Universidade de Lisboa

Faculdade de Farmácia

Medicinal Use of Cannabis

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Abstract

Cannabis is a plant used for thousands of years in various parts of the world, for various purposes, from fiber to fabric or fishing nets, to therapeutic and recreational use. The medicinal uses described include analgesic, powerful relaxant, nausea control and also to stimulate appetite. As time went by, there was an increase in use due to its psychotropic and hallucinogenic properties, so its consumption had to be regulated and was even included in the list of illegal and prohibited substances.

Cannabis sativa plants produce important chemicals called phytocannabinoids that are responsible for the pharmacological properties of the plant. There are about 500 molecules that can be found in these plants, with phytocannabinoids comprising about 100 of these molecules. The phytocannabinoids that appear in greater quantity are Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), and being the ones that produce the most significant effects in humans, they are used as active substances in medicines. When studying the effects of cannabinoids, the endocannabinoid system, composed of cannabinoid receptors and endogenous cannabinoids, was identified. The CB1 receptor can be found extensively in the central nervous system, and the CB2 receptor can be found in peripheral organs and tissues. The molecules classified as endogenous cannabinoids are 2-arachidonoylglycerol and N-arachidonoylethanolamine. As for its therapeutic properties, clinical studies have demonstrated the therapeutic efficacy of using cannabinoids in various dosage forms in various diseases or symptoms of diseases. There are already approved drugs for the treatment of spasms derived from multiple sclerosis in refractory patients to other therapies, chronic neuropathic pain, symptoms associated with neurodegenerative diseases and relief of nausea and vomiting associated with the administration of cytotoxic drugs.

Legislation in Portugal allows the production and use of Cannabis for medicinal purposes and there is already one approved and marketed drug, Sativex. Despite the studies that already exist, clinical practice and evidence is still scarce, so there is still a lot to be studied regarding the therapeutic efficacy of Cannabis and the effects that it can have on human beings in the short and long term.

Keywords: *Cannabis Sativa,* tetrahydrocannabinol, cannabidiol, endocannabinoid system

Resumo

A Cannabis é uma planta utilizada, há milhares de anos em várias partes do mundo, para diversos fins, desde fibra para tecido ou redes de pesca, ao uso terapêutico e recreativo. Os usos medicinais descritos incluem analgésico, poderoso relaxante, controlo de náuseas e enjoos e também para estimular o apetite. Com o passar do tempo, foi-se notando um aumento do uso pelas suas propriedades psicotrópicas e alucinogénias pelo que o seu consumo teve de ser regulado e até foi incluída na lista de substâncias ilegais e proibidas.

As plantas de *Cannabis sativa* produzem químicos importantes denominados fitocanabinóides e que são os responsáveis pelas propriedades farmacológicas da planta. Existem cerca de 500 moléculas que podem ser encontradas nestas plantas, sendo que os fitocanabinóides compreendem cerca 100 dessas moléculas. Os fitocanabinóides que aparecem em maior quantidade, o Δ-9-tetrahidrocanabinol (THC) e canabidiol (CBD), produzem os efeitos mais significativos no ser humano e são as moléculas utilizadas como substâncias ativas em medicamentos. Aquando do estudo dos efeitos dos canabinóides, foi identificado o sistema endocanabinóide, composto pelos recetores de canabinóides e pelos canabinóides endógenos. O recetor CB1 pode ser extensamente encontrado no sistema nervoso central, e o recetor CB2 pode ser encontrado nos órgãos e tecidos periféricos. As moléculas classificadas como canabinóides endógenos são o 2-araquidonoilglicerol e N-araquidonoiletanolamina. Quanto às suas propriedades terapêuticas, estudos clínicos demonstraram eficácia terapêutica do uso de canabinóides em várias formas farmacêuticas em diversas doenças ou sintomas de doenças. Existem já medicamentos aprovados para o tratamento de espasmos derivados de esclerose múltiplas em doentes refratários a outras terapêuticas, dor crónica neuropática, sintomas associados a doenças neurodegenerativas e alívio de náuseas e vómitos associados a administração de citotóxicos.

A legislação em Portugal permite a produção e uso de Cannabis para fins medicinais e já existe 1 medicamento aprovado e comercializado, o Sativex. Apesar dos estudos que já existem, a prática clínica e a evidência ainda é pouca, pelo que ainda existe muito por estudar relativamente à eficácia terapêutica de Cannabis e os efeitos que esta pode ter no ser humano a curto e longo prazo.

Palavras-chave: *Cannabis Sativa,* tetrahidrocanabinol, canabidiol, sistema endocanabinoide

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Certificado de Originalidade

"Certifico que sou responsável pelo trabalho submetido nesta monografia, não sendo este copiado ou plagiado de outra fonte, excetuando o especificado nas referências."

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19 Novembro de 2020

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Acronyms

AD – Alzheimer’s disease
ASD – Autism spectrum disorder
cAMP – Cyclic adenosine monophosphate
CBD – Cannabidiol
CB1 – Cannabinoid receptor 1
CB2 – Cannabinoid receptor 2
CHO – Chinese ovary hamster
DRG – Dorsal root ganglion
FAAH – Fatty acid amide hydroxylase
GABA – Gamma-Aminobutyric acid
GPR55 – G protein-coupled receptor 55
GTPγS – Guanosine triphosphate
HD – Huntington's disease
HG – Hyperemesis gravidarum
IP3 – Inositol trisphosphate
MAA – Marketing Authorization Application
MS – Multiple sclerosis
NTS – Nucleus of the solitary tract
OMC – Office of Medicinal Cannabis
RhoA – Ras homolog family member A
2-AG – 2-arachidonylglycerol
Δ9-THC – Trans-Δ9-tetrahydrocannabinol

1. Introduction

1.1 Historical perspective and the medicinal use of Cannabis sativa L.

For thousands of years, *Cannabis sativa* L. and its extracts have been used for medicinal purposes. From ancient records, its uses have been documented by African, Asian and southern Asian countries like Egypt, China and India, since about 3000 to 5000 years, and later, around the 1500 in the western countries [1]. At that time, its therapeutic indications were to treat malaria, constipation or pain [1], and later also for the treatment of epilepsy [2]. Over time, its use was recorded in British, Canadian and American pharmacies before the concerns about its psychotropic properties raised attention. Around the 70's it was registered as an illicit substance of abuse, however in the last 20 years there has been a growing interest in its therapeutic properties for the treatment of some diseases, with a special focus on its limiting symptoms. [3]

Cannabis sativa L. can be found in a variety of habitats and altitudes, ranging from sea level to the alpine foothills of the Himalayas [1]. Central Asia and South-East Asia have been proposed as potential regions for the natural origin and/or primary domestication of *C. sativa*. In addition, *C. sativa* followed the evolution of the first human societies in the changes that occurred after the glacial Pleistocen epoch. In particular, it seems that in Czech Palaeolithic sites, a variety of ways were used to enable the production of sophisticated braided basketry based on *C. sativa*, making it probably the oldest archaeological evidence of *C. sativa* use [1]. Also, a lot of Neolithic forms of evidence that were found in Taiwan suggest that *C.sativa* was used 12 000 years ago for several different purposes and played a significant role in early cordage and textile manufacturing, being even referred as the oldest known cultivated fibre plant, and even today, it is used as a constituent of fishing nets [1].

The psychotropic effects may have been discovered by accident. American researchers suggest that it happened by accidentally burning parts of *C. sativa*. In South American countries, the use of plants was a common practice. Plant-based preparations were made in order to benefit from its psychotropic properties for rituals and celebrations. These plants were adored as teachers for their ability to cure diseases [1].

Different varieties of the plant will be used for different purposes, such as cultivation, production of textile fibers, in religious rituals and medicinal use. It has been reported in the last 5000 years, when the Chinese emperor Chen Nung, considered the

father of Chinese agriculture, included it on the first Chinese pharmacopeia. *C. sativa* was used in Asia to treat fatigue, rheumatism and malaria. The seeds were used for their vegetable oils and proteins. The seeds contain γ-linoleic acid which was used for skin conditions like eczema or psoriasis, and taken orally, to treat inflammatory diseases. It was also used by other people like the Assyrian, the Egyptian, the Greeks, the Romans and the Indians for many centuries and for many different purposes [1].

In the medieval Europe, the various forms of *Cannabis sativa* were already known. The Italians built the first large-scale cultivation and commercialization of the plant in the Mediterranean area. On the other hand, America was not aware of this plant until the arrival of the Europeans. During this period, *C. sativa* was used for the strength and hardness of its fibers. The English and Spanish colonies imported the ideal botanical varieties for textile manufacturing [1]. Because of its inebriating effects, use in magic and traditional medicine were also known. Pope Innocent VIII issued a papal bull condemning the witchcraft and the use of *C. sativa* in 1484. In the following centuries, the European citizens learned numerous uses of *C. sativa* thanks to travelogues, written by adventurers, sea captains, wealthy travellers, priests, and traders headed to Africa and the East Indies [1]. With the expansion of commerce and navigation capacity, it was possible to discover more properties of *C. sativa* through people who came from different parts of the world. Garcia da Orta and Cristobal Acosta, in the sixteenth century, wrote about the effects of *C. sativa*, which included euphoria, sedation, stimulation of appetite, hallucinations, and aphrodisiac effects [1].

In the middle of the eighteenth century, the Swedish botanist Carl Linnaeus worked on writing the Species Plantarum, writes for the first time the name *Cannabis sativa* (FIG.1). However, thanks to the chronicles of Indies travellers, other botanists began to discuss the existence of distinct types of *Cannabis* based on size, shape and resin contents. In particular, Jean Lamarck reserved the name *Cannabis sativa* for the European plant and *Cannabis indica* for the Indian origin varieties [1].

