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1 Title: Cannabinoid-based Therapies and Brain Development: Potential Harmful Effect of Early  
2 Modulation of the Endocannabinoid System

3

4 Short Title: Cannabinoid-based Therapies and Brain Development

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30

1    **Abstract**

2    The endocannabinoid retrograde signaling pathway is widely expressed in the central nervous system  
3    where it plays major roles in regulating synaptic plasticity (excitatory and inhibitory) through long-term  
4    potentiation and long-term depression. The endocannabinoid system (ECS) components - cannabinoid  
5    receptors, endocannabinoids and synthesis/degradation enzymes - are expressed and are functional from  
6    early developmental stages and throughout adolescence cortical development, regulating progenitor cell  
7    fate, neural differentiation, migration and survival. This may potentially confer increased vulnerability to  
8    adverse outcomes from early cannabinoid exposure. Cannabidiol (CBD) is one of the most studied  
9    exogenous cannabinoid, and CBD-enriched *Cannabis* extracts have been widely (and successfully) used  
10   as adjuvants to treat children with refractory epilepsy, and there is even an FDA-approved product with it.  
11   However, there is not sufficient evidence regarding potential detrimental consequences upon long term  
12   alterations in the central nervous system's development by cannabinoids. As well as the majority of  
13   cannabinoids, CBD is able to exert its effects directly and indirectly through the ECS, which can perturb  
14   the regulatory processes mediated by this system. Besides, CBD has a large number of non-  
15   endocannabinoid targets which can explain CBD's effects. Here, we review the current knowledge about  
16   CBD-based therapies - pure and CBD-enriched *Cannabis* extracts - in studies with pediatric patients, its  
17   side effects, and its mechanisms of action regarding the central nervous system and neurodevelopment  
18   aspects. Since *Cannabis* extracts contain tetrahydrocannabinol ( $\Delta^9$ -THC), we consider that pure CBD is  
19   possibly safer for young patients. Nevertheless, CBD, as well as other natural and/or synthetic  
20   cannabinoids, should be studied in more detail as a therapeutic alternative to CBD enriched-*Cannabis*  
21   extracts during brain developmental stages.

22   **Key points**

- 23       • Cannabidiol (CBD) targets the endocannabinoid system directly via CB1 receptors, or indirectly  
24       by regulating endocannabinoids levels, in both developing and mature brains.
- 25       •  $\Delta^9$ -THC is believed to be responsible for the majority of the potential harmful effects of CBD-  
26       enriched *Cannabis* extracts, although further direct evaluation of the effects of CBD upon brain  
27       development are necessary.
- 28       • For young patients pure CBD, both synthetic or plant derived, produced in accordance with  
29       good manufacturing practices (GMP-grade), is recommended as a therapeutic option instead of

1 CBD-enriched *Cannabis* extracts, and a recently a CBD-based (Epidiolex<sup>®</sup>) product was  
2 approved by FDA for the treatment of Dravet and Lennox-Gastaut syndromes.

- 3 • There is a lack of trials of chronic administration of CBD-based therapies with long term follow-  
4 up periods, which would allow a more realistic comparison of their effects with the current  
5 treatment options.

## 6 7 **1. Introduction**

8 The plant *Cannabis sativa* has been used for medicinal purposes for thousands of years by different  
9 cultures [1]. *Cannabis* extract contains more than 80 components, of which  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -  
10 THC), the main psychoactive ingredient) and cannabidiol (CBD) are the most abundant [2,3]. These  
11 compounds were first identified several decades ago [4], but it is only more recently that the discovery of  
12 cannabinoid receptors and their endogenous homologues, the endocannabinoids [5] such as N-  
13 arachidonylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) [6], has occurred.  
14 Together with their related enzymes, endocannabinoids and their receptors form the endocannabinoid  
15 system (ECS) (Fig 1) [7]. Cannabinoids – both endogenous and plant-derived – target the G protein-  
16 coupled cannabinoid receptors type-1 (CB1), which is widely expressed in the nervous system, and type-2  
17 (CB2), which is mainly expressed in immune cells [8,9]. Presently, it is proposed that the ECS has roles  
18 in the pathological mechanisms of several psychiatric disorders, including schizophrenia [10]. Besides,  
19 cannabinoids such as CBD also interact with a variety of non-endocannabinoid mechanisms, including  
20 numerous classical ion channels, receptors, transporters, and enzymes, as reviewed recently [11].  
21 The effects of isolated cannabinoids and *Cannabis* extracts in different diseases have been studied for  
22 many years [12]. In the United States, recent medical and recreational marijuana legalization increased  
23 *Cannabis* accessibility and use [13]. Additionally, despite widely known deleterious effects during central  
24 nervous system development, medical marijuana usage by minors, with the consent from a legal guardian  
25 and certification from a physician, is approved [14]. Marijuana-derived products have their main effects  
26 against childhood severe epilepsies including Dravet and Lennox-Gastaut syndromes. These early onset  
27 disorders are characterized by frequent, refractory seizures and neurodevelopmental delays, which lead to  
28 impaired quality of life of these individuals. This scenario compels families to seek alternative treatment  
29 methods, such as CBD-based therapies, which include pure synthetic or plant-derived CBD and CBD-  
30 enriched *Cannabis* extracts. In children, plant-derived pharmaceutical-grade isolated CBD has been tested

