LEBANESE AMERICAN UNIVERSITY

The Lebanese Cannabis Oil Extract promotes resilience to Chronic Social Defeat in C57BL/6J male mice

By

Amar Mezher

A thesis

Submitted in partial fulfillment of the requirements
For the degree of Master of Science in Biological Sciences

School of Arts and Sciences

July 2022

LAU
الجامعة اللبنانية الأميركية
Lebanese American University

THESIS APPROVAL FORM

Student Name: Amar Mezher I.D. #: 201401940

Thesis Title: The Crude Lebanese Cannabis Oil Promotes Resilience to Chronic Social Defeat Stress a

Program: M.S. in Biological Sciences

Department: Natural Sciences

School: Arts and Sciences

The undersigned certify that they have examined the final electronic copy of this thesis and approved it in Partial Fulfillment of the requirements for the degree of:

Masters (M.S.) in the major of Biological Sciences

Thesis Advisor's Name: Sama F. Sleiman

Signature: [redacted] Date: 22 / 07 / 2022
Day Month Year

Committee Member's Name: Mohammad Mroueh

Signature: [redacted] Date: 22 / 07 / 2022
Day Month Year

Committee Member's Name: Roy Khalaf

Signature: [redacted] Date: 22 / 07 / 2022
Day Month Year

LAU
الجامعة اللبنانية الأميركية
Lebanese American University

THESIS COPYRIGHT RELEASE FORM

LEBANESE AMERICAN UNIVERSITY NON-EXCLUSIVE DISTRIBUTION LICENSE

By signing and submitting this license, you (the author(s) or copyright owner) grants the Lebanese American University (LAU) the non-exclusive right to reproduce, translate (as defined below), and/or distribute your submission (including the abstract) worldwide in print and electronic formats and in any medium, including but not limited to audio or video. You agree that LAU may, without changing the content, translate the submission to any medium or format for the purpose of preservation. You also agree that LAU may keep more than one copy of this submission for purposes of security, backup and preservation. You represent that the submission is your original work, and that you have the right to grant the rights contained in this license. You also represent that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the submission contains material for which you do not hold copyright, you represent that you have obtained the unrestricted permission of the copyright owner to grant LAU the rights required by this license, and that such third-party owned material is clearly identified and acknowledged within the text or content of the submission. IF THE SUBMISSION IS BASED UPON WORK THAT HAS BEEN SPONSORED OR SUPPORTED BY AN AGENCY OR ORGANIZATION OTHER THAN LAU, YOU REPRESENT THAT YOU HAVE FULFILLED ANY RIGHT OF REVIEW OR OTHER OBLIGATIONS REQUIRED BY SUCH CONTRACT OR AGREEMENT. LAU will clearly identify your name(s) as the author(s) or owner(s) of the submission, and will not make any alteration, other than as allowed by this license, to your submission.

Name: Amar Mezher

Signature: [redacted]

Date: 22 / 07 / 2022
Day Month Year

LAU
الجامعة اللبنانية الأميركية
Lebanese American University

PLAGIARISM POLICY COMPLIANCE STATEMENT

I certify that:

1. I have read and understood LAU's Plagiarism Policy.
2. I understand that failure to comply with this Policy can lead to academic and disciplinary actions against me.
3. This work is substantially my own, and to the extent that any part of this work is not my own I have indicated that by acknowledging its sources.

Name: Amar Mezher

Signature: [redacted] Date: 22 / 07 / 2022
Day Month Year

Acknowledgment

First and foremost, I would like to thank **Dr. Sama F. Sleiman** for the guidance and support she never failed to provide throughout the two years. Working with Dr. Sleiman gave me the opportunity to grow my passion for Neurosciences, which is the main reason why I decided to enroll in this Master's program and join her team. Thank you for giving me the opportunity to push myself outside of my comfort zone, enlarge my knowledge of scientific research and ethics, and to considerably strengthen my critical thinking skills. I'm grateful for the past 2 years, as it has helped me grow, both on the personal and carrier levels.

I am also thankful to have been a part of the SFS lab team. I want to thank Reine Khoury and Litsa Ghayad for welcoming me and helping me learn all the basic techniques. I'm also thankful for the rest of the team and my colleagues Perla El Ahmad, Fady Eid, Diala Masri and Zena Haddad who were always ready to help and assist whenever needed.

I would like to mention **Dr. Mohamad Mroueh** and **Dr. Wassim Shebaby** who offered assistance and support throughout my research work. I would like to thank **Dr Roy Khalaf** for showing support and interest by being part of my committee members. Their intriguing questions, coming from different perspectives encouraged me to widen my knowledge related to the topic.

I would also like to thank **Mrs. Helena Bou Farah**, the graduate laboratory supervisor, who was always ready to help and provide technical support whenever needed.

A big thanks to **Mr. Elias Abi Ramia**, who helped me deal with animals and was always ready to assist whether on weekends or during holidays.

Above all, I would like to express the warmest and heartfelt thanks to my parents, brothers, sister and boyfriend for their tremendous support through this entire process. I am grateful and blessed for having you in my life.

To you my guardian angel, all I want more than anything is to make you proud of me.

The Lebanese Cannabis Oil Extract promotes resilience to Chronic Social Defeat in C57BL/6J male mice.

Amar Mezher

ABSTRACT

Major Depressive Disorder (MDD) has become one of the most common mental disorders around the world. Although antidepressants remain the first-line treatment for moderate to major depression, however, numerous limitations have been reported regarding their efficacy and long-term side effects. Cannabis *sativa* is the most common type of Cannabis which is being extensively used for medical purposes. Conflicting results are reported regarding the effects of Cannabis on depression and anxiety. As a result, more studies are needed to understand how Cannabis affect these behaviors. Therefore, the aim of this study was to investigate the therapeutic effect of the Lebanese Cannabis Oil Extract (COE) in the context of depression and anxiety-like behaviors in C57BL/6J male mice using the Chronic Social Defeat Stress protocol (CSDS). The cotreatment paradigm was applied in order to examine the COE effects when given as a prophylactic treatment, along with stress induction. In a post paradigm, the antidepressant and anxiolytic effects of the COE were assessed after stress induction. In both paradigms, experimental mice received either 20mg/kg, 5mg/kg or 1mg/kg of COE through intraperitoneal injections. Control mice received injections of vehicle solution. Following every treatment, the social interaction test was used to assess depression-like behaviors and social avoidance, whereas elevated plus maze test was used to assess the

anxiety-like behaviors in mice. We found out that the highest dose of COE promoted resilience to chronic social defeat stress and rescued depression- like behaviors, however turned out to be anxiogenic when given as a preventative treatment. the 5mg/kg dose showed positive effects on promoting resilience to stress and rescuing depression-like behaviors in mice with no particular effect on anxiety-like behaviors. The lowest dose turned out to be anxiogenic when given in a preventative treatment without any particular effect on depression-like behaviors in both paradigms. In conclusion, according to the behavioral outcomes of treated mice, the Lebanese COE showed antidepressant properties without affecting anxiety. Further investigations are needed to identify the molecular pathways by which COE exerts its positive effects.

Keywords: Major Depressive Disorder, Depression, Anxiety, Chronic Social Defeat Stress, Cannabis, Cannabis Sativa, Lebanese Cannabis Oil Extract.

TABLE OF CONTENTS

List of Abbreviations

MDD: Major depressive disorder

CSDS: Chronic social defeat stress

SI: Social interaction test

EPM: Elevated plus maze test

COE: Cannabis Oil Extract

SSRIs: Selective serotonin reuptake inhibitors

SNRIs: Serotonin-norepinephrine reuptake inhibitors

SNRIs: Selective norepinephrine reuptake inhibitors

ECS: Endocannabinoid System

THC: Delta-9-tetrahydrocannabinol

CBD: Cannabidiol

AEA: Anandamide or N-arachidonoyl ethanolamine

2-AG: 2 -arachidonoyl glycerol

CB1: Cannabinoids receptor 1

CB2: Cannabinoids receptor 2

FAAH: Fatty acid amide hydrolase

5-HT1A: Serotonin receptors

5-HT: Serotonin

CNS: Central Nervous System

BDNF: Brain-derived neurotrophic factor

List of Figures

Chapter One

Literature Review

1.1 Major Depressive Disorder

The number of people suffering from dysfunctional behavior and mental illnesses is constantly increasing worldwide. Major depressive disorder (MDD) is one of the most diagnosed mental disorders (WHO, 2011). It is a biologically heterogeneous disease characterized by mood changes, anhedonia, social avoidance, poor interest in daily tasks, psychomotor agitation, and many other symptoms (APA, 2013). MDD is a life-threatening mental disorder with specific comorbidity with anxiety and stress disorders (APA, 2013). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), anxiety, phobias, and excessive worry can be associated with depression, affecting the general prognosis, treatment options, and the patient's response. The DSM-V states that to be diagnosed with MDD, the symptoms must not result from specific substance abuse that led to the medical illness. Moreover, the patient must show five of the following eight criteria for two consecutive weeks. The first criterion is a depressed mood that is prevalent most of days. The second criterion is a decreased interest or pleasure in almost all activities. The third criterion is a significant weight fluctuation, or an imbalanced appetite, nearly every day. The fourth criterion is a dormant or slow thought process and a reduction of physical movement. The fifth criterion is unusual fatigue or loss of energy almost every day. The sixth criterion is a helpless feeling and inappropriate guilt. The seventh criterion is

decreased ability to think or concentrate. Finally, recurrent thoughts of death and suicidal thoughts can be noted. The lifetime prevalence of MDD is significantly lower in men compared to women: 26.1% in women and 14.7% in men (DSM-V, 2013). Depression is accompanied by suicidal thoughts in 13.6% of patients (Hasin et al., 2018), which highlights the life-threatening characteristic of MDD.

Numerous factors contribute to the onset of MDD. Environmental factors such as early childhood trauma, post-traumatic stress disorder, or medical disabilities are essential in triggering the onset of MDD (Nabeshima et al., 2016). MDD is also statistically linked to specific genetic predispositions. For instance, an individual is 1.3 to 3 times more likely to develop symptoms of depression when an immediate family member has been previously diagnosed (Weissman et al., 1997). Moreover, studies have shown that an identical twin of a patient with MDD will also develop depressive symptoms in 25% to 93% of cases (Heim et al., 2012). Many studies on animal models have also found that genetic mutations can lead to depressive-like behaviors, supporting that genetic factors play an essential role in MDD (Mouri et al., 2012).

Such conditions alter brain regions' volume and circuits, causing cerebral dysfunction. The underlying neuronal pathways and circuitry of MDD are not fully uncovered yet. Hence scientists are persistently trying to understand its underlying mechanisms and are constantly looking to find effective treatments with minimal side effects (YU Hui et al., 2011).