FIG.1: Cannabis sativa Linnaeus from Franz Eugen Köhler's Medizinal-Pflantzen, 1887, Germany. [1]

On the popular culture, oriental traditions brought the development of private Cannabis consumer clubs. Many Romantic artists and writers frequented these clubs such as Théophile Gautier, Charles Baudelaire, Honoré de Balzac, Alexandre Dumas, and Gustave Flaubert. In 1830, the French physician Jacques Joseph Moreau studied the effects of *C. sativa* in mental illness. He thought that the voluptuary use of *C. sativa* could generate sensations common to the hallucinations and delusions in psychotic individuals. In addition, in the English medicine of the nineteenth century, *C. sativa* was introduced as an analgesic, anti-inflammatory, anti-emetic and anti-convulsant [1].

Despite the benefits described, it was strongly prohibited in the twentieth century due to its remarkable psychoactive effects and was removed from the British Pharmacopoeia in 1932 and included as a banned substance for therapeutic use, in the Act of the Parliament of the United Kingdom, Misuse of Drugs Regulation Act in 1971. In 1937 production, possession or transfer of *C. sativa* was forbidden in the USA due to federal law "The Marihuana Tax Act" [1].

2. Objectives

The objective of this work was to carry out a general investigation on the medicinal use of Cannabis from its historical path to the present, its technical and scientific basis, its mechanism of action and above all the therapeutic indications and the beneficial effects it can have on health of the human being. It was also necessary to understand the physical and chemical parameters in which the active molecules fall and also to ascertain their pharmacological and pharmacokinetic properties. It is also important to make a legal framework and the way in which the use of Cannabis is integrated and legislated in the legal frameworks of the various countries, with a special focus on Portugal.

3. Methods

In this work, a bibliographic review was made about the information already published on the medicinal use of Cannabis. The literature search was made using the most comprehensive platforms such as PubMed, NCBI and Google Scholar in the period between May and November 2020. The choice of research material had as inclusion criteria: the date of publications, with the most recent being preferred; the prestige of the authors, publishers and organizations involved; and the inclusion of the keywords: Cannabis, medicinal use, tetrahydrocannabinol, cannabidiol, endocannabinoid and cannabinoid receptors. The websites of Diário da República, INFARMED and Ordem dos Farmacêuticos were also consulted in order to find the legislation available and currently in force in Portugal in order to regulate the medical and safe use of Cannabis.

4. *Cannabis sativa L.* and the endocannabinoid system

4.1 Cannabis plant taxonomic classification

Regarding the taxonomical classification, the *Cannabis* plants differ in three hypothetical species, *Cannabis sativa Linnaeus*, *Cannabis indica Lamarck*, and *Cannabis ruderalis Janisch*. Genetic-taxonomic analyses of allozyme frequency in different genes have established that there was a previous geographical separation that existed between *C. sativa* ("European"), *C. indica* ("South Asiatic-African") and *C. ruderalis* ("Central Asiatic"). These three species differ fundamentally in terms of height and content of psychoactive molecules [1]. *Cannabis sativa* is distinguishable from other species, for example *Cannabis indica*, in many features like height, stature of the plant, internodal length, leaf size and structure, bud size and density, flowering time, odor smoking ability, and pharmacologic effects. *Indica* plant tend to grow shorter and bushier than the *sativa* plants, and the strains tend to have wide and short leaves, with short wide blades [44].

4.2 Botanical features of Cannabis sativa

C. sativa is a dioecious, rarely monoecious, annual plant of the family *Cannabinaceae*, having erected stems, which depending on the environmental conditions and the genetic variety, can reach up to 5 meters [1]. The leaves are palmate, usually composed of five to seven leaflets, linear-lanceolate, taper at both ends and the margins sharply serrate. The male flowers don't display petals, axillary or terminal panicles, having five yellowish tepals and five anthers. The female flowers germinate in the axils and terminally with one single-ovulate closely adherent perianth. A single, small, smooth, light brownish-grey fruit is produced per flower and propagated, thanks to bird predation. In addition, *C. sativa* is rich in trichomes, epidermal glandular protuberances that cover the leaves, bracts, and stems of the plant [1]. These glandular trichomes surround secondary metabolites as phytocannabinoids, responsible for the defense and interaction with herbivores and pests, and terpenoids, which provide the known smell of the *C. sativa* [1]. The form of the plant varies according to the climate and species. It grows more commonly as a persistent weed at the edge of the cultivated fields on land with high nitrogen content. After 8–12 days, *C. sativa* seeds germinate and their coat splits, opens and exposes the root and two circular embryonic cotyledons grow. During its growth, the plant requires a moderate level of environmental and soil humidity and a good light intensity. *C. sativa* grows vertically and originates continuously new leaves

mostly in pre-flowering phase, with the production of new branches and nodes. It will take about 6–22 weeks and need low light [1].

4.2.1 Phytochemistry of Cannabis sativa L.

Cannabis sativa is a plant that contains in its structure more than 100 phytocannabinoids classified in eleven different chemical classes. These molecules are C21 terpenophenolic constituents and make approximately 24% of the total natural products of the plant [4]. They are compounds chemically related to the terpenes with their ring structure derived from a geranyl pyrophosphate (C10 monoterpene subunit). The cytosolic mevalonate and the plastidial methylerythritol phosphate (MEP) are two independent pathways reported to be responsible for plant terpenoid biosynthesis [42].

The phytocannabinoids content of *C. sativa* is influenced by particular extreme environmental conditions of humidity, temperature, radiation, soil nutrients, and parasites. Phytocannabinoids and several terpenes, present in *C. sativa* leaves and flowers, serve as a barrier to water loss [1]. In addition, sparse rainfall, low humidity, and sunny climate will influence the plant to be richer in psychoactive components. The same *C. sativa* pollen shows an increase in the phytocannabinoids content with decreased humidity. Growing conditions, geographical location and plant processing methods will influence the proportion of each chemical class in the Cannabis plant, and so, the proportions of each phytocannabinoid type or non-cannabinoid content will additionally influence and modulate the pharmacological behavior of whole Cannabis extracts [4].

Terpenes and phytocannabinoids can block other sources of environmental stress such as attacks by bacteria, fungi, and insect, or competition with surrounding vegetation. In particular, the *C. sativa* female plants are often noted for its aromatic quality and many of the terpenes produced (pinene, limonene, terpineol, and borneol), which are known to possess insect-repellent properties and may help to suppress the growth of the surrounding vegetation. Also, the same resin of the glandular trichomes is a sophisticated defensive system to trap insects and may show antibiotic and antifungal properties [1].

The first step in the cannabinoid biosynthesis pathway (FIG.2) is the formation of olivetolic acid, the biosynthesis pathway of which has not been fully elucidated. Olivetolic acid and geranyl diphosphate are coupled under the influence of the prenylase, geranyl diphosphate: olivetolate geranyltransferase, yielding cannabigerolic acid (CBGA). This, in turn, is oxido-cyclized by flavin adenine dinucleotide (FAD)-dependent oxidases,

namely, Δ9-tetrahydrocannabinolic acid (Δ9-THC) synthase, cannabidiolic acid (CBDA) synthase and cannabichromenic acid (CBCA) synthase, producing Δ9-tetrahydrocannabinolic acid (THC), cannabidiolic acid (CBD), and cannabichromenic acid. The type class that we can find in the largest proportion is the Δ9-tetrahydrocannabinol type comprising 17,3% of the total phytocannabinoid content, being followed by the cannabigerol type [42].

