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Review

Review of the oral toxicity of cannabidiol (CBD)

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Highlights

- Potential hazards from long term oral use of CBD are discussed.
- CBD-induced male reproductive toxicity is observed from invertebrates to primates.
- Mechanisms of CBD-mediated oral toxicity are not fully understood.

Abstract

Information in the published literature indicates that consumption of CBD can result in developmental and reproductive toxicity and hepatotoxicity outcomes in animal models. The trend of CBD-induced male reproductive toxicity has been observed in phylogenetically disparate organisms, from invertebrates to non-human primates. CBD has also been shown to inhibit various cytochrome P450 enzymes and certain efflux transporters, resulting in the potential for drug-drug interactions and cellular accumulation of xenobiotics that are normally transported out of the cell. The mechanisms of CBD-mediated toxicity are not fully understood, but they may involve disruption of critical metabolic pathways and liver enzyme functions, receptor-specific binding activity, disruption of testosterone steroidogenesis, inhibition of reuptake and degradation of endocannabinoids, and the triggering of oxidative stress. The toxicological profile of CBD raises safety concerns, especially for long term consumption by the general population.

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Keywords

Up to 6 total): cannabidiol; cannabis; Food safety; Reproductive toxicity; Hepatotoxicity; Review

1. Introduction and background

Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) are the predominant bioactive compounds produced by cannabis (*Cannabis sativa* L.), an annual, herbaceous flowering plant. Cannabis has been used in many parts of the world for centuries for its fiber, edible seeds, and bioactive properties (Ren et al., 2021).

Cannabis varieties have been classified into two broad categories based on their THC content, the substance predominantly responsible for the intoxicating psychological effects associated with the plant (Aizpurua-Olaizola et al., 2016). Cannabis varieties with high THC content, sometimes referred to as “marijuana”, have in many cases undergone selective breeding to increase levels of THC, thereby increasing their intoxicating effects (Gulck and Moller, 2020). Other cannabis varieties, known as hemp or industrial hemp, have relatively low THC content and have historically been cultivated for fiber and seed. The 2018 Agriculture Improvement Act defined hemp as the plant *Cannabis sativa* L. and any part of that plant with a THC concentration of no more than 0.3 percent on a dry weight basis (Congress, 2017–2018). Recently, access to hemp and hemp derivatives, including CBD, has increased in the United States, as the 2018 Agriculture Improvement Act removed hemp from schedule 1 of the Controlled Substances Act (USDA, 2019).

An increasing number of studies have investigated CBD's reported anti-inflammatory and neuropharmacological properties, including antiepileptic, sedative, anxiolytic, and antipsychotic activities (Cunha et al., 1980; Zuardi et al., 2006). These studies have raised the possibility of CBD's use in treating diverse clinical conditions like dementia, cerebral ischemia, diabetes, inflammatory diseases, nausea, and psychiatric disorders (van den Elsen et al., 2014; Wallace et al., 2015; Hayakawa et al., 2010). CBD is the active ingredient in the drug Epidiolex, which the United States Food and Drug Administration (FDA) approved for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in 2018 and with tuberous sclerosis complex in 2020. FDA's approval of this drug is specific to the context of these severe medical conditions and patients are regularly monitored for potential adverse effects.

CBD consumer products, including CBD-containing foods, are now being marketed in many countries for their purported beneficial or general wellness effects. Top self-reported reasons for using CBD include pain, insomnia, and anxiety (Ou et al., 2020). However, toxicity studies indicate that orally consumed CBD can cause significant toxicological effects, including adverse effects on the male reproductive system and liver.

Substances added to food are held to a high safety standard. They are expected to be safe for all consumers over their lifetime. Therefore, evaluation of safety requires considering long-term use and use within various segments of the population, including vulnerable subpopulations such as pregnant women, the

conceptus, the fetus, young children, and the elderly. Foods used as a vehicle to administer pharmacologically active substances are prone to unintentional consumption by both adults and children.

Unlike FDA-approved drugs, risks from a substance added to food are not weighed against benefits demonstrated by adequate clinical trials. The drug regulatory pathway allows for risk management options not in the food ingredient regulatory framework. For example, the drug pathway entails authority to mandate labeling with detailed instructions and warnings, precise dose control, prescription and behind counter dispensation, and a Risk Evaluation and Mitigation Strategy program.

CBD is sometimes added to consumer products in the form of CBD-rich hemp extracts, which contain other plant-derived constituents. The composition of these extracts can vary considerably, depending on the source material and method of manufacturing. The other components in hemp extracts may raise additional safety questions and may interact with CBD toxicologically (Pennypacker and Romero-Sandoval, 2020). This review focuses on studies using relatively pure CBD.

This review summarizes data and information in the scientific literature pertinent to the evaluation of various toxicological concerns associated with oral CBD consumption. Emphasis is placed on *in vivo* studies using orally administered CBD and on *in vitro* studies examining specific cellular effects of CBD. This review is not a risk assessment and does not seek to identify levels of exposure that may result in adverse effects or levels of exposure that are safe for test animals or for humans. This review is intended to enable thorough toxicological hazard identification for orally consumed CBD and to aid in future risk assessments.

1.1. The endocannabinoid system

The receptors that bind to and mediate the physiological effects of THC were first discovered in the 1990s (Sugiura and Waku, 2002). This led to the discovery of the cannabinoid receptors' endogenous ligands. These molecules are referred to as endocannabinoids to distinguish them from plant-derived cannabinoids, or phytocannabinoids. The endocannabinoid system is now known to exist in nearly all animal phyla and consists of two known cannabinoid receptors, cannabinoid receptors 1 and 2 (CB1 and CB2), and two known endocannabinoids, *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) (Silver, 2019). Both AEA and 2-AG have moderate, non-selective binding affinities for CB1 and CB2 (Soethoudt et al., 2017). Since its discovery, the endocannabinoid system has been implicated in many physiological processes in several organ systems. These processes and systems, which could be disrupted by CBD consumption, include neuronal and immune cell signaling and the reproductive systems of both sexes.

