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Potential Therapeutic Effects of Lebanese Cannabis oil in Female Mouse Models of Neuropsychiatric Disorders

By

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DEDICATION

I would like to dedicate my thesis work to the Children Cancer Center of Lebanon (CCCL)

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Potential Therapeutic Effects of Lebanese Cannabis oil in Female Mouse Models of Neuropsychiatric Disorders

Fatina Hatoum

ABSTRACT

The Cannabis plant is the most versatile herbal remedy used for the treatment of various medical conditions, including pain, epilepsy, depression, anxiety, and post-traumatic stress disorder. Stress is a normal part of life, and stress responses can vary in different people. Stress-susceptible individuals become prone to depression, while resilient individuals lead a normal life. Limited literature is available about the pharmacological effects of Lebanese Cannabis oil on depression and anxiety disorders. Accordingly, the current research study examined the therapeutic effects of Cannabis oil extracted from the Lebanese Cannabis sativa plant on resilience to stress and its ability to rescue depression and anxiety-like behaviors in female animal models. Pre-treatment and post-treatment protocols were carried out using Cannabis oil to evaluate its anti-depressive effects. In the pre-treatment protocol, female C57BL/6 mice received daily injections of Cannabis oil (5 mg/kg and 20 mg/kg) for five days before and during the induction of depression-like behavior by using several types of stressors for nine days. At the end of the stress paradigm, the animal behavioral was assessed using the social interaction (SI) test. In the post-treatment protocol, female C57BL/6 mice were subjected to daily Chronic Variable Stress (CVS) for nine days. On day 10, the behavior of the mice was assessed

using the SI test and animals were divided into susceptible or resilient to stress. Only animals that were classified as susceptible to stress received vehicle or Lebanese Cannabis oil i.p injection treatment (doses of 5 mg/kg and 20 mg/kg). The results showed that intraperitoneal injections of Lebanese Cannabis oil at doses of 5 mg/kg and 20 mg/kg rescued depression-like behaviors induced by the CVS. Therefore, Cannabis oil significantly rescues social avoidance behavior and promotes social interaction. These promising findings call for additional studies to gain a better understanding of the potential effect of Lebanese Cannabis oil on stress and anxiety disorders by studying the effects of whole plant or its major phytocannabinoids, Cannabidiol (CBD) and/or tetrahydrocannabinol (THC).

Keywords: Lebanese Cannabis oil, Chronic Variable Stress, Social Interaction, Anxiety, Elevated Plus Maze, Open Field, Depression.

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List of Abbreviations

CB1R cannabinoid CB1 receptor

CB2R cannabinoid CB2 receptor

CBC cannabichromene

CBD cannabidiol

CBE cannabielsoin

CBG cannabigerol

CBL cannabicyclol

CBN cannabinol

CBND cannabinodiol

CBT cannabitriol

CNS central nervous system

COE Cannabis oil extract

CVS chronic variable stress

ECS endocannabinoid system

EPM elevated plus maze

FAAH fatty acid amide hydrolase

GPCR G-protein-coupled receptor

i.p. intraperitoneal

MDD major depressive disorder

MS Multiple Sclerosis

OF open field

PTSD Post-Traumatic Stress Disorder

THC Δ9 -tetrahydrocannabinol

Chapter One

Introduction

1.1 Neuropsychiatric Disorders

Depression is a prominent cause of disability worldwide (Smith, 2014). Major depressive disorder (MDD) is a mental illness described by persistent low mood and a lack of interest or pleasure in enjoyable activities (Feingold & Weinstein, 2020). Furthermore, limited access to mental health care services as well as pharmaceutical and psychological therapies is a critical concern for individuals with mental health problems. Accordingly, their reluctance to seek treatment due to fear of being judged by family, friends and the community remains an impediment to achieving the optimal level of mental health and well-being (García-Gutiérrez et al., 2020a). In 2010, MDD was the most debilitating mental condition globally, responsible for more than 40% of disorder-adjusted life years; a measure of premature death and impairment caused by mental disease (Whiteford et al., 2013). These psychiatric disorders show a high incidence, resulting in a significant deterioration in the quality of life and disruptions in work/school performance, family/social life, and regular daily duties (Jaeschke et al., 2021).

Different psychiatric diseases exhibit similar symptoms and show significant comorbidity, making an appropriate diagnosis complicated. In addition to these issues, current pharmaceutical and psychological therapy options are ineffective, particularly in cases of medium-to high-severity (Chen & Shan, 2019; Maroney, 2020).

Research study has been conducted to investigate the causes and development of mental diseases, and to discover prospective biomarkers to diagnose conditions as well as for pharmaceutical drug development (Hurko, 2009). The therapeutic approach and the introduction of innovative technical tools, such as neuroimaging techniques, are currently booming advancements (Machado-Vieira, 2012). Such findings have led to the development and marketing of drugs such as Esketamine for treatment-resistant depression in adults in the USA and Europe (Daly et al., 2017; Daly et al., 2019). The risk-benefit ratio of antidepressant medications is influenced by side effects such as weight gain, loss of sexual desire, cardiovascular complications, and many others. As a result, new pharmacological remedies must be developed to enhance treatment outcomes for such psychiatric diseases while alleviating the severity of their side effects (García-Gutiérrez et al., 2020b).

1.2 Cannabis and Neuropsychiatric Disorders

Recently, extensive research demonstrated the therapeutic potential of Cannabis in the treatment of several ailments (Borowska et al., 2018). Cannabis-derived components are recognized for their antioxidant, anti-inflammatory, and antinecrotic properties. Additionally, the potential benefits of Cannabis and Cannabinoids has been demonstrated in treating Parkinson's disease, epilepsy, depression, anxiety disorders, schizophrenia, and chronic pain (Campos et al., 2016a). The most prominent constituents of the Cannabis plant are Cannabidiol (CBD) and Tetrahydrocannabinol (THC). The latter is responsible for the psychoactive effect, while CBD lacks this property which could be effective in the treatment of depression and anxiety (Russo et al., 2005). Pain management is a very common use of Cannabis products. Indeed, it has been shown that 9 -THC may

alleviate both acute and chronic pain (ElSohly & Gul, 2014). CBD is the principal non-psychoactive cannabinoid found in Cannabis (especially hemp), which was extracted from Mexican marijuana for the first time (Adams et al., 1940) for its anxiolytic, antipsychotic, and neuroprotective properties. Additionally, there is an indication that CBD might be used for the treatment of post-traumatic stress disorder, depression, bipolar disorder, sleep disturbances, drug abuse and addiction, schizophrenia, social phobia, and Parkinson's disease (Crippa et al., 2018). In addition to CBD and THC and other cannabinoids, other important compounds such as terpenoids, flavonoids, and alkaloids are present in the Cannabis plant, potentiate its pharmacological activities, and may have an impact on a patient's wellbeing (Bonini et al., 2018a).

1.3 Pre-Clinical Studies on Cannabis as an Antidepressant

Cannabis has been proven in pre-clinical studies to be therapeutically efficient in the treatment of depression (Scherma et al., 2018). The agonistic effect of cannabinoids on the central CB1 receptors (CB1Rs) may facilitate this effect and the CB1R regulation of antidepressant-like effects has been investigated (Shearman et al., 2003). According to the latter study, mice were injected with the CB1R inverse agonist AM251 and evaluated their behavior in the tail suspension test (TST) and the forced swim test (FST). In both trials, AM251 considerably reduced immobility without increasing motor activity in the open field and demonstrated an antidepressant effect. Inverse cannabinoid agonism of CB1R may therefore be useful for mood modulation. Pre-clinical animal studies reveal that CBD has therapeutic benefits, such as repairing cognitive deficiencies in Alzheimer's disease mouse models (Cheng et al., 2014), and attenuating 9-THC's detrimental impact on cognition in rhesus monkeys (Jacobs et al., 2016). CBD is beneficial in animal models

of antidepressant effect prediction. In animal studies, CBD promotes both immediate and sustained antidepressant effects. Its potential to interact with numerous neurotransmitter systems implicated in depression, including the serotonergic, glutamatergic, and endocannabinoid systems, appears to be responsible for this effect (Calapai et al., 2019). Animal models suggest a therapeutic potential for Δ9 -THC in several conditions. For instance, Δ9 -THC reduced inflammation and in-vitro motility disturbances in rat colitis. These studies suggest that Δ9 –THC has a biphasic effect on anxiety-like behaviors, with low doses alleviating anxiety while high doses produce anxiogenic effect (Hill et al., 2018a)**.**

1.4 Clinical Studies on Cannabis as a potential antidepressant

Cannabis is commonly used as a self-medication for depression and manic symptoms among Cannabis users (Ashton, C. H. et al., 2005; Grinspoon & Bakalar, 1998). People who have used Cannabis regularly or even daily have fewer depressive symptoms than those who have never tried it (Denson & Earleywine, 2006). Depressed patients have used Cannabis to enhance the quality of their sleep (Babson et al., 2013). Cannabis has been shown in seven cross-sectional studies to enhance depressive mood (Walsh et al., 2017). Among Cannabinoids, the non-

psychoactive phytocannabinoid, CBD is currently the best hope for the treatment of refractory epileptic seizures. Its potent anti-convulsant properties have been thoroughly proven in *in-vitro* and *in-vivo* human studies. Consequently, on August 3rd, 2020, FDA approved an oral solution produced entirely from Cannabis plants **Epidiolex**® for the treatment of pediatric epileptic disorders such as Dravet syndrome and Lennox–Gastaut syndrome in patients aged two years and older (Abu-Sawwa et al., 2020; Szaflarski et al., 2018). This approval has accelerated research into its potential application for additional disorders including anxiety and depression. Cannabinoids have been studied in Post-Traumatic Stress Disorder (PTSD) patients to determine their impact on stress-related biological processes and their possible therapeutic application for stress-related psychopathology (Hill et al., 2018b). Indeed, the US Food and Drug Administration has approved two synthetic analogs of 9 -THC in the form of capsules or as an oral solution that can be prescribed for chemotherapy-induced nausea and vomiting and for the treatment of anorexia associated with weight loss in patients with AIDS (Acquired Immunodeficiency Syndrome). Such approved drugs are **Nabilone** (Valeant Pharmaceuticals North America's Cesamet) and **Dronabinol** (Marinol®, AbbVie Inc.; Syndros®, ®, Insys Therapeutics Inc Solvay Pharmaceuticals) (Murillo-Rodriguez et al., 2020a). In addition, **Nabiximols** (Sativex, GW Pharmaceuticals) containing about equal levels of 9 -THC and CBD, is available as a sublingual spray for the alleviation of Multiple Sclerosis (MS) or cancer pain as well as to lessen MS spasticity (Mechoulam, Raphael et al., 2014). As a result, the majority of scientific evidence has

concentrated on cannabis's ability to modulate pain perception, anxiety, memory, learning, and depression.

