraction and estimation of the cannabinoids in *Cannabis Sativa* L. and its products. *J Pharm*. **1973**, *25*, 150-155.

(6) Bow, E.; Rimoldi, J. The Structure-Function Relationship of Classical Cannabinoids: CB1/CB2 Modulation. *NCBI.* **2016**. *8*, 17-39.

(7) Abrams, DI; Jay, CA; Shade, SB; Vizoso, H; Reda, H; Press, S; Kelly, ME; Rowbotham, MC; Petersen, KL. Cannabis in painful HIV-associated sensory neuropathy: A randomized placebo-controlled trial. *AAN*. **2017**, 515-521

(8) Reiman, A.; Welty, M.; Solomon, P. Cannabis as a Substitute for Opioid-Based Pain Medication. *NCBI,* **2017**.

(9) Maier, C.; Hildebrandt, J.; Klinger, R.; Henrich-Eberl, C.; Lindena, G. Morphine responsiveness, efficacy and tolerability in patients with chronic non-tumor associated pain - results of a double-blind placebo-controlled trial. *Wolters Kluwer*. **2002**. *3959-2*, 223-233.

(10) Perrott, D.; Piira, T.; Goodenough B.; Champion, G. Efficacy and safety of acetaminophen vs ibuprofen for treating children's pain or fever: a meta-analysis. *NCBI,* **2004**. *158-6*, 521-526.

(11) Marsch, L. The Efficacy of Methadone Maintenance Interventions in Reducing Illicit Opiate Use, HIV Risk Behavior and Criminality: A Meta-Analysis. *SSA*, **1998**, *93-4*, 515-532.

(12) NIDA. Overdose Death Rates. *NIDA*. <www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates> **2017**. (Accessed 24 April. 2018)

(13) NIDA. Opioid Overdose Crisis. *NIDA*. <www.drugabuse.gov/drugs-abuse/opioids/opioid-overdose-crisis> **2017**. (Accessed 24 April. 2018)

Supporting Information

Delta-9-Tetrahydrocannibinol, a New Method for the Treatment of Neuropathic Pain

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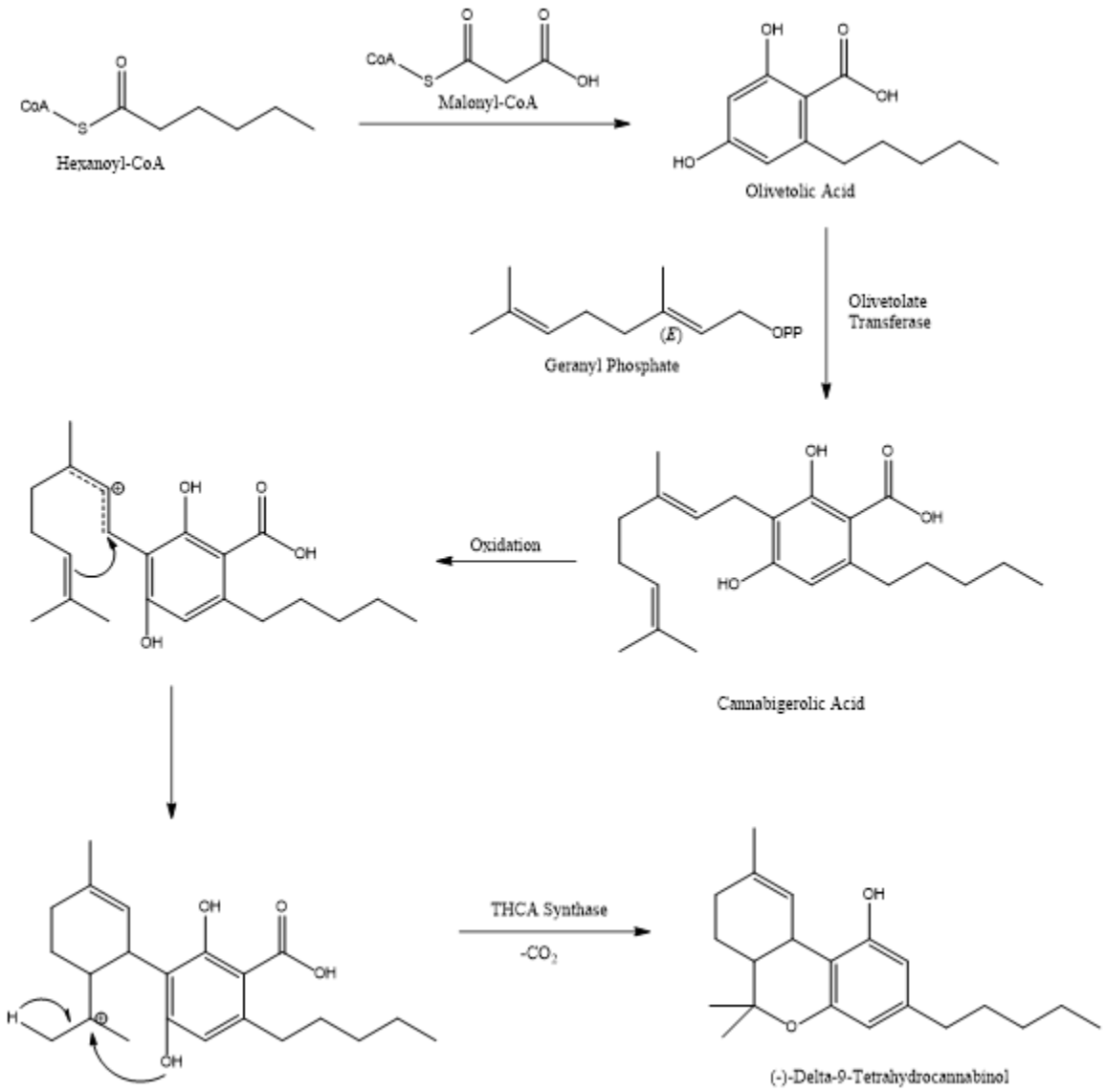
Table of Contents