1.2 Neurobiology of Depression

Several factors including environmental, genetic, and epigenetic factors contribute to the susceptibility and vulnerability of an individual to develop depression (Beck, A. T., & Breidemeier, K., 2016). Hence, multiple neuronal factors may contribute to the mechanisms involved in MDD. Neuronal atrophy and neuronal cell death have been linked to depression in many clinical and animal-based studies. Therefore, it has been hypothesized that low levels of neurotrophic factors can lead to a hippocampal abnormality during the development of depression which can be reversed by antidepressant treatments (Hui Yu et al., 2011). This has been linked to diminished Brain-Derived Neurotrophic Factor (BDNF) levels in patients suffering from depression, which is reversed with antidepressants. BDNF is a secreted protein that belongs to the neurotrophic family of growth factors. It plays a vital role in neurogenesis, the differentiation of new neurons like 5-HT neurons (Martinowich et al., 2008), and in establishing new synaptic connections and cell survival. It significantly impacts the Central Nervous System (CNS) and Peripheral Nervous System (PNS) (Arumugam et al., 2017). BDNF is the most abundant neurotrophin in the CNS and PNS. It is also the most studied neurotrophic factor in MDD (Martinowich et al., 2008). The role attributed to BDNF in the context of depression depends on its location in the neuronal circuitry and brain regions (Hui Yu et al., 2011). Depression and Bipolar Disorder are correlated with low levels of BDNF in human plasma (Cunha AB. et al., 2006). Decreased levels of BDNF in the hippocampus and the prefrontal cortex are associated with depressive-like behaviors and stress susceptibility (Jiang et al., 2014). On the contrary, high levels of BDNF in the NAc and amygdala are correlated to depression-like and anxiety-like symptoms

(Govindarajan et al., 2006). A genetic polymorphism changing valine to methionine at codon 66 (Val66Met) decreases BDNF levels by 35% and is correlated to depression and anxiety symptoms (Notaras et al., 2015). Moreover, it has been shown that BDNF infusion enhances tryptophan hydroxylase (TpOH) in the brain, more specifically in the raphe neurons, and upregulates serotonin (5-HT) release (Goggi et al., 2002). This suggests that 5-HT, a monoamine neurotransmitter involved in mood regulation, is released by serotonergic neurons in the raphe and can also be affected by BDNF expressions (Martinowich et al., 2008). Human-based studies have revealed that BDNF levels are enhanced in individuals treated with antidepressants (Chen et al., 2001). These outcomes have been supported by studies on animal models: antidepressants upregulate transcription of BDNF genes in the hippocampus and cortex of rodents (Martinowich et al., 2008).

1.3 Treatments of MDD

Various treatments are used to manage MDD. Psychotherapeutic and pharmacological treatments remain the initial interventions, and their combination was shown to be the most effective (Cuijpers P et al., 2009). Antidepressants are the first-line treatment for moderate to major depression. Selective Serotonin Reuptake inhibitors (SSRIs) are widely prescribed antidepressants, followed by Serotonin-Norepinephrine Reuptake inhibitors (SNRIs), prescribed for MDD patients with comorbid pain disorders. Atypical antidepressants are a secondary treatment in patients who develop sexual side effects from SSRIs or SNRIs. Other medications include serotonin modulators, tricyclic antidepressants (TCAs), or mood stabilizers (Bains N et al., 2022).

According to the monoamine hypothesis, it is suggested that depression is induced by a deficiency in the neurotransmitters such as dopamine, 5-HT, or norepinephrine. Therefore, antidepressants are designed to target and adequately increase monoamine neurotransmitters' synaptic content. Hence, monoamine reuptake inhibitors (SSRIs, SNRIs, and Norepinephrine Dopamine reuptake inhibitors (NDRIs)) are the most adopted categories of antidepressants (Cleare et al., 2015). However, these antidepressants are effective in only 50% of cases and they usually require 3 to 5 weeks to take effect (YU Hui et al., 2011). Moreover, SSRIs display side effects such as problems in sexual function, gastrointestinal distress, tiredness and weight gain (Cascade et al., 2009). This suggests that novel neurobiological substrates could potentially provide further therapeutic effects leading to the rise of new drugs approved to be used as antidepressants. For instance, Ketamine is an anesthetic that has shown antidepressant effects. Ketamine upregulates glutamate production which allows the brain connections to be reformed. However, studies have revealed that its consumption is addictive and is linked to many psychological disturbances (Shin, C., & Kim, Y., 2020).

Other compounds, such as cannabinoids, are being extensively consumed as mood boosters. Nascent research outcomes also show positive therapeutic effects of Cannabis plant extracts in treating psychiatric diseases such as MDD.

1.4 Background Information on Cannabis

Cannabis *sativa*, Cannabis *indica*, and Cannabis *rudelaris* are all species of the Cannabis plant. Cannabis *Sativa* is the most common type. The dried leaves of

Cannabis *sativa* are widely consumed by the young generation and are referred to as Marijuana (Pereira et al., 2014). In many cultures and for hundreds of years, the medical use of *Cannabis sativa* has shown remarkable results in treating and alleviating symptoms of several diseases and illnesses such as pain, asthma, insomnia, depression, epilepsy, and loss of appetite (Andre et al., 2016). Cannabis is being extensively used for medical purposes, especially in Canada and the USA, where it's legally sold and consumed. With the legalization of Cannabis consumption, consumers are buying it for self-medication (A Asselin et al., 2022). Other users began consuming cannabis to enhance physical performance during exercise. For instance, Ogle, W.L et al. published in 2022 a study conducted on 131 adults to understand why people take Cannabis before physical activity. The survey revealed that Cannabis consumption was mainly correlated with activities such as hiking (60%), yoga (58%), and aerobic machines (50%). When asked about the reason, 66% of consumers explained that cannabis helped them concentrate better, 65% said it helped in enjoying the exercise experience and 65 % claimed that it enhanced the mind-body-spirit connection. The increasing demand for cannabis consumption triggered the interest of scientists to understand the physiological and neurobiological mechanisms of Cannabis, particularly in the brain.

Delta-9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) are considered the primary cannabinoids. THC and CBD represent the main Phytocannabinoids expressed in mature Cannabis flowers or leaves and are extensively studied due to their important range of effects on humans. They are also extracted in high concentrations from Cannabis plants. Scientific resources have reported the identification of 421 substances that are considered to be active compounds of which

100 are recognized as phytocannabinoids (Hebert Jair et al., 2020, Murphy et al., 2017). The number of identified compounds in the *Cannabis sativa* plant has increased from 423 compounds in the 1980s (Turner et al., 1980) to 483 in 1995. To date, new components have been added and researchers have identified 565 substances present in the *Cannabis sativa* plant (M.A. El Solhy et al., 2017). Among all the newly discovered compounds, THC remains the only psychoactive component in the Cannabis plant leading to the 'high' or euphoric effect after administration, whereas CBD, is the non-psychoactive and nontoxic compound when administered at recommended doses (Freeman T.P. et al., 2019).

Four main methods characterize the consumption of Cannabis: oral with capsules, inhalation through smoking, sublingual with oils, or topical with creams and patches. The Drug Policy Alliance in the USA mentions that the administration methods provide different effects and hence could be more or less appropriate to its consumer. For instance, inhalation is the fastest delivery method by which the inhaled marijuana passes through the lungs and directly gains access to the bloodstream giving the desired effect within seconds. Oral administration is mainly adopted for a long-lasting alleviating effect of chronic pain. Indeed, in oral consumption, cannabinoids pass through the digestive system and are subjected to chemical transformation making them stronger and lasting longer. Hence, the effects are felt within 30 to 60 minutes and last up to 6 hours. Sublingual administration allows cannabis compounds to reach the bloodstream of the consumer within a few minutes, similarly to oral consumption. Sativex is one example of a clinically approved medication administered sublingually prescribed to reduce spasticity in patients suffering from neurological conditions (Hebert J. et al., 2020). Another way to use cannabis is through topical administration

by direct application on the skin. Local skin inflammation and pain are mainly treated through topical administration. Indeed, through the very complex mechanism of the skin in absorbing chemicals, cannabinoids penetrate the skin to relieve symptoms within minutes and can last for up to 2 hours.

1.5 The endocannabinoid System

Cannabinoid compounds elicit their effects through the endocannabinoid system (ECS). The ECS is an endogenous neurotransmission system (Bilge S., et al. 2021) composed of CB1 and CB2 receptors, the endogenous cannabinoids Anandamide (N-arachidonoyl ethanolamine, AEA) and 2 -arachidonoyl glycerol (2 -AG), along with catabolizing enzymes (Balapal S. Basavarajappa et al. 2017). AEA and 2-AG bind the CB1 and CB2 receptors in the CNS and PNS. For instance, increases in the concentration of CB1 in the hippocampus, the basal ganglia, the hypothalamus and the cerebellum, is responsible for the appetite regulation, memory functions, motor responses and postures of mammals (Aran et al. 2019; Mc Partlan et al. 2014).

The endocannabinoids are naturally produced when the postsynaptic cells initiate the demand, therefore they are not stocked or stored. When the postsynaptic cells emit the stimulus, presynaptic terminals respond to it. This is known as a retrograde signaling pathway (Aran et al. 2019; Zamberletti et al. 2017). When not needed, AEA is hydrolyzed by the fatty acid amide hydrolase (FAAH) (Bridgemanan and Abazia, 2017).

THC molecules primarily bind to CB1, which are G protein-coupled receptors found on neurons in the CNS. THC also binds to CB2, found in peripheral tissues, and is

correlated to improved mechanisms of the immune system (Mc Partlan et al. 2014). CBD molecules inhibit FAAH, blocking its role in breaking down AEA, which eventually leads to an accumulation of that endogenous cannabinoid in the presynaptic terminal (Bridgemanan and Abazia 2017) and activation of the neuronal signaling pathway. Additionally, CBD has a strong binding affinity to the serotonin receptors 5-HT1A. Hence, they bind to the 5-HT1A receptors, and activate them, leading to the anti-anxiety effect (Lu et al. 2020).

1.6 Cannabinoids as a potential antidepressant

Cannabis was restricted in many countries and its therapeutic roles have been underestimated. However, with the emerging research targeting the medical use of Cannabis, this substance and its derivative compounds have gained significant success across several cultures leading to a rapid change in the policies of its medical applications (Freeman T.P. et al., 2019). Although the Food and Drug Administration (FDA) hasn't yet fully approved the legal consumption of Cannabis, some pharmaceutical compounds have been synthesized to mimic the effect of the two cannabinoids, THC and CBD. These modulators are synthetic agonists of the cannabinoid receptors and are approved for medical consumption in clinical use and under research. For instance, Dronabinol is the worldwide recognized generic name of THC along with Nabilone which is also a *Cannabis sativa* extract similar to THC. In fact, Marinol®, which contains synthetic dronabinol has been approved by the FDA in 1992 to treat anorexia and neuropathy in HIV patients (Abrams, Jay, et al. 2007). Moreover, the Sativex® spray, which contains equal amounts of dronabinol and CBD,

was approved in 2005 for the treatment of spasticity and pain in patients with multiple sclerosis (Hebert J. et al., 2020). Additionally, side effects of chemotherapy in cancer patients, such as nausea and vomiting, are alleviated through Cannabis administration (Machado Rocha et al. 2008).

Aside from the positive therapeutic effects revealed from the medical use of Cannabis, other studies have shown side effects after consumption of Cannabis specifically rich in THC. For example, a positive correlation has been shown between cannabis consumption in adolescents and the onset of schizophrenia (Morgan C.J.A., et al., 2008). Moreover, in a research conducted by Farra Y., et al. in 2019, the locomotor and anxiety-like behaviors of mice receiving inhalation of vaporized cannabis, were assessed. Indeed, mice receiving cannabis spent more time in the corners when compared to the control mice suggesting an enhancement of anxiety like behaviors after cannabis inhalation without any effect on locomotor activity (Farra Y., et al. in 2019). Interestingly, the cannabis used in the previous study was composed of 10.3% of THC and 0.05% of CBD which is mathematically equivalent to 203 times more THC levels than CBD received through the vaporized cannabis compounds. Consistently with previous studies, these results suggest that consumers of high-potency Cannabis (high THC/ low CBD), have higher risks of developing psychotic disorders such as schizophrenia, or cognitive impairments, anxiety and depression (Stuyt E. 2018). Consuming cannabis containing higher THC levels in comparison to compounds containing higher levels of CBD than THC, leads to an impairment in memory skills and the development of depression and anxiety like behaviors (Morgan C.J.A., et al., 2012).