1.5 Cannabis Plant: Versatile Herbal Remedy

1.5.1 Introduction to Cannabis sativa L. ssp. Indica

Cannabis is a botanical genus composed of three species (*C. Sativa, C. Indica, and C. Ruderalis*) that are generally distinguished by their genetic and chemical ability to generate more or less of the two primary phytocannabinoids: THC and CBD. The Cannabis plant is a unique, rich plant in over 554 components including 113 phytocannabinoids (Ahmed et al., 2015) and 120 terpenes (ElSohly & Slade, 2005). Cannabis sativa (or hemp) is an herbaceous plant belonging to the Cannabaceae family and is extensively grown worldwide. Most of our current understanding of the neuro-molecular mechanisms of Cannabis action focusses on the major cannabinoids (THC and CBD). These Phyto-cannabinoids work by binding to the cannabinoid receptors, as well as other receptor systems. Therefore, THC-rich species are utilized for recreational and

therapeutic purposes, whereas species with low THC concentration and high CBD content are cultivated to produce seeds and fiber, as well as for medicinal purposes (Andre et al., 2016a; Hillig, 2005).

1.5.2 The Endocannabinoid System

The Endocannabinoid system (ECS) is a global neuromodulatory system that plays a crucial role in CNS development, synaptic plasticity, and the response to endogenous and environmental stresses (Lu & Mackie, 2015). Extensive studies have demonstrated the therapeutic benefits of molecules targeting the ECS (Morales & Reggio, 2020). Therefore, the function of the ECS in the central nervous system has been the subject of considerable investigation. ECS is comprised of cannabinoid receptors (mainly CB1 and CB2), their ligands (the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG)), and the enzymes responsible for their biosynthesis and degradation (fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)) (Ranieri et al., 2016).

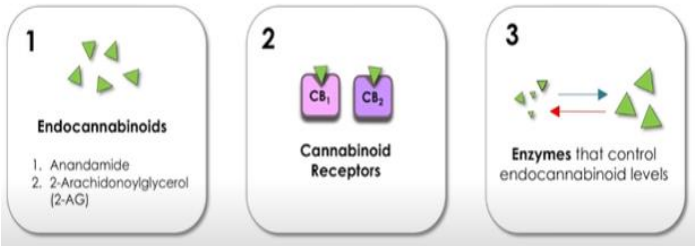


Figure 1: Components of the endocannabinoid system

The two main cannabinoid receptors CB1 and CB2 have been identified as two G-protein-coupled receptors (GPCRs) with unique distribution in the human body. CB1 receptors are mostly concentrated in the central nervous system, while CB2 receptors are primarily located in the immune system as well as other organs and tissues (Demuth &

Molleman, 2006; Matsuda et al., 1990). Preclinical research suggests that modulation in the endocannabinoid system may help people suffering from depression (Feingold & Weinstein, 2020). The endocannabinoid system has the potential to regulate various neurophysiological processes, including neuronal excitation, pain, and inflammation, learning and memory, and emotion modulation (Grotenhermen, 2005). Although CB1 receptors are the most prevalent, CB2, transient receptor potential TRP channels, and peroxisome proliferator-activated receptors (PPARs) all play critical roles and are affected by cannabinoids. Based on previous extensive research, evidence demonstrating considerable benefit, and public interest in cannabis use, the ECS has become the focus of several studies aiming to better understand the physiological and pathological mechanisms implicated (Lu & Mackie, 2015). Building on the current studies, CBD affects the ECS and functions as a noncompetitive antagonist of the CB1 receptor and an inverse agonist of the CB2 receptor (Thomas et al., 2007). Numerous studies have shown that THC's anticonvulsant characteristics are related to its activity at CB1 receptors, while CBD's antiseizure effects are CB1-independent and may be mediated by a distinct, independent mechanism (Wallace et al., 2001; Wallace et al., 2002; Wallace et al., 2003). The ECS's role in depression identifies components of the ECS as possible treatments for antidepressant production (Yin et al., 2019). Furthermore, anxiety disorders are a prevalent worldwide mental disease defined by fear and anxiogenesis, and the Endocannabinoid system plays a bidirectional regulatory role in the regulation of brain anxiety circuits and behavior (Yin et al., 2019). Consequently, further inquiries are required to elucidate the components of Cannabis and which ECS components may be addressed for the treatment of anxiety disorders.

Table 1 summarizes the Different classes of Cannabinoids and their molecular targets.

Class	Cannabinoid	Molecular Target
Endocannabinoids	**2-Arachidonylglycerol 2-AG**	**Non-selective agonist (CB1>CB2)**
	Docosatetraenoyl Ethanolamide DEA	**Selective CB1 receptor agonist**
	N-Arachidonoyl-ethanolamine (Anandamide) AEA	**Non-selective agonist (CB1>CB2)**
Phytocannabinoids	**Δ9–tetrahydrocannabinol THC**	**Non-selective agonist (CB1,CB2)**
	Cannabidiol CBD	**Unclear, not completely elucidated**
	Cannabinol CBN	**Non-selective agonist (CB1,CB2)**
Synthetic cannabinoids	**Methanandamide MA**	**Non-selective agonist (CB1>CB2)**
	HU-210	**Non-selective agonist (CB1>CB2)**

Table 1: Different classes of Cannabinoids and their molecular targets

As previously stated, the Cannabis plant has been used for thousands of years for medical, recreational, seed oil, and industrial applications. Cannabinoids are a class of psychotropic and physiologically active components found mostly in flowers (Andre et al., 2016b). The concentration of cannabinoids in a plant is determined by extreme environmental factors such as humidity, temperature, time of harvest, soil nutrients, and UV light (Jin et al., 2019).

In the United States, 36 states and four territories have legalized Cannabis for medicinal and/or recreational purposes, including marijuana and CBD (Perlman et al., 2021). For instance, many countries such as Canada, the Netherlands, Italy, Germany, Israel, Brazil, Mexico, and Australia have approved the use of medicinal Cannabis

(Bramness et al., 2018). Although Cannabis sativa has been used recreationally and medicinally for centuries, its psychoactive component THC was identified in the mid-1960s (Gaoni & Mechoulam, 1964). Although it has been estimated that around thousands of papers and reviews on Cannabis and its therapeutically active components have been reported in scientific journals, the pharmacological benefits, and therapeutic uses of these molecules is still under investigation.

1.5.3 Cannabis Plant Components

The overall number of chemicals isolated from Cannabis has increased significantly during the past decades. To date, 554 components discovered in Cannabis including 113 phytocannabinoid (Ahmed et al., 2015) and 120 terpenes (ElSohly & Slade, 2005). These cannabinoids include -THC, Δ8 -THC, CBD, cannabigerol (CBG), cannabichromene (CBC), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT), and numerous other cannabinoids (ElSohly & Gul, 2014).

According to the chemical components of Cannabis oil extracted from Lebanese Cannabis sativa L. were examined using GC-MS. The findings of this research indicate

that cannabinoids are the most abundant compounds identified in the Lebanese Cannabis oil extract ingredient of COE which accounts for 85.15 % of the total compounds. As shown in Figure 2, 62% of CBD dominates the cannabinoid portion, and considerable amounts of THC around 21%, followed by limited concentrations of CBN (4%), and β-caryophyllene (2%). Figure 2 provides an overview of the chemical constituents of Lebanese COE.

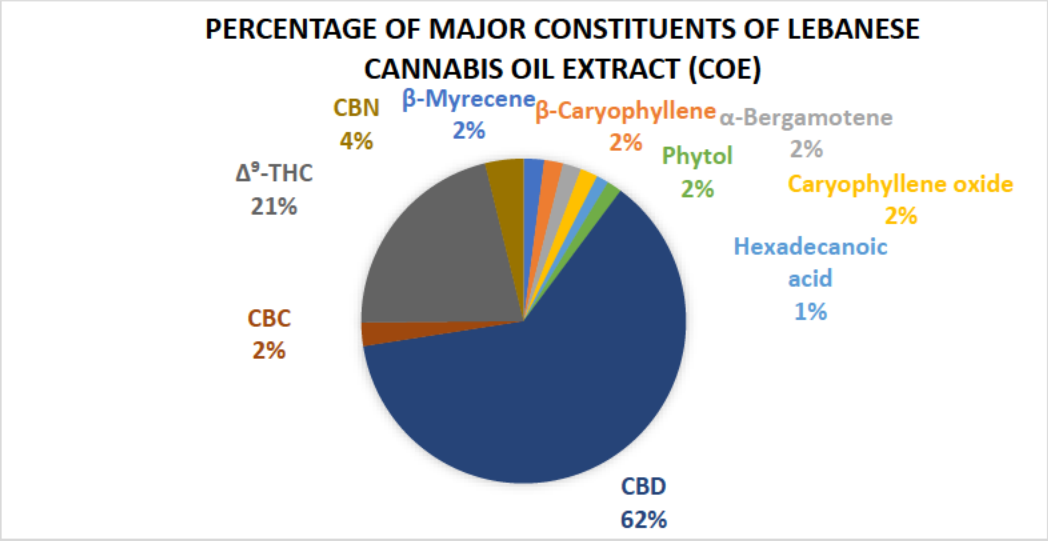


Figure 2: Chemical constituents of COE derived from the Lebanese Cannabis sativa plant, as determined by GC-MS analysis of the extracted oil *(Shebaby, Saliba, Faour et al., 2021b)*

1.5.4 Entourage Effect and Combination Therapy of the Cannabinoids

The most prominent cannabinoids that have been the most researched and acquired knowledge are THC and CBD (Pellati et al., 2018). Studies suggest that combining THC and CBD has greater therapeutic benefits compared to when the cannabinoids are used alone. This phenomenon is described as the "entourage effect," as plants are better at medications than the natural compounds extracted from them (Russo, 2011a). As a result, the pharmacological, psychoactivity, therapeutic, and toxic effects of cannabis types and

"strains" will be determined by the synergistic effects of all of these chemical compounds (Andre et al., 2016; MacCallum & Russo, 2018). Although other cannabinoids are found in less concentrations and their pharmacological efficacy has not been properly reviewed, there is considerable interest in investigating their characteristics. For instance, CBL and CBN which develop with age under certain storage circumstances, lack any psychoactive properties compared to THC. Nonetheless, they are still very poorly studied. Interestingly, CBC is postulated to be the most stable phytocannabinoid and the product formed from the oxidation of CBG and THC (Bonini et al., 2018b). Furthermore, terpenoids are important flavoring components responsible for the fragrance found in the leaves and flowers of the Cannabis plant. More than 200 of these terpenoids have been discovered (Booth et al., 2017a). Terpenoids work along with phytocannabinoid to provide a synergistic mechano-chemical defense mechanism (*The Cannabis Plant: Botanical Aspects*2017). β-caryophyllene and Caryophyllene oxide are the main terpenoids significantly found in the Lebanese Cannabis plant (Shebaby, Saliba, Faour et al., 2021a). β-caryophyllene is the most abundant sesquiterpenoid in Cannabis sativa, and it possesses antimalarial, analgesic, anti-ulcer, and anti-inflammatory properties. Furthermore, Caryophyllene oxide has been proven to be as advantageous, as it exhibits antiplatelet aggregation effects (Bonini et al., 2018). All these chemical compounds of the Cannabis plant are important and evidently, potentiate its therapeutic effects.