Biological Mechanism of THC

THC functions as an antagonist by acting on cannabinoid type 1 receptors and cannabinoid type 2 receptors that are located in the brain and spinal cord. CB1 receptors in the brain are commonly found in the basal ganglia, cerebellum, hippocampus, association cortices, and peripheral nerves. When THC acts on the CB1 receptor, the mental and behavioral affects occur. Additionally, the interaction with CB1 will cause antagonistic effects on 5-HT3 receptors, which leads to anti-emetic effects. The CB2 receptors are predominantly found in peripheral tissues of the hematopoietic system, immune system, and spleen.

CB1 and CB2 are G-protein-coupled receptors that are activated with the signaling of THC. The activation of the G-protein leads to the inhibition of adenylate-cyclase. This leads to inhibition of the release of glutamate and acetylcholine, which indirectly affects the γ-aminobutyric acid (GABA), the N-methyl-D-aspartate, serotonin, and opioid receptors.

Scheme S1: Overall Mechanism of THC Synthesis in Cannabis Sativa



Extraction of THC

THC is extracted from *c. sativa* by gathering the plant material and removing the buds and leaves from the stem. The material is then air-dried at room temperature (~23°C) to reduce moisture content until a 70% loss of mass occurs. The dried material is then heated at a high temperature (105°C) in an oven to remove the remaining water before being crushed into a fine powder. The powder is then extracted with a solvent. The two solvents that proved useful and effective in the extraction of THC are petroleum ether and chloroform. When using petroleum ether, the powder is soaked in 60% to 80% petroleum ether and further agitated mechanically for a few minutes. After the agitation, 88% to 94% of THC is extracted from the solid into the solvent. After two agitations, 94% to 99% of THC is extracted into the solvent layer. The extraction phase of THC can also be conducted using chloroform as the solvent. A single agitation of the powder in chloroform yielded a 98% to 99% THC extraction and a double agitation yielded a 100% extraction. The solvent-THC mixture is boiled off leaving just the THC.[5]