In alignment with the above-mentioned studies, separate and combinatorial treatments of CBD and THC were tested on adolescent (4-6 weeks old) and adult (9 to 12 weeks old) male CD1 mice. Mice were injected with THC or CBD separately (3mg/kg) or a combination injection of 1:1 ratio of THC + CBD (3mg/kg each). THC treatment alone induced behavioral abnormalities such as compulsive behaviors, increased anxiety and impaired working memory. Treating with CBD did not induce any behavioral response. However, combinatorial treatment with CBD+THC revealed positive performances on behavioral testings suggesting that CBD treatment prevents THC-induced impairments (Murphy M., et al., 2017). These findings support the evidence that equal or higher CBD amount in cannabis decreases the risk of cognitive and psychotic disorders.

Since the cultivation of "high potency" Cannabis was revealed as harmful, the growth of "Balanced Cannabis" or high CBD cannabis should be taken into consideration in order to reduce the unwanted negative effects of cannabis and encourage its administration for medical purposes (Murphy M., et al., 2017).

Interestingly, the Lebanese Cannabis oil predominantly contains higher amounts of CBD (59.1%) in comparison to THC (20.2%) (Shebaby et al. 2020). Therefore, using the Lebanese extracts of Cannabis and monitoring its effect on depression and anxiety-like behaviors in mice could have promising therapeutic outcomes.

1.7 The Lebanese Cannabis Oil

Until recently, Cannabis selling and consumption was prohibited in Lebanon even for medical use. However, its cultivation was operated unlawfully in the Bequaa Valley of East Lebanon (Shebaby et al., 2021). In the year of 2020, the Lebanese parliament authorized cannabis cultivation for medical and industrial use (Lebanese Official Gazette; issue 23; 2020). Moreover, Cannabis oil has been used in Lebanon through generations to alleviate symptoms related to many conditions such as cancer, diabetes and chronic pain associated with arthritis (Shebaby et al., 2021). In a study published by Shebaby et al. in 2021, the Lebanese cannabis oil was extracted for in-vivo and in-vitro anti-inflammatory investigations. Eighteen main constituents were identified after extraction. The Lebanese Cannabis oil extracted from the *Cannabis sativa* plant is very rich in CBD which represents 59.1% of the total components. THC constitutes 20.2% of the oil extraction. Other components such as CBN, CBC, Phytol, etc. were also identified with amounts less than 3.63% each.

1.8 Aim of the study

Studies have reported that CBD can lead to an antidepressant-like effect, suggesting the potential of this compound to present novel antidepressant properties. Although CBD oil and combinatorial treatment of CBD and THC revealed promising therapeutic effects on psychiatric diseases, research outcomes remain inconclusive regarding the benign or detrimental effects of cannabinoids on depression and anxiety-like behaviors. Certainly, research regarding Cannabis is still nascent, and more studies should be conducted. To the best of our knowledge, few studies

investigated the effects of Lebanese cannabis in the context of psychiatric diseases such as Depression. Moreover, no research has targeted the effects of Lebanese Cannabis Oil in the context of a Chronic Social Defeat Stress (CSDS) paradigm. We investigated the use of Cannabis oil as a protective factor (along with CSDS) or as a treatment (following CSDS) for depression. Therefore, the aim of this study is to examine whether the administration of specific dosages of the Lebanese Cannabis Oil promotes resilience to CSDS and whether it has an anti-depressant or anxiolytic effect in 6 to 8 weeks old C57BL/6 J male mice.

Chapter Two

Methods and Materials

2.1 Animal housing and experimental paradigms

Male C57BL/6 J mice aged of 6-8 weeks were housed in cages and divided into groups according to the experimental paradigms. Food and water were provided and mice were maintained on a 12h light-dark cycle. Animal use and care were in accordance with the recommended guidelines and as approved by the ACUC. The co-treatment paradigm consisted of male C57BL/6 aged of 6 to 8 weeks old, housed next to an aggressor CD1 mouse to undergo CSDS with simultaneous intraperitoneal (i.p.) injections of COE for a period of 10 days. Behavioral testing and animals' sacrifice were performed on Day 11. The nucleus accumbens, the hippocampus and the prefrontal cortex of mice were collected on dry ice and stored at -80°C.

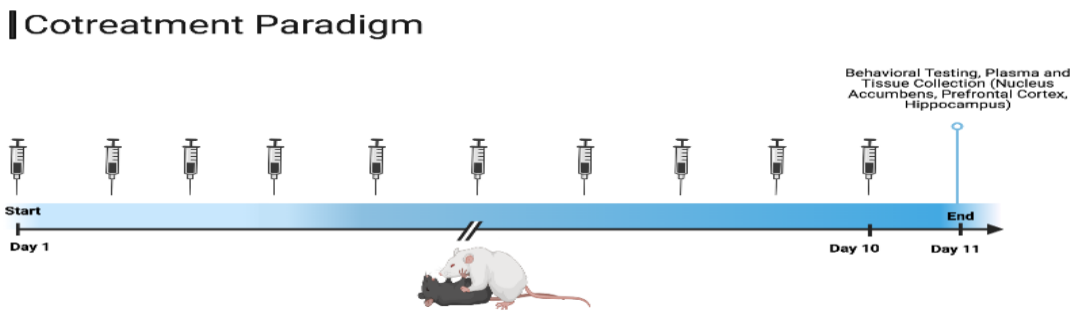


Figure 1. Illustrative schematic of cotreatment paradigm

In the post-treatment paradigm 6 to 8 weeks old male C57BL/6 mice were housed next to an aggressor CD1 mouse to undergo CSDS for 10 days. Behaviors testing was performed on day 11. On the 12th day, Cannabis Oil Extract (COE) injections start to be administered for 2 weeks. Then, on day 26 animals were tested again and sacrificed. The nucleus accumbens, the hippocampus and the prefrontal cortex of mice were collected on dry ice and stored at -80°C.

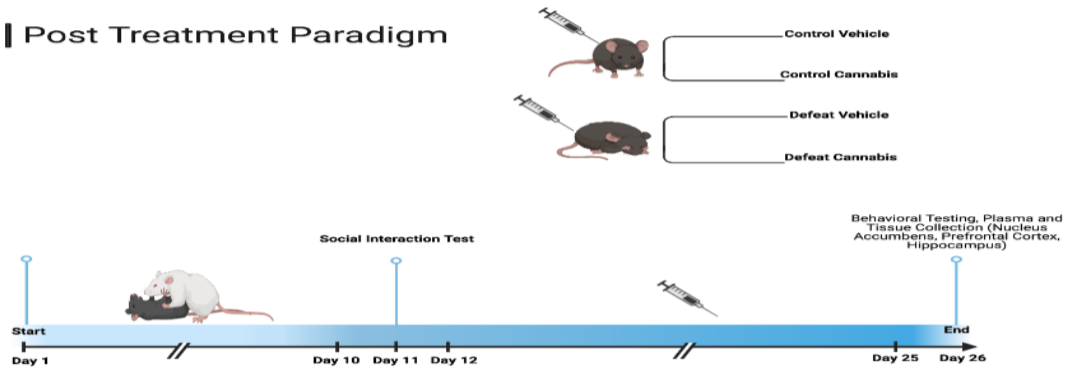


Figure 2. Illustrative schematic of cotreatment paradigm

2.2 Plant Collection and Cannabis oil Extraction

In October 2019, a sample from the cannabis plant was taken from the Yammoune, Beqaa valley in the North-East of Lebanon. The cannabis strains were then procured to the Faculty of Pharmacy of the Lebanese American University by the Drug Enforcement Office in Zahle, Beqaa Governorate, and was kept in a safe location on campus. 10 g of air-dried cannabis flower was used for ethanol extraction for 48 hours (ID, 2019–0011). It was then filtered and concentrated at 45°C under decreased pressure and 1.17 g of cannabis oil extract (COE) was obtained.

2.3 Cannabis Injections

Experimental mice received daily i.p injections of either vehicle solution or the crude Lebanese COE at different concentrations (20mg/kg/day, 5mg/kg/day or 1mg/kg/day). In the cotreatment paradigm, control mice received for a period of 10 consecutive days i.p injections of vehicle solution containing ethanol, Tween 80 and PBS with a ratio of 1:1:18. Experimental mice received either 20mg/kg, 5mg/kg or 1mg/kg daily over 10 days. In the post-treatment paradigm, mice started receiving at day 12 daily i.p injections of COE for a period of 2 weeks post CSDS. Controls were injected with vehicle solution and experimental mice with either 20mg/kg, 5mg/kg or 1mg/kg.

2.4 Chronic social defeat stress (CSDS) model

The CSDS model was used in order to induce and mimic depression and anxiety-like behaviors in experimental C57BL/6 J male mice. The CSDS paradigm was performed as previously described (Karnib et al. 2019; Nasrallah 2019). CD-1 mice were singly housed for a period of 1 to 2 weeks to habituate with constant access to food and water. A screening step of 3 consecutive days was then performed in order to select the aggressors. During this phase, different C57BL/6 J mice were placed in the home cage of the CD-1 mouse for 3 min each day. To be selected and used as an aggressor, the CD- 1 mouse had to initiate at least one aggression episode daily during the three days of screening. Finally, prior to every CSDS experiment, CD-1 mice were screened again and mice that did not meet these criteria were excluded. Then, each CD-1 aggressor mouse and experimental C57BL/6 J mouse were housed in the same

cage, separated by a perforated plexiglass.

To determine whether the Lebanese COE promotes resilience to stress, a co-treatment paradigm consisted of experimental C57BL/6 male mice receiving COE injections daily followed by a defeat session of 5 to 10 minutes by a 3 to 5 months old CD-1 aggressor for 10 days. On Day 11, mice underwent behavioral tests followed by animal sacrifice and tissue collection. In a post treatment paradigm, we investigated the effect of Cannabis after the establishment of stress susceptibility. Hence, male C57BL/6 mice experienced defeat sessions by a CD-1 aggressor for 10 days. On Day 11, mice were subjected to Social Interaction test. Susceptible mice received Cannabis injections daily for 14 days. On day 26, mice undergone a second set of behavioral testing followed by animal sacrifice and tissue collection.

In both paradigms, the control animals were also housed in pairs with a resident mouse and alternated every day, however no physical contact was allowed between the experimental and aggressor mice.

2.5 Social Interaction Test

The social interaction (SI) test was conducted one day following the last defeat session, as previously described by Kaidanovich-Beilin et al. (2011). Experimental C57BL/6J mice were acclimated in a cage with three compartments, two of which had a circular wire enclosure, for 5 minutes. A social stimulus C57BL/6J mouse was restricted to one of the circular enclosures after the habituation phase, and the experimental mouse was restored to the cage in the central chamber. The social stimulus was chosen to match the age and sex of experimental mice. During the test, a camera was used to track the movement of the experimental mouse for 10 minutes

and recorded on ANY-maze which is an application used to track the amount of time spent in each compartment (central, social, and non-social). The mouse showing social avoidance and depressive like behaviors will spend more time in the non-social compartment whereas the resilient mouse will spend more time interacting with the stimulus in the social compartment.

Figure 1. Illustrative schematic of the social interaction test

2.6 Elevated Plus Maze

The Elevated Plus Maze (EPM) is also performed one day after the last CSDS session. It is a validated test to assess anxiety like behaviors in mice. It is composed of two open arms and two closed arms. During the test, mice are allowed to explore the maze for a period of 5 minutes. Time spent in the closed and open arms were tracked by a camera and recorded on the ANY-maze program. The resilient mice are expected to spend more time in the open arms than in the closed arms. On the contrary, mice showing anxiety like behaviors rather spend more time in the closed arms.