1 in clinical trials in patients with such syndromes ([15–17]) and this drug (Epidiolex<sup>®</sup>) has recently been  
2 approved in the US as orphan drug for those syndromes. Clinical trials with synthetic isolated CBD are  
3 ongoing (clinicaltrials.gov website). In addition, reports on the use different form of *Cannabis* extracts in  
4 children with epilepsy have also been published [18–20]. However, only few adequately powered,  
5 placebo-controlled randomized studies have evaluated the safety and efficacy of CBD-based therapies in  
6 children [21]. Nevertheless, most of these have reported a greater reduction in convulsive-seizure  
7 frequency than placebo, however they were also associated with higher rates of adverse events [22].  
8 The constituents of the ECS, receptors and endocannabinoids, are expressed and are functional from very  
9 early developmental stages, whereby they regulate inhibitory and excitatory synapses. Even during  
10 adolescence, the brain and the ECS undergo active development which may confer increased  
11 vulnerability to adverse long-term outcomes from early cannabinoid exposure [23]. Endocannabinoids  
12 have been shown to regulate cortical development throughout life in humans, and exogenous  
13 cannabinoids can alter cortical development of both the somatosensory and the prefrontal cortex [24].  
14 Nevertheless, the current widespread use of CBD-based therapies in children and young adults, without  
15 sufficient studies of the potential consequences upon neuronal and other systems' development, is of  
16 concern to the scientific and medical communities. One area of particular concern is the uncontrolled  
17 amount of  $\Delta^9$ -THC present in such extracts. Moreover, in 2017 an *ad hoc* committee of the National  
18 Academies of Sciences, Engineering, and Medicine presented a report regarding the health effects of  
19 *Cannabis* and CBD use, which revealed no or insufficient evidence to either support or refute the use of  
20 such compounds as an effective treatment for epilepsy [25]. Hence, this article reviews the current  
21 knowledge about the use of CBD-based therapies in pediatric patients, its alleged side effects, and its  
22 mechanisms of action regarding the central nervous system and neurodevelopmental aspects. We  
23 highlight that CBD administration before adulthood must be carefully evaluated, and the use of pure CBD  
24 and/or synthetic cannabinoids as a preferential alternative to *Cannabis* extracts for children and young  
25 adults needs to be studied further.

26

## 27 **2. The Endocannabinoid System**

28 Most cannabinoids exert their therapeutic properties upon the central nervous system primarily *via* the  
29 ECS, although there are other known targets [26]. Here we discuss their effects upon the ECS.

1 Endocannabinoid signaling plays crucial roles in various aspects of both the underdeveloped and the  
2 mature brain [27]. Therefore, disturbances in this system may disrupt neural development.

3 The classical ECS signaling pathway is shown in Figure 1 (for review see [10]). In the mature brain, the  
4 ECS modulates synapses (excitatory and inhibitory) through the release of endocannabinoids AEA and 2-  
5 AG. These act as retrograde messengers, their release by the postsynaptic neuron activating CB<sub>1</sub> receptors  
6 in the pre-synaptic neuron, leading to decreased release of neurotransmitters into the synaptic cleft  
7 [10,28,29]. This process is initiated by increased Ca<sup>2+</sup> influx caused by neurotransmission in the  
8 postsynaptic neuron which activates endocannabinoid synthesis from its precursors in the plasma  
9 membrane. AEA is generated from phospholipase D-mediated hydrolysis of the membrane lipid N-  
10 arachidonoylphosphatidylethanolamine (NAPE), while 2-AG originates from the diacylglycerol lipase-  
11 mediated hydrolysis of diacylglycerol (DAG), derived mainly from membrane-localized  
12 phosphatidylinositol biphosphate (PIP<sub>2</sub>). AEA and 2-AG diffuse towards the pre-synaptic terminals and,  
13 like exogenous cannabinoids such as Δ<sup>9</sup>-THC, bind to and activate the pre-synaptic G-protein-coupled  
14 CB<sub>1</sub> receptors. This binding triggers the activation and release of Gi/Go proteins from the CB<sub>1</sub>, inhibiting  
15 adenylyl cyclase (AC) and thus decreasing cyclic AMP (cAMP) formation and subsequent protein kinase  
16 A (PKA) activity. These events lead to opening of inwardly-rectifying K<sup>+</sup> channels, causing a  
17 hyperpolarization of the pre-synaptic terminal, and closing of Ca<sup>2+</sup> channels, arresting the release of  
18 stored neurotransmitters. Finally, AEA and 2-AG re-enter the post- or pre-synaptic terminals, where they  
19 are catabolized respectively by fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL),  
20 to yield either arachidonic acid (AA) and ethanolamine (ET) in the case of AEA, or AA and glycerol for  
21 2-AG. The transport of endocannabinoids through the plasma membrane is still not completely  
22 understood. Although some studies have proposed the existence of an endocannabinoid transporter, the  
23 trafficking of AEA, which has been most extensively studied, is proposed to occur through facilitated  
24 membrane transport followed by intracellular shuttling and sequestration [30].

25 Additionally, CB<sub>1</sub> receptor activation leads to stimulation of mitogen-activated protein kinase (MAPK)  
26 activity, a mechanism by which cannabinoids affect synaptic plasticity, cell migration, and possibly  
27 neuronal growth [23]. In mature neurons, the MAPK cascade, which leads to the activation of  
28 extracellular signal-regulated kinases (ERK), is stimulated by excitatory glutamatergic signaling.  
29 Subsequently, ERK activity regulates two processes that underlie changes in synaptic transmission — the  
30 activity of postsynaptic AMPA receptors, and structural plasticity [31]. ECS retrograde signaling