Bio-assay Additional Information

Cells derived from human embryonic cells (HEK293) were transfected via electroporation with recombinant human cDNA that contains CB1 and CB2 receptors. The transfected cells are maintained at 37°C and 5% CO_2 in a Dulbecco's Modified Eagle's medium (DMEM). The cells were given F-12 HAM nutrient and supplemented with 2mM of L-glutamine, 10% fetal bovine serum, 0.5% penicillin-streptomycin, and Geneticin (600 mg/mL). A single cell was placed in a fresh plate with the appropriate medium and allowed to proliferate. The grown-in cells were collected in a 50 mM Tris-HCl buffer and centrifuged for 40 minutes at 13,650 rpm at 4°C. The resulting protein concentrate is then used in a Bradford assay where 10 μM of the test compound is added with 0.5 nM THC, and 10 μg of protein membrane. The assay is carried out at 37°C for 90 minutes before vacuum filtrated with a presoaked 0.3% BSA filter. Each assay plate was washed seven times with ice-cold wash buffer containing 50 mM Tris, 154 mM NaCl, 20 mM disodium ethylenediaminetetraacetic acid (EDTA), and 0.2% BSA. The filtered material is allowed to dry at 25°C for 12 hours and then radioactive counts were measured using a scintillation cocktail in a Perkin Elmer TopCount (Perking Elmer Life Sciences Inc., Boston, Mass. U.S.A). The results were calculated using GraphPad Prism to obtain binding affinity and IC_{50} values.[14]

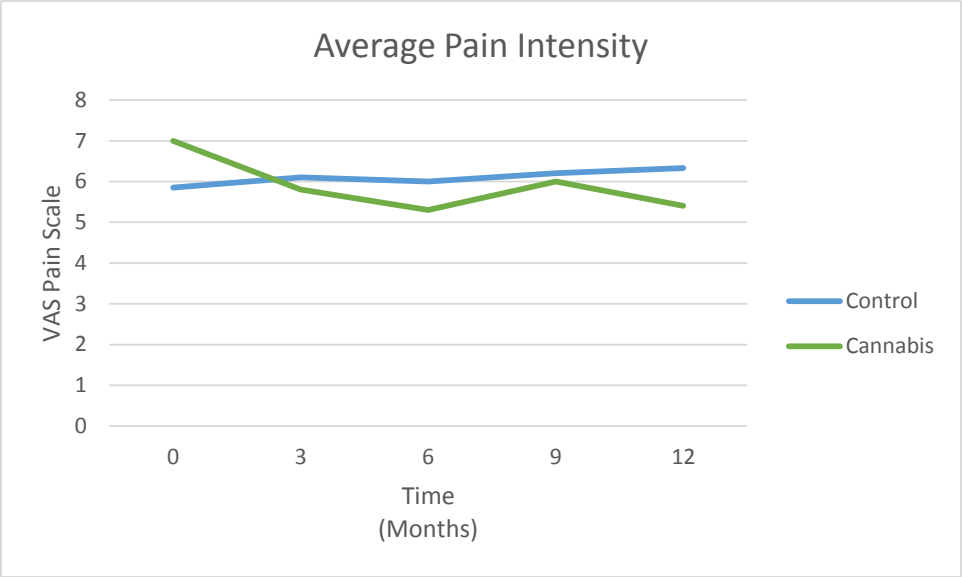


Figure S1: Average pain intensity of THC users vs non-users.

The graph above displays the average pain intensity of 431 people, 215 of which used marijuana and 216 who did not. Each person in the trial experienced chronic neuropathy. Each participant was entered in a trial for a year and every three months they reported their pain using the Visual Analogue Scale. Using the VAS, it allows researchers to view patient's symptoms in a continuous fashion, as opposed to categorizing as none, mild, moderate, and severe. The average pain level for each group was plotted as a graph to determine the overall relationship of THC on pain. The graph shows a decrease in pain for subjects using cannabis, while the control showed average increases in pain throughout the year. This trial is to determine the long-term trend of neuropathic pain through the use of THC.