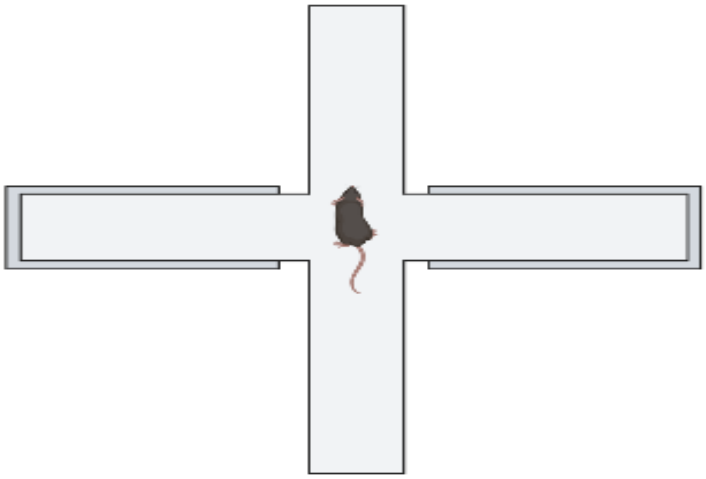


Figure** 4. **Illustrative schematic of the Elevated Plus Maze test

2.7 Statistical Analysis

Results were analyzed using Prism9 application. Two-way ANOVA was performed followed by the Dunnett, Tukey, or Bonferroni multiple comparison tests. A p value below 0.05 was considered to be statistically significant with the following degrees of significance: *p<0.05, **p<0.01, ***p<0.001 and ***p<0.0001.

Chapter Three

Results

3.1. Cannabis promotes resilience to Chronic Social Defeat Stress

In order to assess whether the COE promotes resilience to stress, we first applied a cotreatment paradigm of CSDS. Male C57BL/6 mice aged of 6 weeks old were housed in cages with a CD-1 aggressor separated by a perforated plexiglass. Experimental mice were exposed to a daily defeat session of 5 to 10 minutes for a period of 10 days along with daily i.p injections of either vehicle solution, 20mg/kg, 5mg/kg or 1mg/kg of COE (*Figure 5.A*). Mice were divided into several groups; control mice receiving vehicle solution (n=27), defeat mice receiving vehicle solution (n=23), 3 groups of control mice receiving three different doses of COE (n= 12 in each group) and 3 groups of defeat mice receiving the three different doses of COE (n=12 in each group). On the 11^{th} day, mice were subjected to behavioral testing (*Figure 5.A*). The SI test was used to assess the effect of Cannabis injections on resilience to stress. During the test, mice were habituated for 5 min in a cage divided into three interconnected compartments. Two of these compartments contain a circular wire enclosure. Following the habituation phase, a social stimulus C57BL/6J mouse was introduced to one of the empty enclosures and the experimental mouse was added to the central compartment. The test lasted 10 minutes during which the experimental mouse was left to freely navigate between the three different compartments. The trajectory of the experimental mouse as recorded with a camera. The time spent in each compartment (central, interaction, and non-interaction) was measured by the ANY-maze program. To determine whether the mouse is susceptible

or resilient to stress, the social interaction ratio was calculated as the ratio of the time spent in the interaction compartment over the time spent in the no interaction compartment. A ratio of < 1 indicates susceptibility and > 1 indicates resilience to stress (Henriques-Alves and Queiroz 2015; Kaidanovich-Beilin et al. 2011).

As expected, control mice will spend more time interacting with the stimulus as compared to the defeat mice that showed avoidance behaviors and spend more time in the empty compartment. Indeed, defeat mice receiving vehicle solution interacted significantly less than the controls. The interaction time of control vehicle group is significantly higher than defeat vehicle group (Two-way anova $P<0.0001$) (*Figure 5*). Daily injections of 20mg/kg of COE promoted resilience to CSDS by significantly increasing interaction time in the defeat cannabis group ($p<0.05$) (*Figure 5.C*). Moreover, injections 5mg/kg also significantly increased interaction time of defeat mice that received COE in comparison to defeat mice that received vehicle ($p<0.05$) (*Figure 5.D*). This shows that 20mg/kg or 5mg/kg of COE administered at the same time as the induction of stress promoted resilience to stress by reducing social avoidance behaviors in mice subjected to daily defeat sessions.

In order to select the minimal dose at which this positive effect can be observed, we assessed whether 1mg/Kg of COE also promoted resilience to chronic stress. Injections of 1mg/kg of COE did not increase interaction time of defeat mice as compared to the defeat mice receiving vehicle treatment ($P>0.05$). Our data suggests that daily injections of 1mg/kg COE does not promote resilience to stress nor rescue social interaction behavior (*Figure 5.E*). Moreover, according to the protocol that we applied, the minimal dose at which positive effect can be seen is above 1mg/kg and lower or equal to 5mg/kg of COE.

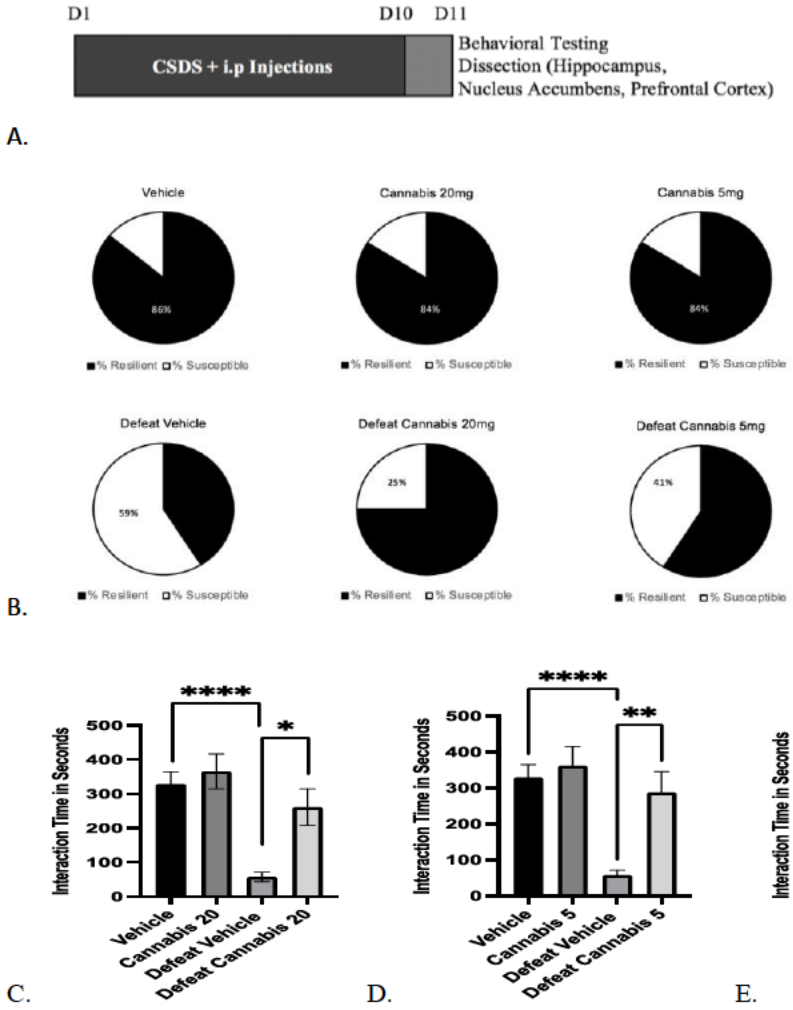


Figure 5: Specific doses of Lebanese COE promote resilience to stress in a cotreatment paradigm of CSDS

A: Timeline representing the cotreatment paradigm of CSDS. The paradigm consists of male mice undergoing CSDSfor 10 days. Prior to every defeat session, mice were intraperitoneally injected with either vehicle or cannabis. 24 hours after the last defeat session, mice underwent the social interaction test to assess depressive-like behavior.

B. Pie charts showing resilience vs susceptibility of mice in each group. The percentage of resilience is presented in black and the percentage of susceptibility is in white. Pie charts of control vehicle vs defeat vehicle clearly indicate that more than 50% of defeat mice are susceptible to stress while 86% of control mice are resilient. Pie charts of control mice treated with 20mg/Kg and 5mg/Kg of COE are 84% resilient. Control mice treated with 1mg/Kg are 67% resilient. Pie charts of defeat mice treated with 20mg/Kg and 5mg/Kg indicate an increase in resilience percentages, 75% and 59% respectively, in comparison to defeat

mice that received vehicle solution. The pie chart of defeat mice that received 1mg/Kg of COE indicates a susceptibility percentage calculated at 79% in comparison to 59% for defeat mice that received vehicle.
C. Bar graph representing the average social interaction ratio measured by the social interaction test. The social interaction ratio equals the time spent in interaction with the stimulus divided by the time the mouse spends not interacting with the stimulus. Results show that i.p injection of 20mg/kg COE significantly increases interaction time in defeated mice as compared to non-treated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 61) = 325.7$, $p<0.0001$ & Treatment: $F(1,61) = 134.2$, $p<0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 20 ($p<0.0137$). The n number of Control Vehicle = 27; Defeat Vehicle = 24; Control Cannabis 20mg = 12 and Defeat Cannabis 20mg = 12.

D: Bar graph representing the average social interaction ratio measured by the social interaction test. Results show that i.p injection of 5 mg/kg COE significantly increases interaction time in defeated mice as compared to non-treated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 61) = 252.1$, $p<0.0001$ & Treatment: $F(1,61) = 146.2$, $p<0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 5 ($p<0.0059$). The n number of Control Vehicle = 27; Defeat Vehicle = 24; Control Cannabis 5 mg = 12 and Defeat Cannabis 5mg = 12.

E: Bar graph representing the average social interaction ratio measured by the social interaction test. Results show that i.p injection of 1 mg/kg COE did not increase interaction time in defeated mice as compared to non-treated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 67) = 752$, $p<0.0001$ & Treatment: $F(1,67) = 14.82$, $p = 0.0003$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 1 ($p<0.5933$). The n number of Control Vehicle = 27; Defeat Vehicle = 24; Control Cannabis 1mg = 12 and Defeat Cannabis 20mg = 12.

3.2. Cannabis acts as an antidepressant by rescuing the social avoidance behaviors of susceptible mice.

In order to determine whether the COE rescues depression-like behaviors and act as a potential antidepressant, we implemented a post treatment paradigm of CSDS. Animals

were divided into 2 groups: a control group (no CSDS) and a defeat group (daily CSDS). Daily defeat sessions of 7 minutes were conducted for a period of 10 days. On day 11, SI test was utilized to help us segregate the defeat group into susceptible or resilient to stress. Only animals that were susceptible to stress were selected for the experiment and received either vehicle or COE i.p injections (20 mg/Kg, 5 mg/Kg and 1 mg/Kg). On average, 50 percent of the defeat animals were susceptible to stress (Golden et al., Nature Protocols, 2011) and selected for the treatments. Four subgroups were formed from the susceptible mice: defeat vehicle (n=23), defeat cannabis that received 20mg/Kg (n=12), defeat cannabis that received 5mg/Kg (n=10) and defeat cannabis that received 1mg/Kg (n=12). The control group was also divided into four subgroups: a control vehicle group (n=22) and 3 control cannabis groups each receiving a different dose of the COE: 20 mg/Kg, 5mg/Kg and 1 mg/Kg (n= 10 in each). Treatments were maintained for a period of 2 weeks after which behavioral testing was performed followed by animal sacrifice and brain tissue collection (*Figure 6.A*). The 2 weeks treatment with 20mg/kg and 5 mg/kg reversed the depression-like behaviors and social avoidance traits induced by the CSDS protocol. Indeed, 87% of defeat mice that received vehicle were still susceptible and only 13% were resilient after the 2 weeks of vehicle injections. Mice that received 20mg/kg of Lebanese COE were only 16% susceptible and 84% resilient (*Figure 6.C*), which reflects a major amelioration due to the cannabis effect. Similarly, mice that received 5mg/kg of Lebanese COE were only 10% susceptible in comparison to 87% for the untreated defeated mice (*Figure 6.D*).

