Safety and Side Effects of Cannabidiol, a *Cannabis sativa* Constituent

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Abstract: Cannabidiol (CBD), a major nonpsychotropic constituent of *Cannabis*, has multiple pharmacological actions, including anxiolytic, antipsychotic, antiemetic and anti-inflammatory properties. However, little is known about its safety and side effect profile in animals and humans. This review describes *in vivo* and *in vitro* reports of CBD administration across a wide range of concentrations, based on reports retrieved from Web of Science, Scielo and Medline. The keywords searched were "cannabinoids", "cannabidiol" and "side effects". Several studies suggest that CBD is non-toxic in non-transformed cells and does not induce changes on food intake, does not induce catalepsy, does not affect physiological parameters (heart rate, blood pressure and body temperature), does not affect gastrointestinal transit and does not alter psychomotor or psychological functions. Also, chronic use and high doses up to 1,500 mg/day of CBD are reportedly well tolerated in humans. Conversely, some studies reported that this cannabinoid can induce some side effects, including inhibition of hepatic drug metabolism, alterations of *in vitro* cell viability, decreased fertilization capacity, and decreased activities of p-glycoprotein and other drug transporters. Based on recent advances in cannabinoid administration in humans, controlled CBD may be safe in humans and animals. However, further studies are needed to clarify these reported *in vitro* and *in vivo* side effects.

Keywords: Cannabidiol, cannabinoid, cannabis sativa, CBD, marijuana, safety, side effects, toxicity.

INTRODUCTION

Cannabidiol (CBD) is a component of *Cannabis sativa* and constitutes up to 40% of the extracts of the plant [1]. However, CBD concentrations are highly variable and depend on the growing conditions, the different phenotypes of illicit cannabis, and on the part of the plant analyzed [2, 3]. Evidence suggests that the potency of CBD has decreased in recent years, while THC concentrations have increased, since the use of varieties such as sensimillia ('skunk'), provided by ilegal cannabis growers, currently dominates the supply of cannabis in many countries [3].

CBD induces markedly different psychological effects compared to the best known marijuana compound, Δ9-tetrahydrocannabinol (THC) [4, 5]. Despite presenting low affinity for CB1 and CB2 receptors, CBD can still interact with these receptors at doses equal to or lower than 1 μM. Therefore, there is no certainty about whether this antagonism is non-competitive. CBD can also act as a CB1 receptor inverse agonist at concentrations below those needed to bind to the CB1 orthosteric site. Moreover, CBD can antagonize THC effects *via* non-CB1/CB2 receptors, such as GPR55, which is activated by THC and blocked by CBD [6]. The time between the intake of CBD and THC, as well as the CBD/THC ratio, seem to play an important role in the interaction between these two cannabinoids. CBD can increase the potency of THC by pharmacokinetic interaction if CBD is administrated before THC, or a pharmacodynamic interaction may occur when both cannabinoids are taken together, mainly at a high dose ratio of CBD/THC [7].

CBD was first isolated by Adams *et al.* in 1940 [8], and its structure was identified 23 years later [9]. Since then, a considerable number of published articles have dealt with its chemistry, biochemistry, pharmacology and clinical effects. By the year 2000, the primary research topics regarding possible therapeutic effects of CBD were related to its antiepileptic, sedative, anxiolytic and antipsychotic activities [10, 11]. The last decade has shown a notable increase in scientific literature on CBD, owing to the identification of its anti-inflammatory and neuroprotective effects. These studies have raised the possibility of therapeutic effects of CBD for diverse conditions, including dementias, cerebral ischemia, diabetes, inflammatory diseases, nausea and psychiatric disorders [12].

This wide range of therapeutic effects can be explained by CBD's multiple mechanisms of action. Despite its low affinity for CB_1 and CB_2 receptors, CBD is capable of antagonizing CB_1 / CB_2 receptor agonists at reasonably low concentrations. At CB_2 receptors, CBD acts as an inverse agonist. Other mechanisms of action include antagonism of the recently discovered GPR55 receptor; transient receptor potential vanilloid type 1 (TRPV1) agonism; transient receptor potential vanilloid type 2 (TRPV2) agonism; 5-HT_{1A} agonism; antagonism of the putative abnormal-CBD receptor; and regulation of intracellular [Ca^{2+}] [13].

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Inhibition of adenosine uptake leads to increased adenosine signaling, which may explain the ability of CBD to decrease inflammation and to present neuroprotective effects [14, 15]. Another similar mechanism has also been reported for CBD, according to which this cannabinoid could block anandamide uptake and inhibit its enzymatic hydrolysis [16].

Few studies have been completed concerning the safety and side effects of CBD after its administration *in vivo* and *in vitro*, but this review will summarize such findings. First, CBD safety in animals and humans will be discussed. Second, side effects of CBD intake will be discussed, as well as the biological parameters affected by CBD interaction with other substances. Finally, some toxicology aspect studied in monkey will be shown.

METHOD

This review was conducted using reports retrieved from Web of Science, Scielo and Medline. The keywords searched were "cannabinoids", "cannabidiol" and "side effects." No time limits were imposed on the search criteria.

We included papers in English, Portuguese and Spanish languages that described research in humans or animals using CBD alone. The reference lists of eligible papers were checked for additional relevant studies. Studies describing mixed cannabinoids or CBD extracts were excluded. A total of 132 papers were selected for the review.

RESULTS

Safety of CBD

Effect on Cell Growth and Embryogenesis

CBD exerts anti-proliferative and pro-apoptotic effects in tumor cell lines. There are several mechanisms by which CBD exhibits its effects, including the production of reactive oxygen species (ROS) and concomitant activation of initiator caspase-8 and caspase-9 [17], inhibition of the procarcinogenic lipoxygenase pathway [18], and induction of apoptosis, inhibition of tumor grown [16].