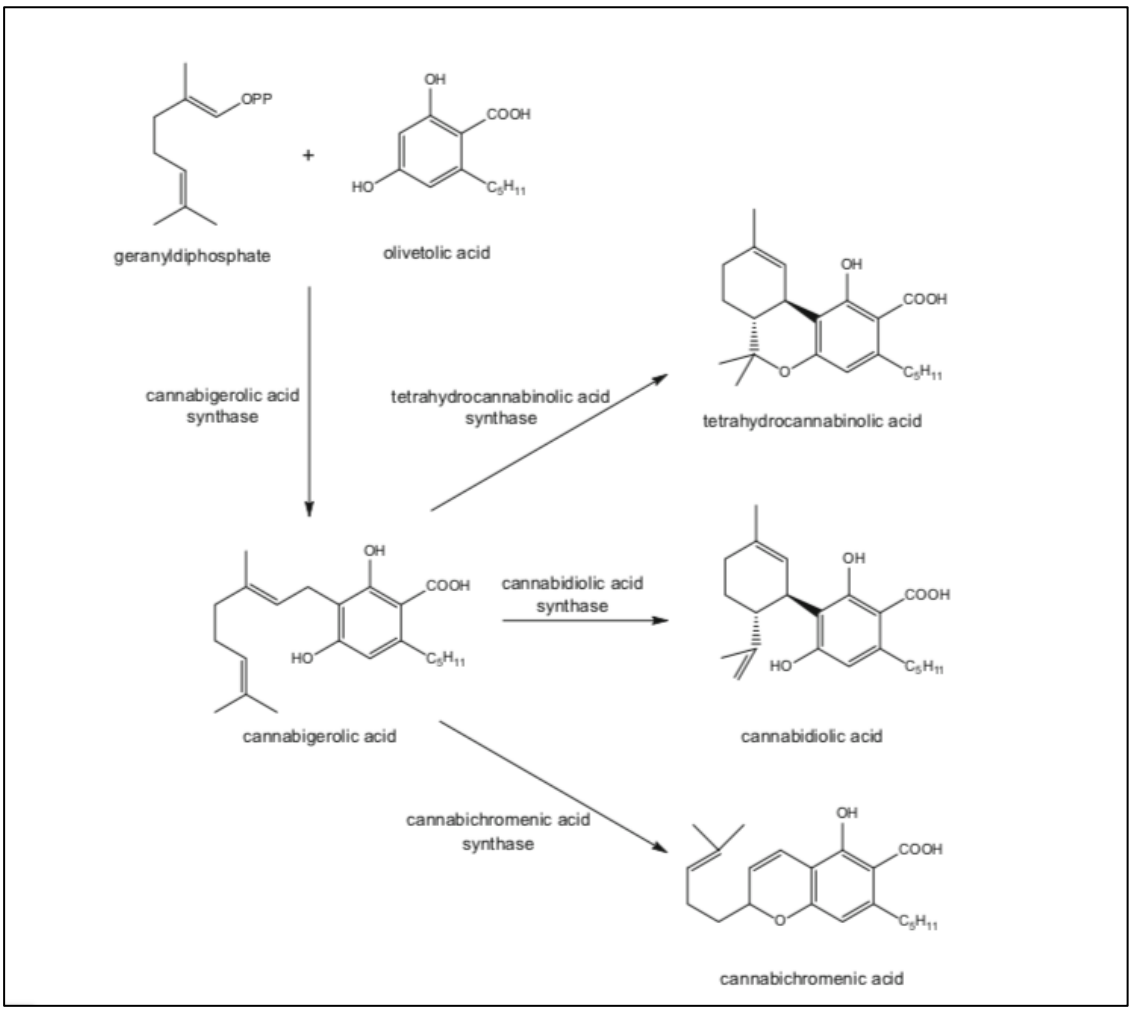

FIG.2: Biosynthesis of tetrahydrocannabinolic acid (THCA) and related cannabinoids. [42]

4.2.2 Δ9-Tetrahydrocannabinol

Trans-Δ-9-tetrahydrocannabinol (Δ9-THC) is the main component of *C. sativa*, responsible for the psychoactive effects [4]. Many Δ9-THC stereoisomers, degradation products or enzymatically generated by products, occur as minor constituents of this class. Δ9-THC is unstable when extracted or chemically purified. It presents itself as an amorphous gum that changes quickly to brown. Δ9-THC degradation is mostly oxidative and it has been estimated in about 10% of the pure product. It has been hypothesized that *C. sativa* may have other metabolic pathways for Δ9-THC degradation [1]. One of the more stable metabolites is Δ8-tetrahydrocannabinol, originated by an acidic isomerization of Δ9-THC with a shift of the endocyclic double bond. In addition, from dihydrocannabinol, Δ9-THC intermediate, could derive tri-hydrocannabinol, identified in the pollen of Cannabis. Other Δ9-THC isomers have been also identified, such as cis-Δ-9-tetrahydrocannabinol, Δ-6a- and Δ-10a-tetrahydrocannabinol, unknown as natural products but synthesized by oxidative aromatization of Δ9-THC. The psychoactive effects of Δ9-THC include anxiety, paranoia, perceptual alterations, and cognitive deficits. Moreover, a low Δ9-THC acute toxicity in murine models has also been observed. When Δ9-THC is taken it can develop hypolocomotion, hypothermia, catalepsy, analgesia, and increased food intake [4].

4.2.3 Cannabidiol

Cannabidiol is a non-psychotropic phytocannabinoid and currently the third most abundant chemical class type in Cannabis, after Δ9-THC and cannabidivarin [4]. Another phytocannabinoid in this class, cannabimovone, was isolated in 2010, thereby increasing the number of phytocannabinoids of this type from seven in 2005 to eight [4]. This class now makes up 7,7% of phytocannabinoid content. Despite the structural similarity between CBD and Δ9-THC, CBD has a low agonism for cannabinoid receptors; in particular, it is considered as an allosteric negative modulator of CB1 and CB2 receptors. Current evidence showed that CBD displays pharmacological effects via specific molecular targets such as adenosine receptors, glycine receptors, opioid receptors, serotonin receptors, non-endocannabinoid G protein-coupled receptors, nicotinic acetylcholine receptors, proliferator-activated receptors [5]. Moreover, CBD shows anticonvulsant, anti-spasmodic, anxiolytic, anti- nausea, anti-rheumatoid arthritis, and neuroprotective properties. Recently, it has been demonstrated that CBD is an inverse agonist for G protein-coupled orphan receptors such as GPR3, GPR6, and GPR12,

suggesting new therapeutic uses of CBD for Alzheimer's disease, Parkinson's disease, cancer, and infertility [4].

4.3 Endocannabinoid system

4.3.1 Cannabinoid Receptors

The interest for phytocannabinoids has lasted for thousands of years, but its main structure was only reported in 1971 [4]. This discovery gave rise to research that found the cannabinoid receptors, CB1 in 1990, largely located in the central nervous system (they are present in very high levels in several brain regions), and CB2 in 1993, in many peripheral organs and tissues, e.g., immune cells, spleen, adrenals, sympathetic ganglia, pancreas, skin, heart, blood vessels, lung, and parts of the urogenital tract and gastrointestinal tract [6].

Howlett *et al*. (1990) provided definitive proof for a cannabinoid receptor. Their work established that cannabinoids activate a G protein-coupled receptor (GPCR) that inhibits adenylyl cyclase [6]. The cloning of a cannabinoid receptor by Matsuda *et al.* provided the final evidence for the existence of a cannabinoid receptor and allowed the identification of cannabinoid receptor-expressing neurons. This cloning was swiftly followed by the cloning of a second cannabinoid receptor, designated CB2, from a promyelocytic cell [7].

The mammalian endocannabinoid system was then established, when the endogenous cannabinoid receptor ligands were found: arachidonylethanolamide (AEA-anandamide) and 2-arachidonylglycerol (2-AG), and was found evidence that this molecules have important and numerous physiological actions [6]. The endocannabinoid system consists of the endogenous cannabinoids, cannabinoid receptors and the enzymes that synthesize and degrade endocannabinoids. It is on CB1 receptor that the psychotropic activity of Δ9-THC happens, being the Δ9-THC, partial agonist on this receptor, and on CB2, a modulator of immunological and anti-inflammatory effects. The CB1-mediated effects (psychotropic and neuromodulator effects) are caused by the perturbation of GABA/glutamatergic neurotransmission and dopamine release, and above all, they are generally acute, transient, and self-limited. Overtime, other molecular targets outside of the endocannabinoid system were identified, such as ion channels, non-CB1 or CB2 G-protein coupled receptors, enzymes, and transporters [43].

4.3.2 Mechanism of action

Amid and Declan have written, and according to the studies of Pertwee *et al*. (2008), that both CB1 and CB2 receptors are mostly negatively linked to adenylate cyclase activity. When the receptors are expressed in cell lines, they start a pertussis toxic mediated event that requires Gi/o signaling, resulting in a reduced production of cAMP (FIG.3) [3].

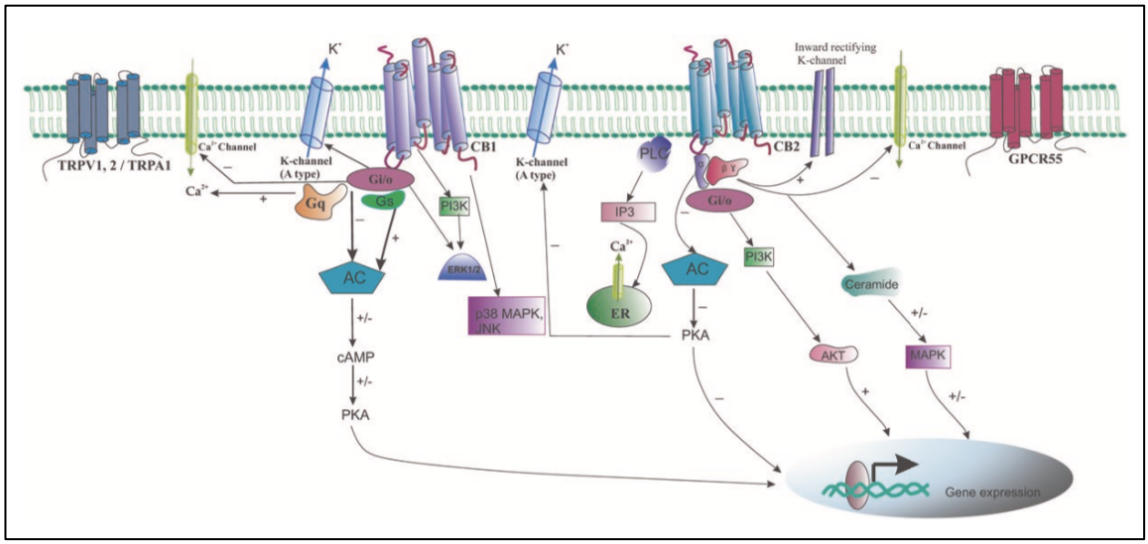

FIG.3 Schematic outline for some of the possible receptors for phytocannabinoids and endocannabinoids. [3]

The prototypical G-protein coupled receptors for cannabinoids are CB1 and CB2, but GPR55 has been suggested to be a possible third cannabinoid receptor. CB1 and CB2 are negatively coupled to adenylate cyclase (AC) via-Gi/o, while GPR55 is potentially linked to the IP3/DAG/Ca2+ system. Cannabinoids are also known to bind to transient receptor potential channels such as TRPV1, TRPV2 and TRPA1. Possible downstream effects include the regulation of genes and ion channel activity (A-type K+ channels).