In order to determine the minimal dose at which positive effects could be seen in the post treatment paradigm, we administered the 1 mg/kg treatment. This dose did not rescue

depression-like behaviors since the percentage of mice that were still susceptible to stress was calculated at 70% following the 2 weeks of treatment (*Figure 6.E*)

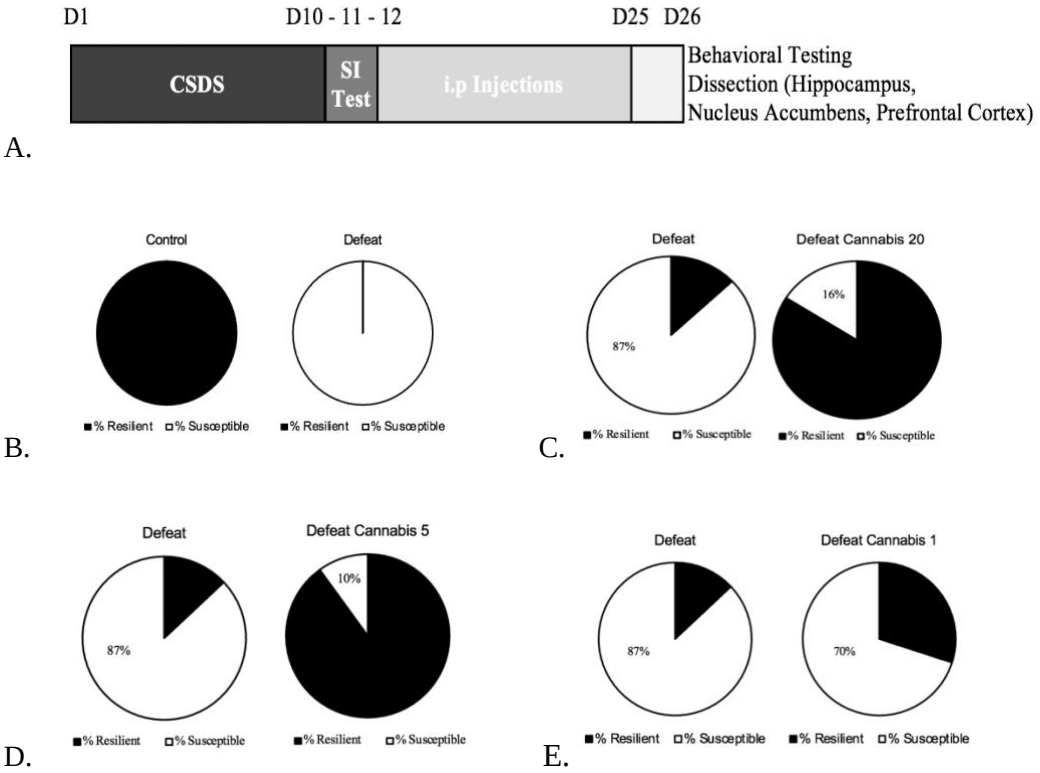


Figure 6. Specific doses of the Lebanese COE rescue depressive like behaviors, therefore showing antidepressant properties.

A: Timeline representing the post treatment paradigm of the CSDS. It consists of subjecting C57BL/6 6-week-old mice to daily CSDS. On the 11th day, mice are divided into susceptible and resilient mice according to their social interaction ratio. Then, vehicle or cannabis are administered. After two weeks of treatment, animals' behaviors are analyzed through behavioral tests. Then, mice are sacrificed and parts of the brain are extracted for further molecular analysis.

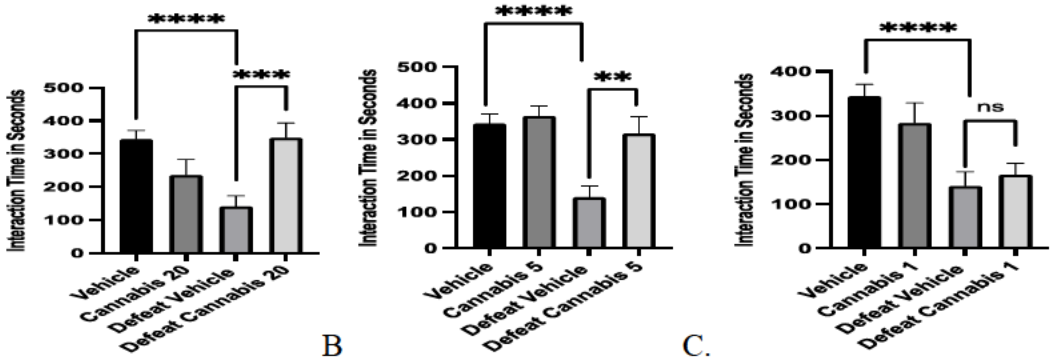
B: Pie charts showing Control vs Defeat groups before the 2 weeks of Cannabis treatment. The percentage of resilience is presented in black and the percentage of susceptibility in white. Since only susceptible mice are chosen to undergo treatment, pie charts clearly indicate that before treatment, mice were completely susceptible to stress.

C: Pie charrs showing 20mg/kg treatment rescues depressive like behaviors in defeat mice that received COE: 16% susceptibility presented in white, as compared to untreated mice with 87% susceptibility.

D: Pie Charts showing 5 mg/kg treatment rescues depressive like behaviors in defeated treated mice (10% susceptibility) as compared to untreated defeated mice (87% susceptibility).

E: Pie charts showing that the 1 mg/kg treatment does not rescue depressive like behaviors in defeated treated mice (70% susceptibility) as compared to untreated defeated mice (87% susceptibility).

The Lebanese COE also showed positive effects by increasing interaction time measured during the social interaction test after the 2 weeks treatment, which reflects a rescue of social avoidance behaviors. Defeat vehicle group, the mice that were exposed to defeat sessions but did not receive any dose of cannabis treatment, were interacting significantly less than animals of the control vehicle group ($p<0.0001$; *figure 7 A. B and C*). Mice that received 20mg/kg and 5mg/kg daily treatment of Lebanese COE spend significantly more time in the interaction zone as compared to defeat mice receiving vehicle treatment ($p<0.05$; *figure 7. A and B*). Concerning the lowest administered dose, 1mg/kg did not rescue depression like behaviors and did not increase interaction time in comparison to the defeat vehicle group ($p>0.05$; *Figure 7.C*).



A. B C.

Figure 7: Specific doses of the Lebanese COE significantly rescues depressive like behaviors by increasing social interaction time.

A: Bar graph showing the average social interaction ratio as measured by the social interaction test. Results showed that the defeated mice treated with Cannabis 20mg had a significantly higher social interaction ratio compared to the defeated mice treated with vehicle. Statistical significance was measured by 2way Anova. Defeat: $F(1, 63) = 61.20$, $p<0.0001$ & Treatment: $F(1,63) = 68.12$, $p<0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 20 (<0.0008). n number of control vehicle = 22, defeat vehicle = 23, control cannabis 20 =10 and defeat cannabis 20 = 12.

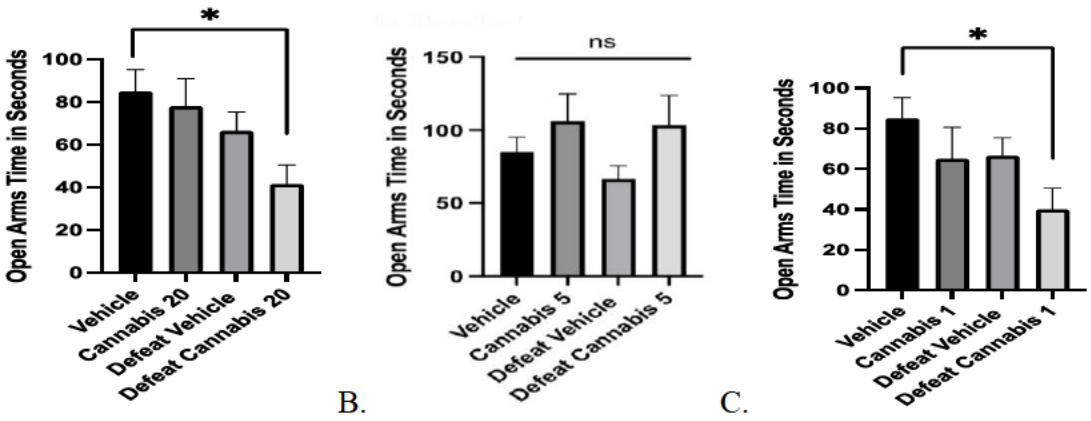
B: Bar graph showing the average social interaction ratio as measured by the social interaction test. Results showed that the defeated mice treated with Cannabis 5 mg had a significantly higher social interaction ratio compared to the defeated mice treated with vehicle. Statistical significance was measured by 2way Anova. Defeat: $F(1, 61) = 163.7$, $p<0.0001$ & Treatment: $F(1,61) = 101.6$, $p<0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 5 ($p=0.0053$). n number of control vehicle = 22, defeat vehicle = 23, control cannabis 5 and defeat cannabis 5 = 10.

C: Bar graph showing the average social interaction ratio as measured by the social interaction test. Results showed that the defeated mice treated with Cannabis 1 mg did not have a significantly higher social interaction ratio compared to the defeated mice treated with vehicle. Statistical significance was measured by 2way Anova. Defeat: $F(1, 62) = 64.78$, $p<0.0001$ & Treatment: $F(1,62) = 12.77$, $p < 0.0001$.

Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 1 ($p=0609$). n number of control vehicle = 22, defeat vehicle = 23, control cannabis 1 = 10 and defeat cannabis 1 = 12.

3.3. Cannabis shows anxiogenic effects in preventative treatment

The CSDS paradigm also induces anxiety-like behaviors in mice subjected to defeat (Golden et al., 2011). Following the cotreatment paradigm of CSDS, where mice are subjected to 10 days of defeat sessions along with i.p injections of either COE or vehicle, anxiety behaviors were measured by the EPM test on the 11th day. The time spent in the open arms was recorded by the ANY Maze program and then analyzed. Mice that exhibit anxiety-like behaviors are expected to spend more time in the closed arms, looking for a protected and safer area; whereas unaffected mice are expected spend more time exploring the open arms zones of the maze (Walf and Frye, 2007). Our results showed that the Lebanese COE did not prevent anxiety like behaviors in the defeat mice. In fact, COE administered at 5mg/Kg dose does not have any effect on anxiety-like behaviors ($p > 0.05$; *Figure 8.B*). Interestingly, defeat mice that received either 20mg/kg or 1 mg/kg of COE spend significantly less time in the open arms when compared to the control vehicle group ($p<0.05$; *Figure 8. A and C*). Our data indicates that the highest and lowest doses of COE injected along with the daily defeat sessions present anxiogenic effects while 5mg/Kg does not affect anxiety like behaviors in mice.



A. B. C.

Figure 8: The Lebanese Cannabis Oil Extract shows an anxiogenic effect in prophylactic treatments.