1 mediates synaptic plasticity through three classical mechanisms: depolarization-induced suppression of  
2 inhibition or excitation, metabotropic-induced suppression of inhibition or excitation, and  
3 endocannabinoid-mediated short-term depression or long-term depression (STD/LTD) [10]. Also, CB1  
4 agonists can prevent long-term potentiation (LTP) of synaptic transmission, but the influence of  
5 endogenously formed cannabinoids on hippocampal LTP remains ambiguous [32]. Both LTP and LTD  
6 have roles in learning and neural development [24].  
7 Thus, the central component of the ECS in neurons is the CB<sub>1</sub> receptor (Fig. 1). In the central nervous  
8 system, CB<sub>1</sub> is particularly enriched in the cortex, hippocampus, amygdala, basal ganglia outflow tracts,  
9 and cerebellum. This distribution corresponds to the most prominent behavioral effects of *Cannabis*  
10 and helps to predict neurological and psychological effects of ECS manipulation [33]. CB<sub>1</sub> receptors are  
11 also observed in intracellular compartments such as the mitochondrial surface, where they are able to  
12 activate G protein-dependent signaling and modify intracellular levels of ATP, Ca<sup>2+</sup>, and reactive oxygen  
13 species, all of which impact upon synaptic transmission [34].  
14 In the developing nervous system and the remaining neurogenic areas in the adult brain (the hippocampal  
15 subgranular zone and subventricular zone), the ECS exerts a regulatory role on neural progenitor cell  
16 survival, proliferation, differentiation and migration *via* CB<sub>1</sub> [35,36], thus possibly affecting the  
17 formation of adult specialized tissues [37]. Recently, the ECS has also been shown to regulate  
18 proliferation and differentiation of mesoderm-derived hematopoietic and mesenchymal stem cells, with a  
19 key role in determining the formation of several cell types in peripheral tissues [38].  
20 The importance of the ECS during embryonic development has been investigated through many  
21 experimental models and approaches, mainly focusing upon the deleterious effect of early Δ<sup>9</sup>-THC  
22 administration. For example, Δ<sup>9</sup>-THC administration to pregnant mice interfered with sub-cerebral  
23 projection neuron generation, thereby altering corticospinal connectivity, and produced long-lasting  
24 alterations in the fine motor performance and seizure susceptibility of the adult offspring. These  
25 deleterious consequences were solely attributed to Δ<sup>9</sup>-THC's ability to disrupt the neurodevelopmental  
26 role of CB<sub>1</sub> signaling [39].  
27 During adolescence, the ECS has a role in the development of the cortex, amygdala, hippocampus and  
28 hypothalamus, and exogenous cannabinoids have long-term effects on cognition, anxiety and stress-  
29 related behaviors, leading to mood disorders and substance abuse [24]. At this age, cannabinoids may  
30 produce abnormal LTD in prefrontal cortex by disrupting LTD mediated by metabotropic glutamate

1 receptors and CB<sub>1</sub> [40]. The ECS maintain the homeostasis of prefrontal cortex interactions with the  
2 amygdala and hippocampus, which are responsible for behaviors such as emotional memory and anxiety-  
3 related behaviors. Endocannabinoids are required for the normal stress response, a process which matures  
4 during adolescence [24]. Besides, as the prefrontal cortex is the last brain region to finish development  
5 after adolescence, the abundance of CB<sub>1</sub> receptors may explain the negative effects of *Cannabis* use in  
6 this age range [27]. Finally, endocannabinoids are necessary for the normal regulation of neuronal  
7 excitation and inhibition, hence disturbances in this delicate equilibrium likely result in changes in the  
8 balance of excitation/inhibition in individual neurons and networks, processes which are necessary for  
9 normal cortical development [24].

10 For therapeutic purposes, regarding the mature central nervous system, the ECS has shown to modulate  
11 anxiety, depression, neurogenesis, reward, cognition, learning, and memory [23]. Moreover, its retrograde  
12 signaling acts to regulate seizure activity and neuronal hyper-excitability – cannabinoids have shown CB<sub>1</sub>  
13 activity in experimental models of seizure and epilepsy [41,42]. However, the use of CB<sub>1</sub> agonists such as  
14  $\Delta^9$ -THC, or even *Cannabis* extract, as a therapeutic strategy is unfeasible due to their psychoactive  
15 effects, abuse potential and development of tolerance [42]. On the other hand, antagonism of CB<sub>1</sub> can also  
16 exacerbate seizure activity in the epileptic phenotype [43].

17 Thus, the modulation of the ECS as a therapeutic approach is challenging because its blockage or its  
18 exacerbation could lead to undesired outcomes, especially during neuronal development. More studies are  
19 required to clarify its physiological functions and to predict the effect of CB<sub>1</sub> agonists and antagonists,  
20 both in adult and pediatric patients, to support its targeting for therapeutic purposes.

21

### 22 **3. Therapeutic uses and mechanisms of action of CBD**

23 *Cannabis* causes many psychotropic effects, mainly mediated by  $\Delta^9$ -THC agonism of CB<sub>1</sub> [44], which  
24 makes it unlikely to be used *in natura*. On the other hand, experimental studies have demonstrated several  
25 therapeutic properties of isolated cannabinoids in a number of *in vitro* and *in vivo* models [45]. Here, we  
26 discuss the therapeutic uses of the most prominent of these cannabinoids, CBD, and its mechanisms of  
27 action, highlighting its activity towards the CB<sub>1</sub> receptor.

28 Although only a limited number of studies have focused upon CBD, recently it has been shown to be a  
29 potent anti-inflammatory and antioxidant agent and to attenuate the memory-impairing effects produced  
30 by  $\Delta^9$ -THC, amongst other effects [23]. This opens a wide range of possible therapeutic uses in



1 neurodegenerative disorders including Parkinson's disease, Alzheimer's disease and cerebral ischemia  
2 [46]. Moreover, CBD is anti-emetic [47], has antitumoral properties against many types of cancer [48]  
3 and is also suggested to have antipsychotic, anxiolytic and antidepressant effects [49]. Finally, as already  
4 mentioned above, numerous studies have shown CBD to have anticonvulsive properties [50].  
5 CBD has been reported to have a large number of possible molecular targets other than the ECS in a wide  
6 range of medical conditions, raising the possibility of significant off target effects [26]. For instance,  
7 CBD is described as a full 5-HT<sub>1A</sub> agonist, a weak partial 5-HT<sub>2A</sub> agonist and a non-competitive 5-  
8 HT<sub>3A</sub> antagonist [51]. The ability of CBD to activate the A<sub>1A</sub> adenosine receptor has also been reported  
9 [52]. CBD may play a role in the regulation of T-type calcium channels and the activity of nuclear  
10 peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), both of which have been implicated in seizure  
11 activity [53]. Other molecular targets have also been studied, among them the PPAR $\gamma$  nuclear receptors  
12 [54], glycine receptors [55], GABA<sub>A</sub> receptors [56], and transient receptor potential (TRP) channels [57].  
13 Studies focused on the possible epigenetic regulation of skin differentiation genes by CBD revealed that it  
14 can act as a transcriptional repressor, controlling cell proliferation and differentiation through DNA  
15 methylation [58]. Hence, the molecular mechanistic basis for the effects of CBD appear to be complex,  
16 and thus remain to be fully elucidated.