A feature that distinguishes endocannabinoids from many other neuromodulators is that they are not synthesized in advance and stored in vesicles. Rather, their precursors exist in cell membranes and are cleaved by specific enzymes. This form of synthesis is often referred to as ‘on demand’. Many of the effects of cannabinoids and endocannabinoids are mediated by the two G protein-coupled receptors (GPCRs), CB1 and CB2, although additional receptors may be involved [3].

Concerning the endocannabinoid system, ligand binding studies have shown that anandamide is capable of inhibiting adenylate cyclase activity in membranes possessing

CB1 receptors [8]. However, anandamide showed markedly less effectiveness on CHO cells expressing CB2 receptors, suggesting that anandamide has differential effects on CB1 and CB2 receptors. In the other hand, the other main endocannabinoid, 2-arachidonoylglycerol (2-AG), will take action as a full agonist at the cannabinoid receptors when inhibiting, forskolin-induced cAMP accumulation [3].

The isoform of the adenylate cyclase that associates with the receptor will be an important modulator of the downstream effects of CB receptor activation. For example, ligand binding to CB receptors co-expressed with adenylate cyclase isoforms 1, 3, 4, 5 or 8 leads to inhibition of cAMP, while co-expression with adenylate cyclase isoforms 2, 4 or 7 leads to stimulation of cAMP production [3]. Therefore, CB1/CB2 are capable of activating Gq in addition to Gi/o, even though much of the endogenous or physiological activity appears to lead to an inhibition of cAMP. Amin and Declan (2019) conclude that the description of these mechanisms, that underlie key interactions between the cannabinoid receptors, and their agonists and antagonists, was further increased with the elucidation of the crystal structure of the human CB1 receptor in 2016 [3].

In 2007, Ryberg E *et al.* reported that the orphan receptor GPR55 is also a cannabinoid receptor [9]. His work showed that the CB1/CB2 receptor ligand [H^3] GPR55940 exhibited high specificity for GPR55 when cloned, sequenced and expressed. Furthermore, Δ9-THC, anandamide, 2-AG and the CB1 selective agonist noladin ether can also activate GPR55 receptor. It was also found that 2-AG can display almost 200-fold greater potency as an agonist at GPR55 compared with the prototypical CB1 and CB2 receptors, and that Δ9-THC has a greater efficacy at GPR55 compared with CB1 or CB2 [3]. Ryberg's studies also found that the GPR55 receptor couples to Ga13, a G protein that acts as a cellular signal transducer but has also been linked to increases in intracellular Ca2+ via a mechanism that involves Gq, G12, Rhoa, actin, phospholipase C and Ca2+ release from IP3-gated stores [9].

Amid and Declan concluded that the cannabinoid receptors' activity is connected to multiple second messenger systems that have the potential to couple enzyme activity to ion channel behavior, to gene activation, and more [3]. Sylantyev S *et al.* (2013) drew an investigation into the role of GPR55 at pre-synaptic terminals of CA3-CA1 (subfields of the hippocampus) synapses show that activation of GPR55 by L-alfa-lysophosphatidylinositol (LP1) transiently will improve calcium release probability by elevating presynaptic Ca2+ through activation of local Ca2+ stores, implying a possible role in short-term potentiation in hippocampus [3]. Amid and Declan (2019) state that

there have been suggestions that the GPR55 receptor could be classified as a type 3 cannabinoid receptor, CB3, because of its significant characteristics and distribution within the body, subcellular localization, temporal expression patterns and downstream signaling pathways. The study of this receptor will lead to a greater understanding of the function of endocannabinoids and effects of phytocannabinoids [3].

Another kind of receptors were studied by Zheng J (2013), that has shown significant evidence for a direct interaction between cannabinoids and transient receptor potential channels such as the transient receptor potential of vanilloid type 1 and 2 (TRPV1 and TRPV2) and transient receptor potential of ankyrin type 1 (TRPA1) [10]. TRPV1 and V2 channels are cation channels that are permeable to the passage of Na2+, K+ and Ca2+ across cell membranes and are activated by capsaicin or heat above temperatures of 40ºC and above ~50ºC, respectively, whereas TRPA1 are menthol and cold activated cation channels [3]. TRPV1 are activated by the endocannabinoids 2-AG and anandamide, while TRPV2 and TRPA1 activated by Δ9-THC and CBD. TRPV1 can be found in the cerebellum, basal ganglia, hippocampus, diencephalon and DRG neurons. TRPV2 tend to be localized to sensory neurons of the DRG, spinal cord, and trigeminal ganglia, but are also found in the cerebellum [3]. TRPA1 is manly adjacent to TRPV1 in sensory neurons. Activation of these receptors will lead to depolarization of the membrane and its activation, although TRPV1 and TRPA1 are known to exhibit functional desensitization [3]. Amid and Declan's (2019) explanation is that the activation of TRPV1 and TRPA1 by cannabinoids may lead to an immediate depolarization, but this will be followed by sensitization and subsequently inhibition because further activation by ligands, heat or cold will be muted as the channels are in a desensitized state [3].

5. Medicinal use of *Cannabis sativa* L.

5.1 Therapeutic Indications

5.1.1 Pain

A strong desire to find alternatives to other pain medication such as opioids has pushed cannabinoids-based therapies to the front line, and while there is a general lack of well-designed studies on the effects of medical Cannabis as pain medications, there is data indicating that smoking Cannabis is effective for some forms of pain [3]. Cannabinoids and cannabinoid-based products are used to alleviate neuropathic pain, which is a severe form or chronic pain caused by the lesions or disease affecting the somatosensory system. Evidence shows that specifically THC can be effective in reducing neuropathic pain, however the data is inconsistent and the potential side effects are concerning [3].

Studies conducted by Borgelt LM *et al* (2013) showed that participants generally reported effective pain relief with increased efficiency linked to higher THC content [11]. Overall the pain relief was modest, and not as effective as medications prescribed specifically for pain such as, the GABA receptor agonists gabapentin and pregabalin. As a general rule, more effective pain relief tends to occur when cannabinoids are taken together with existing pain medications as opposed to being taken on their own. For instance, oromucosal sprays such as Nabiximols (equal mixtures of Δ9-THC and CBD), taken along with existing pain medication results in a significant reduction in pain intensity. Similarly, Δ9-THC/CBD spray was found to be better than placebo when comparing mean pain relief [3].

5.1.2 Dystonia and dyskinesia

Benbadis *et al.* (2014) have studied the mechanism on which endocannabinoid system plays a role in modulation dyskinesia. The endocannabinoid receptors that we can find in the basal ganglia, especially the globus pallidus interna, pars reticulata, and cerebellum may regulate the tone and motor function through the effect of the endogenous cannabinoid ligand, arachidonylethanolamida (anadamide), on modulation of GABA transmission [12]. Many studies have been done with primate or rat models to determine if cannabinoid agonists or antagonists could act to suppress dyskinesias

without exacerbating hypokinesis; however, translation to patients has proved difficult [13].

Dystonia happens when an overactivity of muscles required for normal movement occurs with more strength or activation than necessary, usually recurring to unnecessary muscles, including those that supposedly would not be involved in these movements. These events are often painful and uncomfortable in addition to interfering with normal body functions. It can be primary, as in torticollis and blepharospasm/orofacial dyskinesias or dystonias (Meige syndrome) or as part of another condition such as Huntington's disease (HD) and tardive dyskinesia after dopa-blocking drugs. The globus pallidus and substantia nigra pars reticularis contain CB1 receptors, with cannabinoids acting as neuromodulators and enhancing GABA release and decreasing its reuptake]13].

Koppel BS *et al.* (2015) [13] speak on Marsden's (1981) reports on a patient with torticoliis that improved his condition by smoking Cannabis [14]. An open-label series followed, and self-reported improvement with smoking marijuana was described in 2002 in a patient with central pain and dystonia and in a patient with Wilson disease [13].

5.1.3 Epilepsy

Amin MR *et al.* (2019) have summoned in their reviews many studies that were performed to assess the impact cannabinoids can have in the treatment of severe symptoms in epilepsy. The therapeutic potential of Cannabis has been studied for many years, with a special focus on the ability to reduce seizures in patients with epilepsy, not only in intensity but also in frequency, and to decrease spasticity [3]. Some studies have been done using animal models, showing that CBD has anticonvulsant abilities when tested in audiogenic seizure models, pilocarpine models and electroshock models. The tests performed by Wallace MJ *et al*. (2001) were designed to assess the efficacy of Δ9-THC and CBD in animal models of epilepsy, showing that both Δ9-THC and CBD have anticonvulsant effects in rodents [15]. The same team also studied the mechanism of the endocannabinoid system on modulating the intensity of seizures and concluded that the "endocannabinoid anandamide produces anticonvulsant effects in rodents as well" [3]. A synthetic agonist of CB1 receptors, WIN55212, was developed to evaluate the synergistic effect when given in conjunction with standard epileptic drugs, reveling that a greater degree of relief from seizures was obtained [3].

The conclusions taken by Amin MR *et al.* (2019) were that “when it comes to animal models, the evidence is overwhelmingly in support of the anticonvulsant effects of cannabinoids”, but nowadays we lack evidence on well-constructed, randomized clinical trials in humans [3].