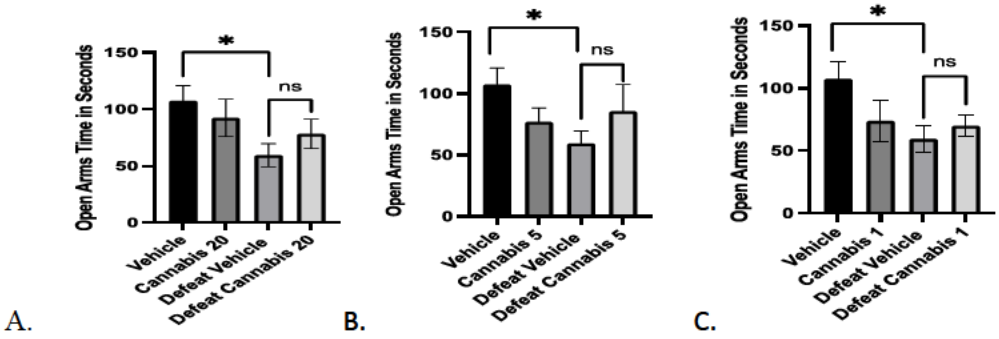
A: Bar graph showing the average open arms time in seconds as measured by the EPM test. Results showed that the defeated mice treated with Cannabis 20 mg spent significantly less time in the open arms compared to the control vehicle mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 71) = 120.9$, $p<0.0001$ & Treatment: $F(1,71) = 40.53$, $p=0.0003$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.4772$); vehicle vs Defeat Cannabis 20 ($p<0.0395$). n = 27 Vehicle, n = 24 Defeat Vehicle, n =12 Cannabis 20 & Defeat Cannabis 20.

B: Bar graph showing the average open arms time in seconds as measured by the EPM test. Results showed that the defeated mice treated with Cannabis 1 mg spent significantly less time in the open arms compared to the control vehicle mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 77) = 72.87$, $p<0.0001$ & Treatment: $F(77) = 82.32$, $p<0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0001$); Defeat vs Defeat Cannabis 1 ($p<0.3784$). n = 27 Vehicle, n = 24 Defeat Vehicle, n = 15 Cannabis 1 and n=14 Defeat Cannabis1.

3.4 Cannabis does not show anxiolytic properties

We also assessed the anxiety-like behaviors in the post treatment paradigm, following stress induction. As represented in the panel A of figure 6, the post treatment paradigm consists of inducing stress using the daily CSDS for a period of 10 days, followed by a social interaction test to segregate different groups according to their treatments. The Cannabis treatments were administered over a period of 14 days, after which a second round of behavioral testing was performed, followed by brain dissection and tissue collection. Here, we also used the EPM test to assess the effect of the cannabis treatment on anxiety-like behaviors in defeat mice. As expected, we saw that susceptible mice that received vehicle injections spent significantly less time in the open arms as compared to the control resilient mice ($p< 0.05$; *figure 9, A, B and C*). However, the three doses of the Lebanese cannabis oil extract, 20mg/kg, 5mg/kg

and 1mg/kg, did not affect anxiety like behaviors in susceptible mice in comparison to the defeat mice that received only vehicle solution (p>0.05; *figure 9 A, B and C*). This suggests that the Lebanese COE does not rescue anxiety traits in mice, hence it does not act as an anxiolytic.



A. B. C.

Figure 9: Cannabis treatment does not rescue anxiety-like behaviors.

A. Bar graph showing the average open arms time in seconds as measured by the EPM test. Results showed that the untreated defeated spent significantly less time in the open arms as compared to the control vehicle group. Defeated mice treated with Cannabis 20 mg did not differ from the untreated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 63) = 86.92$, $p<0.0001$ & Treatment: $F(1,63) = 0.3317$, $p = 0.05667$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0195$); Defeat vs Defeat Cannabis 20 ($p=0.7582$). n = 22 Vehicle, n = 23 Defeat Vehicle, n = 10 Cannabis 20 & n=12 Defeat Cannabis.

B. Bar graph showing the average open arms time in seconds as measured by the EPM test. Results showed that the untreated defeated spent significantly less time in the open arms as compared to the control vehicle group. Defeated mice treated with Cannabis 5 mg did not differ from the untreated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 61) = 28.6$, $p<0.0001$ & Treatment: $F(1,61) = 0.3496$, $p = 0.556$

Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p< 0.0264$); Defeat vs Defeat Cannabis 5 ($p = 0.6038$). n = 22 Vehicle, n = 23 Defeat Vehicle, n = 10 Cannabis 5 & n=10 Defeat Cannabis 5.

C. Bar graph showing the average open arms time in seconds as measured by the EPM test. Results showed that the untreated defeated spent significantly less time in the open arms as compared to the control vehicle group. Defeated mice treated with Cannabis 1 mg did not differ from the untreated defeated mice. Statistical significance was measured by 2way Anova. Defeat: $F(1, 62) = 64.78$, $p<0.0001$ & Treatment: $F(1,62) = 12.77$, $p < 0.0001$. Followed by Tukey's multiple comparison test; significance was measured versus the defeat group: Vehicle vs Defeat ($p<0.0129$); Defeat vs Defeat Cannabis 1 ($p=0.9322$). n = 22 Vehicle, n = 23 Defeat Vehicle, n = 10 Cannabis 1 & n=12 Defeat Cannabis 1.

Chapter Four
Discussion

The number of people encountering mental illnesses keeps on increasing and MDD has become one of the most commonly diagnosed mental disorder (APA, 2013). Underlying causes of MDD are not fully uncovered yet. The predisposition of an individual to develop psychiatric disorders such as MDD is mainly due to an interaction between environmental and genetic factors (Beck et al., 2016). Antidepressants remain the first-line treatment for moderate to major depression. However, numerous limitations have been revealed regarding their efficacy and long-term side effects (Kato et al., 2021). Hence, finding novel effective treatments with minimal side effects is primordial.

In Canada and the US, teenagers and young adults are relying on novel substances as mood boosters. For instance, THC and CBD, cannabinoids extracted from Cannabis, are being extensively used in youth and young adults. The cannabis compounds, also known as Marijuana, are known to provide a distressing, analgesic and soothing effect to its consumers. Its selling has been legalized in several countries and their consumption have significantly increased (Andre et al., 2016). In many cultures, THC and CBD have been widely prescribed for medical purposes in cancer patients as an anti-suffering drug in terminal phases and individuals diagnosed with neurological disorders such as multiple sclerosis, Huntington Disease, Alzheimer and Parkinson's Disease (Barrales-Cureño, H.J.; et al., 2020).

In Lebanon, cannabis has been cultivated unlawfully for decades until it was legalized for medical purposes (Shebaby et al.,2020). The beneficial therapeutic effects of cannabis in treating psychiatric disorders such as Generalized Anxiety Disorder or MDD are supported by a vast wealth of scientific resources (Bahji et al., 2022).

Therefore, the aim of this project was to assess the therapeutic effects of the Lebanese Cannabis Oil Extracts on depressive and anxious like behaviors in male mice. Indeed, our results provided evidence that the Lebanese COE possesses potential antidepressant properties and promotes resilience to chronic stress. Moreover, our results also show that Cannabis can have anxiogenic effects in high and low doses when administered as a preventative treatment whereas it does not have any specific effect on anxiety like behaviors when given as a curative treatment.

Several human and animal-based studies have shown the positive effects of the cannabinoids compounds on improving social avoidance behaviors and depression symptoms. Combined treatment of THC and CBD have effective and safe therapeutic effects in mental illnesses such as depression and schizophrenia. In fact, it is put forward that CBD inhibits THC psychoactive effects and improves symptoms of depression (Newton et al., 2020). In the brain, cannabinoids interact with the CNS through the CB1 receptors and activate the endocannabinoids signaling. The ECS exerts a major role in maintaining general body homeostasis, enhancing neuroplasticity and refining neuronal connections. The exogenous cannabinoids were shown to mediate their effect through the endocannabinoids system, hence improving emotion regulation and reducing stress response (Katona et al., 2012; Befort K. 2015).

Cannabis composed of high levels of THC and low CBD was correlated to major memory dysfunctions in addition to depression and anxiety symptoms (Morgan et al., 2012). Moreover, THC-induces impairments were reversed by CBD treatment in mice (Murphy M., et al., 2017). These studies support our results in the present study. Indeed, the COE, which naturally contains higher levels of CBD to THC (59.1%: 20.2 %), increased social interaction behaviors in the cotreatment protocol. This suggests that the Lebanese COE can be effective in preventing the onset of social stress and avoidance behaviors, which are one of the main symptoms related to MDD. Moreover, our treatment rescued depressive like behaviors in the post treatment paradigm by increasing the interaction time of treated defeated mice in comparison to the untreated defeated mice which reveals the effective antidepressant properties of the Lebanese cannabis crude oil.

In the second part of our study, we assessed the effects of the Lebanese COE on anxiety like behaviors. In fact, we found out that high levels (20mg/kg) and low levels (1mg/kg) of cannabis treatment triggered anxiety in the cotreatment paradigm of the CSDS. In the 5mg/kg dose, the treatment did not induce anxiety however, no preventative effect was noted. This suggests that Cannabis oil does not prevent the onset of anxiety like symptoms and can be anxiogenic when given at very high or very low doses. A possible reason to which high concentration of the Lebanese COE have induced anxiety like behaviors could be due to a more elevated THC level in the 20mg/kg doses in comparison to the 5mg/kg. Inhalation of Cannabis augmented anxiety behaviors in mice (Farra et al., 2020). In human studies, THC has been linked to anxiogenic response; high consumption of Δ^9-tetrahydrocannabinol can trigger paranoia, elevate anxiety and increase panic attacks (Sharpe et al., 2020).

However, why the middle dose did not have anxiogenic effect whereas the low concentration induced anxiety, is not very clear. Numerous work have mentioned the biphasic property of cannabis in treating anxiety (Braida et al., 2009). Studies have also indicated that the combination of low doses of CBD with THC leads to an intoxication effect whereas high doses of CBD attenuate this effect and reverse the intoxication induced by THC (Solowij et al., 2019). It is thought that CBD reverses the side effects of THC in many human-based and animal studies and that this mechanism could be dose dependent. This supports the fact that different doses of THC or CBD can show different effects. It is suggested that the cannabis effect on anxiety depends on the interaction of its different compounds within the brain: cannabis compounds that act on the GABAergic terminals induce anxiety like behaviors whereas cannabinoids interacting on the glutamatergic terminals could decrease anxiety behaviors and act an anxiolytic (Rey et al., 2012).

By the present study, we also reported by that cannabis treatment does not have anxiolytic properties in all administered doses (20mg/kg, 5mg/kg and 1mg/kg). This suggests that the Lebanese COE not rescue anxiety like behaviors and does not have anxiolytic effects. In fact, studies outcomes regarding the therapeutic effects of Cannabis on anxiety behaviors remain very contradictory. The lack of lucidity concerning cannabis consumption and its effects, is explained by either the heterogeneity of the cannabis compositions (Burggren et al., 2019) or the administration mode (Farra et al., 2020). Despite the wide range of papers targeting the relation between cannabis and anxiety, the mechanism of action behind the anxiogenic or anxiolytic effects of Cannabis remain unknown (Sharpe et al., 2020). Furthermore, treatments involving a combination of cannabis compounds in comparison to treatments with isolated compounds are crucial.

Indeed, the vast amount of chemicals reported in the cannabis plant have been found to have different effects, and to be less active when purified than when combined with other compounds (Namdar et al., 2019). Further behavioral research and molecular investigations on specific brain tissues targeting the proteins levels and epigenetic modifications remain necessary to address these questions and provide clearer and more accurate evidence.