17 Although current evidence suggests that CBD does not directly interact with the ECS except *in vitro* at  
18 supraphysiological concentrations [11], it can also indirectly act as agonist or antagonist of the CB<sub>1</sub>  
19 receptor. In the nanomolar range (below the reported affinity ( $K_i$ ) for CBD to these receptors), CBD can  
20 antagonize the pharmacological effects of CB<sub>1</sub> agonists such as  $\Delta^9$ -THC and AEA, despite having low  
21 direct affinity in the micromolar range for CB<sub>1</sub> *in vitro* [59,60]. McPartland *et al* reviewed *in vitro* and *ex*  
22 *vivo* mechanistic studies of CBD and found one study that reported slight agonism, and one study that  
23 reported slightly inverse agonism comprising binding to the inactive form of the receptor, blocking  
24 agonist effects, both of which occurred at high concentrations of CBD ( $\geq 10 \mu\text{M}$ ). Surprisingly, in some  
25 mechanistic studies, the effects of CBD could be reversed by CB<sub>1</sub> receptor inverse agonists, or were  
26 absent in CB<sub>1</sub> receptor knockout mice [59]. This suggests that CBD may exert indirect agonism,  
27 comprising the enhancing of the effect of a receptor's agonist without having any direct agonist effect  
28 itself, at CB<sub>1</sub> receptors – either augmenting CB<sub>1</sub> constitutive activity [61], or augmenting  
29 endocannabinoid tone through inhibition of AEA hydrolysis, inhibition of the putative AEA transporter  
30 and increase of 2-AG levels [59].

1 Recent evidence supports the hypothesis that CBD also binds to an allosteric site on CB<sub>1</sub> receptors that is  
2 functionally distinct from the orthosteric site for its agonists. CBD reduced the potency and efficacy of  
3 CB<sub>1</sub> agonists at concentrations lower than the predicted affinity of CBD for the orthosteric site of CB<sub>1</sub>  
4 receptors [62]. The presence of this allosteric site is still to be directly demonstrated due to difficulties in  
5 the resolution of the crystallographic structure of this receptor [63]. Despite such methodological issues,  
6 *in vitro* pharmacological experiments have demonstrated that, at very low concentrations, CBD is a  
7 negative allosteric modulator of CB<sub>1</sub> [62].

8 Therefore, depending on the conditions, CBD seems to be able to interact both directly and indirectly  
9 with the CB<sub>1</sub> receptor, *via* the regulation of endocannabinoids levels. Thus, since the ECS has a broad  
10 spectrum of physiological functions during neural development, it is reasonable to assume that CBD is  
11 potentially able interfere with processes regulated by CB<sub>1</sub> when administered in infants. In fact,  
12 depending on the dosage and the clinical condition, potential CBD activity over CB<sub>1</sub> (agonism or  
13 antagonism) results in different outcomes – either therapeutic or harmful [27], therefore its use must be  
14 very carefully considered in such ages. Besides, as CBD has effects upon other targets at lower  
15 concentrations, the mechanisms underlying its therapeutic properties are not yet clearly understood [42].  
16

#### 17 **4. Studies with CBD-enriched *Cannabis* extracts and pure CBD in pediatric patients**

18 Currently, CBD is clinically used in association with Δ<sup>9</sup>-THC in a *Cannabis*-based preparation (Sativex<sup>®</sup>)  
19 that contains equimolar content of both, for the management of neuropathic symptoms associated with  
20 multiple sclerosis [64]. Relieve of spasticity and pain have been reported for multiple sclerosis patients  
21 that smoke *Cannabis*, but, for these patients, structural MRI scans have suggested reduced brain volume  
22 is associated with cognitive impairment [65]. Likewise, in recreational users, *Cannabis* has been shown to  
23 result in volumetric, gray matter and white matter structural changes in the brain, in particular in the  
24 hippocampus and the amygdala [66], further evidence that *Cannabis* (smoked and possibly in extracts)  
25 can be harmful in adult brain.

26 In 2016, GW pharmaceuticals reported the first results of pure CBD (Epidiolex<sup>®</sup>) in phase III clinical  
27 trials for use in treatment-resistant seizure disorders, including Lennox–Gastaut and Dravet syndromes  
28 [17,22]. More recently, the same authors have released a further results of a randomized, double-blind,  
29 placebo-controlled trial using pure CBD [67,68]. Moreover, CBD-enriched *Cannabis* extract is still  
30 widely used as a therapeutic option. In this section, we review the available data on clinical trials, case

1 reports and parental surveys available from January 2000 to May 2018. We focused on literature  
2 containing data about isolated CBD administration and relevant oral *Cannabis* extracts with high CBD  
3 content in pediatric and young patients, as well as relevant studies on adult volunteer.

4 The use of common *Cannabis* extracts is not recommended in children and adolescent patients due to the  
5 potential for deleterious effects. Fetal development is affected by prenatal maternal *Cannabis* use, while  
6 during infancy there is a negative impact upon cognitive and behavioral outcomes [69]. Early exposure to  
7 cannabinoids, mainly  $\Delta^9$ -THC, can impair all stages of memory, from encoding to consolidation and  
8 retrieval [70]. Additionally, *Cannabis* usage during adolescence increases the risk of developing  
9 psychotic disorders such as schizophrenia later in life [71,72]. Nevertheless, these effects are mainly  
10 associated with  $\Delta^9$ -THC, and CBD is able to counteract such effects [73]. This indicates that pure CBD  
11 would be a better therapeutic option instead of CBD-enriched or common *Cannabis* extracts. Careful  
12 consideration and attention should be taken when using CBD-enriched *Cannabis* extracts, in particular  
13 within pediatric contexts. In a recent case report, for example, two children presented typical symptoms  
14 of  $\Delta^9$ -THC intoxication (inappropriate laughter, ataxia, reduced attention, and eye redness) after using a  
15 CBD-enriched *Cannabis* extract. The extract was replaced by the same dose of purified CBD, resulting in  
16 decreased intoxication symptoms and seizure remission [74].