In order to investigate the selectivity of CBD's effects in tumoral and nontumoral cells, several concentrations of CBD (1-25μM) were tested *in vitro* on different stabilized nontumor cell lines, such as human keratinocyte, rat preadipocytes, and mouse monocytemacrophages. CBD does not affect the vitality of nontumor cell lines, contrary to what occurs with human breast carcinoma cells, human prostate carcinoma cells, human colorectal carcinoma cells, human gastric adenocarcinoma cells, rat glioma cells, rat thyroid cells transformed with the v-K-*ras* oncogene, and rat basophilic leukemia cells [16]. Glial cells were also tested against CBD toxicity and their viability was not affected by the treatment with CBD up to 50μM. The safety of CBD on non-transformed cells may be explained by the lack of ROS damage in glial cells [17].

Analysis of CBD's effects on embryo development is also important, because it raises the question whether expectant mothers can take CBD, and, consequently, whether it affects fetal development. *In vitro* results revealed that CBD did not significantly alter embryonic development at concentrations of 6.4, 32 and 160 nM [19].

Effect on Food Intake

One common effect of THC is increased food intake [20-22], which is mediated by CB1 and induced by stimulation of dopamine release in the nucleus accumbens [6].

CBD has a low affinity for the CB1 receptor, and concentrations of 3 to 100 mg/kg body weight (bw) administered intraperitoneally (i.p.) resulted in no significant effects on food intake in mice [23, 25] or rats. However, CBD (20mg/kg bw i.p.) decreased hyperphagia induced by CB1 and 5-HT1A receptor agonists in rats [26].

Conversely, chronic use of CBD for up to 14 days reduced body weight gain in rats at doses of 2.5 and 5 mg/kg bw. This effect was prevented by co-administration of a CB2 receptor antagonist [27].

Cataleptic Effects and Motor Changes

Typical antipsychotic drugs exhibit catalepsy as a side effect, which is mediated by the blockade of dopamine receptors in the dorsal striatum. These drugs may counteract the stereotypical actions of dopaminergic agents in rodents, including d-amphetamine, and hyperlocomotion induced by dopaminergic agents or antagonism of the N-methyl-d-aspartate (NMDA) glutamate-receptor subtype. Moreover, these dopaminergic agents cause decreased social interaction and disruption of the prepulse inhibition of the startle reflex. The antagonism of these effects is predictive for compounds with antipsychotic activity [28].

Several studies have evaluated the antipsychotic-like properties of CBD in animal models. This cannabinoid has not been shown to induce catalepsy, even at doses as high as 480 mg/kg bw [12, 29, 32].

Motor changes were investigated in studies of possible anxiolytic and antidepressant effects of CBD. Antidepressant drugs activate the 5-HT1A receptors [33], and CBD may also exhibit agonist properties at 5-HT1A receptors [34]. CBD shows anxiolytic-like and antidepressant-like effects with an inverted U-shaped profile, but does not induce motor changes [23, 28, 35, 36].

Effects on Physiological Parameters in Animals

Several studies administering CBD by different routes have shown it to be safe, in regards to the effects on physiological parameters.

At a wide range of doses (3-30mg/kg bw i.p.), CBD does not affect blood pressure, heart rate, body temperature, glucose levels, pH, Pco_2, Po_2, hematocrit, K^+ or Na^+ levels, gastrointestinal transit or rectal temperature in rodents [24, 37, 42]. The results were the same, even after 14 days of treatment [43]. An *in vitro* study showed that the cannabinoid failed to induce contraction in mouse small intestine at concentrations ranging from 0.01μmol/L to 10.0μmol/L [37]. Furthermore, CBD has not shown significant effects on open-field physiological activity (defecation and urination) nor on vocalization behavior [39]. Mice treated with 60 mg/kg bw CBD i.p. three times per week for 12 weeks did not experience significant side effects

such as ataxia, kyphosis, generalized tremor, swaying gait, or tail stiffness [44]. Finally, CBD at 10 and 20mg/kg bw i.p. did not produce emesis in mice [45].

Another study performed to determine whether CBD is an agonist at rat TRPV1 receptors *in vivo* demonstrated the safety of this cannabinoid in other physiological parameters. Rats received a CBD injection (0.003-6.36μmol; 1-2,000μg intra-arterially), but did not exhibit appreciable effects on mean blood pressure, arterial blood gas tensions, pH, ventilatory responses or respiratory minute volume. This study provided evidence that CBD does not affect ventilation [46].

Cannabinoids interact at different degrees with TRP channels, being CBD most potent at TRPV1 [47]. Stimulation of vanilloid receptors induces vasodilation and inflammation. CBD has been shown to be a full agonist of human TRPV1 at concentrations lower than those needed to bind to CB1/CB2 receptors, usually at doses ranging from 10 to 50 mg/kg in humans, followed by a quick desensitization of TRPV1 receptors, which leads to the depletion of sensory nociceptors [48].

CBD (0.1-30mg/kg bw intravenously (i.v.)) had no effect on the rate of intestinal transit or the rate of gastric emptying, or cardiovascular, antinociception, hypothermia or respiratory parameters [29, 49, 50]. An evaluation of the neuroprotective activity of CBD revealed that CBD was not only free from significant side effects, but also associated with cardiac, hemodynamic, and ventilatory benefits in piglets [51].