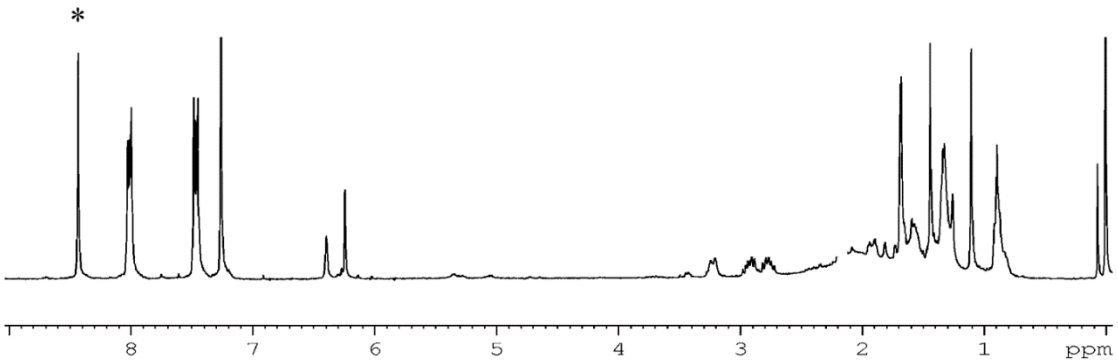


Figure S2: ^{1}Hydrogen NMR spectrum of THC.

HNMR of Cannabis extract with 1mg of Anthracene standard. The solvent used in the system is deuterated chloroform.

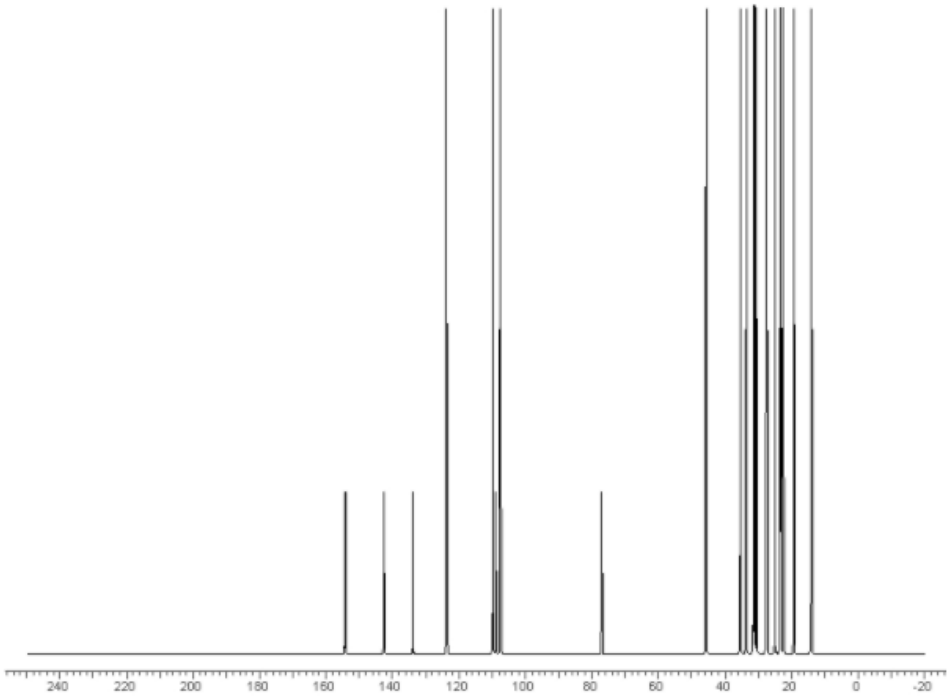


Figure S3: ^{13}Carbon NMR spectrum of THC.

The figure above shows a ^{13}C NMR spectra of THC, from the plant, cannabis sativa. The standard for CNMR is tetrachlorosilane and the solvent used in the system is deuterated chloroform.