Antiepileptic drugs work by either reducing excitation (via blocking voltage-gated Na+ channels or Ca2+ channels, usually T-type), or by increasing inhibition (often by modulation GABA related activity) in the CNS. CB1 receptors are known to modulate neuronal excitability by decreasing presynaptic neurotransmitter release [3]. CB1 receptors are also known to play homeostatic roles since increased levels of activity will result in the release of endocannabinoids that feedback on presynaptic CB1. Ligand binding to these presynaptic receptors activate Gi/o or Gq will lead to a reduction in transmitter release [3]. Activation of the CB1 receptors by endocannabinoids is involved in retrograde inhibition of transmitter release, the control of neuronal excitability and even in the regulation of some forms of synaptic plasticity [3]. On that note, Amid MR *et al.* (2019) concluded that there is a possibility that increased levels of CB1 receptor activity might make less intense neuronal excitation [3]. The tests previously mentioned revealed that the specific CB1 agonist WIN55212, and the cannabinoid Δ9-THC were both able to abolish spontaneous epileptic seizures in rats, and levels of 2-AG and expression of CB1 protein increased in the hippocampus of pilocarpine-induced seizure animals [3].

On a study driven by Monory *et al.* (2003), the experimenters introduced conditional mutants lacking CB1 receptors in specific neuronal populations and used a kainic acid model of seizures to show that the CB1 receptors localized to hippocampal glutamatergic neurons are necessary for the CB1-dependent protection against kainic acid-induced acute excitotoxic seizures [16]. Interestingly the CB1 receptors associated with GABAergic neurons did not appear to play a significant neuroprotective role against KA-induced seizures, only the CB1 receptors localized to glutamatergic neurons. Additionally, virus-mediated conditional overexpression of CB1 receptors in pyramidal and mossy fiber cells of the mouse hippocampus confers neuroprotection and reduces convulsions in an acute kainic acid seizure model [3]. The seizures induced the release of anandamide followed by activation of CB1 receptors. Protection against epileptic-like synchronous activity and overexcitability in neural networks may also be given by activation of CB1 receptors. In healthy individuals, the endocannabinoid system working through CB1 confers neuroprotection, and in those afflicted with refractory epilepsy, activation of CB1 might constitute an important avenue for medical intervention [3].

Amid MR *et al.* (2019) also wondered how activation of CB1 leads to a downregulation of neural activity and concluded that it could happen via a number of mechanisms. They refer a study driven by Mackie K *et al.* (1992) that showed that presynaptic activation of CB1 reduces presynaptic Ca2+ entry through N-type Ca2+ channels and lowers glutamate release [17]. Activation of CB1 also leads to an enhancement of A-type voltage gated K+ channels as well as an enhancement of inward rectifying K+ channels conductance [18]. The overall effect of activation of either of these K channel types could lead to a reduction in excitation [3].

5.1.4 Neurodegenerative diseases

Multiple sclerosis and Alzheimer's disease are now new targets to the use of medical cannabinoids. Additionally, a role in schizophrenia and other psychiatric conditions is being also studied. Multiple sclerosis shares a number of pathological mechanisms with other neurodegenerative diseases such as link with neurodegeneration, neuroinflammation and excitotoxicity [3]. Demyelination and degeneration of motor neurons often cause neuropathic pain, aberrant neuronal activity and debilitating and painful muscle spasms. Cannabis plant extracts have been used successfully in order to alleviate the symptoms of MS [3]. The administration of Sativex (1:1 ratio of Δ9-THC:CBD) via the oral-mucosal route has analgesic effects and limits neuropathic pain while also reducing muscle spasms. CBD has shown to be capable of relieving neuropathic pain associated with Multiple Sclerosis. In patients with MS, endocannabinoid levels in the circulating plasma are increased [3]. Experimental animal models for MS created by Jean-Gilles L *et al* (2009) where the animals were induced with experimental autoimmune encephalomyelitis (EAE), the endocannabinoid levels in the brain have actually been downregulated. In fact, animals in which CB1 receptors are decreased and are then induced with EAE tend to develop neurodegeneration more rapidly than those that express CB1 receptors reflecting on a neuroprotective role for CB1 [3].

Alzheimer's disease is, according to Amin MR *et al*. (2019), an "age-related neurodegenerative disease in which a pathological hallmark is the onset of neurofibrillary tangles and amyloid beta plaques in the brain" [3]. Neurodegeneration occurs and the individual will develop a progressive decline in cognition and memory. An activation of microglia will happen in plaque, filling regions along with neuroinflammation and oxidative stress. Cell death occurs via multiple mechanisms but in large part caused by excitotoxicity. CB1 receptor expression is high in basal ganglia and hippocampus, where

beta-amyloid plaques tend to occur most often in AD [3]. Neuronal CB1 expression is reduced in these two regions [19] while expression of CB1 and CB2 expressing microglia is increased [20]. These studies made by Westlake *et al*. (1994) and Ramirez BG *et al*. (2005) conclude that medications that protect from excitotoxicity and neuroinflammation have the potential to offer therapeutic benefits to individuals afflicted with AD because they relieve secondary pathologies rather than the direct cause of the disease [3]. Later in time, the studies driven by Benito C *et al.* (2003) and Pazos MR *et al.* (2004) conclude that there is a link between the endocannabinoid system and Alzheimer's disease [21,22]. Amid MR *et al.* (2009), point out the evidence existing THC's ability to actively inhibit Aβ aggregation. By the studies published by Eubanks LM *et al.* (2006), that show that "Δ9-THC has been shown to be directly linked to AD" [3]. In this study, Eubanks and colleagues found that Δ9-THC competitively inhibits acetylcholinesterase activity and reduces Aβ aggregation in vitro. In addition, Amid MR *et al.* (2009) also mention the ability of CB1 receptor agonists anandamide and nolandin are both capable of inhibiting Aβ toxicity in a differentiated human teratocarcinoma cell line Ntera 2/c1-D1-neurons [3].

Their important conclusion is that in parallel with epilepsy, "there may be a connection in decrease in glutamate release and downregulation of N-type Ca channel activity, or an upregulation of K-channel activity", both of which are associated with reduced synaptic transmitter release. [3]

5.1.5 Nausea and vomits caused by chemotherapy administration

Controlling emesis in chemotherapy has been done efficiently over the years, since 5-HT receptor blockade was developed. Other protocols using dexamethasone in combination, 5-HT3 antagonists and NK1 receptor antagonists have also been shown to be effective in reducing vomiting and nausea. Meanwhile, several studies have been done in order to understand if the use of cannabinoids can also be beneficial for the control of these adverse effects [23].

A Canadian team led by Parker L *et al.* (2010) reviewed the existing literature on the mechanism of action of Cannabis in controlling nausea and vomiting. In their conclusions they state that evidence showed that by manipulating the endocannabinoid system we are able to reduce nausea and vomiting in humans and other animals. The anti-emetic effect of cannabinoids has been shown across a wide variety of animals that are capable of vomiting in response to a toxic challenge [24]. Agonists of the CB1 receptor will suppress vomiting, which is reversed by CB1 antagonism, and CB1 inverse

agonism promotes vomiting [24]. Evidence from animal experiments suggests that cannabinoids may be especially useful in treating the more difficult to control symptoms of nausea and anticipatory nausea in chemotherapy patients. Although rats and mice are incapable of vomiting, they display a distinctive conditioned gaping response when re-exposed to cues (flavours or contexts) paired with a nauseating treatment. Cannabinoid agonists (Δ9-THC, HU-210) and the fatty acid amide hydrolase (FAAH) inhibitor, URB-597, suppress conditioned gaping reactions (nausea) in rats, as they suppress vomiting in emetic species. Inverse agonists, but not neutral antagonists, of the CB1 receptor promote nausea, and at subthreshold doses potentiate nausea produced by other toxins (LiCl) [24]. They also suggest that CBD suppresses nausea and vomiting within a limited dose range. The anti-nausea/anti-emetic effects of CBD may be mediated by indirect activation of somatodendritic 5-HT1A receptors in the dorsal raphe nucleus; activation of these autoreceptors reduces the release of 5-HT in terminal forebrain regions. Preclinical research indicates that cannabinoids, including CBD, may be effective clinically for treating both nausea and vomiting produced by chemotherapy or other therapeutic treatments [24].

Rocha Machado *et al.* (2008) evaluated the efficacy of cannabinoids for their antiemetic efficacy in cancer patients receiving chemotherapy. In the meta-analysis, 13 double blind and crossover randomized clinical trials were reviewed regarding the antiemetic effect of cannabinoids versus either placebo or neuroleptics for cancer patients receiving chemotherapy [23]. The drugs used were nabilone (a synthetic cannabinoid), dronabinol (an enantiomer form of tetrahydrocannabinol), and levonantradol (a synthetic analog of dronabinol) were reviewed for antiemetic effects. In short, the results showed that dronabinol was more effective compared with the neuroleptics [23]. All three drugs had a more favorable side effects profile when compared with conventional antiemetics (prochlorperazine, chlorpromazine, domperidone, haloperidol, alizapride, and metoclopramide) and placebo. It was noted that most dropouts in these studies were due to causes other than cannabinoid effects. The reasons for dropouts were mainly due to change of chemotherapeutic strategy during the study, death due to cancer, protocol violation, toxicity, use of concomitant antiemetic medication, and inadequate data [23]. The side effects associated with the medical cannabinoids were also seen across various patient populations. Side effects observed included paranoid delusions, hallucinations, dysphoria, and/or depression. More frequent side effects included a “high” sensation, sleepiness, sedation, and euphoria, which patients preferred to the control drugs (i.e., prochlorperazine,

chlorpromazine, domperidone, haloperidol, alizapride, metoclopramide, and placebo). The meta-analysis therefore concluded that while more research is needed, the superiority of the antiemetic efficacy of cannabinoids in cancer patients receiving chemotherapy was established [23].