It is important to note that the lack of CBD side effects was observed during studies whose primary objectives were not to evaluate CBD´s safety, but to study cannabinoid activity. Furthermore, several other studies that evaluated the anxiolytic effects of CBD in rodents demonstrated the safety and tolerability of this drug in rodents [52-57].

Effects on Monoamine Oxidase Activity

CBD (0.3-300μg/mg protein) was ineffective at inhibiting porcine monoamine oxidase activity of brain and liver mitochondria after 1 hr of incubation with mitochondrial preparation [58].

Effects on Memory

Short-term memory and other cognitive deficits have been reported in humans after smoking marijuana. In rats tested against a delayed match to sample task, THC showed a correlation between delay and dose-dependent behavioral deficit produced in this task. This performance was selectively impaired by a lack of discharge of hippocampal neurons. However, CBD at doses of 0.75-2.0mg/kg bw (i.p.) were tested in the same task and no significant effect on performance was observed [59].

Effects at Estrogen Receptors

Compounds possessing the tricyclic cannabinoid structure, including CBD, have been reported to interact with rodent estrogen receptors. To test the hypothesis that cannabinoids produce a direct activation of estrogen receptors, Ruh *et al.* [60] investigated whether cannabinoid compounds exhibit estrogen-induced mitogenesis in MCF-7 breast cancer cells. CBD (1 and 10μM) did not significantly stimulate the proliferative response or transcriptional activity compared to controls. As a result, CBD failed to behave as an estrogen receptor agonist *in vitro*.

Studies in Humans

In human studies, CBD administration did not induce side effects across a wide range of dosages, including acute and chronic dose regimens, and tolerance to CBD did not develop.

Acute Studies

In the 1970s, human studies showed that oral CBD intake from 15 to 160mg [61-63], inhalation of 0.15mg/kg bw [64] or intravenous injection from 5 to 30mg [4, 61] were not followed by ill effects.

CBD does not interfere with several psychomotor and psychological functions in humans. CBD does not affect heart rate, blood pressure, or performance in the verbal paired-associate learning test as measured by recall score at doses up to 600mg [52, 62, 65, 74].

Subsequent studies concerning the antipsychotic effects of CBD have not reported any side effects after CBD intake [75-77].

Chronic Studies

Chronic oral administration of 10mg CBD daily for 21 days did not induce any changes in neurological (including electroencephalogram (EEG)), clinical (including electrocardiogram (EKG)), psychiatric, blood or urine examinations [78]. Likewise, oral CBD administration in healthy participants (3mg/kg bw daily for 30 days) and in epileptic patients (200-300mg daily for 135 days) was well tolerated and no signs of toxicity or serious side effects were detected on neurological and physical examinations, blood and urine analysis, or EKG and EEG, which were performed at weekly intervals [10].

CBD was evaluated for symptomatic efficacy and safety in 15 neuroleptic-free patients with Huntington's Disease. Effects after oral CBD (10mg/kg bw /day for 6 weeks) or placebo (sesame oil for 6 weeks) intake were evaluated weekly under a double-blind, randomized crossover design. CBD showed no significant or clinical differences compared to placebo in the *Cannabis* side effect inventory, clinical lab tests or other safety outcome variables. Also, weekly plasma levels of CBD (mean range 5.9 to 11.2 ng/ml), assayed by GC/MS, did not differ significantly over the 6 weeks of CBD administration [79].

A previous case report of a teenager diagnosed with schizophrenia who experienced severe side effects after treatment with conventional antipsychotics demonstrated significant improvement of symptoms with no adverse effects after hospitalization and 4 weeks of treatment with increasing doses of CBD up to 1,500mg/day [80]. More recently, CBD monotherapy was administered to three patients with treatment-resistant schizophrenia (initial oral dose of 40 mg, increased to 1,280mg/day) for up to 4 weeks with no side effects reported, even at the highest dose [81]. A similar result was observed in two patients with bipolar affective disorder who received CBD (600-1,200mg/day) for up to 24 days [82]. A double-blind study with 42 patients

diagnosed with schizophrenia or schizophreniform disorder (DSM-IV) in an acute episode showed that CBD (800mg) significantly reduced psychotic symptoms after 2 to 4 weeks of treatment and induced fewer side effects, such as extrapyramidal symptoms, increased prolactin levels, and weight gain, compared to amilsupride [83].

The efficacy and safety of CBD on Parkinson's disease patients with psychotic symptoms were study in a 4-week open trial. A flexible oral dose of CBD, ranging from 150mg/day to 400mg/day in the last week, plus patients' usual treatments showed that psychotic symptoms were significantly reduced; cognitive and motor symptoms were not affected by the cannabinoid and no serious side effects were reported [84]. A double-blind placebo controlled trial is currently underway by our group to evaluate the efficacy, safety, and tolerability of CBD in patients with Parkinson's disease and psychosis.

Finally, a 19-year old female with a history of cannabis addiction received CBD 300mg on day 1, 600mg/day divided into two doses days 2 through 10, and CBD 300mg on day 11. During treatment with CBD, the patient did not report any marijuana withdrawal symptoms, and she did not experience anxiety or dissociative symptoms [67] or improved sleep quality, as assessed by standardized rating scales.

We did not include in this review studies on cannabis extracts or CBD-rich extracts, as the other several compounds may have multiple interactions with CBD. However, some clinical trials in multiple sclerosis have shown that the 1:1 mix of THC and CBD, which is available as an oromucosal spray (Sativex®) at doses ranging from 2.5 to 120 mg of each cannabinoid, showed no adverse effects on cognition or mood [85] or other than those observed with psychoactive drugs for pain treatment [86].

These studies concerning the safety of CBD administration are summarized in Tables **1** and **2**.