Mental illnesses such as MDD are associated with major epigenetic modifications that can alter the brain structure and functions on the long term. In order to apprehend the mechanism of action of existing antidepressants such as SSRIs or potential new antidepressants such as Cannabis, we need to understand the pathogenesis of MDD. Concerning the different brain structures, the PFC, the amygdala and the hippocampus are suggested to be directly linked to MDD induced modifications (Trifu et al., 2020). Magnetic Resonance Imaging suggests a shrinkage of these brain areas in depressed patients as compared to healthy individuals (Palazidou et al., 2012). MDD is also known to reduce brain neuroplasticity which is the structural and functional modifications that occur in the nervous system in attempts to make adaptive changes (Fuchs & Flügge, 2014). Concerning epigenetic mechanisms, DNA methylation is a commonly studied epigenetic modification occurring in the depressed brain. Indeed, deoxyribonucleic acid methylation increases vulnerability to psychopathological illnesses (Carbadello et al., 2012).

It is still adopted that depression is mainly explained by a decreased level of monoamine in the brain such as 5-HT, dopamine and noradrenaline (Tifu et al., 2020). Hence antidepressants such as SSRIs and SNRIs exert their effect by increasing the levels of the neurotransmitters and enhance the synaptic transmission to improve depressive symptoms

(Chai et al., 2016). More studies have outlined that the activation of 5-HT1a receptors mediates the antidepressant effect of CBD (Sartim et al., 2016). To further support this evidence, it has been shown that the administration of 5-HT1a antagonists blocked the antidepressant effect of CBD, suggesting that the positive therapeutic effect is achieved through an enhancement of the serotonin neurotransmission. This is succeeded by an activation of post synaptic 5-HT1a receptors (Sartim et al., 2016). Moreover, an elevated extracellular level of Serotonin (5-HT) in the ventromedial prefrontal cortex was correlated with an acute intake of CBD (Linge et al., 2016). Another study elucidated that chronic high doses of CBD augmented levels of serotonin in the hippocampus. Moreover, 30mg/kg and 100mg/kg of CBD treatment were found to have antidepressant effects in mice which is mediated through a downregulation of NF-kB in the hippocampus. Therefore, NF-kB holds a major role in mediating antidepressants effects (Caviedes et al.,2017). This suggests that CBD may be mediating its effect through the NF-kB signaling pathway (Abame et al., 2021).

BDNF is greatly involved in the neurobiology of depression. Antidepressant drugs and neurotransmitters functions are highly correlated to BDNF (Palazidou et al. 2012). BDNF is enriched in the hippocampus and is crucial for neuronal survival, maturation and plasticity. BDNF is downregulated in the hippocampus and PFC of depressed brains and antidepressants increase their levels and alleviate depressive symptoms. Studies on post-mortem brains revealed that hippocampal BDNF levels were more elevated in treated patients than untreated patients diagnosed with MDD supporting the fact that antidepressants elevate BDNF levels in specific brain parts and improve symptoms (Trifu et al., 2020). An interesting number of studies have mentioned the synergy between the ECS and BDNF in invitro and invivo (Maison et al., 2009; De Chiara et al., 2010; Galve-

Roperh et al., 2013; Zhao et al., 2015). A study has elucidated that a genetic aberration of CB1R is correlated with a reduced expression of BDNF. On the contrary, a protective trait of the CB1R is correlated to an induction of BDNF expression (Aso et al., 2018). Moreover, THC upregulates BDNF in mice brain (Butovsky et al., 2005) and downregulates BDNF expression in plasma of cannabis consumers compared to non-users (Lisano et al., 2020).

With the above-mentioned data and studies, we provide several possible molecular pathways through which the Lebanese COE might be mediating its antidepressant effects. Further research is essentially required in order to clarify the mechanisms of actions and the neuronal pathways involved in the promising behavioral results reported in the present study.

Chapter Five
Conclusion

The target of this study was to analyze the behaviors of mice and to evaluate the therapeutic effect of the Lebanese COE. We showed promising results regarding the antidepressant effect of the Lebanese COE. Our treatment boosted resilience to chronic stress and rescued depressive like behaviors in male mice. However, the Lebanese COE can be anxiogenic in preventative treatments and does not show anxiolytic properties. The lack of consistency among researches concerning the impact of cannabis on anxiety may be due to the heterogeneity of the cannabis plant, to different rodent species used across studies or to the adopted cannabis administration methods. Extensive molecular examination is needed in order to understand the underlying mechanisms behind which the Lebanese COE is mediating its antidepressant effects. Identifying which neuronal pathways are implicated in the cannabinoids effect is crucial in order to better elucidate the clinical impact and uses of cannabis compounds in the treatment or prevention of mental illnesses such as MDD.

Bibliography

Abrams, D. I., Jay, C. A., Shade, S. B., Vizoso, H., Reda, H., Press, S., ... & Petersen, K.L. (2007). Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo controlled trial. *Neurology*, *68*(7), 515-521.

Absollhi, M. (Ed.). (2014). *Encyclopedia of toxicology*. Elsevier.

Andre, C. M., Hausman, J. F., & Guerriero, G. (2016). Cannabis sativa: the plant of the thousand and one molecules. *Frontiers in plant science*, *7*, 19.

Aran, A., Cassuto, H., Lubotzky, A., Wattad, N., & Hazan, E. (2019). Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems a retrospective feasibility study. *Journal of Autism and Developmental Disorders*, *49*(3), 1284-1288.

Arumugam, V., John, V. S., Augustine, N., Jacob, T., Joy, S. M., Sen, S., & Sen, T. (2017). The impact of antidepressant treatment on brain-derived neurotrophic factor level: An evidence-based approach through systematic review and meta-analysis. *Indian journal of pharmacology*, *49*(3), 236.

Aso, E., Ozaita, A., Valdizán, E. M., Ledent, C., Pazos, A., Maldonado, R., & Valverde, O. (2008). BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. *Journal of neurochemistry*, *105*(2), 565-572.

Asselin, A., Lamarre, O. B., Chamberland, R., Mc Neil, S. J., Demers, É., & Zongo, A. (2021). A description of self-medication with cannabis among adults with legal access to cannabis.

Barrales-Cureño, H. J., López-Valdez, L. G., Reyes, C., Cetina-Alcalá, V. M., Vasquez-García, I., Diaz-Lira, O. F., & Herrera-Cabrera, B. E. (2020). Chemical characteristics, therapeutic uses, and legal aspects of the cannabinoids of Cannabis sativa: a review. *Brazilian Archives of Biology and Technology*, *63*.

Bains, N., & Abdijadid, S. (2022). Major depressive disorder. In *StatPearls [Internet]*. StatPearls Publishing.

Burggren, A. C., Shirazi, A., Ginder, N., & London, E. D. (2019). Cannabis effects on brain structure, function, and cognition: considerations for medical uses of cannabis and its derivatives. *The American journal of drug and alcohol abuse*, *45*(6), 563-579.

Basavarajappa, B. S., Shivakumar, M., Joshi, V., & Subbanna, S. (2017). Endocannabinoid system in neurodegenerative disorders. *Journal of neurochemistry*, *142*(5), 624-648.

Bahji, A., Breward, N., Duff, W., Absher, N., Patten, S. B., Alcorn, J., & Mousseau, D. D. (2022). Cannabinoids in the management of behavioral, psychological, and motor symptoms of neurocognitive disorders: a mixed studies systematic review. *Journal of Cannabis Research*, *4*(1), 1-19.

Beck, A. T., & Bredemeier, K. (2016). A unified model of depression: Integrating clinical, cognitive, biological, and evolutionary perspectives. *Clinical Psychological Science*, *4*(4), 596-619.

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

Bilge, S., & Ekici, B. (2021). CBD-enriched cannabis for autism spectrum disorder: an experience of a single center in Turkey and reviews of the literature. *Journal of cannabis research*, *3*(1), 1-11.

Braund, T. A., Tillman, G., Palmer, D. M., Gordon, E., Rush, A. J., & Harris, A. W. (2021). Antidepressant side effects and their impact on treatment outcome in people with major depressive disorder: an iSPOT-D report. *Translational psychiatry*, *11*(1), 1-8.

Braida, D., Capurro, V., Zani, A., Rubino, T., Viganò, D., Parolaro, D., & Sala, M. (2009). Potential anxiolytic-and antidepressant-like effects of salvinorin A, the main active ingredient of Salvia divinorum, in rodents. *British journal of pharmacology*, *157*(5), 844-853.

Bridgeman, M. B., & Abazia, D. T. (2017). Medicinal cannabis: history, pharmacology, and implications for the acute care setting. *Pharmacy and therapeutics*, *42*(3), 180.

Cascade, E., Kalali, A. H., & Kennedy, S. H. (2009). Real-world data on SSRI antidepressant side effects. *Psychiatry (Edgmont)*, *6*(2), 16.

Butovsky, E., Juknat, A., Goncharov, I., Elbaz, J., Eilam, R., Zangen, A., & Vogel, Z. (2005). In vivo up-regulation of brain-derived neurotrophic factor in specific brain areas by chronic exposure to Δ9-tetrahydrocannabinol. *Journal of neurochemistry*, *93*(4), 802-811.

Cascade, E., Kalali, A. H., & Kennedy, S. H. (2009). Real-world data on SSRI antidepressant side effects. *Psychiatry (Edgmont)*, *6*(2), 16.

Carballedo, A., Lisiecka, D., Fagan, A., Saleh, K., Ferguson, Y., Connolly, G., ... & Frodl, T. (2012). Early life adversity is associated with brain changes in subjects at family risk for depression. *The World Journal of Biological Psychiatry*, *13*(8), 569-578.

Caviedes, A., Lafourcade, C., Soto, C., & Wyneken, U. (2017). BDNF/NF-κB signaling in the neurobiology of depression. *Current pharmaceutical design*, *23*(21), 3154-3163.

Chai, G. S., Wang, Y. Y., Yasheng, A., & Zhao, P. (2016). Beta 2-adrenergic receptor activation enhances neurogenesis in Alzheimer's disease mice. *Neural regeneration research*, *11*(10), 1617.

Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biological psychiatry*, *50*(4), 260-265.

Cleare, A., Pariante, C. M., Young, A. H., Anderson, I. M., Christmas, D., Cowen, P. J., ... & members of the Consensus Meeting. (2015). Evidence-based guidelines for treating depressive disorders with antidepressants: a revision of the 2008 British Association for Psychopharmacology guidelines. *Journal of Psychopharmacology*, *29*(5), 459-525.

Cuijpers, P., Dekker, J., Hollon, S. D., & Andersson, G. (2009). Adding psychotherapy to pharmacotherapy in the treatment of depressive disorders in adults: a meta-analysis. *The Journal of clinical psychiatry*, *70*(9), 401.

Cunha, A. B., Frey, B. N., Andreazza, A. C., Goi, J. D., Rosa, A. R., Gonçalves, C. A., ... & Kapczinski, F. (2006). Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neuroscience letters*, *398*(3), 215-219.

Hasin, D. S., Sarvet, A. L., Meyers, J. L., Saha, T. D., Ruan, W. J., Stohl, M., & Grant, B. F. (2018). Epidemiology of adult DSM-5 major depressive disorder and its specifiers in the United States. *JAMA psychiatry*, *75*(4), 336-346.

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene–environment interactions, and epigenetics. *Experimental neurology*, *233*(1), 102-111.

Alliance, D. P. (2011). Drug policy alliance and global commission on drug policy June 2011 media report.

Stratton, K., Kwan, L. Y., & Eaton, D. L. (2018). Public health consequences of e-cigarettes: consensus study report. *Public health consequences of e-cigarettes: consensus study report.*

De Chiara, V., Angelucci, F., Rossi, S., Musella, A., Cavasinni, F., Cantarella, C., & Centonze, D. (2010). Brain-derived neurotrophic factor controls cannabinoid CB1 receptor function in the striatum. *Journal of Neuroscience*, *30*(24), 8127-8137.

Devinsky, O., Marsh, E., Friedman, D., Thiele, E., Laux, L., Sullivan, J., ... & Cilio, M. R. (2016). Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *The Lancet Neurology*, *15*(3), 270-278.