The first identified cannabinoid receptor, CB1, was cloned from the rat brain, structurally classified as a seven-transmembrane G-protein coupled receptor (GPCR), and shown to be THC-responsive (Matsuda et al., 1990). The human and rat cannabinoid receptor nucleotide sequences are 90% identical and the amino acid sequences are 98% identical (Gerard et al., 1990), suggesting CB1 has a conserved physiological function across species. CB1 is expressed throughout the body, but at the highest levels in brain, endocrine, respiratory, connective, and male and female reproductive tissues (Uhlen et al., 2015). The central nervous system's response to THC is believed to be mediated exclusively by CB1, which is found locally at the axon terminals of central and peripheral neurons (Munro et al., 1993). Activation of CB1 can inhibit the release of several different excitatory and inhibitory neurotransmitters.

CB2 was cloned a few years later, and its amino acid sequence is 44% identical to that of CB1 (Munro et al., 1993). Like CB1, CB2 is also expressed throughout the body, but has a different tissue-expression pattern, where the highest levels occur in endocrine, gastrointestinal, kidney, female reproductive, skin, and lymphoid tissues (Uhlen et al., 2015). Differences in amino acid sequence and in tissue expression patterns between the two receptors may reflect a functional specialization, with CB1 having been shown to regulate neurotransmitter signaling, and CB2 having implications in immune and inflammatory signaling (Pertwee et al., 2010).

Endocannabinoids are produced and released by cells in response to an external stimulus (e.g., pain), act locally, and are inactivated by cellular reuptake and intracellular hydrolysis. The two enzymes responsible for hydrolyzing and inactivating AEA and 2-AG, are fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL), respectively (Barrie and Manolios, 2017). Fatty acid binding proteins (FABPs) can act as intracellular carriers that present AEA to FAAH for reuptake and turnover (Deutsch, 2016). CBD was reported to indirectly inhibit reuptake and catabolism of AEA by binding to FABPs, thereby inhibiting their activity in AEA turnover (Fig. 1). CBD-mediated inhibition of AEA and 2-AG hydrolysis, reuptake, and turnover can prolong the antinociceptive and anti-inflammatory effects of both endocannabinoids (Barrie and Manolios, 2017).

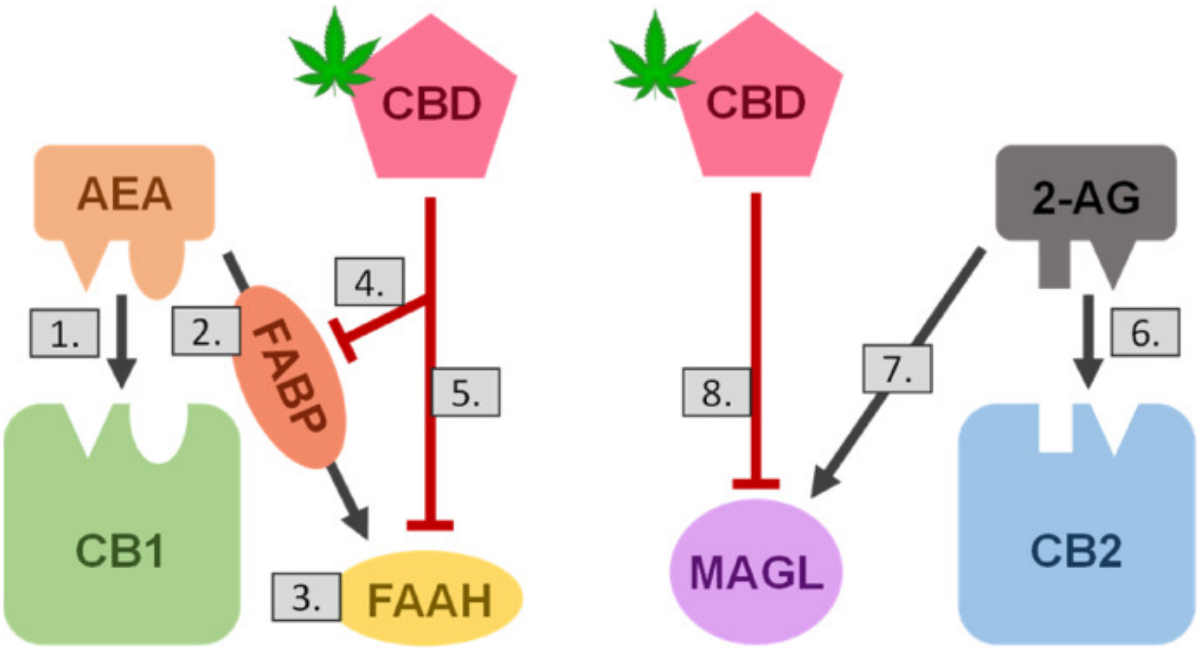

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Fig. 1. **CBD disrupts physiological endocannabinoid signaling.** The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are released locally by cells in response to an external stimulus and can act through two known pathways. Under normal conditions, AEA binds to the cannabinoid receptor 1 (CB1) to elicit a cellular response (**1.**) and is then presented via fatty acid binding proteins (FABP; **2.**) to fatty acid amide hydrolase (FAAH) for hydrolysis (**3.**). CBD has been shown to inhibit both FABP presentation (**4.**) and FAAH hydrolysis (**5.**) of AEA. 2-AG, which has a stronger affinity for CB2 than CB1, first binds to CB2 to elicit a cellular response (**6.**) and is then inactivated by monoacyl glycerol lipase (MAGL; **7.**). CBD has been shown

to inhibit MAGL activity (**8.**). These disruptions of CBD to the endocannabinoid system could result in prolonged endocannabinoid signaling due to decreased hydrolysis, reuptake, and turnover of AEA and 2-AG.

1.2. Receptor binding profile of CBD

Although there is a high structural similarity between THC and CBD, CBD has a weak binding affinity for either CB1 or CB2 (McPartland et al., 2015; Shahbazi et al., 2020), and to date, there is no substantive evidence that CBD causes THC-like psychotropic effects (WHO, 2018). Thus, CBD's mechanism of action must occur, in part, through targets other than canonical cannabinoid receptor binding. Despite having weak affinity for CB1 and CB2, CBD can act as a non-competitive negative allosteric modulator for CB1 in the presence of THC and is able to reduce the efficacy and potency of both THC and the endocannabinoid AEA (Laprairie et al., 2015; Thomas et al., 2007).