17 Table 1 summarizes the main findings in children and young adult patients treated with pure CBD and  
18 CBD-enriched *Cannabis* extract. As most studies that established safety and dose tolerance were  
19 performed in adults, they were also reviewed (supplementary table 1). The majority of published articles  
20 focused on neurological and neuropsychiatric conditions. In adult volunteers, CBD presented few adverse  
21 events and appeared to be safe, although its effectiveness was not always confirmed. In most of these  
22 studies, CBD was administered in a single dose. A recent article on the safety and tolerability of pure  
23 CBD in 34 children between 4 – 10 years old with Dravet Syndrome showed that CBD did not alter  
24 plasma antileptic drug levels, when randomized into different dosages or placebo for 3 weeks of treatment  
25 followed by a 4 week follow-up period [16]. The main adverse effects were pyrexia, somnolence,  
26 decreased appetite, sedation, vomiting, ataxia, and abnormal behavior. As observed in the studies above  
27 mentioned, and reviewed by Wong and Wilens (2017), the methodological quality of those clinical  
28 studies varied significantly (*e.g.* studies lacking control groups; limited by small sample size). Studies are  
29 also heterogeneous in the dosage and duration of treatment, and many lack any long-term follow-up

1 reviews to identify potential adverse effects [13]. This variability in protocols employed makes it difficult  
2 to evaluate the real benefits and risks of CBD-based therapies.

3 Until a few years ago, the suggested beneficial outcomes of CBD-based therapies for pediatric patients  
4 were based mainly upon case reports and surveys of parents with epileptic children (supplementary table  
5 2). Such anecdotal studies were the first to report improvement in general condition of children with  
6 refractory epilepsies by *Cannabis* extracts, and so they attracted the interest of the scientific community  
7 for cannabinoids-based treatments. Many surveys of parents of children with refractory seizures who self-  
8 administered CBD-enriched *Cannabis* extracts have been published in the last decades. One such survey,  
9 involving a small cohort of patients, showed that 42% of children had a greater than 80% reduction in  
10 seizure frequency [75]. Another survey, using a larger cohort of 75 pediatric patients, reported that 38%  
11 of children achieved greater than 50% reduction in seizures [20]. An online survey of 117 parents of  
12 children with epilepsy reported that 85% of children had a reduction in seizure frequency, whilst 14%  
13 reported complete freedom from seizure after CBD-enriched *Cannabis* treatment [19]. These surveys,  
14 even though not controlled, reported general improvements in cognitive and motor function in patients  
15 undergoing CBD-based therapies, along with some mild side effects.

16 On the other hand, not all studies have reported favorable results (e.g. CBD-enriched *Cannabis* extract  
17 resulted in no improvement in general condition or seizure relief of an 18-year old male with severe  
18 refractory epilepsy) [76]. Moreover, case reports and parent surveys rarely describe side effects or even  
19 drug administration issues. For this reason, clinical trials are indispensable for investigating both the  
20 therapeutic and toxicological aspects of CBD-based therapies, as well in standardizing drug  
21 administration protocols to allow direct study comparisons.

22 However, as anecdotal studies have stimulated a growing interest in the anticonvulsive properties of  
23 CBD, pure CBD or CBD-enriched *Cannabis* extracts are now being tested in controlled clinical trials,  
24 with relevant positive outcomes thus far reported (table 1). Such studies are still somewhat limited in  
25 number, however a brief survey on [clinicaltrials.gov](https://clinicaltrials.gov) website report at least 20 clinical trials that are  
26 currently recruiting young patients or already in progress [77]. An open-label clinical trial of 214 patients  
27 (aged 1–30 years) with severe, intractable, childhood-onset, treatment-resistant epilepsy investigated the  
28 efficacy and safety of pure CBD. Patients in the efficacy analysis group reported a median reduction in  
29 monthly motor seizures of 36.5% compared to the placebo group. Adverse events were reported in 79%  
30 of the safety analysis group, and serious adverse events were reported in 30% of patients, including one

1 death — a sudden, unexpected death due to the patient’s epilepsy which was determined as unrelated to  
2 CBD. Twelve percent of patients had severe adverse events possibly related to CBD use, the most  
3 common of which was *status epilepticus* (6%). Three percent of patients discontinued treatment because  
4 of an adverse event [17].

5 A randomized placebo-controlled clinical trial of pure CBD reported a significant reduction in  
6 total seizures of all types. Although there was no significant reduction in non-convulsive seizures, they  
7 did demonstrate a greater reduction in convulsive seizure frequency, with 62% of patients reporting an  
8 improvement in overall condition, with 5% of patients becoming seizure-free. Adverse events included  
9 diarrhea, vomiting, fatigue, pyrexia, somnolence, and abnormal liver function tests [22]. This report,  
10 however, did not evaluate possible drug-drug interactions between CBD and Clobazam, of which 65%  
11 of patients enrolled on the study were prescribed. CBD can increase plasma Clobazam concentrations  
12 [78], hence the beneficial effects of CBD may have arisen indirectly due to the increased pharmacological  
13 effects of Clobazam and not as a direct pharmacological effect of CBD itself.

14 In 2018, a randomized, double-blind, placebo-controlled trial encompassing 24 clinical sites in the USA,  
15 the Netherlands, and Poland, was published. In this study, pure CBD (20 mg/kg/day) or placebo was  
16 administered to patients with treatment-resistant Lennox-Gastaut syndrome (aged 2–55 years) for 14  
17 weeks. Of the 171 randomly assigned patients that received CBD (n = 86) or placebo (n = 85), 14 patients  
18 in the CBD group and one in the placebo group discontinued study treatment. The monthly drop in  
19 seizure frequency was reduced by 43.9% in the CBD group and 21.8% in the placebo group. Adverse  
20 events, which were mostly mild or moderate, occurred in 86% of patients in the CBD group and in 69%  
21 of patients in the placebo group [67].