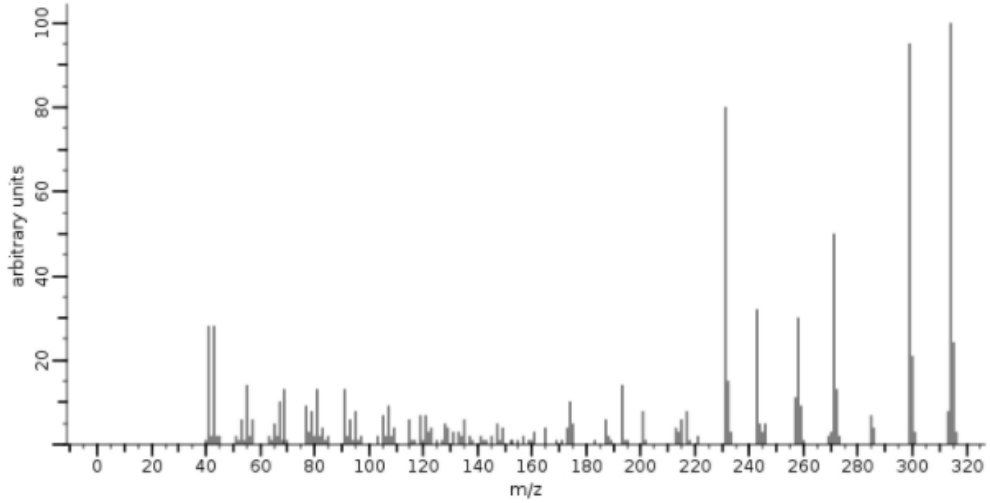


Figure S4: Mass spectrum of THC.

The figure above displays a 70eV electron ionization mass spectrum of THC.

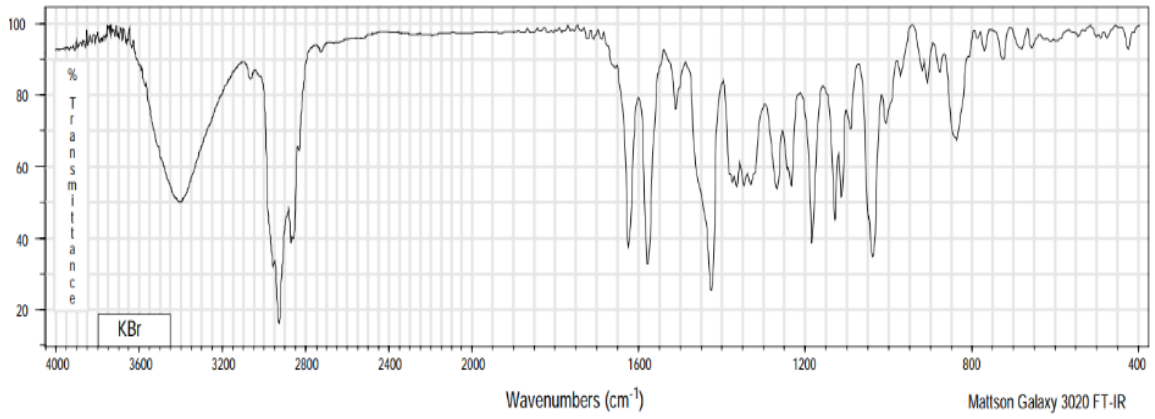


Figure S5: Fourier-Transform infrared spectroscopy (FT-IR) of THC.

Above, the FT-IR spectra of THC is shown. Solvents are not used in FT-IR. The salt crystals used was made from KBr.