1.5.5 Lebanese Cannabis Plant

Lebanon is well-known for its species diversity, which is characterized by the existence of plants with distinct biogeographical origins (Medail & Quezel, 1997). Limited research has been conducted to provide a clear overview of Lebanese

Cannabis (Nadeen Hilal, Lama Bou-Karroum, Noor Ataya FEJ, 2018). Cannabis cultivation in Lebanon was present as far as Roman times, as proven by the finding of a Cannabis leaf engraved in a Roman temple in the ancient city of Baalbek (Basma, 2018). *Cannabis sativa* and *Cannabis indica* are the most common Cannabis strains identified in Lebanon. In 1963, Mechoulam and Shvo extracted and analyzed CBD structure from Lebanese hashish. (Mechoulam, R. & Shvo, 1963). Cannabis has been a plant of interest cultivated and traditionally used in Lebanon to treat many medical conditions, most commonly cancer, diabetes, and chronic pain associated with arthritis (Shebaby et al., 2021). Medical Cannabis (MC) was first applied therapeutically in Arab medicine in the ninth century to treat ear disorders. Later on, Arabic physicians identified more medicinal advantages and applications for Medical Cannabis, including gout, otitis, and infections. Lebanon, an Arabic country, established a formal prohibition on all illegal drugs in 1926, during the French mandate period (Fakhry et al., 2021). Figure 3 highlights significant events in Lebanon's Cannabis history.

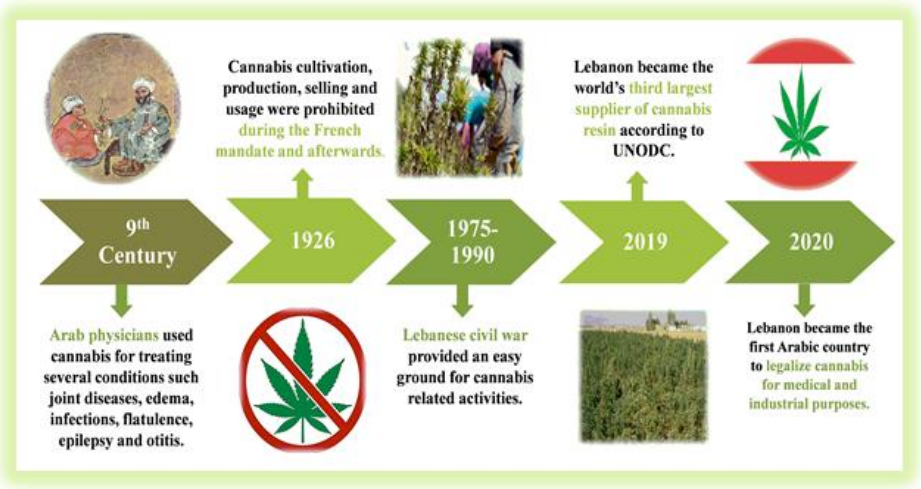


Figure 3: Major events in the history of Cannabis in Lebanon (*(Fakhry et al., 2021)*

Despite being prohibited, Lebanon's Cannabis plant was illegally produced for many years in the Beqaa Valley in Eastern Lebanon, where it had been present as early as Roman Times. The Lebanese *Cannabis sativa L. ssp. indica* has not been properly studied, although it possesses impressively high concentrations of the two main phytocannabinoids CBD and THC (59.1% and 20.2% in Cannabis oil) respectively (Shebaby et al., 2021). In April 2020, the Lebanese Parliament approved a bill legalizing Cannabis growth for **medicinal and industrial** purposes (Lebanese Official Gazette; issue 23; 2020). Due to the substantial medical properties of Cannabis, its legal status and potential for legalization is a debatable topic. Accordingly, this decision made Lebanon the first Arab country to legalize Cannabis cultivation for only therapeutic and industrial uses.

1.6 Rationale, Aim, and Objectives

Medicinal Cannabis has attracted substantial research attention in recent years and has been shown to alleviate pain, muscle spasms, and epilepsy. However, its potential application in the field of neuropsychology hasn’t been fully investigated. As mentioned before, Lebanese Cannabis oil was traditionally used in Lebanon to cure many medical conditions, most commonly cancer, diabetes, and chronic pain associated with arthritis. The Lebanese *Cannabis sativa L. ssp. indica* has not been properly investigated, although it possesses impressively high concentrations of the two main phytocannabinoids CBD and THC (59.1% and 20.2%) respectively (Shebaby et al., 2021). Therefore, according to the previous findings mentioned, cannabinoids are the most abundant compounds identified in the Lebanese Cannabis oil extract which accounts for mainly CBD dominating the cannabinoid portion, and considerable amounts of THC followed by

limited concentrations of CBN and CBC (Shebaby et al., 2021). CBD has been tested for its potential therapeutic applications in a variety of neurologic and psychiatric conditions as it exhibits anxiolytic, anti-inflammatory, neuroprotective, anti-convulsant, and anti-psychotic properties (Fasinu et al., 2016). While THC was identified to be the cannabinoid responsible for the psychoactive and euphoric effects of cannabis, CBD accounts for its lack of psychotropic properties. Since pure CBD or THC was previously tested on neuropsychiatric disorders, the combination of these phytocannabinoids along with other components including terpenes in the Lebanese Cannabis oil might have an additional promising effect.

Available literature reveals that the therapeutic effects of Lebanese cannabis oil on resilience to stress and its ability to rescue depression and anxiety-like behaviors have not been previously studied. Therefore, further investigations are required to evaluate the potential effect of Lebanese Cannabis on stress and anxiety disorders using a whole plant extract or individual phytocannabinoids such as pure extracted CBD or THC.

The present research study aims to examine the effect of Lebanese Cannabis oil on stress resilience and its potential to alleviate depression and anxiety-like behaviors in female mouse models of neuropsychiatric disorders, using the Chronic Variable Stress (CVS) paradigm by evaluating the pre-treatment and post-treatment protocols.

1.6.1 Specific Objectives

The specific objectives of this study are the following:

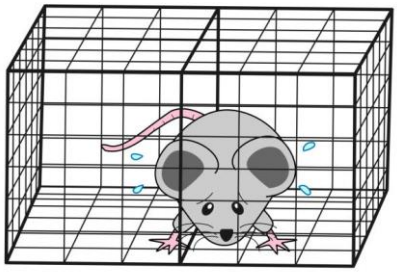
I. To investigate the **dose-response relationship** of Lebanese Cannabis oil at different doses (5 mg/Kg and 20 mg/Kg) on social avoidance behavior.

II. To evaluate the social avoidance behavior of the mice by performing the **Social Interaction (SI)** test.

III. To assess the anxiety-like behaviors of the mice by performing the **Elevated Plus Maze (EPM)** and **Open Field (OF)** tests.

Chapter Two

Materials and Methods



2.1 Experimental Subjects

Female C57BL/6 mice (6–8-week-old, approximately 20gr) were provided by the animal facility at the Lebanese American University (L.A.U). For the Cannabis oil experiments, the female mice were housed separately in their home cages with free access to food and water according to the protocol approved by *the Animal Care and Use Committee.*

2.2 Lebanese Cannabis Oil Extraction

Dried samples of the Lebanese Cannabis strain (Cannabis sativa L. ssp. indica) were supplied by the Beqaa Governorate's Drug Enforcement Office. Cannabis plant material was securely stored in a storage facility on the LAU Byblos campus. Approximately 1.2 g of Cannabis oil was extracted from 10 g of air-dried Cannabis flowers (Shebaby et al., 2021). The extraction procedure was performed using ethanol for 48 hours. The natural extract obtained was then filtered and concentrated at 45°C under reduced pressure to yield pure COE that was stored in a dark, cool place.

A Cannabis oil extraction conducted by **Dr. Mohammad Mroueh** in his laboratory at the Lebanese American University, Byblos campus

2.3 Chronic Variable Stress Paradigm (CVS)

2.3.1 Experimental Setup

The CVS paradigm consists of exposing a female mouse to one or two variable stressors per day for a total of 9 days. This paradigm was used to mimic the symptoms of anxiety and depression like behaviors (Borrow et al., 2019). On days in which two stressors were administered, a minimum of two rest hours between the stressors were enforced. After each stressor is removed, the adult female mice were left undisturbed in their home cages until the next stressor was administered.

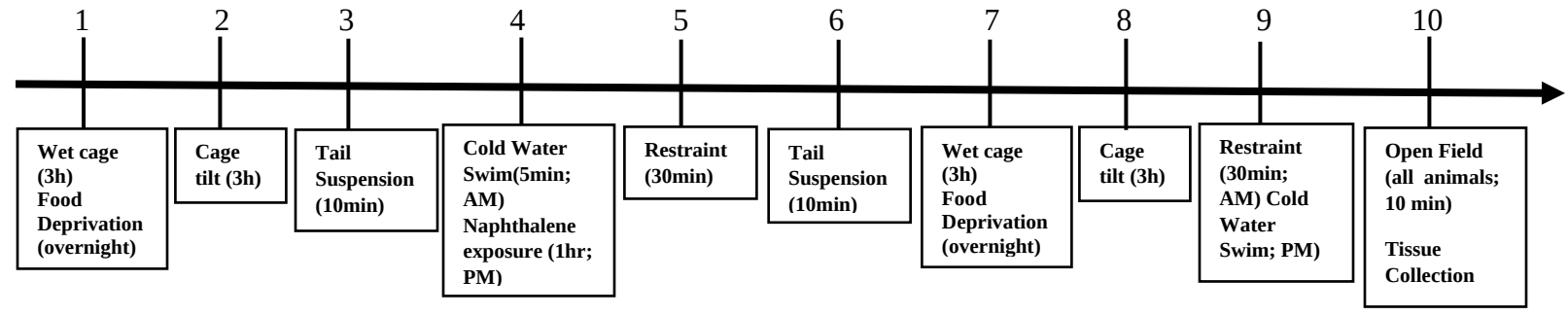


Figure 3: Experimental timeline. Schematic representation of the timeline for Chronic Variable Stress (CVS).

Female mice were exposed to various types of daily stressors for nine consecutive days followed by tissue collection (Figure 3). Briefly, stressors included wet cage (3h), food deprivation (overnight), cage tilt at 45° (3h), tail suspension (10 min), cold water swim (5min), naphthalene exposure (1h), and restraint stress (30min). To prevent predictability and habituation, stressors were enforced in a randomized manner to the mice (Jung et al., 2017).