22 Another recent double-blind, placebo-controlled trial, in which 225 patients with the Lennox–Gastaut  
23 syndrome (age range of 2 to 55 years) were randomly assigned to receive CBD at 10 mg/kg/day, 20  
24 mg/kg/day, or placebo administered in two equally divided doses daily for 14 weeks, showed significant  
25 decreases in seizure frequency [68]. Seizure frequency decreased by 41.9% in the 20 mg cannabidiol  
26 group, 37.2% in the 10-mg cannabidiol group, and 17.2% in the placebo group. Six patients in the 20 mg  
27 cannabidiol group and one patient in the 10 mg cannabidiol group were withdrawn from the trial because  
28 of adverse events. Fourteen patients who received cannabidiol (9%) had elevated plasma liver  
29 aminotransferase levels. The most common adverse events among the patients in the cannabidiol groups  
30 were somnolence, decreased appetite, and diarrhea; these events occurred more frequently in the higher-

1 dose group. Yet, even in these two recent clinical trials, although they are scientifically relevant and  
2 reliable, a longer treatment and follow-up period was missing.

3 In general, in pediatric patient clinical trials, the most common side effects reported were either mild  
4 (somnolence, fatigue, altered appetite, weight gain/loss, diarrhea and other gastrointestinal disturbances,  
5 irritability) or serious (drowsiness/dizziness, ataxia, tremor, mental sedation), with severe adverse effects  
6 such as increased seizure frequency and worsening seizure phenotype also being observed. Alimentary  
7 effects can be explained by the presence of the ECS in the gastrointestinal tract, where it has effects on  
8 motility, inflammation and immunity, intestinal and gastric acid secretion, nociception and emesis  
9 pathways, and appetite control [79]. In the brain, ECS modulates several brain functions, such as  
10 memory, mood, food intake, pain perception and the sleep-wake cycle [80], which may explain, at least  
11 partially, the CNS-mediated adverse effects observed in clinical trials. Besides, as discussed above, other  
12 cannabinoids present in *Cannabis* extracts as well as CBD are able to interact and possibly disturb the  
13 important roles played by the ECS during neurodevelopmental stages.

14 It is likely that non-endocannabinoid targets of CBD may explain some of the positive and adverse effects  
15 observed [11]. For example, in a mouse model of Dravet Syndrome, the beneficial effects of CBD on  
16 inhibitory neurotransmission were mimicked and blocked by an antagonist of the orphan G protein-  
17 coupled receptor 55 (GPR55), suggesting that the therapeutic effects of CBD are mediated through this  
18 lipid-activated G protein-coupled receptor and thus identify it as a third cannabinoid receptor [81].

19 A careful case-to-case evaluation on the risk/benefit balance of CBD usage must be taken, as in the most  
20 serious cases repetitive infantile seizures can cause severe developmental, cognitive and motor  
21 impairment. These are obviously more detrimental than the adverse effects and possible  
22 neurodevelopmental implications of CBD, hence CBD may be an attractive therapeutic option in these  
23 cases.

24 Finally, CBD therapy does not always work for all patients. Also, some of the studies used CBD-enriched  
25 *Cannabis* extracts, which contains  $\Delta^9$ -THC. Even the ones with pure CBD, in controlled clinical trials,  
26 have short treatment periods and short follow-up periods, which will not reveal the possible long-term  
27 effects of CBD as well as possible developmental adverse effects. Hence, more clinical trials, with larger  
28 population sizes and longer chronic pure CBD administration, are warranted in order to clarify under  
29 which conditions it is worthwhile and safe to use. In addition, it is still unknown how CBD acts on  
30 hormones, hepatic enzymes, drug transporters, and its interactions with other drugs [12].

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## 5. CBD during development: effects in cell culture and animal models for the developing brain

Despite the increasing use of CBD-based therapies in children and adolescents whose brains are still developing, most *in vitro* and *in vivo* studies use mature cells or adult animal models and are thus not faithful mimics of the juvenile CNS. Experiments with immature animals or cells have greater potential for identifying CBD's effects and the molecular mechanisms by which such effects are mediated with greater relevance to juveniles. However, few studies have evaluated the developmental phases which are equivalent to human CNS development. Here, we present some of the recent studies using pure CBD in relevant cellular and animal models of the developing brain.

In a genetic mouse model of Dravet Syndrome, caused by loss-of-function mutations in the voltage-gated sodium channel NaV1.1, CBD treatment from postnatal day 21 to 27 decreased the duration and severity of thermally-induced seizures and the frequency of spontaneous seizures. Lower doses of CBD also improved autistic-like social interaction deficits [81]. This mouse model represents a very specific cause of children refractory epilepsy, a single mutation in a sodium channel subunit, and its positive outcomes must be considered carefully when extrapolated to other pathologies.

Single dose administration of CBD to newborn piglets shortly after hypoxia-ischemia had a protective effect upon neurons and astrocytes, preserved brain activity, prevented seizures and improved neurobehavioral performance [82,83]. In newborn rat brains, CBD administration also prevented necrotic and apoptotic cell death in an *in vivo* model of hypoxia-ischemia damage [84], and rescued neuron function after sciatic nerve transection [85]. However, both studies used a single dose of CBD at a very specific moment, namely immediately after an intensive brain injury, to evaluate its acute effects. Thus, these results may not be representative of long-term treatments with CBD.