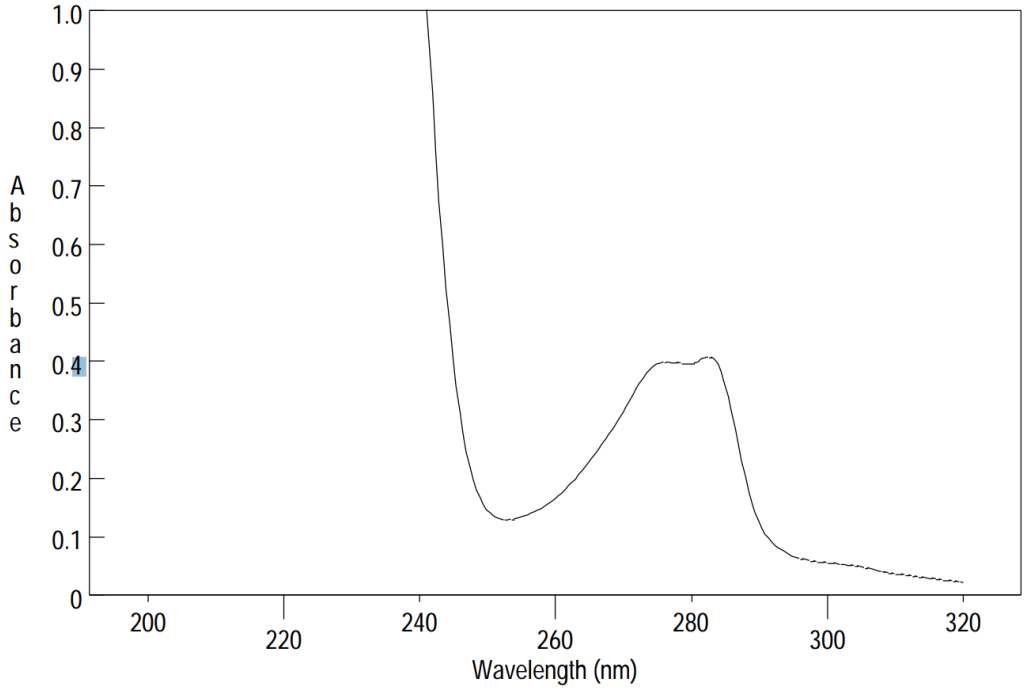


Figure S6: Ultraviolet spectrum of THC.

Pictured above is the UV Spectrum of THC. This spectrum was obtained using methanol as a solvent. The peak is displayed 276.5nm with absorbance at ~0.399. The dilution1:9 (v/v)

Bibliography

Abrams, DI; Jay, CA; Shade, SB; Vizoso, H; Reda, H; Press, S; Kelly, ME; Rowbotham, MC; Petersen, KL. Cannabis in painful HIV-associated sensory neuropathy: A randomized placebo-controlled trial. American Academy of Neurology. **2017**, 515-521.

Befort, K. Interactions of the opioid and cannabinoid systems in reward: Insights from knockout studies. Frontiers in Pharmacology. **2015**, 6, 1-10.

Bow, E.; Rimoldi, J. The Structure-Function Relationship of Classical Cannabinoids: CB1/CB2 Modulation. *NCBI.* **2016**, 8, *17-39.*

Fairbairn, J.; Liebmann, J. The extraction and estimation of the cannabinoids in *Cannabis Sativa* L. and its products. *Journal of Pharm*. **1973**, 25, *150-155.*

Husni, A.; McCurdy, C.; Radwan, M.; Ahmed, Safwat.; Slade, D.; Ross, S.; ElSohly, M.; Cutler, S. Evaluation of Phytocannabinoids from High Potency Cannabis sativa using In Vitro Bioassays to Determine Structure-Activity Relationships for Cannabinoid Receptor 1 and Cannabinoid Receptor 2. NCBI, **2015**, 23(9), 4295-4300.

Maier, C.; Hildebrandt, J.; Klinger, R.; Henrich-Eberl, C.; Lindena, G. Morphine responsiveness, efficacy and tolerability in patients with chronic non-tumor associated pain - results of a double-blind placebo-controlled trial. Wolters Kluwer. **2002**. 3959-2, 223-233.

Oberbarnscheidt, T; Miller, N.S. Pharmacology of Marijuana: Marijuana: Clinical, Research, Policy. J Addict Res Ther. **2016**.

Perrott, D.; Piira, T.; Goodenough B.; Champion, G. Efficacy and safety of acetaminophen vs ibuprofen for treating children's pain or fever: a meta-analysis. NCBI, **2004**. 158-6, 521-526.

Product Information: Δ^9-TETRAHYDROCANNABINOL. *Sigma Aldrich.* **2004**. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Datasheet/1/t4764-091k8801dat.pdf

Qi, Longwu; Yamamoto, Noboru; Meijler, Michael M.; Altobell, Laurence J., III; Koob, George F.; Wirsching, Peter; Janda, Kim D.; Haptens, Monoclonal Antibodies, and a Convenient Synthesis of Radiolabeled Δ9-Tetrahydrocannabinol. *Journal of Med. Chem.* **2005**, 48, 23, 7389-7399

Reiman, Amanda; Welty, Mark; Solomon, Perry. Cannabis as a Substitute for Opioid-Based Pain Medication: Patient Self-Report. *Cannabis and Cannabinoid Research*. **2017**, 160-166.

Ware, Mark A; Wang, Tongtong; Shapiro, Stan; Collet, Jean-Paul; Cannabis for the Management of Pain: Assessment of Safety Study. *The Journal of Pain*. **2015**, 16, 1233-1242.

Waterman, W; Frappier, A. Opioid Epidemic: Uses, Abuses and Innovation. **2018**. 1-2