CBD has been reported to activate the vanilloid type 1 receptor (TRPV1), a non-specific cation channel involved in nociceptive signaling (Bisogno et al., 2001). When activated by CBD (EC_{50}=3.2–3.5 μM), TRPV1 causes an increase in intracellular Ca^{2+}. *In vitro*, this can cause desensitization of TRPV1 to its natural ligand, capsaicin, suggesting that TRPV1 receptors could mediate some of the toxicological effects of CBD (Bisogno et al., 2001).

Recently, CBD was demonstrated to interact with the D1-like dopamine receptors in the hippocampus (Nouri et al., 2021), which are known to play a crucial role in regulating the reward properties of methamphetamine and other drugs. The authors reported that CBD's interaction with D1-like dopamine receptors inhibited the reward effect of methamphetamine in rats.

Studies suggest additional receptors could be involved in CBD's mechanisms of action, such as G-protein coupled receptor 55 (GPR55), serotonin receptor subtype 1A (5-HT_{1A}), and dopamine receptor (D2) (Galaj and Xi, 2020). In rats, CBD reduced neuronal hyperexcitability and inflammation through modulation of adenosine-mediated signaling and intracellular calcium levels via interaction with GPR55 and TRPV1 (FDA, 2018a). In mice, CBD exerted an analgesic effect through positive allosteric modulation of α3 glycine receptors by directly binding to the receptor; this effect was not observed in α3 glycine receptors-null mice (Xiong et al., 2011). Other studies suggest CBD interacts with many other receptors, such as PPAR-γ, α1-adrenoceptors, $GABA_A$, and μ- and δ-opioid receptors, with varying efficacy (McPartland et al., 2015).

Together, these data suggest CBD has a non-selective receptor binding profile. Since the mechanisms by which CBD elicits its toxicological effects are still poorly understood, it remains unclear exactly which receptors are implicated for which endpoints, but it is likely multifarious.

It is important to note that CBD occurs as both a (+) and (−) enantiomer. The (−) enantiomer is the plant-derived form of CBD, the primary subject of study in the literature, and the active ingredient in the drug Epidiolex. Chemical synthesis of CBD can result in either the (+) and (−) enantiomer, depending on the starting material. Interestingly, CBD's (+) enantiomer demonstrates a different receptor binding profile than described above. The K_i of the CBD (+) enantiomer for CB1 and CB2 receptors is much lower than the (−) enantiomer, suggesting that the (+) enantiomer has a far greater binding affinity for the cannabinoid receptors (Morales et al., 2017). The CBD (+) enantiomer was also found to be more active than the CBD (−) enantiomer as an anticonvulsant agent in a mouse seizure model (Leite et al., 1982). These noted

pharmacological differences between (+) and (−) CBD enantiomers will be important to consider should synthetically derived CBD become prevalent.

1.3. Toxicokinetics of CBD

Millar et al. (2018) conducted a systematic review of published articles retrieved from PubMed and EMBASE that reported toxicokinetic (TK) data on CBD in humans. The mean half-life ($t_{1/2}$) of CBD was reported to be 1.44–10.86 h after oromucosal spray (5–20 mg), 1.09–1.97 h after single oral administration (10 or 20 mg), 2–5 days after chronic oral administration, 24 h after intravenous administration, and 31 h after inhalation. The area-under-the-curve (AUC) and maximum serum concentration achieved (C_{max}) increased in a dose-dependent manner and were higher following inhalation relative to oromucosal spray or oral administration. Administration of CBD with a meal or in a lipid formulation also increased C_{max}. The time to C_{max} (T_{max}) was reached between 0 and 5 h and was not dose dependent. CBD was rapidly distributed into tissues, and appears to distribute to adipose tissue due to its high lipophilicity (WHO, 2018). Mean apparent volume of distribution for CBD was estimated to be 2520 L following intravenous administration, and around 30,000 L following oromucosal spray (Millar et al., 2018).

Some TK parameters of CBD in humans were identified in studies on pharmaceutical grade CBD as the active ingredient in the drug Epidiolex. In these studies, oral exposure to CBD resulted in a nonlinear increase in plasma CBD concentration with a dose of up to 6000 mg under fasting conditions, with the median T_{max} being 2.5–5 h. Consuming CBD with a high-fat meal increased the C_{max} approximately 5-fold and increased the AUC approximately 4-fold. The estimated V_d in healthy volunteers ranged from 20,963–42,849 L, similar to the oromucosal spray administration. The average elimination $t_{1/2}$ was 56–61 h, and CBD was shown to be primarily excreted in feces (84%), with a small fraction (8%) excreted in urine (FDA, 2018a; FDA, 2018b). In rats, the $t_{1/2}$ of CBD was within the same range as humans (4–5 h) after a single oral dose (FDA, 2018b).

The oral bioavailability of CBD in humans is low, varying between approximately 6 and 19% (Mechoulam et al., 2002; Cherniakov et al., 2017; Millar et al., 2020; Perucca and Bialer, 2020). Oral bioavailability is lowest (~6%) under fasting conditions, and highest (~19%) when consumed with a high fat meal (Perucca and Bialer, 2020). Low oral bioavailability of CBD is likely due to its highly lipophilic nature (logP=6.3), which results in poor gastric solubility (Millar et al., 2020). The oral bioavailability of CBD in rats is lower than humans, reaching only 2.8% after a single dose (FDA, 2018b).

Taken together, these data demonstrate that the toxicokinetics of CBD markedly vary depending on route of administration and food matrix in both rats and humans. Importantly, alterations in the conditions of use or formulations of CBD that enhance its oral bioavailability in consumer products or significantly impact other toxicokinetic parameters for CBD, could increase the potential for adverse toxicological outcomes.