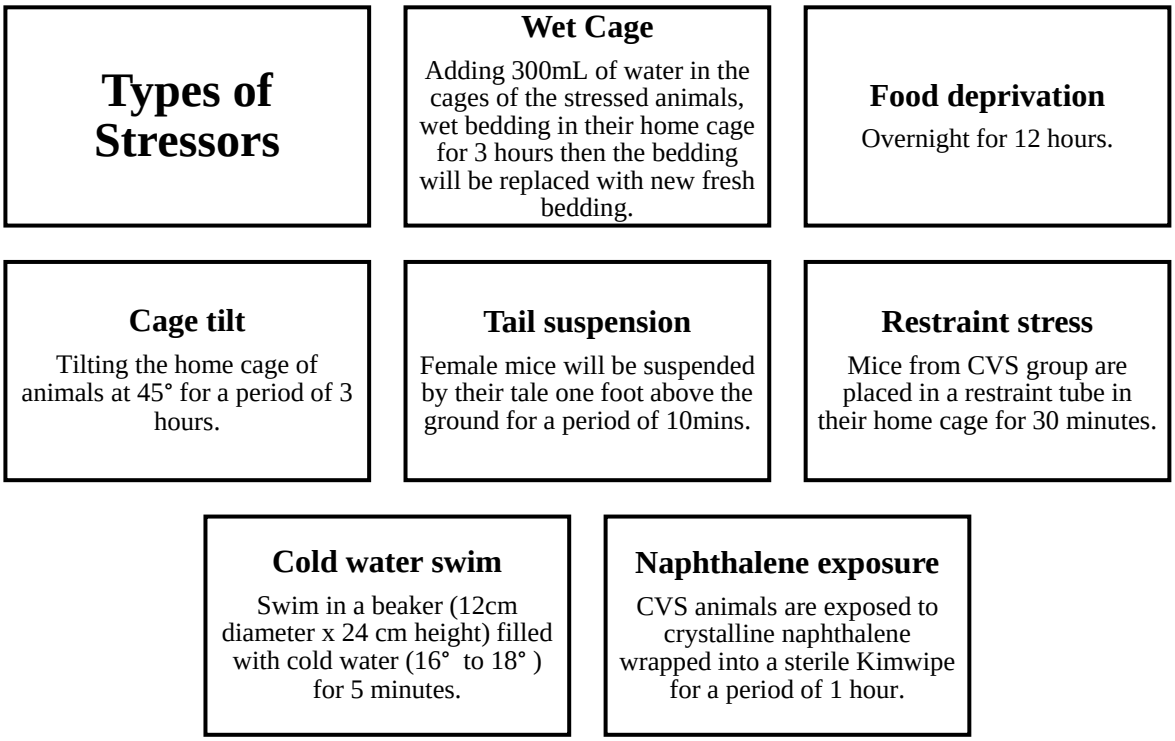


Figure 4: Various types of stressors

2.4 Behavioral Tests

a. To evaluate the **social avoidance behavior** of the mice induced by CVS, the Social Interaction (SI) test was performed.

b. To assess the **anxiety-like behaviors** of the mice induced by CVS, the Elevated Plus Maze (EPM) and Open Field (OF) tests were performed.

2.4.1 Social Interaction Test

The social interaction test is performed to assess depression-like behaviors in mice.

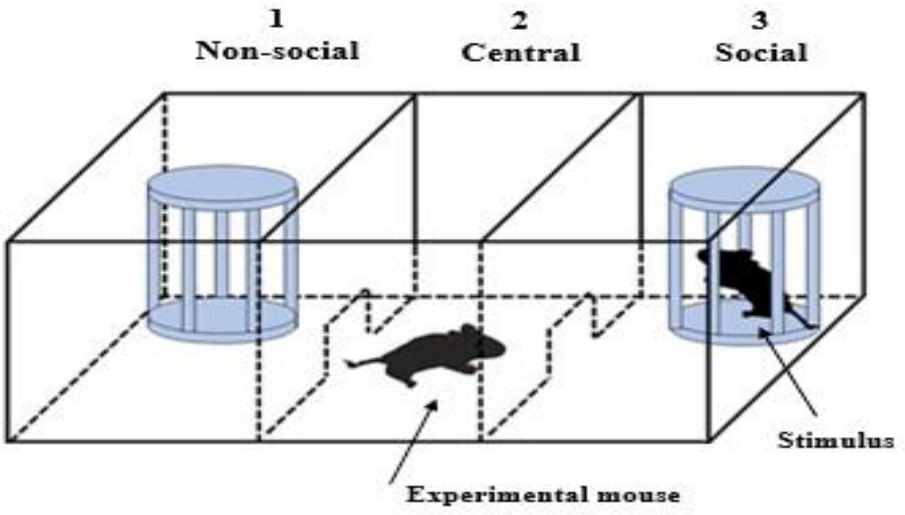


Figure 5: Experimental design for the social interaction test

The SI test consists of a **habituation phase** in which mice were habituated for 5 min in a cage divided into three compartments (social, center, and non-social) with two compartments containing circular wire enclosures. Following the habituation phase, a social stimulus C57BL/6J mouse (6-8 weeks) was introduced to one of the circular enclosures. Then, the experimental mouse was reintroduced to the cage in the central chamber and allowed to reexplore the maze for ten minutes. The movement of the experimental mouse was recorded with a camera and the time spent in each compartment was measured by the **ANY-maze program** (Figure 5).

To determine whether the mouse was resilient or susceptible to stress, the Social interaction (SI) ratio was calculated as the ratio of the time spent in the interaction zone interacting with the stimulus over the time spent in the non-interaction zone (Henriques-Alves & Queiroz, 2015).

The Social Interaction Ratio =

$$\frac{\textbf{The total time spent by the mouse in the social compartment}}{\textbf{The total time spent in the non-social compartment}}$$

Mice exposed to CVS are classified as susceptible if the ratio is less than 1, whereas mice exposed to CVS are classified as resilient if the ratio is greater than 1 (Henriques-Alves & Queiroz, 2015).

2.4.2 Elevated Plus Maze

The Elevated Plus Maze (EPM) is the validated test for anxiety (Sidor et al., 2010). The EPM test was performed to analyze anxiety-related behavior in C57BL/6J mice (Jawhar et al., 2012). The mice were allowed to discover the maze for five minutes and the amount of time spent in the closed arms was recorded using the ANY-maze software (File, 2001). A significant increase in the time spent exploring the closed arms of the maze is associated with anxious behavior.

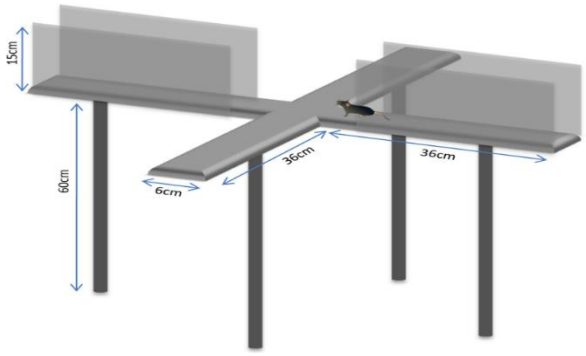


Figure 6: Elevated Plus Maze test

2.4.3 Open Field

In the open field test, the mice can freely explore a box for 5 minutes (Seibenhener & Wooten, 2015). The average distance travelled was measured with a camera using the ANY-maze program. Therefore, a simple analysis of general locomotor ability & anxiety-related emotional behaviors was performed.

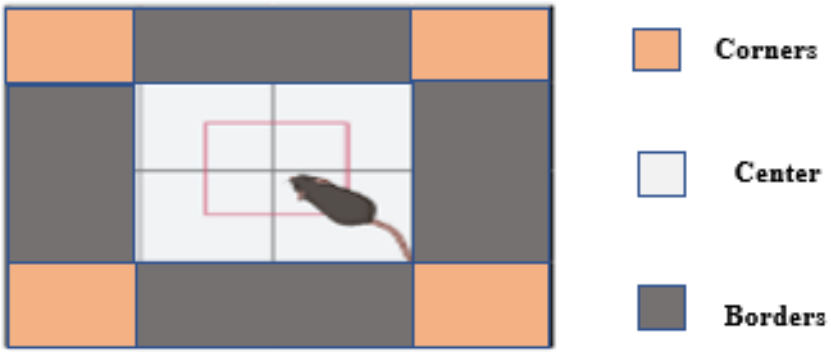


Figure 7: Open Field test

2.5 Experimental Design

The experimental paradigms consist of pre-treatment and post-treatment protocols. Female C57BL/6 mice were divided according to the below experimental groups (n=10) as shown in Table 2.

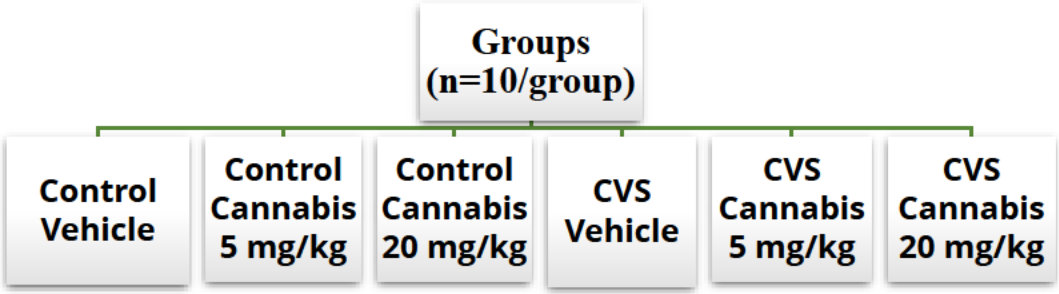


Figure 8: Experimental groups

Number of Groups	Groups	i.p. injections
Group 1	Control	Vehicle
Group 2	CVS	Vehicle
Group 3	Control	5 mg/Kg Cannabis oil
Group 4	CVS	5 mg/Kg Cannabis oil
Group 5	Control	20 mg/Kg Cannabis oil
Group 6	CVS	20 mg/Kg Cannabis oil

Table 2: Distribution of control and CVS groups per i.p. injections.

2.5.1 Pre-Treatment Protocol

The pretreatment experiment consisted of six groups of 6–8-week-old C57/BL6 female mice (10 animals/group). According to Table 2, each group received intraperitoneal injections of vehicle or Lebanese Cannabis oil at different doses (5 mg/kg, and 20 mg/kg). Stress was induced using the chronic variable stress (CVS) paradigm. To determine whether the Lebanese Cannabis oil promotes resilience to stress, mice received daily injections of Cannabis oil for five days. Two hours after the injections on day 6, the mice were exposed to CVS for 9 days along with the Cannabis treatment. On day 15, behavioral tests were conducted. These included the social interaction (SI), open field (OF), and elevated plus maze (EPM) tests. After euthanizing the animals, we collected the

nucleus accumbens, the cortex, and the hippocampi. The schematic diagram of experimental design for the pre-treatment protocol is shown in Figure 9.