5.2 Side effects

The side effects developed by Cannabis and cannabinoid receptor agonists are mostly very similar with small differences. The main goal of recreational use is to obtain those side effects that occurs at doses above the individual consumer's psychotropic threshold, with them being the need to feel pleasure of relax sensation. The sensory perception is altered and heightened, which may lead to dysphoria and anxiety or panic attacks. Other significant side effects are impairment of memory, reductions in psychomotor and cognitive performance, disordered perception of the passage of time, and euphoria [33].

Grotenhermen *et al.* (2012) bring the discussion if high consumption of Cannabis has long-term consequences on cognitive performance and, based on the data brought by Grant I *et al.* (2003) and Bolla KI *et al*. (2002), they state that "only extremely high consumption at levels hardly ever used for therapeutic purposes leads to irreversible cognitive impairments" [25,26]. They also perceived that the risk is much higher in children and adolescents particularly those who start use before going through puberty, concluding that the medical use in this age group should be "weighed up very carefully" [33].

One of the most significant side effects is the Cannabis ability to develop schizophrenic psychosis in individuals who regularly consume it. Data brought by Moore TH *et al.* (2004) showed that Cannabis consumption may double the risk of schizophrenia in adolescents [27], concluding that psychosis should be seen as a contraindication to treat patients with cannabinoid medicines. However, cases have been recorded where the use of THC proved to be an efficient treatment to refractory schizophrenia symptoms [33].

Despite the psychiatric side effect that can happen, it was also reported that some physical effects related to the cannabinoids use, with them being mostly tiredness, dizziness, tachycardia, orthostatic hypotension, dry mouth, reduced lacrimation, muscle relaxation, increased appetite and increase the risk of myocardial. Also, epidemiological

studies drawn by Hézode C *et al.* (2008) show that frequent cannabinoids user may be more likely to develop cirrhosis in patients with hepatitis C [28. Despite the diverse side effects caused by the use of cannabinoids, Grotenhermen *et al.* (2012) also stated that "no acute deaths have been described that could be unequivocally attributed solely to Cannabis consumption or treatment with cannabinoids" [33].

It has also been described that tolerance to some side effects like tiredness, dizziness, cardiovascular and psychoactive effects, may also happen in a period of days or weeks. On the other hand, frequent consumers may experience withdrawal symptoms after abrupt cessation of consumption, and they are described to be proximate in "character and intensity to those experienced after sudden cessation of cigarette smoking and include uneasiness, irritability, sleeplessness, increased perspiration, and loss of appetite" [33].

5.3 Pharmacokinetics

According to data brought by Amid and Declan (2019), the adipose tissue and spleen will be THC's main long-term storage sites due to the fact that it is a highly lipophilic molecule. It readily crosses the blood-brain barrier and can be found in high quantities in the brain [3]. THC released from fat has a half-life of several days and in some circumstances can lead up to several weeks to definitely clear from adipose tissue [29,30].

In the smoked way, it is estimated that up to 37% of Δ9-THC present in cigarettes can be delivered to the body, while up to 30% is destroyed via pyrolysis [3]. Huestis MA (2007) made experiments to access the Δ9-THC behavior in the human body, and determined that, by smoking, the cannabinoids will enter the blood stream extremely rapidly with rising levels detected in blood plasma within 1-2min of the first inhalation. In his experiments, puffs of a 3,5% Δ9-THC cigarette resulted in peak Δ9-THC blood plasma levels of approximately 270ng/ml, and in experiments where the THC content of cigarettes was kept at either a "low" dose of 1,75% or a "high" dose of 3,55%, the blood plasma levels obtained from individuals smoking the higher dose cigarettes were variable and ranged from <90ng/ml to >250ng/ml [29].

Further data indicated that the bioavailability varies within each individual, and factors such as weight, gender, age, health and physiological background, will most certainly alter the way to which Δ9-THC and other cannabinoids affect an individual.

Studies have shown that Δ9-THC taken orally usually peaks in the circulation within 1-2h, with blood plasma levels lower than those obtained during smoking [3].

The metabolism Δ9-THC occurs manly in the liver, and it is converted quickly, within minutes, into 11-hydroxy-THC which is psychotropically active, and to 11-nor-9-carboxy-THC which is not. The second metabolite is the main component found in the urine analysis and it is used to identify Cannabis consumption [30].

5.4 Interactions

Grotenhermen et al. (2012) comprised some data concerning Cannabis interactions. They state that THC can interfere with metabolism of other substances also metabolized by cytochrome P450, because it is manly metabolized in the liver (manly by CYP2C) [33].

Kosel BW *et al.* (2002) studied the impact of cannabinoids in patients receiving antiretroviral drugs [31], and Engels FK *et al.* (2007) studied the same impact in oncological patients [32], and both concluded that cannabinoids did not influence plasma level of antiretroviral and cytostatic drugs, when received simultaneously. Cannabinoids may interact mostly with drugs that share the same mechanisms of action, cell signal transducers or ligands, which may lead to mutual modulation of effect. Clinically, the most visible evidence is the increased manifestation of symptoms such as tiredness when cannabinoids are taken together with other psychotropic agents such alcohol and benzodiazepines, or interactions with medications that also act on the cardiovascular system (such as amphetamines, atropine, and beta-blockers). In the smoked way, Cannabis may reduce the plasma concentration of antipsychotics (clozapine, olanzapine [33].

Patients may also benefit from interaction effects when cannabinoids are given concurrently with antispastic drugs, broncholytics, analgesics, and antiemetics, as well as in the treatment of glaucoma [33].

5.5 Special Populations

5.5.1 Pediatric use

A review by Koren G and Cohen R in Israel, provided a first approach on the use of medical Cannabis in children and pregnant women [36]. They studied the effects of Cannabis-based preparations in children diagnosed with Autism Spectrum Disorder (ASD) and pregnant women with nausea caused by Hyperemesis Gravidarum (HG). They provide a first insight at the results obtained in children and pregnant women. Despite the various approved drugs and their use for few years now, there is still little evidence and few studies on the safe use in children and pregnant women.

One of the problems of children with ASD is the treatment of symptoms such as aggression, self-harm, restlessness, sleep problems, hyperactivity, anxiety, and other comorbidities [36]. Conventional medical treatment includes various psychotropic medications such as atypical antipsychotics, selective serotonin reuptake inhibitors (SSRIs), stimulants, and anxiolytics. Koren G *et al.* (2020) conducted an observational retrospective study, where they reviewed children diagnosed with ASD and receiving their Cannabis from an Israel company. The children were given oral oil-based cannabinoid extract drops, after permission was granted by the Israeli Ministry of Health and under the supervision of the children's pediatricians. It was used a cannabinoid oil solution with a concentration of 30% and at 1/20 ratio of CBD and THC. In a first instance, the parents were instructed by a nurse on how to administer the preparation. Then, a biweekly follow-up telephone interview was conducted, where parents were asked to report the improvement and side effects of the Cannabis given to their children. The regimen started with one or two drops given under the tongue, with the dose increased or decreased based on the child's reported response. The population of this study were fifty-three children, ages between 4 and 22, and were followed up for changes in ASD-related symptoms [36;37]. The study ran between 30 to 588 days, and the daily doses of CBD and THC were between 1,5mg to 315mg, and 0,5mg to 49,5mg, respectively. According to the study by Koren G *et al.* (2020), the "parents reported a notable improvement in self- injury and rage attacks, hyperactivity, sleep problems, anxiety and mood problems, and social communication and reciprocity issues". Concerning the side effects, the ones that were most reported were somnolence, nausea and change in appetite [36]. The conclusions taken by the study were 43.1% of the parents reported significant improvement, mild to moderate improvement was reported in 31.4%, and no

change was reported in 21.6%; in the case of 2 children (3.9%), parents reported a worsening of symptoms, suggesting that Cannabis-based preparations may have benefits in improving ASD symptoms, with mild and transient side effects. Nevertheless, there is still little evidence of the effectiveness and safety of these preparations because there are not yet enough studies to prove them [36].

5.5.2 Pregnant women use

Concerning pregnant women, the same team studied the effects of Cannabis on women who had nausea and vomiting, a condition called Hyperemesis Gravidarum. Some benefits of using Cannabis in nausea caused by chemotherapy have been presented and this team suggested that those benefits could also be usefull to this condition. The team evaluated four Motherisk-counseled cases of HG, following Cannabis use (Motherisk is a clinical and research program at Yitzhak Shamir Medical Center in Be'er Ya'akov, Israel). The team report a highly significant improvement in symptoms: the validated Pregnancy-Unique Quantification of Emesis (PUQE) (Appendix 1) [38] score improved from 14.5±1 to 7.5±0.58 (P=0.0004). Cannabis use was associated with a significant increase in PUQE Quality of Life scale, from 2±0.82 to 7±0.82 (P=0.0012) [36]. Their conclusion is that Cannabis may be effective for HG and should be further research in controlled trials.

Another relevant information that these investigators point out is that, according to Metz and Stickrath's studies is that Δ9-tetrahydrocannabinol crosses the placental barrier and can lead to smaller birth weights, and still and pre-term births [39].