1.4. Species-specific metabolism

In humans, CBD is metabolized in the gut and liver, where it undergoes phase I metabolism by multiple cytochrome P450 (CYP) isoforms (primarily CYP2C19 and CYP3A4) and undergoes phase II metabolism (glucuronidation) by UDP-glucuronosyltransferases (UGT; primarily UGT1A7, UGT1A9, and UGT2B7) (FDA, 2018a; Jiang et al., 2011). Human liver microsomes can metabolize CBD to produce monohydroxylated metabolites (6α–OH–, 6β–OH–,7-OH-, 1”–OH–, 2”–OH–, 3”–OH–, 4”–OH–, and 5”–OH–CBDs) of which 6α–OH–, 6β–OH–, 7-OH-, and 4”–OH–CBDs are the most abundant (Harvey and Mechoulam, 1990). Some of

these hydroxylated metabolites are further oxidized into carboxylic acid derivatives (WHO, 2018). The predominant CBD metabolite in humans is 7-COOH-CBD (Harvey et al., 1991). Consistent with this, clinical studies associated with the CBD-based drug Epidiolex reported that the plasma AUC for 7-COOH-CBD was about 40-fold higher than that of the parent compound, indicating CBD undergoes rapid metabolism in humans (FDA, 2018a).

In vitro studies using liver microsomes from a wide variety of mammalian species, including humans, have detected more than 50 different CBD metabolites with complex profiles and significant interspecies differences (Harvey and Brown, 1991). CBD metabolites were evaluated *in vivo* in dogs, rats, and humans. In dogs, 7-COOH-CBD was identified only as a minor urinary metabolite, where 4”- and 5”-hydroxy and 6-oxo-CBD were dominant. In rats, C-6 or C-7 hydroxylated metabolites predominated. No intact glucuronide or glucoside metabolites were found in either dog or rat urine (Harvey et al., 1991). In contrast, *in vivo* human studies report that a large portion of the administered CBD was excreted as 7-COOH-CBD or as a glucuronide-conjugate (Ujvary and Hanus, 2016).

These data add to the body of literature suggesting both similarities and differences in the metabolism of CBD among mammalian species. Importantly, in the nonclinical review of Epidiolex, it is noted that these studies did not adequately assess the safety of 7-COOH-CBD due to its relatively low abundance in the animal test species compared to humans, and that this gap represents a significant deficiency that should be addressed (FDA, 2018b).

1.5. Transport proteins

CBD is not a substrate for the major drug efflux transporters breast cancer resistance protein (BCRP) and permeability glycoprotein (P-gp) (Iffland and Grotenhermen, 2017; Feinshtein et al., 2013). These findings could suggest CBD has poor efflux from blood-tissue barriers, which raises questions as to CBD levels in various tissues such as brain, testes, or placenta. Additionally, CBD is not a substrate for the important human hepatic uptake transporters OATP1B1 and OATP1B3, the major renal uptake transporters OAT1, OAT2, OCT2, or the major renal efflux transporter MATE1 (FDA, 2018a), which may account for its low percentage of urinary elimination.

2. Toxicological profile of CBD

2.1. Developmental and reproductive toxicity

2.1.1. Non-human primates

A 90-day (subchronic) oral toxicity study for CBD was performed in Rhesus monkeys (Rosenkrantz et al., 1981). Animals were administered 0, 30, 100, or 300 mg/kg bw/day of CBD (99% purity) by oral intubation. Significant changes in organ weights were seen in all CBD dose groups in both sexes, although a clear dose-response was not observed for all organs. Relative organ weights were 13–56% higher for liver and 16–22% higher for kidneys compared to controls. Relative heart weight was 16–22% higher in the highest dose group (300 mg/kg bw). Significantly lower gonadal weight was observed in both sexes at all doses, including for relative ovary weight (25–75% lower) and testes size (8–25%) at day 90. After a 30-day washout period, the weight of most organs returned to normal, suggesting that the changes in relative organ weights were CBD

treatment-related, and partially reversible with discontinued use. However, the liver weights remained slightly elevated, and testes size remained depressed by 20–25%. No changes in organ weights were associated with any functional impairment, except for the testes. Inhibition of spermatogenesis was observed in 2 of 4, 3 of 4, and 4 of 4 monkeys given 30, 100 and 300 mg/kg bw of CBD, respectively. This change was not observed in the control animals. Functional impairment of testes was accompanied by histological changes, including smaller seminiferous tubules, lower mitotic index, fewer germ cells per tubule, and decreased number of spermatocytes, spermatids, and spermatozoa. These data strongly suggest that orally administered CBD exerts toxic effects on the male reproductive system in primates, some of which, like testes size, may be irreversible. To note, the same researchers also conducted an acute intravenous toxicity study in Rhesus monkey, which established an LD_{50} for CBD (approximately 212 mg/kg bw) and reported serious adverse effects on the testes, which significantly decreased in weight (~57%) after seven days of exposure (Rosenkrantz et al., 1981).

2.1.2. Rodents

In mice, a perinatal exposure study to CBD and other cannabinoids was used to evaluate their impact on testicular function and fertility (Dalterio and deRooij, 1986). In this study, one group of females received a single oral dose of 50 mg/kg BW of CBD on day 12 of gestation, and another group of females received a single oral dose of 50 mg/kg BW of CBD within 12 h of parturition to evaluate the developmental effects of *in utero* and lactational exposure to CBD. In a previous study, this dose did not produce overt maternal toxicity or pup mortality (Dalterio, 1980). Female mice receiving an oral dose of 50 mg CBD/kg BW on day 12 of gestation produced male offspring with a significant reduction in testicular weight. Male mice with lactational exposure to CBD had a reduced rate of successful impregnations in adulthood compared to controls and sired significantly fewer live pups. These males had about 20% fewer viable spermatids than the controls. These findings are consistent with earlier *in vitro* explant studies in human and animal tissue that report cannabinoids, specifically THC, can disrupt testicular function (Bloch et al., 1978). The same group also reported that male mice exposed to CBD in adulthood had altered spermatogenesis, reduced fertility, and produced litters with significantly more prenatal and postnatal mortality (Dalterio et al., 1982).