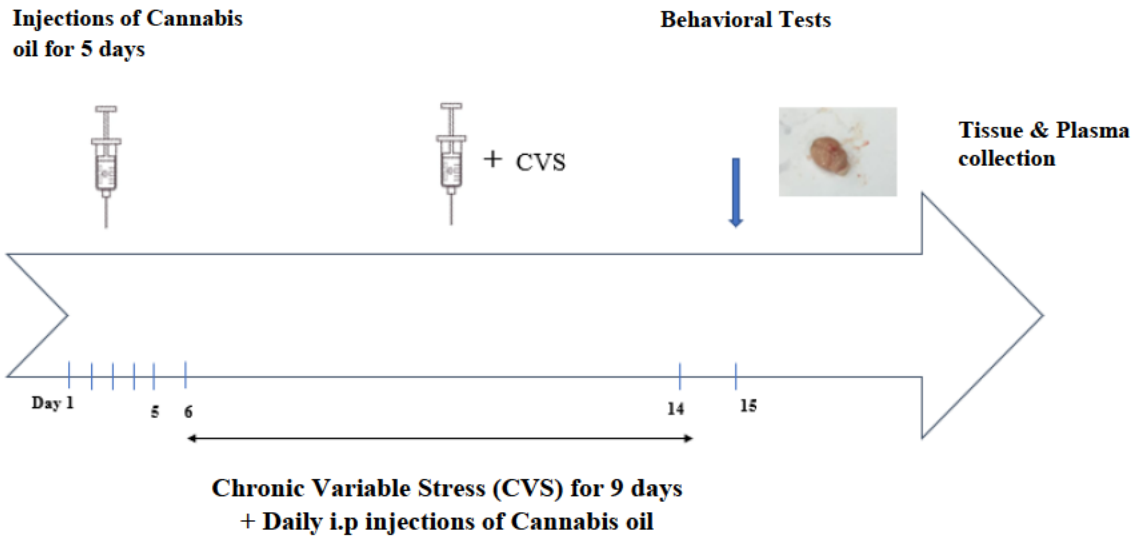
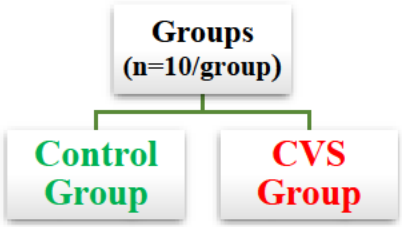


Figure 9: Experimental design for the pre-treatment protocol.

2.5.2 Post-Treatment Protocol



In the post-treatment protocol, animals were divided into two groups: Control and CVS groups. To determine whether Lebanese Cannabis oil rescue social avoidance behavior, we subjected C57BL/6J female mice (6–8weeks) to a CVS paradigm for 9 days only. On day 10, we conducted the social interaction test to divide the animals: Mice exposed to CVS and that exhibited social avoidance behaviors were classified as

susceptible to stress, whereas mice exposed to CVS and that exhibited normal behavior were classified as resilient to stress. Only animals that were susceptible to stress were used and either received i.p injections of vehicle or Lebanese Cannabis oil (5mg/Kg, and 20mg/Kg). In addition, the control groups also received i.p injections of vehicle and the different doses of the Lebanese Cannabis Oil. The schematic diagram of experimental design for the post-treatment protocol was shown in Figure 10.

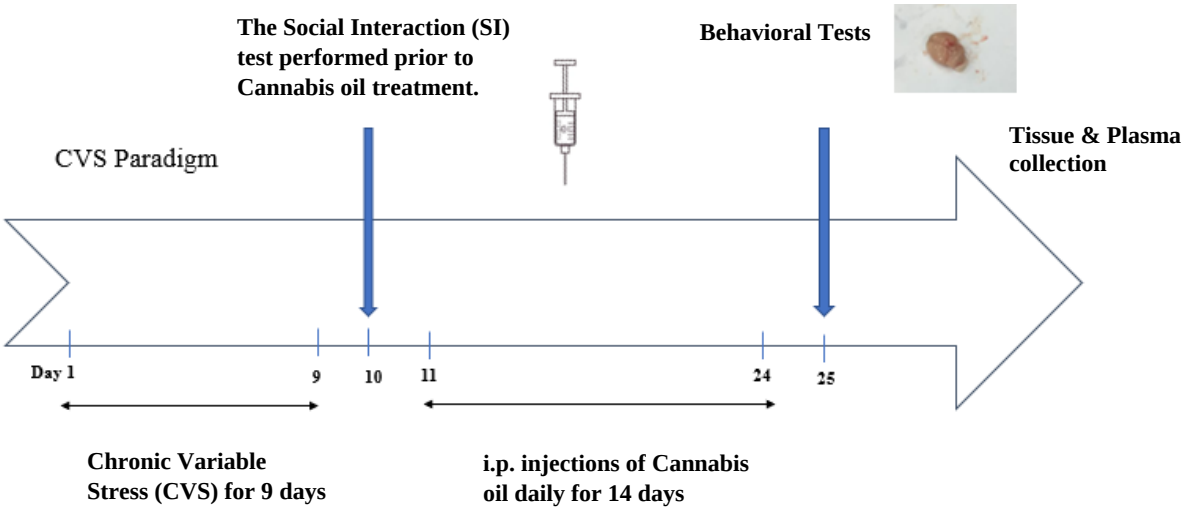


Figure 10: Experimental design for the post-treatment protocol.

Chapter Three

Results

3.1 Lebanese Cannabis oil at doses of 5 mg/kg, and 20 mg/kg promotes resilience to Chronic Variable Stress (CVS) and social interaction

Our initial interest was focused on testing whether Lebanese Cannabis oil mediates resilience to chronic variable stress and rescues social avoidance behavior in the CVS model of depression. First, we treated animals with two doses of Lebanese Cannabis oil to assess the dose dependent effects on social behavior.

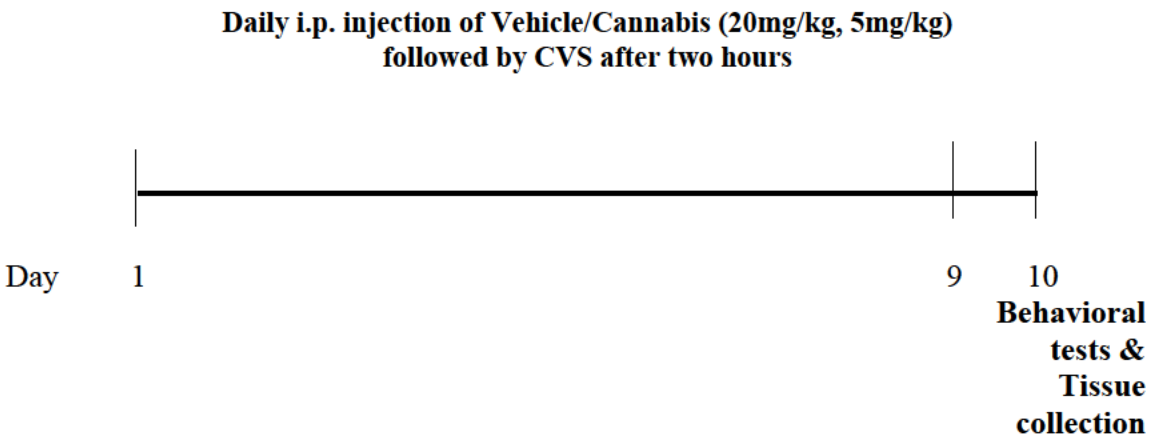
The mice was divided into six experimental groups; control mice that received vehicle, control mice that received Cannabis (5 mg/kg), control mice that received Cannabis (20 mg/kg), CVS mice that received vehicle, CVS mice that received Cannabis (5 mg/kg), and CVS mice that received Cannabis (20 mg/kg). Mice received the intraperitoneal injections of either vehicle or Cannabis (20 mg/kg, 5 mg/kg) two hours before the daily Chronic Variable Stress (CVS) sessions and on day 10, behavioral analysis was conducted (Figure 11a.).

Results showed that Lebanese Cannabis oil (20 mg/kg and 5 mg/kg) promote resilience to chronic variable stress (Figure 11 b). The percentage of CVS animals resilient to stress reached 90% and 100% upon injection of 20 mg/kg and 5mg/kg of Cannabis oil, respectively, as compared to 20% in CVS animals receiving the vehicle injection. Furthermore, Cannabis promoted social interaction (Figure 11c and d). Control animals receiving vehicle or Cannabis injections spent most of their time in the interaction zone. The susceptible CVS animals that received the vehicle spent most of their time in the non-interaction zone. This effect was reversed by Cannabis (20 mg/kg and 5 mg/kg). Indeed, CVS animals that received Cannabis at doses of 20 mg/kg and 5 mg/kg spent significantly

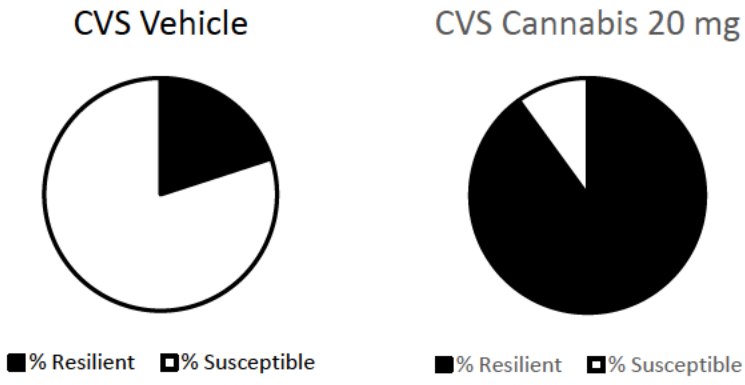
more time in the interaction zone as compared to CVS animals that received vehicle (Figure 11c).