Although recent literature has primarily searched for protective and therapeutic potentials of CBD, a recent research paper has reported negative effects. Zebrafish, exposed from blastula through to larval stage to micromolar concentrations of  $\Delta^9$ -THC (1-16  $\mu$ M) or CBD (0.25-4  $\mu$ M), presented similarity in dysmorphologies to both compounds (*i.e.*, edemas, curved axis, eye/snout/jaw/trunk/fin deformities, swim bladder distention, and behavioral abnormalities), whilst the  $LC_{50}$  for CBD was nearly seven times lower than  $\Delta^9$ -THC. The authors also reported teratogenic effects of low concentrations of CBD. [86]. In contrast, another research found no malformation in development of zebrafish embryos exposed to CBD 20–300  $\mu$ g/L, although the maximal dosage caused delay in embryo hatching. Besides, they were

1 temporarily more active than control. The authors discussed that the effects observed are intimately  
2 related to CB1 receptor [87]. Again, the chosen doses may be responsible for the difference in results  
3 observed in these two studies. Additionally, 10  $\mu$ M of  $\Delta^9$ -THC, but not 10  $\mu$ M of CBD, arrested the  
4 development of preimplantation mouse embryos [88].  
5 Notwithstanding that very few studies offer insight into CBD toxicity, some deleterious effects have been  
6 reported for CBD *in vitro* and *in vivo*. These include alterations in cell viability, reduced fertilization  
7 capacity, and inhibition of hepatic drug metabolism and drug transporters [89]. Our research group  
8 showed, in a study using an *in vitro* model of human neurons (human neuroblastoma SH-SY5Y cells  
9 differentiated with retinoid acid), that a sublethal dose of CBD with antioxidant activity did not exhibit  
10 neuroprotection against the neurotoxic effect of glycolaldehyde, methylglyoxal, 6-hydroxydopamine, and  
11 hydrogen peroxide in terminally-differentiated neurons. When SH-SY5Y cells undergoing neuronal  
12 differentiation were exposed to the same dose of CBD, besides the lack of neuroprotection and  
13 antioxidant activity, CBD potentiated the neurotoxicity induced by all redox-active drugs tested [90].  
14 These results suggest a possible hidden negative effect of CBD during neuronal development, reinforcing  
15 the observation that effective dosages for CBD and the resulting pathologies observed can vary widely  
16 according to the experimental model used.  
17 Thus, pure CBD present both positive and deleterious effects in animal and cellular models of early  
18 stages of development. We recommend that the therapeutic use of CBD and other cannabinoids during  
19 brain developmental stages must be always supported by experimental studies in appropriate cellular and  
20 animal models, with a special attention to the therapeutic window of CBD. It is particularly important to  
21 consider that the effect of CBD in humans follows an inverted U-shaped dose-effect curve pattern of  
22 effectiveness observed in many animal studies [91,92].

23

## 24 **6. Therapeutic perspectives**

25 Although a number of physiological effects of CBD in the brain have been identified, the mechanism(s)  
26 underlying its therapeutic properties in neurological diseases and during neurodevelopment are not yet  
27 clearly understood. Depending on the experimental model, the dosage used and the protocol, CBD can act  
28 upon CB1 as an agonist, or as an antagonist of endogenous ligands, or as an allosteric modulator, as well  
29 as acting upon non-endocannabinoid targets. Nevertheless,  $\Delta^9$ -THC, which is able to interact with the  
30 ECS, is present in CBD-enriched *Cannabis* extracts used in some studies. Since the ECS performs



1 primordial functions during embryonic development and neurodevelopment, in addition to neurogenesis  
2 in adults, it makes sense to hypothesize that any molecule that disturbs ECS activity, such as  $\Delta^9$ -THC  
3 (and potentially CBD), might disrupt the processes regulated by this cellular signaling system.

4 Regarding CBD therapeutic use for the treatment of children, there are several positive results in clinical  
5 trials and case reports in children with refractory epilepsy. However, for CBD-enriched *Cannabis* extracts  
6 the controversial effects of  $\Delta^9$ -THC points to a possible risk of adverse effects for its use in young  
7 patients. *Cannabis* has been associated with development of psychotic symptoms later in life, and a recent  
8 publication was able to establish a causal role of *Cannabis* use during adolescence and the emergence of  
9 such symptoms in the subsequent year [72]. Such effects are attributed to  $\Delta^9$ -THC activity on CB<sub>1</sub>. As  
10 CBD has low affinity for CB<sub>1</sub>, although it interferes in other steps of ECS signaling, this cannabinoid may  
11 be preferable and safer. Thus, formulations containing  $\Delta^9$ -THC should be avoided. Moreover, adverse  
12 effects of CBD and its extracts – even though they are mainly not severe – as well as absence of  
13 therapeutic effects were also reported. Seizure reduction has a significant effect on the patient’s quality of  
14 life, but the need to take into account other changes that CBD could cause in social behavior, cognitive  
15 function, or motor skills, is also important. Another concern is that the use of CBD-based therapies for  
16 pediatric epilepsy and anxiety (see table 1 and supplementary table 2), together with the common belief  
17 that natural products are always harmless, could represent a precedent for its use to treat other  
18 neurological diseases. It is not completely clear how CBD affects children’s brain development and how  
19 it could represent any probability of developing diseases later in adulthood. Thus, despite evidences for  
20 potential benefits in pediatric patients, pediatricians and families must balance the decision to use CBD  
21 with the associated risks [13]. An evaluation must occur on a case-to-case basis, with each instance  
22 considering the damage to the patient that may arise from uncontrolled epileptic seizures, the adverse  
23 effects of the established antiepileptic drugs and the uncertainties in the effects of CBD during brain  
24 development.

25 Recently, natural and synthetic derivatives of CBD have attracted the attention of both industry and  
26 academia. Indeed, some of these molecules are being studied for a variety of purposes, most of them  
27 aiming to improve the potency, efficacy, or pharmacokinetic properties of CBD [93]. For instance, a  
28 natural CBD derivative, cannabidiolic acid (CBDA), does not have effect on inhibition of anandamide  
29 uptake while keeping the low CB<sub>1</sub> affinity [93]. Thus, CBDA probably does not interfere in ECS  
30 signaling, what lowers the risk for adverse effects during brain development. The conversion of oral CBD

1 into  $\Delta^9$ -THC in an acidic environment (*e.g.* the stomach) is another concern, although it has not been  
2 observed *in vivo* thus far [94]. A novel CBD derivative, HU-444, is a potential novel drug which cannot  
3 be converted by acid cyclization into a  $\Delta^9$ -THC-like compound. *In vitro*, HU-444 has an anti-  
4 inflammatory activity, leading to the suppression of TNF- $\alpha$  production and amelioration of liver damage,  
5 whilst not causing  $\Delta^9$ -THC-like effects in mice [95]. Another synthetic cannabinoid, HU-320, produced  
6 strong anti-inflammatory and immunosuppressive effects in an *in vivo* model of collagen-induced arthritis  
7 [95].