Consistent with these findings on the male reproductive toxicity of CBD, three other studies by a different group also observed that CBD administered orally to male mice during the juvenile period of postnatal development caused reproductive toxicity (Carvalho et al., 2018a, 2018b, 2022). Here, 21-day old male Swiss mice were administered CBD (99.9% pure) by gavage for 34 consecutive days at doses of 0, 15, or 30 mg/kg BW/day. Sexual behavior was analyzed for 10 days using observations like first mount, number of intromissions, ejaculation latencies, and postejaculatory mount intervals. These analyses revealed that chronic administration of 15 mg/kg BW/day of CBD reduced the frequency of mounts and the number of intromissions and ejaculations. At 30 mg/kg BW/day, CBD exposure resulted in a statistically significant reduction in fertility rate, including pre- and post-implantation embryo loss, and in the total number of litters. The average percent loss of pre- and post-implantation embryos tended to be higher in the highest dose group (30 mg/kg BW), although the inter-dose differences were not statistically significant (Carvalho et al., 2018a). In a second study by the same researchers using the same dosing strategy but with a 35-day washout period (Carvalho et al., 2018b), CBD exposure did not affect body weight or the weight of reproductive tissue such as testis, epididymis, and seminal vesicle. In the 30 mg/kg BW group, there was a significant decrease in total circulating plasma testosterone compared to controls. A decrease in total

circulating plasma testosterone was also observed in the 15 mg/kg BW group, but the decrease was not statistically significant. In the 30 mg/kg BW group, a statistically significant decrease in the number of Sertoli cells was observed. However, this was not accompanied by significant differences in testicular sperm counts, although the number of spermatozoa in the epididymis tail was reduced. Spermatozoa from both dose groups had head and tail abnormalities, and a large number of cytoplasmic droplets were observed in the medial region of flagella. The authors stated that the steady state blood levels of CBD for the doses used (15 and 30 mg/kg BW) would be about 80–160 ng/ml. In a third study by the same researchers using the same dosing strategy in 21-day-old male mice (Carvalho et al., 2022), marked effects in spermatogenesis and spermatozoa performance were noted at both doses. Such effects included increases in DNA damage and number of abnormal acrosomes in mature spermatozoa and decreases in spermatozoa straight-line and average pathway velocities and in enzymatic activity of superoxide dismutase and catalase — enzymes that function to protect spermatozoa from oxidative stress. Changes in testicular morphology, including a reduced seminiferous tubule epithelium height, and changes in spermatogenesis in a stage-specific manner were also noted in CBD-exposed animals. Additionally, the highest dose (30 mg CBD/kg BW/day) resulted in reductions in spermatozoa motility, curvilinear velocity, and number of mature spermatozoa with intact acrosomes. While these three studies were not formally integrated, findings following a washout period suggest CBD-induced male reproductive toxicity effects in mice are reversible with discontinued use, which differs from reports in monkey. However, the findings on spermatogenesis are similar to those observed in adult non-human primates (Rosenkrantz et al., 1981).

In a recent study, prenatal CBD exposure (20 mg CBD/kg BW/day) in mice, which occurred via maternal oral exposure 2-weeks prior to mating and continued through gestation and lactation, resulted in increased anxiety-like behavior in F_1 female offspring (Wanner et al., 2021). Additionally, differentially methylated loci in both the cerebral cortex and hippocampus brain regions were identified. Taken together, these findings suggest that developmental exposure to CBD via maternal oral exposure may be associated with undesirable behavioral outcomes in female offspring and perturbations in the brain epigenome.

In rats, three studies were reviewed by FDA to support approval of the CBD-based drug Epidiolex, including (I) an embryofetal development study, (II) a fertility and early embryonic developmental study, and (III) a pre- and postnatal developmental toxicity study (FDA, 2018b). In all three studies, CBD was administered at doses of 0, 75, 150, and 250 mg/kg BW/day via oral gavage. Summarized below are results from these studies; conclusions are reported in the Epidiolex drug label (FDA, 2018c).

(I) Pregnant females were exposed from gestational day (GD) 6 through 17. Two females in the high-dose group (250 mg/kg BW/day) had CBD treatment-related total litter loss *in utero.* There were statistically significant differences compared to controls in the occurrence of fetal structural variations, like supernumerary liver lobe in the high dose group, which was associated with maternal plasma levels greater than those expected in humans.

(II) Male and female rats were exposed from 2 weeks prior to mating through GD 6. Lower overall body weight gains were observed in mid- and high-dose males; body weight gain was not affected in females. One mid-dose male and one high-dose male failed to impregnate a female. At necropsy, there were decreases in male reproductive organ weights at all doses tested.

(III) Pregnant females were exposed from GD 6 through pregnancy and lactation (to postnatal day [PND] 21). Here, fur staining in the urogenital area was observed at all doses; increased incidences of pups with

attached umbilical cords, small pup size, shorter gestation, noisy respiration, delayed achievement of developmental landmarks (i.e., pinna unfolding, eye opening, and pupillary reflex), delayed sexual maturation, neurobehavioral changes, and adverse effects on the male reproductive system occurred in the mid- and high-dose groups.

The Epidiolex label states that no adverse effects on fertility in rats occurred after CBD administration prior to and throughout mating and into early gestation. However, it does conclude that administration of CBD to pregnant rats produced evidence of developmental toxicity in the absence of maternal toxicity, at maternal plasma cannabidiol levels greater than that in humans at therapeutic doses. These outcomes include decreased growth; delayed sexual maturation; decreased neurobehavioral activity; and reduction in size and development of the testes in males (FDA, 2018c).

Male reproductive toxicity outcomes are commonly observed following CBD exposure and have been the topic of a recent review article (Carvalho et al., 2020). These effects have been reported across multiple different species, despite inherent interspecies differences in CBD's metabolism and toxicokinetic profile as a whole.

2.1.3. Lagomorphs

Nonclinical studies reviewed by FDA for Epidiolex also include an embryofetal development study in New Zealand White rabbits (FDA, 2018b). In this study, mated female rabbits were administered CBD (0, 50, 80, or 125 mg/kg BW/day) by oral gavage throughout organogenesis (GD 7 through 19). Animals were sacrificed on GD 29, at which time pregnancy outcomes were evaluated. Common structural variations indicative of delayed development were more frequently observed in fetuses from the high-dose group (125 mg/kg BW/day). These structural variations appear to be associated with decreased fetal and maternal body weights. The Epidiolex label concludes that developmental toxicity outcomes in rabbits occurred at maternal plasma exposures similar to that in humans at therapeutic doses (FDA, 2018c).