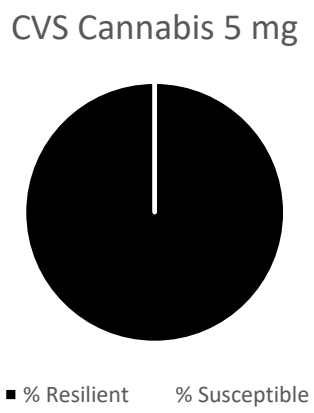
Taken together, our results demonstrate that 20 mg/kg and 5 mg/kg of Cannabis promote resilience to chronic variable stress and social interaction.

a)

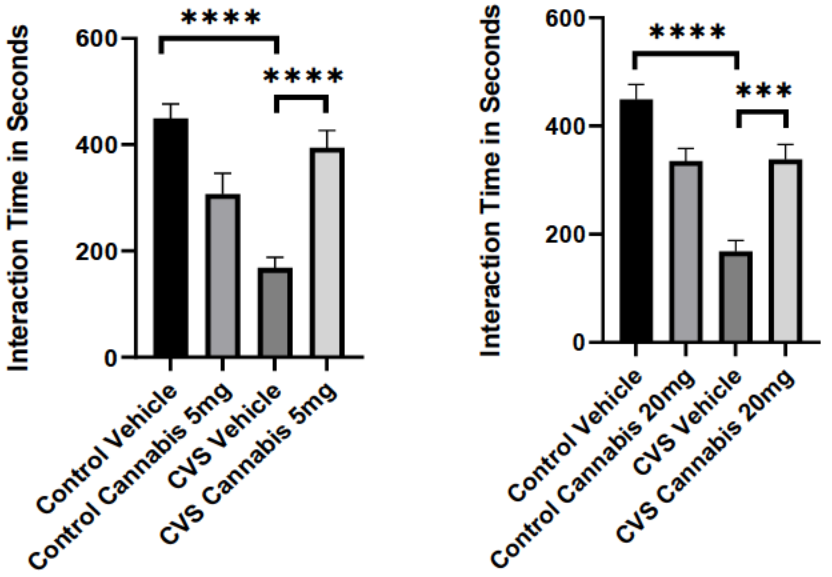


b)




CVS Cannabis 5 mg
% Resilient
% Susceptible

c)



d)

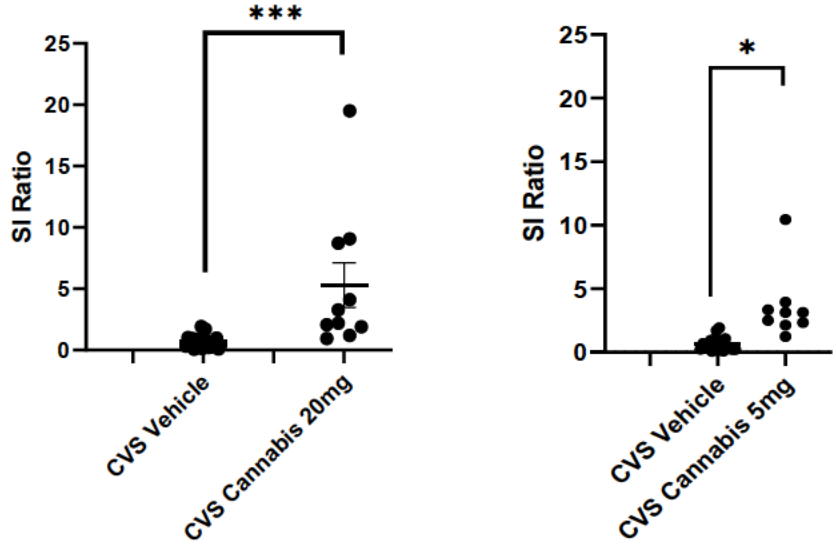


Figure 11: Cannabis oil extract 20 mg/kg and 5 mg/kg promotes resilience to chronic stress and social interaction.

a) The pre-treatment paradigm consists of dividing the mice into six groups: control receiving the vehicle injection, control receiving the Cannabis (20 mg/kg or 5 mg/kg) injection, CVS group receiving the vehicle injection and CVS group receiving the Cannabis injection (20 mg/kg or 5 mg/kg). The mice were injected with either the vehicle or the 20 mg/kg or the 5 mg/kg Cannabis injection for five days. On day 6, two hours after the Cannabis/vehicle injection, the chronic variable stress was performed for 9 days. On the tenth day, behavioral tests were performed, and tissues were collected.

b) Cannabis promotes resilience to stress. Pie chart showing the percentage of animals resilient (in black) or susceptible (in white) to chronic variable stress. The observed results showed an increase in the percentage of resilient animals: 20 % in the CVS group that received the vehicle injection compared to 90% and 100% in CVS group that received 20 mg/kg and 5 mg/kg of Cannabis injection .

c) Bar graph showing the time spent by each of the experimental group in the interaction zone. CVS group that received 20 mg/kg and 5 mg/kg of Cannabis spent most of the time in the interaction zone compared to the CVS susceptible mice that received the vehicle injection. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the CVS group. The average time spent interacting with the social stimulus was significantly higher in the CVS mice receiving the 20mg of Lebanese cannabis. ***$p<0.0001$ and ****<0.0001. Interaction time: Interaction: $F(1, 55)= 27.96$, $p<0.0001$; CVS: $F(1, 55)= 26.78$, $p<0.0001$ and

treatment: F (1, 55)=1.107, p=0.2974 n = 18 Vehicle, n = 10 Cannabis 20 mg/kg, n = 21 CVS Vehicle, n = 10 CVS Cannabis 20 mg/kg. Besides, CVS mice receiving vehicle spent significantly less time interacting with the social stimulus as compared to the control mice. The average time spent interacting with the social stimulus was significantly higher in the CVS mice receiving the 5mg of Lebanese cannabis. . ****p<0.0001 and ****<0.0001. Interaction time: Interaction: F(1.58)= 39.53, p<0.0001; CVS: : F(1.58)=10.91, P=0.0016; and treatment: : F(1.58)= 2.065, p= 0.1561. n = 18 control vehicle, n = 10 Cannabis 5 mg/kg, n = 21 CVS vehicle, n = 13 CVS Cannabis 5 mg/kg.

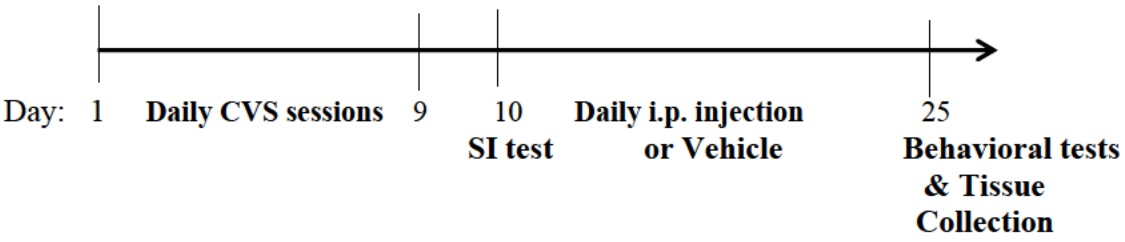
d) **SI ratio:** The social interaction ratio is equal to the time the mouse spent in the interaction zone, interacting with the stimulus, over the time spent in the non-interaction zone. Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Results showed an increase in the interaction ratio between the CVS vehicle and CVS Cannabis 20 mg/kg groups. Statistical significance was measured by unpaired T-test ***p<0.001 n=21 for CVS vehicle and n=10 for CVS Cannabis 20 mg/kg. Also, results computed an increase in the interaction ratio between the CVS vehicle and CVS Cannabis 5mg/kg groups Statistical significance was measured by unpaired T-test *p=0.02 n= 21 for CVS vehicle and n=13 for CVS Cannabis 5mg/kg.

3.2 Lebanese COE acts potentially as an antidepressant treatment

Our results demonstrate that Cannabis promotes resilience to chronic variable stress and social interaction and reveal that it has prophylactic and preventative effects against the development of depression-like symptoms in mice subjected to CVS. Consequently, we wanted to test if Lebanese COE can serve as a treatment and rescue depression-like phenotypes, thus acting as an antidepressant. We induced depression-like behaviors using the CVS paradigm for 9 days. Mice were divided into control and CVS group. On day 10, the SI test was performed to test the susceptibility versus the resilience of the mice. The resilient mice were removed and only the susceptible mice were treated with vehicle or Cannabis for 14 consecutive days and on the 25th day, behavioral tests were conducted. (Figure 12a). We showed that Lebanese Cannabis oil rescued depression-like behavior. Indeed, while 100% of mice were CVS susceptible to stress prior to Lebanese Cannabis oil treatment, 100% became resilient to stress after treatment with Lebanese Cannabis oil (20mg/Kg) and 92% became resilient to stress after treatment with Lebanese Cannabis oil (5mg/Kg) (Figure 12 b). Moreover, animals treated with Lebanese Cannabis oil (20mg/Kg) had a significantly higher SI ratio as compared to animals treated with vehicle (Figure 12c). Finally, Lebanese Cannabis oil (5mg/Kg and 20mg/Kg) treatment enhanced social behavior since animals receiving these treatments spent significantly more time interacting with the social stimulus as compared to animals receiving the vehicle (Figure 12d). Taken together, our results demonstrate that Lebanese

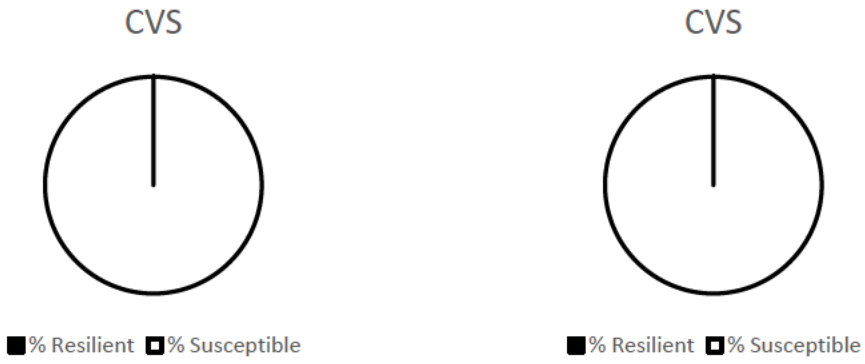
Cannabis oil has antidepressant effects and rescues depression-like behavior in female mice subjected to CVS.

a)

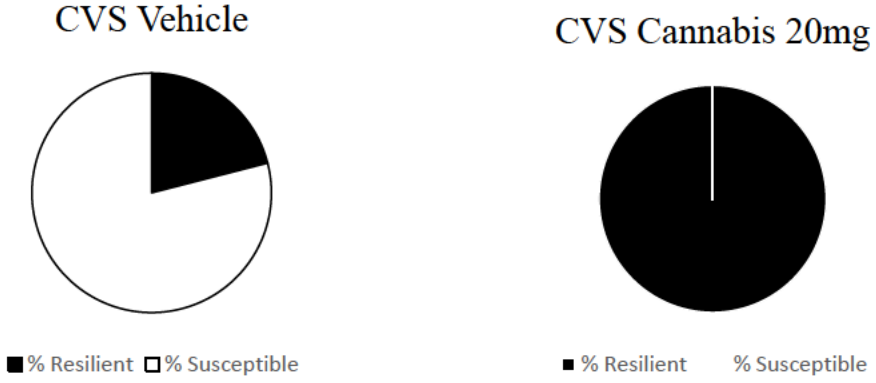


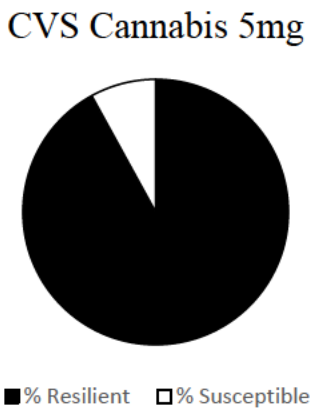
b)