5.6 Legislation

5.6.1 Portugal

It is believed that in Portugal, the use of Cannabis has been occurring since the 8th century, having been imported by Muslims. Already in the time of Garcia de Orta, its use has been described as appetite stimulant and euphoric. The introduction of approved Cannabis-based drugs in Portugal takes place first in 2012 with Sativex. Other relevant dates are legalization of the manufacture, processing and distribution of Cannabis for medicinal use that takes place in 2018, and the reach of drug status in 2019. When publishing the draft laws presented by the Portuguese "Bloco de Esquerda" party, a working group of the professional organization of Portuguese pharmacists – "Ordem dos

Farmaceuticos" issued an opinion on the use of Cannabis for medicinal purposes [34]. In that document, it is written that "drugs containing cannabinoids are classified as psychotropic substances, must be subject to a special medical prescription and cannot be subject to publicity with the public". It was also mentioned that "although pre-clinical trials have shown pharmacological activity, most effects need clinical evaluation", with the aim of alerting to the scarcity of information that still exists due to the fact that the drug is extremely recent and little used at large-scale. They also raise awareness of side effects and lack of evidence for some indications [34].

In Portugal, Cannabis-based preparations are not considered medications as they do not have enough information to be given Marketing Authorization Aplication (MAA), and according to Law 33/2018, medicines, preparations and substances based on the Cannabis plant are always subject to authorization issued by INFARMED. In the following year, Decree-Law 8/2019 complemented the previous law that regulates the use of drugs, preparations and substances based on the Cannabis plant for medicinal purposes. In this document, the principles and objectives regarding prescription, dispensation in pharmacy, possession and transportation, scientific research, information for professionals, as well as regulation and supervision of activities related to the use of the Cannabis plant for medicinal purposes were established. It also defines and fits the activities of cultivation, production, extraction and manufacturing, wholesale trade, import and export, transit, acquisition, sale and delivery of medicines, as well as the placing on the market of medicines and plant-based preparations and substances of Cannabis intended for human use for medicinal purposes. Later it is also reinforced by INFARMED that in relation to Cannabis-based drugs, these should be prescribed only in cases where it is determined that conventional treatments with authorized drugs are not producing the expected effects or cause relevant adverse effects. It should also be noted that in Portugal there is a tight and extensive legislation to prevent illegal drug trafficking, and Cannabis is also under Decree-Law nº. 15/93, of 22 January, which establishes the legal aspects of drug trafficking and consumption of psychotropic drugs and improves the fight against illegal drugs in Portugal. In addition, on January 31 of 2019, a decree was also issued regulating the price regime for preparations and substances based on the Cannabis plant for medicinal purposes [34].

Despite the drugs that have already been granted MAA, other drugs outside Portugal can be granted special authorization as long as the therapeutic benefit is justified and proven. In other countries, like the United States of America or Canada, in addition to Sativex®, other drugs whose active substance is dronabinol (Marinol® and

Syndros®), and nabilone (Cesamet®) were also approved. These are synthetic analogues of THC and are approved in the form of capsules (Marinol® and Cesamet®) or oral solution (Syndros®) [34]. These synthetic analogues of THC are approved to treat loss of appetite that causes weight loss in people with AIDS and treat severe nausea and vomiting caused by cancer chemotherapy.

5.6.2 Spain

Concerning the medicinal Cannabis use, Spain is somewhat behind compared to other European countries. In addition, the possession and use of Cannabis is still illegal, but it has been decriminalized. People are allowed to grow and use the plant for personal and medical purposes and will not be criminally charged. Catalonia (which is an autonomous community on the northeastern corner of Spain) is putting pressure on the Spanish government to legalize medical marijuana for use to treat pain and nausea resulting from cancer-or-chemotherapy treatments, and other diseases that cause chronic pain or appetite loss. They also seek to end Barcelona's popular Cannabis consuming clubs, which started as non-profit cooperatives for people who wanted to use marijuana medicinally. Overtime it became a popular tourist spot that contribute to the sale of black market, and uncontrolled and dangerous consumption of Cannabis. Their intentions to the future are to legally control the supply of medical Cannabis and shut down these clubs [35].

5.6.3 Italy

In Italy, the use of medicinal Cannabis was authorized in 2007. In 2014 was published a legislation that abolished the previously bureaucratic process required in order to obtain a prescription for the use of medicinal Cannabis, which allowed Cannabis to be available to patients with prescription from primary care physicians. A new law was introduced to make available the use of medicinal Cannabis, because "mode of delivery of drugs and galenic preparations based on cannabinoids for therapeutic purposes" made it possible, in regions like Sicily, and then Abruzzo, Puglia, Tuscany, Liguria, Veneto, Lombardy and Piemonte. In these notes, the national Government did not make concerns on the distribution of Cannabis, but tried to shield patient's rights and safety, stating that Cannabis-based drugs should be prescribed only "when other available medications have proven to be ineffective or inadequate to the therapeutic need for the patient"[35].

The Regional Health System will cover the costs through a hospital pharmacy or the patient's health insurance. The Italian Ministry of Health also started a pilot project to grow Cannabis plants and manufacture the products directly in Italy at the Military Chemical-Pharmaceutical Factory, in order to decrease import costs [35].

5.6.4 Germany

In Germany, the Federal Institute for Drugs and Medical Devices has authorized the medicinal use of Cannabis for special cases, which was strongly encouraged by the Federal Administrative Court in 2005. Currently, about 300 German patients with severe medical conditions are allowed to buy Cannabis products in any pharmacy to relieve their pain. The issue of costs is highly relevant in Germany, however, as palliative Cannabis treatment, which can amount to between €800 and €1,000 every month, is not covered by the health insurance system. The issue solved in July last year, when the Administrative Court of Cologne ruled that severely ill people who suffer from chronic pain can cultivate their own Cannabis plants. The court's reasoning was based on economic arguments: patients without adequate insurance to cover the costs of imported Cannabis products should nonetheless have access to treatment. The court also stressed that the requirements of the patients need to be assessed on a case-by-case basis [35].

5.6.5 Netherlands

Currently, the Office of Medicinal Cannabis (OMC) has approved three medicinal Cannabis products, being them Bedrocan, Bedrobinol, and Bediol which contain a standardized content of THC (18%, 13% and 5%, respectively) and CBD (0,8%, 0,2% and 6%, respectively). The cultivation and production of the Cannabis plants is assured by a single cultivator, approved and selected by the OMC. Each batch is analysed according to monograph formulated by the National Institute for Public Health and Environment, to ensure the quality control of the Cannabis production. It has specific guidelines that have to be followed regarding not only the THC and CBD content, but also to control the use of pesticides and heavy metals and adequate microbiological purity. At the end of the circuit, the patient can get his medicinal Cannabis at the pharmacy by presenting a prescription from the doctor [35].

6. Future Perspectives

Nowadays, the medicinal use of Cannabis is growing and making its place. There are many patient organizations, industry lobbyists, and political forces trying to impose the early use of medical Cannabis for management many diseases or alleviating their symptoms. Many places already recommend the use of Cannabis or Cannabis-based preparations for a variety of situations, without consulting a health professional or undergoing a medical evaluation to check effectiveness and safety. This has given rise to a phenomenon in which patients go to an appointment and confront health professionals with substances that they know could improve their condition, and ask or demand it to be prescribed, without having all the necessary information for administration in safety. Despite some clinicians are receptive to trying these therapies, they should warn of the lack of information and data that still exists. It is important to collect high-quality basic information promoting informed prescribing, such as proper indications, absolute and relative contraindications, dosages, formulations, and data regarding specific patient groups. Among the various routes of administration, smoked or injected should not be considered or recommended as therapeutic options [40].

Recently, opioid addiction and abuse has become a public health problem so it is thought that in the future cannabinoids may work as replacement therapy or to mitigate side effects, since they share similar mechanisms, only with a different pharmacological family. However, it is crucial to understand the variations between different plant strains and the effects of cannabinoids other than THC/CBD. The little information that exists does not yet allow us to safely confirm side effects and other factors that may be influenced by taking these substances such as such as the rates of addiction, the leakage of medical Cannabis to the recreational market, effects on driving and manipulating heavy machinery or effects on patients with duties that require high cognitive demand [41].

In the years to come, it is necessary to pursue major research effort aimed at developing pharmaceutical grade cannabinoid medications, documenting and publishing real-world data and large-scale registries reflecting ongoing clinical experience and collecting high quality evidence, to better inform all those who wish to improve the care of patients while still maintaining the professional standards that separate good practices from false information.

7. Conclusions

Despite the studies that have been done and the secular history of Cannabis use, we can conclude that there is still much to be discovered and evaluated. The use of Cannabis as a medicine date back thousands of years and many of the indications for which it was used are still practiced today, now with more scientific evidence and with more associated clinical practice.

The endocannabinoid system is a complex system, recently discovered, that still needs a lot of studies and a lot of research. Its mechanisms of action allow us to realize that new therapeutic targets may be in sight, bringing solutions for the treatment of some diseases and their associated symptoms. Additionally, the way in which the endocannabinoid system is located in the central nervous system put the neurological diseases at focus for studies on the efficacy of therapies using Cannabis.

Cannabis-based preparations have already proved being effective in some therapeutic indications such as the treatment of spasms in patients with multiple sclerosis, chronic neuropathic pain, symptoms associated with neurodegenerative diseases and relief of nausea and vomiting associated with the administration of cytotoxic drugs. Approved Cannabis-based drugs already show beneficial results in patients refractory to first-line therapies such as tricyclic antidepressants, serotonin–norepinephrine reuptake inhibitors, gabapentinoids and opioids. Although some of its medical uses have already been put into practice, there is still not enough evidence to support its safe and efficacy use in all therapeutic indications that are often proposed.