Side Effects of CBD

Effect of Cannabidiol in the Human Immune System

The majority of available literature shows inhibitory capacities of cannabinoids, including CBD, on cells of the human immune system. CBD (2.5-10μg/ml) strongly inhibited interleukin (IL)-10 production in a virus-negative T-cell line, and increased IL-8, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β production in an eosinophilic leukemia cell line and inhibited IL-8 production by B-cells. Since CBD decreased production of IL-8 and CC chemokines (MIP-1α and MIP-1β) by B-cells, a patient's risk of infection with human immunodeficiency virus – 1 (HIV-1) or other infectious organisms may increase, along with a risk of disease progression. Previous reports suggested that IL-10 inhibits HIV-1 expression by infected macrophages [87-89]. Therefore, the strong inhibition of IL-10 production by CBD could be another mechanism by which this cannabinoid can up regulate HIV-1 production [90].

In summary, although these effects are of potential benefit in some conditions, they may worsen disease progression, HIV infection, tumor genesis, and metastases, and exacerbate allergic inflammation in the lung [90]. However, some results suggested that CBD could yield a biphasic response in the immune system with stimulatory capacity at lower doses (nanomolar concentrations) and inhibitory activity at higher doses (micromolar concentrations). Accordingly, an enhancement of mitogen-induced indoleamine 2,3-dioxygenase activity and secretion of interferon (IFN)-γ by CBD (10-100ng/ml) and suppression of these activities at higher doses (1-10μg/ml) were observed in human peripheral blood mononuclear cells [91].

In *in vivo* evaluations of CBD in humans, significant correlations were found between IFN-γ blood levels, neopterin, and the kynurenine-to-tryptophan ratio in various diseases, including human immunodeficiency virus

Table 1. Effects of CBD Administration in *In Vitro* Studies

Study	Reference	Cell Lines	Dose	Relevant Information
Ligresti *et al.* (2006)	[16]	tumoral cell lines	1-25μM	no significant effect on non-transformed cells
Massi *et al.* (2006)	[17]	U87 human glioma cells	0-50μM	no significant effect on non-transformed cells
Massi *et al.* (2008)	[18]	U87 human glioma cells	10-16μmol/L	no significant effect on non-transformed cells
Paria *et al.* (1995)	[19]	mouse's embryo	6.4 -160nM	no significant effect on embryonic development
de Filippis *et al.* (2008)	[37]	mouse's small intestine muscle strips	0.01-10μmol/L	no significant effects on inducing contraction
Schurr *et al.* (1976)	[58]	porcine's brain and liver mitochondria	0.3-300μg/mg protein	no inhibition on porcine monoamine oxidase activity
Ruh *et al.* (1997)	[60]	MCF-7 breast cancer cells	1–10μM	no significant effect on estrogen receptors
Gallily *et al.* (2003)	[101]	human PBMC	1-15μg/ml	no significant effect on non-transformed cells
Steger *et al.* (1990)	[127]	rat's pituitaries	0.1-10mg/kg bw	no significant effect on luteinizing hormone secretion

Abbreviations: bw, body weight; PBMC, peripheral blood mononuclear cells.

Table 2. Effects of CBD Administration in *In Vivo* Studies

Study	Reference	Species	Route	Dose	Relevant Information
Zuardi *et al.* (1982)	[5]	human	oral	1mg/kg bw	no significant effects on heart rate and bodily symptoms
Cunha *et al.* (1980)	[10]	human	oral	3mg/kg bw; 200 and 300mg/day	no significant effects on neurological and physical examinations, blood and urine analysis, electrocardiogram and electroencephalogram
Ligresti *et al*. (2006)	[16]	mouse	intratumor	5mg/kg bw	lower potency in noncancer cells
Massi *et al*. (2008)	[18]	mouse	peritumoral	0.5 mg/mouse	no significant effect on non-transformed cells
Riedel *et al.* (2009)	[23]	mouse	intraperitoneal	10mg/kg bw	no significant effects on weight gain and on locomotor activity
El-Remessy *et al.* (2006)	[24]	mouse	intraperitoneal	10mg/kg bw	no significant effect on weight gain and on blood glucose levels
Wiley *et al.* (2005)	[25]	mouse	intraperitoneal	0-100mg/kg bw	no significant effect on weight gain
Scopinho *et al.* (2011)	[26]	rat	intraperitoneal	20mg/kg bw	decreased induced-hyperphagia
Varvel *et al.* (2006)	[29]	mouse	intravenous	1–30mg/kg bw	no significant effects on catalepsy, antinociception and hypothermia
Zuardi *et al.* (1991)	[30]	rat	intraperitoneal	15-480mg/kg bw	no significant effect on catalepsy
Fairbairn *et al.* (1979)	[31]	mouse	oral	3.13-100mg/kg bw	no significant effects on catalepsy
Pertwee *et al.* (1972)	[32]	mouse	intraperitoneal	5-100mg/kg bw	no significant effect on catalepsy
Zanelati *et al.* (2010)	[35]	mouse	intraperitoneal	3-100mg/kg bw	did not induce motor changes
Guimarães *et al.* (1990)	[36]	rat	intraperitoneal	2.5-20mg/kg bw	did not induce motor changes
de Filippis *et al*. (2008)	[37]	mouse	intraperitoneal	10mg/kg bw	no significant effects on gastrointestinal motility
Hayakawa *et al.* (2007)	[38]	mouse	intraperitoneal	3mg/kg bw	no significant effects on blood pH, $PaCo_2$, PO_2, hematocrit, K+ and Na+ levels, glucose, blood pressure, heart rate and rectal temperature
Hiltunen *et al.* (1988)	[39]	rat	intraperitoneal	10and 30mg/kg bw	no significant effects on rectal temperature, open-field physiological activity and on vocalization behavior
Hampson *et al.* (2000)	[40]	rat	intraperitoneal	20mg/kg bw	no significant effects on $PaCo_2$, PO_2, glucose, blood pressure and rectal temperature
Resstel *et al.* (2006)	[41]	rat	intraperitoneal	10mg/kg bw	no significant effects on blood pressure and heart rate
Chesher *et al.* (1973)	[42]	mouse	oral	6-30mg/kg bw	no significant effects on gastrointestinal motility
Hayakawa *et al.* (2007)	[43]	mouse	intraperitoneal	3mg/kg bw	no significant effects on blood pH, $PaCo_2$, PO_2, hematocrit, K+ and Na+ levels and rectal temperature
Dirikoc *et al.* (2007)	[44]	mouse	intraperitoneal	60mg/kg bw	no significant effects on ataxia, kyphosis, generalized tremor, swaying gait, tail stiffness
Darmani (2002)	[45]	shrew	intraperitoneal	10 and 20mg/kg bw	did not induce motor changes
McQueen *et al.* (2004)	[46]	rat	intra-arterial	0.003-6.36µmol; 1-2,000µg	no significant effects on blood pressure, arterial blood gas tensions, pH, ventilatory responses and respiratory minute volume
Shook *et al.* (1989)	[49]	mouse	intravenous	0.1 - 100mg/kg bw	no significant effects on the rate of intestinal transit and of gastric emptying