Test day 1: Before treatment

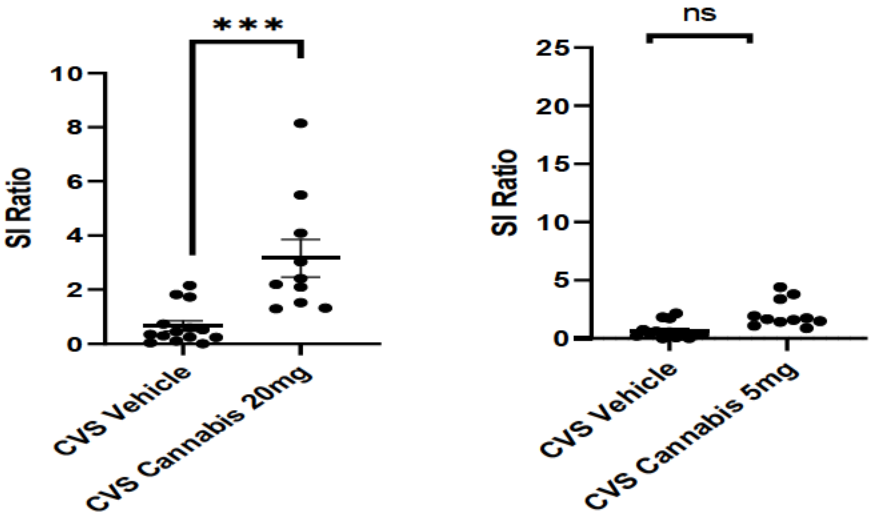


Test day 2: After treatment




CVS Cannabis 5mg
% Resilient
% Susceptible

c)


***
SI Ratio
10
8
6
4
2
0
CVS Vehicle
CVS Cannabis 20mg
ns
SI Ratio
25
20
15
10
5
0
CVS Vehicle
CVS Cannabis 5mg

d)

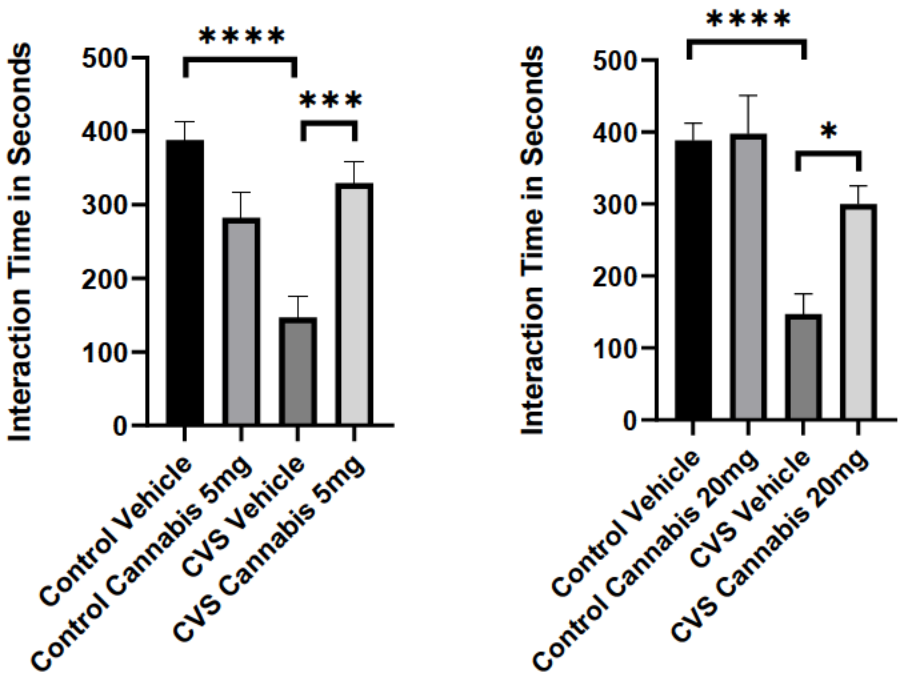


Figure 12: Cannabis oil extract acts potentially as an antidepressant drug

a) The post treatment paradigm consists of inducing depression-like symptoms first using the CVS for 9 days and then treating the susceptible mice either with vehicle or Cannabis (5 mg/kg or 20 mg/kg). Mice were divided into two groups: control and CVS. On day 10, the SI was performed to identify the susceptible versus resilient to stress. Based on the SI ratios, only susceptible mice were used and received treatment for 14 consecutive days. On the 25th day, behavioral tests were performed to assess the depression-like behaviors.

b) Cannabis rescued susceptibility to stress. Pie chart showing the percentage of animals resilient (in black) or susceptible (in white) to chronic variable stress before and after treatment. Only the susceptible mice were treated. After treatment, mice receiving the vehicle showed very high susceptibility to stress. Indeed, in the group of mice receiving vehicle only 21% were resilient to stress. Nevertheless, the behavior of the susceptible CVS group that received 20 mg/kg and 5 mg/kg of Cannabis was 100% and 92% rescued, respectively.

c) **SI ratio:** The social interaction ratio is equal to the time the mouse spent in the interaction zone, interacting with the stimulus, over the time spent in the non-interaction zone. Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Results showed an increase in the interaction ratio between the CVS vehicle and CVS Cannabis 20 mg/kg groups. Statistical significance was measured by unpaired T-test ***$p<0.001$ n=14 for CVS vehicle and n=10 for CVS Cannabis 20 mg/kg. Nevertheless, no statistical significance in the interaction ratio is observed between the CVS vehicle and CVS Cannabis 5 mg/kg groups.

d) Bar graph showing the time spent by each of the experimental group in the interaction zone. CVS group that received 20 mg/kg and 5 mg/kg of Cannabis spent most of the time in the interaction zone compared to the CVS susceptible mice that received the vehicle injection. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the CVS group. The average time spent interacting with the social stimulus was significantly higher in the CVS mice receiving the

20mg of Lebanese cannabis.****p<0.0001. Interaction time: Interaction: F(1,42)=4.582, p=0.003; CVS: F(1,42)= 25.34, p<0.0001 and treatment: F(1,42)=5.787, p=0.002; n = 12 Vehicle, n = 10 Cannabis 20 mg/kg, n = 14 CVS Vehicle, n = 10 CVS Cannabis 20 mg/kg. Besides, CVS mice receiving vehicle spent significantly less time interacting with the social stimulus as compared to the control mice. The average time spent interacting with the social stimulus was significantly higher in the CVS mice receiving the 5 mg of Lebanese cannabis 5 mg/kg.****p< 0.0001. Interaction time: Interaction: F(1,40)=21.79, p<0.0001, CVS: F(1,40)=9.844, p=0.003 and treatment: F(1,40)=1.525, p=0.022 n = 12 Vehicle, n = 6 Cannabis 5 mg/kg, n = 14 CVS Vehicle, n = 12 CVS Cannabis 5 mg/kg.

3.3 COE at low dose shows an anxiogenic effect

Our results reveal that Lebanese COE has antidepressant effects and rescue social avoidance behavior. Next, we tested the potential effect of Lebanese COE on anxiety-like behaviors by performing the Elevated Plus Maze test (EPM). A significant increase in the time spent exploring the closed arms of the maze was associated with anxious behavior.

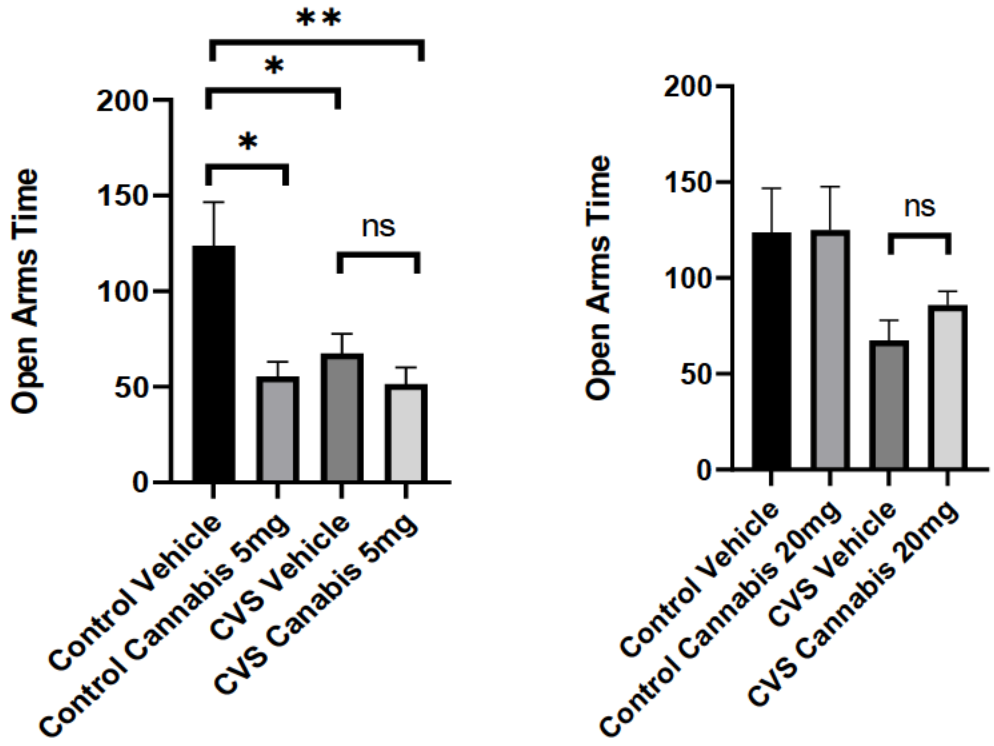


Figure 13: Cannabis at low dose (5 mg/kg) has an anxiogenic effect

Control mice receiving i.p. injection of Lebanese Cannabis oil at low dose (5 mg/ Kg) spent significantly less time exploring the open arms of the EPM. This suggests that low dose of the Lebanese Cannabis oil promotes anxiety-like behavior. This effect was also observed in mice subjected to CVS. Interestingly, our results reveal the independent anxiogenic effects of both the low dose of Lebanese Cannabis oil and CVS. In contrast, the 20 mg/ Kg did not have an anxiogenic effect. Indeed, Lebanese Cannabis oil treatment (20mg/Kg) had no effect on anxiety-like behavior since animals receiving this dose spend the same time as controls exploring the open arms of the EPM. Taken together, we observed a significant anxiogenic effect in the control group of female mice receiving i.p. injection of Lebanese Cannabis oil at low dose (5 mg/ Kg), whereas, at higher dose (Cannabis 20 mg/ Kg), the anxiogenic effect is not seen.

Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the CVS group. The observed results showed a significant decrease in the time spent exploring the open arms of the maze in the control groups receiving Cannabis 5 mg/kg of which is associated with anxious behavior: *p= 0.003. In contrast, no anxiogenic effect is seen in the control groups receiving Cannabis 20mg/kg. Therefore, higher dose is considered better and safe in female mice (Figure 13).