2.1.4. Phylogenetically distant organisms and in vitro studies

The trend of CBD-induced male reproductive and developmental toxicity also holds true for organisms phylogenetically distant from humans, such as sea urchins, zebrafish, and chickens, as well as in *in vitro* studies. In sea urchins (*Strongylocentrotus purpuratus*), pre-incubation of spermatozoa with THC or CBD inhibited the acrosomal reaction, which is necessary for fertilization (Schuel et al., 1991). Zebrafish exposed to THC or CBD from the blastula stage through the larval stage (96 h post-fertilization) exhibited developmental toxicity, as reported by edemas, curved axis, physical deformities (eye/snout/jaw/trunk/fin), swim bladder distention, and behavioral abnormalities. Importantly, CBD was found to be a more potent developmental toxicant in zebrafish than THC (LC_{50} for CBD=0.53 mg/L; LC_{50} of THC=3.65 mg/L) (Carty et al., 2019).

An *in ovo* study examined the effects of CBD, synthetic cannabinoids (HU 210 and HU 211), and AEA on the viability and development of chick embryos (Gustafsson and Jacobsson, 2019). Fertilized chicken egg yolks were injected on days 1, 4, and 7 with the test compounds or vehicle. After day 9, CBD was demonstrated to be embryotoxic at the highest dose tested (50 μM), reducing the number of viable embryos by 80%. The antioxidant α-tocopherol (a form of vitamin E) protected against CBD's embryotoxic effects, suggesting oxidative stress is involved in mediating this toxicity outcome. This study demonstrated that exposure to

CBD during early embryonic development decreases embryo viability, highlighting another potential risk of cannabinoid use during pregnancy.

In vitro, CBD, and to a lesser extent THC, inhibited testosterone production in rat Leydig cells (Jakubovic et al., 1979; Gorzalka et al., 2010). In human primary and in mouse immortalized Sertoli cells, CBD and its main human metabolites, 7-COOH-CBD and 7-OH-CBD, induced cytotoxicity resultant from the inhibition of the G1/S-phase cell cycle transition, inhibition of DNA synthesis, and downregulation of key cell cycle and functional proteins. Importantly, CBD and its main human metabolites were more cytotoxic to human Sertoli cells than to mouse Sertoli cells (Li et al., 2022). Exogenous CBD exposure *in vitro* also interfered with cellular processes involved in human placental development and function (Alves et al., 2021), endometrial stromal cell differentiation (Almada et al., 2020), and trophoblast-endometrial crosstalk (Neradugomma et al., 2019); processes necessary for uterine receptivity, implantation, and successful pregnancy. These data demonstrate that the reproductive toxicity of CBD may not be limited to male fertility parameters but could also include effects on female fertility and pregnancy outcomes.

In total, there are clear reproductive toxicity effects following CBD exposure in adult males and gestational CBD exposure in male offspring, specifically on spermatogenesis, which can result in abnormal sperm morphology and the subsequent prevention of oocyte fertilization. In concordance with CBD's male reproductive toxicity across phylogenetically distant species, developmental toxicity effects were frequently observed across animal models (FDA, 2018b).

2.2. Hepatotoxicity

Hepatotoxic effects of orally administered CBD, such as increased liver weights and elevated liver enzymes, have been reported in both animal and human studies. In clinical trials for the CBD-based drug Epidiolex, elevated liver enzymes were observed in 5–20% of epileptic patients treated with CBD, and a few patients were withdrawn for having elevated liver aminotransferase enzymes that exceeded 3 times the upper limit of normal (Devinsky et al., 2017, 2018a; Thiele et al., 2018). The majority of epileptic patients who presented with elevated liver enzymes were also concomitantly prescribed other anti-epileptic medications, like valproate, which itself carries a risk for elevating liver aminotransferases. However, in a more recent publication, elevated liver enzymes were also noted in healthy adults given CBD, who were not on other medications at the time. These findings suggest that CBD consumption could also cause drug-induced liver injury in non-epileptic individuals (Watkins et al., 2021).

In mice, a two-part acute (24 h) and subacute (10-day) exposure study was performed using CBD-rich cannabis extract (CRCE; ~58% CBD) administered by gavage (Ewing et al., 2019a). In the acute study, the authors chose a single dose of 246, 738 or 2460 mg CRCE/kg BW/day. Results reported a dose-dependent, statistically significant increase in both liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as a marked elevation of total bilirubin at the highest dose; indicating that CBD can cause a dose-dependent increase in hepatotoxicity. In the 10-day study, doses of 61.5, 184.5, and 615 mg/kg BW/day for ten non-consecutive days were used. The lowest dose of 61.5 mg/kg BW was determined equivalent to a human CBD dose of 5 mg/kg BW, the recommended daily starting dosage of Epidiolex, based on the CBD content of the extract and the use of allometric scaling between mouse and human. Histopathological evaluation revealed pan-hepatic cytoplasmic swelling at the highest dose (615 mg/kg BW), with foci of cytoplasmic swelling in the mid-dose (184.5 mg/kg BW) group. At the lowest

dose (61.5 mg/kg BW), an increase in the relative liver and kidney weights, and increases in ALT, AST, and serum bilirubin were reported. Additionally, in both parts of the study, gene expression analysis revealed a dose-dependent increase in several CYP and UGT isoforms. This study suggests that oral exposure to CBD through CRCE, at a therapeutically relevant dose, can result in overt signs of hepatotoxicity.