(Table 2) contd…..

Study	Reference	Species	Route	Dose	Relevant Information
Graham *et al.* (1973)	[50]	rat	intravenous	1mg/kg bw	no significant effect on cardiovascular and respiratory parameters
Alvarez *et al.* (2008)	[51]	piglet	intravenous	0.1mg/kg bw	no significant effects on blood pH, PaCo2, PO2, heart rate, blood pressure, hemodynamic and respiratory parameters
Heyser *et al.* (1993)	[59]	rat	intraperitoneal	0.75-2.0mg/kg bw	no effect delayed match to sample task performance
Hollister (1973)	[61]	human	oral	20-100mg	no significant side effect
Hollister (1973)	[61]	human	intravenous	5-30mg	no significant side effect
Karniol *et al.* (1974)	[62]	human	oral	15-60mg	no significant effects on heart rate, psychological reactions and on time prduction tasks
Bergamaschi *et al.* (2011)	[65]	human	oral	600mg	no significant effects on heart rate, blood pressure, skin conductance, bodily symptoms and psychological measurements
Crippa *et al.* (2011)	[66]	human	oral	400mg	no significant effects on subjective and psychological measurements
Crippa *et al.* (2010)	[67]	human	oral	300-600mg/day	no significant side effect
Fusar-Poli *et al.* (2009)	[68]	human	oral	600mg	no significant effects on heart rate, blood pressure, task performance and psychological measurements
Fusar-Poli *et al.* (2009)	[69]	human	oral	600mg	no significant side effect
Bhattacharyya *et al.* (2009)	[70]	human	oral	600mg	no significant effects on verbal learning task and psychotic symptoms
Borgwardt *et al.* (2008)	[71]	human	oral	600mg	no significant effects on intoxication, sedation, psychotic symptoms and motor inhibition task
Crippa *et al.* (2004)	[72]	human	oral	400mg	no significant effects psychological measurements
Zuardi *et al.* (1993)	[73]	human	oral	300mg	no significant effects on heart rate, blood pressure, psychomotor performance, bodily symptoms and psychological measurements
Consroe *et al.* (1979)	[74]	human	oral	200mg	no significant impairments of motor and mental performances
Hallak *et al.* (2011)	[75]	human	oral	600mg	no significant effects on heart rate, blood pressure and behavior measurements
Bhattacharyya *et al.* (2010)	[76]	human	oral	600mg	no significant effects on heart rate and psychotic symptoms
Hallak *et al.* (2010)	[77]	human	oral	300 and 600mg	no significant side effect
Mincis *et al.* (1973)	[78]	human	oral	10mg	no significant change in neurological, clinical, psychiatric, blood and urine examinations
Consroe *et al.* (1991)	[79]	human	oral	10mg/kg/day	no significant side effect
Zuardi *et al.* (1995)	[80]	human	oral	1,500mg/day	no significant side effect
Zuardi *et al.* (2006)	[81]	human	oral	40-1,280mg/day	no significant side effect
Zuardi *et al.* (2010)	[82]	human	oral	600-1,200mg/day	no significant side effect
Leweke *et al.* (2007)	[83]	human	oral	800mg/day	less side effect than amisulpride
Zuardi *et al.* (2009)	[84]	human	oral	150-400mg/day	no significant side effect
Steger *et al.* (1990)	[127]	rat	oral	0.1-10mg/kg bw	no significant effect on gonadal hormone levels

Abbreviations: bw, body weight; PaCo2, carbon dioxide partial pressure;PO2, oxygen partial pressure; K+, potassium; Na+, sodium.

infection, malignancy and autoimmune syndromes [92-94]. Moreover, there are significant correlations between the decrease of tryptophan levels and the increased susceptibility of patients to mood disturbances and depression [95-97]. Activation of indoleamine 2,3-dioxygenase could represent a link between the immunological network and the pathogenesis of depression, when the availability of tryptophan limits serotonin biosynthesis [91, 96, 98].