Chapter Four

Discussion

Cannabis sativa has been medicinally and recreationally used since ancient times due to the extensive reports of its remarkable medical benefits such as pain, anxiety, epilepsy, anxiety, and post-traumatic stress disorder (Andre et al., 2016; Murillo-Rodriguez et al., 2020b). This herbal remedy is known to possess rich chemical components consisting of cannabinoids, terpenoids, flavonoids, and alkaloids (Andre et al., 2016c). Nowadays, 554 components discovered in Cannabis including 113 phytocannabinoid (Ahmed et al., 2015) and 120 terpenes (ElSohly & Slade, 2005). Interestingly, combining the two main phytocannabinoids THC and CBD has been demonstrated to have enhanced therapeutic effects when compared to each of the cannabinoids alone (Russo, 2011b). As such, the Lebanese *Cannabis sativa L. ssp. indica* possesses impressively high concentrations of the two main phytocannabinoids CBD and THC (59.1% and 20.2%) respectively (Shebaby et al., 2021) along with terpenes. However, the Lebanese *Cannabis sativa L. ssp. indica* is not adequately studied.

Stress is a ubiquitous risk factor for most neuropsychiatric disorders such as depression, Post-Traumatic Stress Disorder (PTSD), and anxiety (Pitman et al., 2012 McEwen et al., 2015; Murrough et al., 2017;) . Indeed, recent observational studies indicate that patients who use medicinal cannabis to treat anxiety, depression, chronic pain, and sleep, exhibit improvements not only in their mood, quality of life, and sleep but also in brain activity (Gruber et al., 2017). However, many aspects of Cannabis research still require more investigation. In this study, we showed that the Lebanese Cannabis oil

promotes resilience to CVS and prevents social avoidance behavior in female mice models of neuropsychiatric disorders.

The Lebanese Cannabis oil possesses high concentrations of the two main phytocannabinoids CBD and THC (59.1% and 20.2%) respectively, which prompted us to its effects on resilience to CVS, and whether it increases social interaction in stressed mice. Our results suggest that Cannabis oil (5 mg/kg and 20 mg/kg) mediates resilience to stress and social interaction and can potentially act as an antidepressant treatment (Figures 10 and 11). However, a significant anxiogenic effect is seen in the control group of female mice receiving i.p. injection of Lebanese Cannabis oil at a low dose (5 mg/ kg body weight). Whereas, at a higher dose (20 mg/ Kg), the anxiogenic effect is not seen. Therefore, a higher dose is considered better and safe in female mice (Figure 12). Yet, additional studies for the validation of the results and a better understanding of the potential effect of Lebanese Cannabis oil on stress and anxiety disorders are required.

In addition to the therapeutic effect of Cannabis oil as a potential antidepressant drug, research study indicated that Cannabis users experienced better sleep, higher quality of life, and reduced average pain (National Academies of Sciences, Engineering, and Medicine et al., 2017). The participants who started using medicinal cannabis during the follow-up period had a substantial reduction in both depression and anxiety symptoms. (Martin et al., 2021). One report has been published outlining the good safety profile of CBD as a promising candidate for the treatment of neuropsychiatric disorders and presenting a wide range of therapeutic effects (Pisanti et al., 2017). Indeed, studies have reported that CBD possesses many pharmacological activities and benefits such as anti-cancer, anti-epileptic, anxiolytic, anti-inflammatory, and anti-psychotic properties

(Esposito et al., 2013;Campos et al., 2016b) Interestingly, a previous report demonstrated a dose-dependent antidepressant-like effect of the non-psychoactive cannabinoid CBD and a significant antidepressant-like action of THC at 2.5 mg/Kg dose in adult male mouse models of behavioral despair (El-Alfy et al., 2010a). These promising results are in line with our current research outcomes that the phytocannabinoids component of the Cannabis sativa exert antidepressant-like actions and thus may contribute to the mood-enhancing properties of Cannabis in female mouse models.

On the other hand, THC which is one of the most potent psychotropic components of the Cannabis plant, responsible for its euphoric effect and has been subjected to extensive studies (Ashton, C. Heather, 2001). Therefore, along with the emergence of literature on this molecule, there has been a comparable increase in the use of cannabis for medicinal purposes, with the most often reported reasons for its use being the management of pain, anxiety, and depression (Diehl et al., 2010). A handful of studies have previously demonstrated the anxiolytic effects of THC and most of these findings have exhibited a positive effect in clinical populations (Bergamaschi et al., 2011; Masataka, 2019). Furthermore, both community and laboratory research has shown that the relative amounts of cannabinoids in the plant might affect its pharmacological efficacy when consumed. For instance, when combining CBD with THC, CBD may potentially offset some of THC's adverse effects including memory impairment and paranoia (Englund et al., 2013; Morgan et al., 2010a). This goes in line with previous findings which reported that high doses of CBD reverse some of the anxiogenic effects of THC in rats (Malone et al., 2009). According to a previous study, the two main components in Cannabis, CBD, and THC appear to have opposing effects, with CBD associated with

anxiolytic outcomes and THC attributed to anxiogenic effects (Zuardi et al., 1982). Nevertheless, a recent publication has revealed that THC's impact is dose-dependent, with lower doses having the opposite effect. Based on this evidence, high CBD and low THC cannabis preparations would be the most effective in treating anxiety in male rats (Rodríguez-Manzo & Canseco-Alba, 2015). This supports the important characteristic of Lebanese COE which impressively possess high concentrations of the two main phytocannabinoids CBD and THC which is approximately close to (CBD: THC) 3:1 ratio (59.1% and 20.2%) respectively. Interestingly, many studies have shown that the mood-improving benefits of cannabis usage might be attributed to the CBD concentration in the Cannabis plant, modulating several targets in the neurobiology of depression and supporting the biphasic nature of cannabinoids in both anxiety and behavioral responses including motor activity and aggression (Braida et al., 2009; Onaivi et al., 1990). CBD was primarily investigated for its potential to reduce stress-induced emotional effects by enhancing behavioral adaptability. Furthermore, systemic CBD injection before restraint stress exposure reduced the anxiogenic and cardiovascular effects observed in rat models (Sales et al., 2018). CBD has been shown to exhibit an acute antidepressant effect in animal models usually not sensitive to acute monoaminergic treatment, thus suggesting that CBD might be a fast-acting antidepressant component of the Cannabis plant and considered for the first time as an antidepressant-like effect of CBD (Silote et al., 2019).

Several pieces of evidence support that low dose of THC specifically reduced stress-induced anxiety-like behavior in stress-susceptible male and female mice (Bluett et al., 2017). Therefore, these data are consistent with our current results which shows an improvement in stress rescue in female mouse models subjected to Chronic Variable

Stress (CVS) paradigm. Furthermore, a previous report indicated that THC works differently in animals and people according to whether it is administered alone or in combination with other cannabinoids or terpenes. Morgan et al (2010) revealed that CBD used in combination with THC in smoked Cannabis, exhibited a protective effect against some of THC's adverse psychological effects (Morgan et al., 2010b). Additionally, there is a positive correlation between cannabinoid-induced neurogenesis and behavioral improvement in animal models of anxiety, psychosis, and depression (Manoela Viar Fogaca et al., 2013; Marchalant et al., 2009). Furthermore, the variety of chemical components involved in the cannabis plant as a whole has been discovered to be more effective than isolated purified phytocannabinoids only (Russo, 2011c). This is in accordance with our findings which indicate the antidepressant potential effect of Cannabis crude extract on susceptibility to stress induced by the CVS paradigm in female mouse models of neuropsychiatric disorders. Furthermore, a recent study reported the potential effect of Cannabis *sativa* on the motility, anxiety, and social effects in mouse models (Mastinu et al., 2022) which also supports that Cannabis has preventive effects in the development of depression-like symptoms in mice subjected to chronic variable stress. Another interesting finding from our work focuses on the effect of Lebanese Cannabis oil in mediating social interaction. Indeed, Lebanese Cannabis at doses (5 mg/ kg and 20 mg/kg) prevented social avoidance behavior. These outcomes suggest that phytocannabinoids along with the terpenes present in the Cannabis plant may mediate social interaction. This goes in line with a previous report indicating that Cannabis users exhibited prosocial behaviors and enhanced social interactions (Rahm-Knigge et al., 2019). Furthermore, in a survey of college students, more than 70% said that cannabis consumption made them feel more eager to communicate with others due to increased

emotions of cohesiveness (Wei et al., 2017). In addition to cannabinoids, secondary metabolites of cannabis have been demonstrated to have anxiolytic properties. Comprehensive preclinical research using multiple animal models of anxiety-like behavior revealed that plant-derived chemicals such as alkaloids, terpenes, flavonoids, and phenolic acids exhibit anxiolytic properties (Farzaei et al., 2016). As previously stated, terpenes are an essential class of chemicals generated by C. sativa that contribute to its distinctive scent (Booth et al., 2017b). Importantly, terpenes have been proposed not only to convey the aroma of various cannabis flowers, but also to have certain medicinal properties, either by themselves or as coactivating agents, improving the effective approach of phytocannabinoids in humans (Russo, 2011). This fundamental phenomenon is described as the "entourage effect", a positive contribution derived from the addition of terpenes to the effect of unique cannabinoids. In a previous study, β-Caryophyllene may be effective in treating depression and stress-related mental disorders in animal models (Hwang et al., 2020). This compound which is present in the Lebanese COE (2%) may contribute to our current results indicating stress rescue effect and mediating social avoidance behavior. Moreover, β-Myrcene is the important monoterpene present in the Lebanese COE (2%); It possesses the antipsychotic, antioxidant, analgesic, muscle relaxant, and anti-inflammatory properties (Hanuš & Hod, 2020). Additionally, a recent publication study indicates that CBC exhibited a significant antidepressant-like effect in animal models of behavioral despair (El-Alfy et al., 2010b). The latter compound present in the Lebanese COE (2%) may also support our current results indicating rescue depression effect. This reveals the importance of the Lebanese COE which exhibits a unique combination of CBD and THC along with terpenes and potentiates their therapeutic effects.

Due to the scarce pool of data available on the therapeutic effect of Lebanese COE, more studies are needed to uncover promising outcomes to further validate the current results and gain a better understanding of the therapeutic outcomes. To the best of our knowledge, such innovative combinations between cannabinoids mainly CBD and THC along with terpenes might reduce the adverse effects of available antidepressants, particularly for patients who are non-responsive to conventional therapy.

Chapter Five

Conclusion

The Cannabis plant has been a resurgence of interest consisting of rich chemical compounds, including phytocannabinoids, terpenes, and phenolics. To the best of our knowledge, this work is the first research study that demonstrates the potential therapeutic effects of Cannabis crude oil extracted from the Lebanese *Cannabis sativa L. ssp. indica* plant on stress and anxiety disorders in animal models. Further studies are required to explore the importance and effect of specific Cannabis components including the phytocannabinoid alone, such as CBD or THC on stress and anxiety disorders. Furthermore, we will also focus on different parts of the brain mainly the nucleus accumbens to elucidate the mechanism of action involved in the regulation of social behavior. These promising findings revealed that Cannabis (5 mg/kg and 20 mg/kg) promotes resilience to stress and social interaction and can potentially act as an antidepressant. Further investigations are required to gain a better understanding of the potential therapeutic effects of the various cannabinoids.

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