El Hayek, L., Khalifeh, M., Zibara, V., Abi Assaad, R., Emmanuel, N., Karnib, N., ... & Sleiman, S. F. (2019). Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF). *Journal of Neuroscience*, *39*(13), 2369-2382.

ElSohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life sciences*, *78*(5), 539-548.

ElSohly, M. A., Radwan, M. M., Gul, W., Chandra, S., & Galal, A. (2017). Phytochemistry of Cannabis sativa L. *Phytocannabinoids*, 1-36.

Farra, Y. M., Eden, M. J., Coleman, J. R., Kulkarni, P., Ferris, C. F., Oakes, J. M., & Bellini, C. (2020). Acute neuroradiological, behavioral, and physiological effects of nose-only exposure to vaporized cannabis in C57BL/6 mice. *Inhalation toxicology*, *32*(5), 200-217.

Funk, M. (2016). Global burden of mental disorders and the need for a comprehensive, coordinated response from health and social sectors at the country level. *Retrieved on*, *30*.

Freeman, T. P., Hindocha, C., Green, S. F., & Bloomfield, M. A. (2019). Medicinal use of cannabis based products and cannabinoids. *Bmj*, *365*.

Galve-Roperh, I., Chiurchiù, V., Díaz-Alonso, J., Bari, M., Guzmán, M., & Maccarrone, M. (2013). Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. *Progress in lipid research*, *52*(4), 633-650.

Goggi, J., Pullar, I. A., Carney, S. L., & Bradford, H. F. (2002). Modulation of neurotransmitter release induced by brain-derived neurotrophic factor in rat brain striatal slices in vitro. *Brain research*, *941*(1-2), 34-42.

Govindarajan, A., Rao, B. S., Nair, D., Trinh, M., Mawjee, N., Tonegawa, S., & Chattarji, S. (2006). Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proceedings of the National Academy of Sciences*, *103*(35), 13208-13213.

Jiang, B., Wang, F., Yang, S., Fang, P., Deng, Z. F., Xiao, J. L., ... & Chen, J. G. (2015). SKF83959 produces antidepressant effects in a chronic social defeat stress model of depression through BDNF-TrkB pathway. *International Journal of Neuropsychopharmacology*, *18*(6).

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., & Woodgett, J. R. (2011). Assessment of social interaction behaviors. *JoVE (Journal of Visualized Experiments)*, (48), e2473.

Kato, M., Hori, H., Inoue, T., Iga, J., Iwata, M., Inagaki, T., ... & Tajika, A. (2021). Discontinuation of antidepressants after remission with antidepressant medication in major depressive disorder: a systematic review and meta-analysis. *Molecular psychiatry*, *26*(1), 118-133.

Katona, I., & Freund, T. F. (2012). Multiple functions of endocannabinoid signaling in the brain. *Annual review of neuroscience*, *35*, 529.

Kocsis, R. N. (2013). Book review: diagnostic and statistical manual of mental disorders: (DSM-5).

Lisano, J. K., Kisiolek, J. N., Smoak, P., Phillips, K. T., & Stewart, L. K. (2020). Chronic cannabis use and circulating biomarkers of neural health, stress, and inflammation in physically active individuals. *Applied Physiology, Nutrition, and Metabolism*, *45*(3), 258-263.

Lu, H. C., & Mackie, K. (2021). Review of the endocannabinoid system. *Biological Psychiatry: Cognitive Neuroscienc*

Machado Rocha, F. C., Stefano, S. C., De Cassia Haiek, R., Rosa Oliveira, L. M. Q., & Da Silveira, D. X. (2008). Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *European journal of cancer care*, *17*(5), 431-443.

Maison, P., Walker, D. J., Walsh, F. S., Williams, G., & Doherty, P. (2009). BDNF regulates neuronal sensitivity to endocannabinoids. *Neuroscience letters*, *467*(2), 90-94.

Malone, D. T., Hill, M. N., & Rubino, T. (2010). Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. *British journal of pharmacology*, *160*(3), 511-522.

McPartland, J. M., Guy, G. W., & Di Marzo, V. (2014). Care and feeding of the endocannabinoid system: a systematic review of potential clinical interventions that upregulate the endocannabinoid system. *PloS one*, *9*(3), e89566.

Morgan, C. J., & Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *The British Journal of Psychiatry*, *192*(4), 306-307.

Mouri, A., Sasaki, A., Watanabe, K., Sogawa, C., Kitayama, S., Mamiya, T., ... & Nabeshima, T. (2012). MAGE-D1 regulates expression of depression-like behavior through serotonin transporter ubiquitylation. *Journal of Neuroscience*, *32*(13), 4562-4580.

Murphy, M., Mills, S., Winstone, J., Leishman, E., Wager-Miller, J., Bradshaw, H., & Mackie, K. (2017). Chronic adolescent Δ9-tetrahydrocannabinol treatment of male mice leads to long-term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. *Cannabis and cannabinoid research*, *2*(1), 235-246.

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., ... & Mathew, S. J. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *American Journal of Psychiatry*, *170*(10), 1134-1142.

Morgan, C. J. A., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T. P., ... & Curran, H. V. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological medicine*, *42*(2), 391-400.

Nabeshima, T., & Kim, H. C. (2013). Involvement of genetic and environmental factors in the onset of depression. *Experimental neurobiology*, *22*(4), 235.

Namdar, D., Voet, H., Ajjampura, V., Nadarajan, S., Mayzlish-Gati, E., Mazuz, M., ... & Koltai, H. (2019). Terpenoids and phytocannabinoids co-produced in Cannabis sativa strains show specific interaction for cell cytotoxic activity. *Molecules*, *24*(17), 3031.

Newton, M., & Newton, D. W. (2020). Cannabidiol or CBD Oil: Help, Hope, and Hype for Psychiatric and Neurologic Conditions. *Journal of the American Psychiatric Nurses Association*, *26*(5), 447-457.

Notaras, M., Hill, R., & van den Buuse, M. (2015). The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Molecular psychiatry*, *20*(8), 916-930.

Ogle, W. L., Gold, G. J., Coppen, L. E., & Copriviza, C. (2022). How and why adults use cannabis during physical activity. *Journal of Cannabis Research*, *4*(1), 1-10.

Palazidou, E. (2012). The neurobiology of depression. *British medical bulletin*, *101*(1), 127-145.

Rey, A. A., Purrio, M., Viveros, M. P., & Lutz, B. (2012). Biphasic effects of cannabinoids in anxiety responses: CB1 and GABAB receptors in the balance of GABAergic and glutamatergic neurotransmission. *Neuropsychopharmacology*, *37*(12), 2624-2634.

Linge Méndez, R., Jiménez Sánchez, L., Campa, L., Pilar Cuéllar, M. F., Vidal Casado, R., Pazos Carro, Á., ... & Díaz Martínez, Á. (2016). Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: role of 5-HT1A receptors.

Sales, A. J., Fogaça, M. V., Sartim, A. G., Pereira, V. S., Wegener, G., Guimarães, F. S., & Joca, S. R. (2019). Cannabidiol induces rapid and sustained antidepressant-like effects through increased BDNF signaling and synaptogenesis in the prefrontal cortex. *Molecular neurobiology*, *56*(2), 1070-1081.

Sartim, A. G., Guimarães, F. S., & Joca, S. R. L. (2016). Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex—Possible involvement of 5-HT1A and CB1 receptors. *Behavioural brain research*, *303*, 218-227.

Schleicher, E. M., Ott, F. W., Müller, M., Silcher, B., Sichler, M. E., Löw, M. J., ... & Bouter, Y. (2019). Prolonged cannabidiol treatment lacks on detrimental effects on memory, motor performance and anxiety in C57BL/6J mice. *Frontiers in behavioral neuroscience*, *13*, 94.

Schreiber, S., Bader, M., Lenchinski, T., Meningher, I., Rubovitch, V., Katz, Y., ... & Pick, C. G. (2019). Functional effects of synthetic cannabinoids versus Δ9-THC in mice on body temperature, nociceptive threshold, anxiety, cognition, locomotor/exploratory parameters and depression. *Addiction Biology*, *24*(3), 414-425.

Sharpe, L., Sinclair, J., Kramer, A., de Manincor, M., & Sarris, J. (2020). Cannabis, a cause for anxiety? A critical appraisal of the anxiogenic and anxiolytic properties. *Journal of translational medicine*, *18*(1), 1-21.

Shebaby, W., Saliba, J., Faour, W. H., Ismail, J., El Hage, M., Daher, C. F., ... & Mroueh, M. (2021). In vivo and in vitro anti-inflammatory activity evaluation of Lebanese Cannabis sativa L. ssp. indica (Lam.). *Journal of Ethnopharmacology*, *270*, 113743.

Shrivastava, A., Kuzontkoski, P. M., Groopman, J. E., & Prasad, A. (2011). Cannabidiol Induces Programmed Cell Death in Breast Cancer Cells by Coordinating the Cross-talk between Apoptosis and AutophagyCBD Induces Programmed Cell Death in Breast Cancer Cells. *Molecular cancer therapeutics*, *10*(7), 1161-1172.

Solowij, N., Broyd, S., Greenwood, L. M., van Hell, H., Martelozzo, D., Rueb, K., ... & Croft, R. (2019). A randomised controlled trial of vaporised Δ9-tetrahydrocannabinol and cannabidiol alone and in combination in frequent and infrequent cannabis users: acute intoxication effects. *European archives of psychiatry and clinical neuroscience*, *269*(1), 17-35.

Stuyt, E. (2018). The problem with the current high potency THC marijuana from the perspective of an addiction psychiatrist. *Missouri medicine*, *115*(6), 482.

Trifu, S. C., Trifu, A. C., Aluaş, E., Tătaru, M. A., & Costea, R. V. (2020). Brain changes in depression. *Romanian Journal of Morphology and Embryology*, *61*(2), 361.

Turner, C. E., Mole, M. L., Hanus, L., & Elsohly, H. N. (1981). Isolation of a new cannabinoid from an Indian variant of Cannabis sativa L. *Activitas nervosa superior*, *23*(4), 266-267.

Weissman, M. M., Wickramaratne, P., Nomura, Y., Warner, V., Pilowsky, D., & Verdeli, H. (2006). Offspring of depressed parents: 20 years later. *American journal of Psychiatry*, *163*(6), 1001-1008.

Xu, C., Chang, T., Du, Y., Yu, C., Tan, X., & Li, X. (2019). Pharmacokinetics of oral and intravenous cannabidiol and its antidepressant-like effects in chronic mild stress mouse model. *Environmental Toxicology and Pharmacology*, *70*, 103202.

Yu, H., & Chen, Z. Y. (2011). The role of BDNF in depression on the basis of its location in the neural circuitry. *Acta Pharmacologica Sinica*, *32*(1), 3-11.

Zamberletti, E., Gabaglio, M., & Parolaro, D. (2017). The endocannabinoid system and autism spectrum disorders: insights from animal models. *International journal of molecular sciences*, *18*(9), 1916.

Zanelati, T. V., Biojone, C., Moreira, F. A., Guimarães, F. S., & Joca, S. R. L. (2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *British journal of pharmacology*, *159*(1), 122-128.

Zhang, H. Y., Shen, H., Gao, M., Ma, Z., Hempel, B. J., Bi, G. H., ... & Xi, Z. X. (2021). Cannabinoid CB2 receptors are expressed in glutamate neurons in the red nucleus and functionally modulate motor behavior in mice. *Neuropharmacology*, *189*, 108538.

Zhao, L., Yeh, M. L. W., & Levine, E. S. (2015). Role for endogenous BDNF in endocannabinoid-mediated long-term depression at neocortical inhibitory synapses. *Eneuro*, *2*(2).