Effects on Cell Viability

Studies of CBD evaluating cell viability and apoptosis have been conducted for decades [99]. The induction of apoptosis by the cannabinoids has been demonstrated primarily in leukemia, breast carcinoma, and glioma cells [100], but little information pertaining to primary cells is available. Some reports have shown a differential sensitivity between transformed and nontransformed monocytes and glia cells to CBD-induced apoptosis [16, 17, 101], implicating the potential use of CBD as an anticancer agent against sensitive tumors [102].

However, exposure of thymocytes to CBD (4–16μM) for 2h increased the mean fluorescence of 2',7'-dichlorofluorescin (DCF) in a concentration-related manner, indicating an elevated cellular ROS production. Nonetheless, CBD treatment significantly increased the DCF fluorescence in thymocytes and EL-4 thymoma cells. Time-course analyses revealed that CBD-mediated apoptosis occurred earlier in EL-4 cells than in thymocytes [102]. Several studies have reported a crucial role for ROS in CBD-induced apoptosis in glioma and leukemia cells [17, 100].

Primary monocytes and glia cells are reportedly non-sensitive to CBD-induced apoptosis [17, 101], but an enhancement of apoptosis by CBD treatment was observed in normal lymphocytes. CBD also increased splenocyte apoptosis *via* ROS-dependent activation of caspase-8 [102]. Exposure of splenocytes to CBD (4–8μM) elicited an early production of ROS with peak response at 1h post-CBD treatment and a parallel gradual decrease in cellular glutathione. In addition, CBD treatment (8μM) significantly stimulated caspase-8 activation. Although it did not demonstrate a positive impact on ROS production, pretreatment of splenocytes with a cell-permeable inhibitor for caspase-8 significantly attenuated CBD-mediated apoptosis in a concentration-dependent manner [103].

This pro-apoptotic property induced by CBD in normal lymphocytes could contribute to the immunosuppressive effects induced by this cannabinoid. The repercussions of this effect in patients with infectious diseases need to be investigated.

Inhibition of Hepatic Drug Metabolism

Cannabidiol is a potent inhibitor of hepatic drug metabolism and this effect raises the question of whether CBD can inhibit the metabolism of other drugs *in vivo*, affecting their metabolite concentration in the central nervous system [104, 105].

The CBD-mediated inhibition of drug metabolism is likely a result of the covalent binding of a reactive CBD metabolite to hepatic microsomal P450 [106], which affect specific isozymes. Acute treatment with CBD in male rats decreased hepatic cytochrome P450 content [107]. A similar effect was observed in mice, showing inactivation of specific cytochrome P450 isoforms belonging to the 2C and 3A subfamilies [108. 109]. Orthologs of these P450s are also found in human liver microsomes, and immune inhibition studies show that their metabolite profiles are qualitatively similar to those of their mouse counterparts [110]. Furthermore, CBD can inactivate human P450 3A4 [111], which is responsible for metabolizing more than 60% of clinically prescribed drugs [112].

The metabolism of the main active constituent of *Cannabis*, THC, and the endogenous cannabinoid anandamide are inhibited by CBD. To determine the effect of CBD in P450-catalyzed anandamide metabolism, mice were treated with CBD (120mg/kg bw) before hepatic microsomes were prepared and incubated with anandamide. CBD treatment significantly inhibited the formation of two anandamide metabolites. Thus, mouse hepatic P450s 2C and 3A, which are selectively inactivated by CBD [113], may be involved in the formation of some, but not all, anandamide metabolites [114].

Vitamin A and the cannabinoids are metabolized by P450s 2C, and CBD-mediated inhibition of this enzyme may alter vitamin A metabolism. This interaction may be clinically important, especially when large doses of vitamin A are therapeutically employed in xerophthalmia treatment [108].

Compounds that inhibit or inactivate cytochrome P450s after acute treatment can also induce P450s after long-term exposure. For example, CBD can inactivate cytochrome P450s after acute administration and can also induce P450s after repeated use in mice. In fact, Bornheim and Correia [115] showed that acute CBD treatment decreased the mouse hepatic cytochrome P450 content, while multiple CBD treatment regimens induced cytochrome P450s, which was indistinguishable from induction by phenobarbital, suggesting the involvement of the 2B subfamily [116]. Mice treated with CBD showed initial inactivation of P450s 3A and 2C, with a subsequent increase in mRNA encoding P450s 3A, 2C, and 2BlO after repeated administration [117].

In summary, the metabolism of drugs by cytochrome P450s 3A, 2C and 2B subfamilies can be affected when CBD in simultaneously administered. On the other hand, CBD extracts or Sativex® do not seem to inhibit or induce hepatic CYP450, probably because the administration of CBD and THC is simultaneous, which avoids the pharmacokinetic interaction, in addition to the fact that the dose ratios are very low (=1) to induce pharmacodynamic blockade [118, 119].

Effects on P-Glycoprotein Activity and Other Drug Transporters

P-glycoprotein (P-gp) is a protein that plays an important role in the disposition of many endogenous and exogenous compounds. P-gp is an ATP-dependent efflux transporter coded by the multidrug resistance 1 (MDR1) gene. Usually, P-gp activity is measured in the distal region of the small intestine where basal expression levels of this protein are higher than in other regions of the body. Human polymorphisms in the MDR1 gene can alter P-gp expression

and function, yielding altered drug pharmacokinetics and pharmacodynamics. MDR1 polymorphisms are one of the primary mechanisms responsible for the low oral bioavailability and limited brain penetration of many therapeutic drugs [120].

An *in vitro* P-gp activity assay was performed using different CBD concentrations (0.1, 1, 25, 50 and 100μM). Depending on the P-gp substrates, CBD (3-100μM) exhibited potent inhibitory effects on P-gp efflux and on P-gp ATPase activity, leading to an increased intracellular accumulation of these substrates [116, 120]. One hour of CBD exposure did not inhibit P-gp activity in drug-selected human MDR leukemia cells that over-expressed P-gp, but 3 days of repeat exposure to CBD decreased P-gp expression in these cell lines [121].