8 For the generation of another class of CBD derivatives, the introduction of the DMH alkyl chain in the (-  
9 )-DMH-CBD series did not alter the lack of CB<sub>1</sub> and CB<sub>2</sub> receptor affinity [96]. (-)-DMH-CBD analogs  
10 have displayed anxiolytic, analgesic, anti-inflammatory, and antiproliferative effects in diverse assays  
11 [93]. (-)-DMH-CBD has been shown to have anti-inflammatory and antiproliferative properties in human  
12 acute myeloid leukemia [97]. Interestingly, (-)-7-OH-DMH-CBD exhibited potent inhibition of  
13 electrically-evoked contractions of the mouse *vas deferens* that was not mediated through CB<sub>1</sub>, CB<sub>2</sub>,  
14 TRPV1, opioid, or  $\alpha$ 2-adrenergic receptors [98,99].

15 Measurements of the binding affinities for the CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors yielded unexpected  
16 outcomes of some CBD enantiomers. Contrary to naturally occurring (-)-CBD analogs, some synthetic  
17 derivatives, such as (+)-CBD, H2-CBD, H4-CBD, and HU-465 bind to CB<sub>1</sub> and several of them have  
18 shown interesting pharmacological properties for various pathologies [93]. However, as CB<sub>1</sub> activity is  
19 not desirable for an antiepileptic drug due to all the ECS roles at developmental stages, such derivatives  
20 might not be an alternative in these cases. Thus, likewise natural occurring cannabinoids, different CBD  
21 derivatives vary in their pharmacological and therapeutic properties, evidencing the need for a better  
22 understanding of their mechanism of action.

23

## 24 **7. Conclusion**

25 As *Cannabis* extracts contain  $\Delta^9$ -THC, which has psychoactive effects and is a CB<sub>1</sub> agonist and may  
26 potentially disturb the ECS processes during brain development, pure GMP-grade CBD, synthetic or  
27 plant derived, is probably a safer option for use in pediatric and juvenile patients. Recently, a CBD oral  
28 solution, purified from a *Cannabis* extract, and developed and tested by GW Research has been approved  
29 by the Food and Drug Administration agency of United States (FDA), as an adjuvant in the treatment of  
30 seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in patients 2 years of age and

1 older. According to the released document, the approval was based on CBD's effectiveness in preclinical  
2 and clinical trials and due to its mechanisms of action (low CB<sub>1</sub> affinity, reduction of neuronal  
3 hyperexcitability and inflammation) [100].

4 However, since CBD can potentially affect the ECS also, further studies are recommended in order to  
5 clarify its mechanisms of action and developmental implications. Besides, longer chronic treatment and  
6 follow-up periods are recommended in clinical trials and animal studies in order to evaluate CBD's long-  
7 term effects, as well as the most effective dosage and the age which the therapeutic use of pure CBD is  
8 not only effective but also safe.

9 At the moment, we consider that CBD is recommended as the last option for the treatment of non-  
10 responsive epileptic children. For other neurological or psychiatric diseases, such as childhood anxiety,  
11 there is insufficient evidence to support the effectiveness of CBD. Besides, we suggest that more studies  
12 should use adequate experimental models to focus on pure CBD, in order to establish its safe and  
13 effective dosage and therapeutic targets, as well as synthetic CBD derivatives, aiming to identify a CBD  
14 analog with therapeutic properties but with fewer risks to the developing brain.

## 16 **Compliance with Ethical Standards**

## 18 **Funding**

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## 24 **Conflict of interest statement**

25 AWZ, JECH and JAC are co-inventors of the patent “Fluorinated CBD compounds,  
26 compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.:  
27 PCT/IL2014/050023” Def. US no. Reg. 62193296; 29/07/2015; INPI on 19/08/2015  
28 (BR1120150164927). The University of São Paulo has licensed the patent to *Phytecs Pharm* (USP  
29 Resolution No. 15.1.130002.1.1). The University of São Paulo has an agreement with *Prati-Donaduzzi*

1 (Toledo, Brazil) to “develop a pharmaceutical product containing synthetic cannabidiol and prove its  
2 safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson’s disease, and  
3 anxiety disorders.” JECH and JAC have received travel support from and are medical advisors of BSPG-  
4 Pharm. AWZ is medical advisor of BSPG-Pharm.

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19

## 20 **Figure Legends:**

21 **Fig. 1** Retrograde endocannabinoid signaling. Endocannabinoids are produced on demand in the post-  
22 synaptic neuron, released in the synaptic cleft and activate CB1 receptor in the pre-synaptic neuron.  
23 Reactive oxygen species (ROS); endocannabinoid system (ECS); anandamide (AEA); 2-  
24 arachydonoiglycerol (2-AG); N-arachidonoylphosphatidylethanolamine (NAPE); N-  
25 arachidonoylphosphatidylethanolamine phospholipase-D (NAPE-PLD); diacylglycerol (DAG);  
26 diacylglycerol lipase (DAGL); phosphatidylinositol biphosphate (PIP<sub>2</sub>); CB1 (CB1 receptor); adenylyl  
27 cyclase (AC), cyclic AMP (cAMP); protein kinase A (PKA); fatty acid amide hydrolase (FAAH);  
28 monoacylglycerol lipase (MAGL); arachidonic acid (AA); ethanolamine (ET); mitogen-activated protein  
29 kinase (MAPK).

30

1 **Table 1.** Clinical trials using pure CBD published until May 2018 with children and young patients

2

3 **Supplementary Table 1.** Adult volunteer studies on adverse effects of pure CBD and relevant CBD-  
4 enriched extract (CBD-based therapies)

5

6 **Supplementary Table 2.** Pure CBD and relevant CBD-enriched extract (CBD-based therapies) case  
7 reports, parent surveys and retrospective chart reviews published until May 2018 with children and young

8 adults