More clinical trials are needed to support the therapeutic evidence. Future clinical trials should accommodate more population and more specific groups, such as pregnant women, infants or children and also realize the effects that this medication can have in the short and long term on humans.

On a final note, we need to warn of the dangers to recreational and unruly Cannabis use. The damaging effects of psychotropic properties are real and can lead to health problems. Illegal consumption is a public health problem insofar as it can generate addictions, crimes and ultimately death. Properly regulated, everything has its space and time. Freedom is useful if we know what to do with it.

Bibliography

[1] Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M et al. Cannabis sativa: a comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol 227 300-315. 2018.

[2] Thomas RH, Cunningham MO. Cannabis and Epilepsy. Pract Neurol 18(6):465-471. 2018.

[3] Amin MR, Ali DW. Pharmacology of Medical Cannabis. Adv Exp Med Biol. 1162 151-165. 2019.

[4] Elsohly M, Gul W. Constituents of Cannabis sativa. In: Pertwee RG (ed) Handbook of Cannabis. Oxford University Press, Oxford Chapter 1: 3–22. 2014.

[5] Laun AS, Shrader SH, Brown KJ, Song ZH. GPR3, GPR6, and GPR12 as novel molecular targets: their biological functions and interaction with cannabidiol. Acta Pharmacol Sin. 40(3):300-308. 2019.

[6] Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54 161–202. 2002.

[7] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561-564. 1990.

[8] Howlett AC, Mukhopadhyay S. Cellular signal transduction by anandamide and 2-arachidonoylglycerol. Chem Phys Lipids 108(1-2) 53-70. 2000.

[9] Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 152(7) 1092-110. 2007.

[10] Zheng J. Molecular mechanism of TRP channels. Compr Physiol 3(1) 221-242. 2013.

[11] Borgelt LM, Franson KL, Nussbaum AM, Wangs GS. The pharmacologic and clinic effects of medical cannabis. Pharmacoterapy 33(2) 195-209. 2013.

[12] Benbadis SR, Sanchez-Ramos J, Bozorg A, Giarratano M, Kalidas K, Katzin L, Robertson D, Vu T, Smith A, Zesiewicz T. Medical marijuana in neurology. Expert Rev Neurother 14 1453-1465. 2014.

[13] S Koppel Barbara . Cannabis in the Treatment of Dystonia, Dyskinesias, and Tics. Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics vol. 12,4; 790-792. 2015.

[14] Marsden CD. Treatment of torsion dystonia. In: Barbeau A (ed.) Disorders of movement, current status of modern therapy, Vol 8. Lippincott, Philadelphia, PA, 81-104. 1981.

[15] Wallace MJ, Wiley JL, Martin BR, DeLorenzo RJ. Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. Eur J Pharmacol 428(1) 51-57. 2001.

[16] Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wölfel B, Dodt HU, Zieglgänsberger W, Wotjak CT, Mackie K, Elphick MR,

Marsicano G, Lutz B. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51(4) 455-466. 2006.

[17] Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastomaglioma cells. Proc Natl Acad Sci U S A 89(9) 3825-3829. 1992.

[18] Mackie K, Lai Y, Westenbroek R, Mitchell R. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J Neurosci 15(10) 6552-6561. 1995.

[19] Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63(3) 637-652. 1994.

[20] Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. J Neurosci 25(8) 1904-1913. 2005.

[21] Benito C, Núñez E, Tolón RM, Carrier EJ, Rábano A, Hillard CJ, Romero J. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic paque-associated glia in Alzheimer's disease brains. J Neurosci 23(35) 11136-11141. 2003.

[22] Pazos MR, Nunez E, Benito C, Tolon RM, Romero J. Role of the endocannabinoid system in Alzheimer's disease: new perspectives. Life Sci 75(16) 1907-1915. 2004.

[23] Machado Rocha FC, Stefano SC, De Cassia Haiek R, Rosa Oliveira LMQ, Da Silveira DX. Therapeutic use of Cannabis Sativa on Chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. Eur J Cancer Care;17(5) 431-443. 2008.

[24] Parker LA, Rock EM, Limebeer CL. Regulation of nausea and vomiting by cannabinoids. Br J Pharmacol 163(7) 1411-1422. 2011.

[25] Grant I, Gonzalez R, Carey CL, Natarajan L, Wolfson T. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. J Int Neuropsychol Soc 9 679-689. 2003.

[26] Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. Neurology 59 1337-1343. 2002.

[27] Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, Lewis G. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370 319-328. 2007.

[28] Hézode C, Zafrani ES, Roudot-Thoraval F, Costentin C, Hessami A, Bouvier-Alias M, Medkour F, Pawlostky JM, Lotersztajn S, Mallat A. Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C. Gastoenterology 134 432-439. 2008.

[29] Huestis MA. Human cannabinoid pharmacokinetic. Chem Biodivers 5(8) 1770-1804. 2007.

[30] Huestis MA, Mitchell JM, Cone EJ. Urinary excretion profiles of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in humans after single smoked doses of marijuana. J Anal Toxicol 20(6) 441-452. 1996.

[31] Kosel BW, Aweeka FT, Benowitz NL, Shade SB, Hilton JF, Lizak PS, Abrams DI. The effects of cannabinoids on the pharmacokinetics of Indinavir and nelfinavir. AIDS 16 543-550. 2002.

[32] Engels FK, de Jong FA, Sparreboom A. Medicinal cannabis does not influence the clinical pharmacokinetics of Irinotecan and Docetaxel. Oncologist 12 291-300. 2007.

[33] Grotenhermen F, Muller-Vahl K. The therapeutic potential of cannabis and cannabinoids. Deutsch Arztebl Int 109(29-30) 495-501. 2012

[34] Fonseca B, Mota Filipe H, Carvalho F, Campos MG. "Parecer do Grupo de Trabalho da Ordem dos Farmacêuticos sobre a Utilização de Canábis com Fins Terapêuticos". OF. 2015.

[35] Bifulco M, Pisanti S. Medicinal use of cannabis in Europe: the fact that more countries legalize the medicinal use of cannabis should not become an argument for unfettered and uncontrolled use. EMBO Rep 16(2) 130-132. 2015.

[36] Koren G, Cohen R. Medicinal Use of Cannabis in Children and Pregnant Women. Rambam Maimonides Med J, 11(1), e0005, 1-3. 2020

[37] Barchel D, Stolar O, De-Haan T, Ziv-Baran T, Saban N, Fuchs DO, Koren G, Berkovitch M. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and co-morbidities. Front Pharmacol. 9:1521, eCollection, 2-4 2018. 2019.

[38] Koren G, Boskovic R, Hard M, Maltepe C, Navioz Y, Einarson A. Motherisk-PUQE (pregnancy-unique quantification of emesis and nausea) scoring system for nausea and vomiting of pregnancy. Am J Obstet. Gynecol. 186 (5 Suppl Understanding): S228-231. 2002.

[39] Metz TD, Stickrath EH. Marijuana use in pregnancy and lactation: a review of the evidence. Am J Obstet. Gynecol. 213, 761–8. 2015.

[40] Sharon H, Brill S. Cannabis-based medicines for chronic pain management: current and future prospects. Curr Opin Anaesthesiol. 32(5) 623-628. 2019.

[41] Häuser W, Finn DP, Kalso E, Krcevski-Skvarc N, Kress HG, Morlion B, Perrot S, Schäfer M, Wells C, Brill S. European Pain Federation (EFIC) position paper on appropriate use of cannabis-based medicines and medical cannabis for chronic pain management. Eur J Pain 22 1547–1564. 2019.

[42] ElSohly MA, Radwan MM, Gul W, Chandra S, Galal A. Phytochemistry of Cannabis sativa L. Prog Chem Org Nat Prod 103 1-36. 2017.

[43] Turner SE, Williams CM, Iversen L, Whalley BJ. Molecular Pharmacology of Phytocannabinoids. Prog Chem Org Nat Prod. 103 61-101. 2017.

[44] Makary P, Parmar JR, Mims N, Khanfar NM, Freeman RA. Patient Counseling Guidelines for the Use of Cannabis for the Treatment of Chemotherapy-Induced Nausea/Vomiting and Chronic Pain. J Pain Palliat Care Pharmacother. 32(4) 216-225 2018.

Appendix

PUQE form:

Pregnancy-Unique Quantification of Emesis and nausea

Circle the answer that suit the best your situation for the last 24 hours.

1. On average in a day, for how long do you feel nauseated or sick to your stomach?

> 6 hours 5 points	4-6 hours 4 points	2-3 hours 3 points	≤1 hour 2 points	Not at all 1 point

2. On average in a day, how many times do you vomit or throw up?

≥7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

3. On average in a day, how many times have you had retching or dry heaves without bringing anything up?

≥7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

Total score (sum of replies to 1, 2, and 3): mild NVP ≤6; moderate NVP, 7-12; severe NVP ≥13.

Quality of life question:
On a scale of 0 to 10, how would you rate your well-being:_____
0 (worst possible) 10 (As good as you felt before pregnancy)

1. Koren G, Boskovic R, Hard M, Maltepe C, Navioz Y, Einarson A. Motherisk PUQE (pregnancy-unique quantification of emesis and nausea) scoring system for nausea and vomiting of pregnancy. Am J Obstet Gynecol 2002;186(5 Suppl Understanding):S228-31