Cannabis and cannabinoids could interact with a range of cancer drugs, due to the overlapping substrate specificities of the multidrug transporters. Multidrug resistance-related protein 1 (ABCC1/MRPP1) is a membrane-bound, energy-dependent efflux transporter, which transports several drugs used clinically for cancer treatment. Additionally, breast cancer resistance protein (ABCG2/BCRP) is a transport protein found in cancer cell lines. CBD increased the intracellular accumulation of these substrates *in vitro* [122, 123].

These findings are important since cannabinoid preparations are used to attenuate nausea and vomiting induced by cancer chemotherapy and are likely to be co-administered with anticancer drugs. Although inhibition of these transporters may be considered a side effect, this CBD-transporter interaction may lead to an increased bioavailability of cancer treatment drugs. However, it is important to remember that some pharmacokinetic and pharmacodynamic interactions may occur with these anticancer drugs, leading to undesirable effects, such as overdosing and toxicity.

Effects on Sex Steroids and Reproduction

CBD can inhibit fertilization in sea urchin *Strongylocentrotus purpuratus* by decreasing sperm fertilizing capacity and by inhibiting acrosome reaction in a concentration- and time-dependent manner. The receptivity of eggs to sperm is likely not affected [124, 125].

Suppression of follicular steroidogenesis (production of testosterone, progesterone and estradiol-17β) has been demonstrated *in vitro* at a wide range of CBD concentrations (100-200μM). Luteinizing hormone-stimulated accumulation of progesterone and testosterone decreased, while estradiol accumulation was only slightly affected. A probable mechanism is that cannabinoids modulate the release of cholesterol from its ester storage in lipid droplets and, thus, limit the availability of the substrate for steroidogenesis [126]. Contradicting these results, no significant effect of CBD (0.1, 1 and 10 mg/kg bw) treatment was observed on luteinizing hormone levels, plasma follicle-stimulating hormone levels or testosterone levels in rats. None of the treatments altered rat luteinizing releasing hormone content. Moreover, CBD administration did not change luteinizing hormone secretion after *in vitro* luteinizing releasing hormone stimulation [127].

The enzyme progesterone 17α-hydroxylase generates precursors for the synthesis of glucocorticoids and sex steroids. It was inhibited by a high concentration of CBD (1mM), but was not significantly affected at lower concentrations (100μM), which can lead to time- and concentration- dependent inactivation. CBD treatment (10 and 120mg/kg bw) in rats showed inhibition of hepatic testosterone hydroxylase [107, 108, 128].

Toxicology

High Doses of *Cannabidiol in Monkeys*

The acute (i.v.) and subchronic (oral) effects of CBD at high doses were studied in rhesus monkeys [129]. CBD was injected at doses of 150, 200, 225, 250, or 300mg/kg bw i.v. Tremors were evident at all doses and the central nervous system inhibition progressed from sedation to prostration within 30min. Convulsions and emesis occurred at intermediate doses. Hyperpnoea was observed at the lowest dose and hypopnoea at higher doses. Changes in rectal temperatures were of borderline significance, but declined rapidly at higher doses. A dose- and time-related bradycardia occurred, which terminated in cardiac failure at the higher doses. Respiratory arrest and cardiac failure accounted for the death of the monkeys at doses above 200mg/kg bw. After smaller doses, survivors recovered in one to three days and liver weights increased from 19 to 142%; no changes in liver weight were observed at 300mg/kg bw, a dose that caused rapid death. There was a marked 57% decrease in relative testicular weight at 200mg/kg bw and a 33% increase in ovarian weight at this same dose.

In a study of the effects of subchronic CBD, four monkeys/sex/dose received oral treatment with CBD at doses of 30, 100, or 300mg/kg bw daily for 90 days. Clinical measures, growth rates, rectal temperatures and EKG recordings were within normal limits. Significant changes were observed in organ relative weights (ratio to brain weight). Liver weights of both sexes increased 13 to 56% and kidney weights increased 16 to 22%. These increases were not strictly related to the dose administered. Heart weights increased 16 to 22% at the highest dose. A dose-related decrease in testicular size was observed after 90 days. After a 30-day recovery interval, testicular size remained diminished. Inhibition of spermatogenesis occurred in all monkeys that received the highest dose of CBD.

A brief summary of these reported side effects are described in Tables **3** and **4**.

CONCLUSION

Several studies suggest that CBD is well tolerated and safe in humans at high doses and with chronic use. However, *in vitro* and *in vivo* studies showed potential drug metabolism interactions, cytotoxicity, and decreased receptor activity. This data highlights the need for careful monitoring of CBD use in humans, especially when CBD is used in clinical practice, such as in the treatment of psychiatric disorders or as an option for drug abuse treatment [130].