The same researchers conducted another study in mice to investigate liver effects following exposure to a combination of CRCE and a common over the counter anti-inflammatory medication, acetaminophen (APAP) (Ewing et al., 2019b). APAP is known to cause liver injury through glutathione depletion and oxidative stress. In this study, female mice were orally administered CRCE at doses of 116 or 290 mg/kg BW/day for three consecutive days, followed by an intraperitoneal challenge with APAP. The lower dose (116 mg/kg BW+APAP) produced liver injury (i.e., sinusoidal obstruction) and mortality. These effects were not observed in the control APAP group. Paradoxically, in the high-dose group (290 mg/kg BW+APAP), no mortality was observed, and liver injury was noted in only 25% of mice. The lower incidence of hepatotoxicity in the 290 mg/kg BW group was associated with a rapid re-synthesis of glutathione such that the total glutathione level was similar to that in controls. These findings highlight the potential for CBD-drug interactions as revealed by the paradoxical effect of the combination of CBD and APAP. This is consistent with other evidence suggesting that CBD can interact with drugs and lead to serious adverse health effects, including liver injury (Devinsky et al., 2018b).

For the CBD-based drug Epidiolex, a 26-week oral toxicity study in rats and a 39-week oral toxicity study in dogs were conducted (FDA, 2018b). In rats, centrilobular hypertrophy was observed in the mid-dose (50 mg/kg BW) and high-dose (150 mg/kg BW) groups in both sexes, and was associated with increased ALT, ALP, and relative liver weights. In dogs, decreases in absolute body weight were observed at all doses (low-10 mg/kg BW, mid-50 mg/kg BW, and high-dose 100 mg/kg BW) in both sexes. Hepatocellular hypertrophy was also detected at all doses in both sexes, coupled with a slight increase in ALT and an 8-fold increase in ALP. The observed liver hypertrophy was associated with increased liver weights and macroscopic enlargement. Additionally, a decreased heart rate was observed in high-dose males only.

These studies clearly establish that orally consumed CBD has the potential to elicit hepatotoxic effects in rodents, dogs, and humans, as evidenced by increases in various biomarkers of hepatotoxicity, like liver enzymes and bilirubin, and histopathological changes.

2.3. Immunotoxicity

CBD has been shown to suppress the immune function of splenocytes (T and B lymphocytes) directly exposed *in vitro* or isolated from CBD-exposed mice. Direct exposure caused these cells to become apoptotic in a concentration dependent manner after 12 h of exposure (Wu et al., 2008).

Similarly, CBD induced apoptosis in normal murine thymocytes (T lymphocytes) and in EL-4 thymoma cells (Lee et al., 2008). Here, the induction of apoptosis occurred in a time- and concentration-dependent manner in both cell types, with a 50% effective concentration (EC_{50}) of about 7 μM in normal murine thymocytes. This work demonstrates that normal thymocytes are more sensitive to CBD-induced damage than thymoma cells *in vitro*. Further investigation revealed that the cellular damage was caused by reactive oxygen species and oxidative stress induced by CBD treatment. In an attempt to reverse the CBD-induced oxidative stress, Lee et al. co-treated thymocytes with CBD and *N*-acetyl-L-cysteine, a glutathione precursor, which was able

to attenuate this effect. These results are consistent with the similar findings in splenic lymphocytes (Wu et al., 2008) and leukemia cells (McKallip et al., 2006).

Several *in vitro* studies and one *in vivo* study suggest that CBD can induce damage to both normal and cancer-derived lymphatic cells. These findings suggest oxidative stress caused by a reduction in the level of intracellular glutathione and apoptosis caused by the activation of multiple caspases may be involved in the immunotoxicity potential of CBD.

In support of the potential for CBD to suppress immune cell function, clinical studies on the CBD-based drug Epidiolex show a potential dose-dependent increase in infections, particularly pneumonia (FDA, 2018d).

2.4. Mutagenicity and genotoxicity

The genotoxicity potential of CBD has been reported in several published studies with conflicting conclusions as reviewed and discussed below.

Genotoxicity assays conducted on the CBD-based drug Epidiolex, inclusive of Ames assay, *in vivo* micronucleus assay in rat, and *in vivo* alkaline COMET assay, reported that CBD is non-mutagenic and non-genotoxic under the conditions of the assays (FDA, 2018b). Additionally, in a double-blind placebo-controlled study in humans, there were no statistically significant changes in chromosome damage after administering 1200 mg CBD/day to study volunteers for 20 consecutive days (Matsuyama and Fu, 1981).

However, in mice, intraperitoneal administration of 10 mg/kg BW/day of CBD for 5 consecutive days produced statistically significant increases in the percentage of micronuclei in bone marrow polychromatic erythrocytes compared to controls, with a noted increase in the incidence of chromosomal abnormalities (Zimmerman and Raj, 1980). Additionally, exposure of human-derived HepG2 and TR146 cells to highly purified CBD resulted in single and double-stranded DNA breaks (Russo et al., 2019).

Conflicting reports on CBD's potential genotoxicity and clastogenicity have been previously reviewed (Zimmerman and Zimmerman, 1990). The reviewers opined that the conflicting reports on the cytogenetic effects can be partially explained by the different experimental protocols, cell types, and/or the animal species used by the investigators.

2.5. Inhibition of Drug/Xenobiotic Metabolizing Enzymes and Transport Proteins, and CBD-Drug Interaction Potential

In animal models, CBD has been shown to inhibit hepatic microsomal drug metabolism through inhibition of specific CYP enzymes (Paton and Pertwee, 1972), based on the observation in mice that pentobarbitone-induced sleep was significantly prolonged by CBD treatment in a dose-dependent manner. This suggests that CBD-induced inhibition of microsomal enzymes prevented pentobarbitone metabolism. An additional study in mice reported that CBD treatment resulted in a decrease in testosterone 6β-hydroxylation and erythromycin *N*-demethylation; both reactions are markers of CYP3A activity, indicating that CBD could inhibit CYP3A (Yamamoto et al., 1995). As with mice, in male rats, CBD inhibited CYP2C11 function, resulting in decreased 2α- and 16α-hydroxylation of testosterone (Narimatsu et al., 1988). Interestingly, work by other authors in rats demonstrated that CBD may disrupt hormone homeostasis and steroidogenesis through selective inhibition of male specific CYPs, like CYP2C11, in the liver (Watanabe et al., 2005).