Nonetheless, some pharmacokinetic data regarding CBD should be highlighted. High inter-individual variability was

Table 3. Effects of CBD Administration in *In Vitro* Studies

Study	Reference	Cell lines	Dose	Relevant Information
Srivastava *et al.* (1998)	[90]	HUT-78	2.5-10μg/ml	inhibited IL-10 production
Srivastava *et al.* (1998)	[90]	SRIS-EOSL	2.5-5μg/ml	increased IL-8, MIP-1α and MIP-1β production
Srivastava *et al.* (1998)	[90]	SRIH-B (ATL)	2.5-10μg/ml	decreased IL-8, MIP-1α and MIP-1β production
Jenny *et al.* (2009)	[91]	human PBMC	10-100ng/ml	increased mitogen-induced indoleamine 2,3-dioxygenase and IFN-γ activity
Jenny *et al.* (2009)	[91]	human PBMC	1–10μg/ml	decreased mitogen-induced indoleamine 2,3-dioxygenase and IFN-γ activity
Lee *et al.* (2008)	[102]	mouse thymocyte and EL-4 thymoma line	12–16μM	induced apoptosis in non-transformed cells
Wu *et al.* (2008)	[103]	mouse splenocytes	4-8μM	induced apoptosis in non-transformed cells
Paton *et al.* (1972)	[105]	mouse liver homogenate	12.7-254.8μmol/L	inhibition on hepatic drug metabolism
Zhu *et al.* (2006)	[120]	human P-gp membranes	5-100μM	decreased P-gp ATPase activity
Holland *et al.* (2006)	[121]	T lymphoblastoid leukaemia cell line	1-10μM	decreased P-gp expression
Holland *et al.* (2008)	[122]	human ovarian carcinoma cell line	50-200μM	decreased ABCC1 activity
Holland *et al.* (2007)	[123]	mouse embryonic fibroblasts	10-50μM	decreased ABCG2 activity
Schuel *et al.* (1987)	[124]	sea urchin sperms	0.1-10μM	decreased fertilizing capacity
Schuel *et al.* (1991)	[125]	sea urchin sperms	0.1-100μM	inhibited acrosome reaction
Reich *et al.* (1982)	[126]	rat Graafian follicle	100-200μM	decreased steroid accumulation
Watanabe *et al.* (2005)	[128]	rat testis microsomes	1mM	decreased progesterone 17-hydroxylase activity
Watanabe *et al.* (2005)	[128]	rat liver microsomes	100-1000μM	decreased testosterone metabolism

Abbreviations: HUT-78, HTLV-1 genome positive, virus negative T cell line; SRIS-EOSL, eosinophilic leukemia cell line; SRIH-B (ATL), HTLV-1 positive B cell line; PBMC, peripheral blood mononuclear cells; IL-10, Interleukin-10; IL-8, Interleukin-8; MIP-1α, Macrophage inflammatory protein-1α; MIP-1β, Macrophage inflammatory protein-1β; IFN-γ, Interferon gamma; P-gp, P-glycoprotein; ABCC1, ATP-binding cassette transporter; ABCG2, ATP-binding cassette sub-family G member 2.

noted by the smoked route, with an average of 31% (11-45%). CBD has a half-life of 24 hours on average, with a twofold in the time noted by i.v. route and average of 31 hours by smoke route. CBD is cleared from a plasma at rates between 960 and 1560 ml/min and its distribution volume is estimated to be around 30L/kg [131].

Since several studies on CBD involve animals, the different metabolic profiles between species must be taken into account. CBD metabolism seems to follow the same pathways across species, although variations may occur, such as the involvement of different enzymes leading to diverse positions of hydroxylated compounds, or still the enrollment of a different type of sugar (or more than one) during conjugation, which could explain some slight differences in CBD effects or in metabolites between species [132].

Owing to advances in legislation concerning *Cannabis* use and newly available phytocannabinoid-based drugs for the treatment of chronic diseases, including multiple sclerosis, the public and scientific interest in *Cannabis* research and administration in humans has increased. Thus, *in vivo* studies, as well as randomized, double-blind placebo-controlled clinical studies, are still needed to assess cannabinoid effects in biological systems.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Table 4. Effects of CBD Administration in *In Vivo* Studies

Study	Reference	Species	Route	Dose	Relevant Information
Jones *et al.* (1972)	[104]	mouse	intraperitoneal	50mg/kg bw	inhibition on hepatic drug metabolism
Paton *et al.* (1972)	[105]	mouse	intraperitoneal	12.5-50mg/kg bw	inhibition on hepatic drug metabolism
Bornheim *et al.* (1994)	[106]	mouse	intraperitoneal	120mg/kg bw	changed hepatic cytochrome P450 content
Narimatsu *et al.* (1990)	[107]	rat	intraperitoneal	10mg/kg bw	changed hepatic cytochrome P450 content, decreased testosterone metabolism
Bornheim *et al.* (1990)	[108]	mouse	intraperitoneal	120mg/kg bw	changed hepatic cytochrome P450 content
Bornheim *et al.* (1991)	[109]	mouse	intraperitoneal	120mg/kg bw	changed hepatic cytochrome P450 content
Jaeger *et al.* (1992)	[111]	mouse	intraperitoneal	120mg/kg bw	inhibition on hepatic drug metabolism
Bornheim *et al.* (1993)	[114]	mouse	intraperitoneal	120mg/kg bw	inhibition on hepatic drug metabolism
Bornheim *et al.* (1989)	[115]	mouse	intraperitoneal	120mg/kg bw	changed hepatic cytochrome P450 content
Comelli *et al.* (2008)	[116]	rat	oral	10mg/kg bw	changed hepatic cytochrome P450 content
Bornheim *et al.* (1994)	[117]	mouse	intraperitoneal	120mg/kg bw	changed hepatic cytochrome P450 genetic expression
Rosenkrantz *et al.* (1981)	[129]	monkey	Intravenous	150-300mg/kg bw	tremors, hypopnoea, hyperpnoea, convulsion, emesis, bradycardia, liver and testicular weights changes
Rosenkrantz *et al.* (1981)	[129]	monkey	oral	30-300mg/kg bw	heart, kidney and liver weights changes, testicular size reduced and Inhibition of spermatogenesis

Abbreviations: bw, body weight.

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