An *in vitro* study using human liver microsomes showed that CBD is a potent inhibitor of specific human CYPs: CYP1A1, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 (Jiang et al., 2011). Importantly, a study associated with the CBD-based drug Epidiolex reported that CBD can inhibit (IC_{50}<10mM) the function of multiple CYP isoforms *in vitro*, including CYP1A2, CYP2B6, CYP2C8, CYP2C19, and CYP3A4 (FDA, 2018a). These data suggest CBD may compete with pharmaceuticals and xenobiotics that use a similar metabolism pathway; in turn, raising the possibility for drug-drug interactions.

In addition to inhibiting CYP enzymes, CBD can also inhibit crucial efflux transporters like P-gp and BCRP. *In vitro* studies have shown that CBD (3–100μM) can inhibit P-gp mediated efflux, leading to an increased intracellular accumulation of undesirable P-gp substrates, like phenobarbital and digoxin (Iffland and Grotenhermen, 2017). Additionally, in an *in vitro* model of the placental barrier using choriocarcinoma BeWo and Jar cells, both of which express the efflux transporter BCRP, exposure to 10 and 25μM CBD resulted in a dose-dependent accumulation of mitoxantrone, a BCRP substrate, in both cell types (Feinshtein et al., 2013). This demonstrated that CBD inhibits BCRP-mediated efflux of mitoxantrone. CBD's inhibitory effects on efflux transporters like P-gp and BCRP can cause aberrant accumulation of various compounds, such as xenobiotics and therapeutic drugs, that would otherwise be transported out of the cell. Greater penetration and accumulation of these chemicals in the brain and other tissues raises safety concerns for the general population. It is also important to note that P-gp and BCRP play critical roles in placenta-mediated efflux of pharmaceuticals and xenobiotics. Dysregulation of this fetal protective mechanism by CBD raises safety concerns regarding gestational exposure to CBD in the presence of other pharmacologically active agents or environmental toxicants.

3. Conclusions

The studies and data reviewed herein show potential hazards associated with oral exposure to CBD for the general population. Observed effects include organ weight alterations; developmental and reproductive toxicities in both males and females, including effects on neuronal development and embryo-fetal mortality; hepatotoxicity; immune suppression, including lymphocytotoxicity; mutagenicity and genotoxicity; and effects on liver metabolizing enzymes and drug transport proteins.

CBD can cause adverse effects on the male reproductive system from exposure during gestation or adulthood. These effects have been attributed to dysregulated endocannabinoid-modulated steroidogenesis and/or dysregulated hormonal feedback mechanisms, primarily involving testosterone. Available data indicate additional concerns for developmental effects, and suggest the reproductive toxicity of CBD includes female- and pregnancy-specific outcomes. Toxicities observed from gestational exposure to CBD in both sexes, such as delayed sexual maturity, increased pre-implantation loss, and undesirable alterations to the brain epigenome are of particular concern, as these effects could be transgenerational.

CBD can also cause adverse effects on the liver. These findings highlight the potential for CBD-drug interactions as revealed by the effect of CBD on multiple drug metabolizing enzymes, and the paradoxical effect of the combination of CBD and APAP. While the impact of CBD on drug metabolizing enzymes is well established, further studies would be needed to investigate the mechanism of CBD's paradoxical interaction with APAP and similar pharmaceuticals.

The diverse and disparate effects observed following CBD exposure suggest multiple potential mechanisms of toxicity. Analysis of identified CBD cellular targets and their native functions suggests the following possible mechanisms of CBD-mediated toxicity: (I) inhibition of, or competition for, several metabolic pathway enzymes, including both phase I and II drug metabolizing enzymes, (II) receptor binding activity, (III) disruption of testosterone steroidogenesis, (IV) inhibition of the reuptake and breakdown of endocannabinoids, and (V) oxidative stress via depletion of cellular glutathione in the liver or inhibition of testicular enzymatic activity. CBD may additionally act though secondary mechanisms to impact reproduction and development. For instance, CBD was shown *in vitro* to inhibit TRPV1, dysregulation of which has been observed in placentas from preeclamptic pregnancies (Martinez et al., 2016).

Although CBD's mechanisms of action remain unclear and are likely multifarious, many proposed mechanisms relate to the endocannabinoid system. Physiological processes controlled by the endocannabinoid system are areas of potential concern for CBD toxicity. It bears noting that the endocannabinoid system is still poorly understood, and future elucidation of its intricacies may provide new insight into safety concerns for perturbation of this biological system and the mechanisms of CBD's effects. Demonstrated differences between THC's and CBD's biological effects and toxicities highlights the complexity of this system. While this review focuses on relatively pure CBD, many other phytocannabinoids with structural similarity to CBD exist for which there is little or no toxicological data to evaluate their safety.

Potential adverse effects from CBD use may not be immediately evident to users of CBD-containing consumer products. For example, early signs of liver toxicity would go undetected without monitoring for such effects. Additionally, effects observed on the male reproductive system in animal models involve damage to testicular structure and function, including effects on the development and abundance of spermatozoa, in the absence of any outwardly visible damage. If these effects are relevant to humans, they imply that chronic consumption of CBD could interfere with male reproductive function in a way that may only manifest as a reduction, or non-recurrent failure, in reproductive success (i.e., subfertility). Thus, it would be difficult to identify such outcomes through typical post-market monitoring and adverse event reporting systems.

The available data clearly establish CBD's potential for adverse health effects when consumed without medical supervision by the general population. Some risks, such as the potential for liver injury, will likely be further characterized with ongoing clinical observations. Other observed effects from the toxicology data, such as male and potential female reproductive effects, have not been documented in humans but raise significant concerns for the use of CBD (in oral consumer products) by the broad population. Importantly, the degree of reproductive effects and the wide range of species impacted further contributes to the concerns around CBD consumption by the general population.

Adverse health effects have been observed in humans and animals at levels of intake that could reasonably occur from the use of CBD-containing consumer products (Dubrow et al., 2021). CBD's lengthy $t_{1/2}$ following chronic oral administration makes long-term consumption of CBD products by the broad population concerning. Available data from multiple oral toxicity studies raise serious safety questions about the potential for reproductive and developmental toxicity effects, which could be irreversible, and support particular concerns about the use of CBD during pregnancy or in combination with other drugs.

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Jeremy Gingrich: Investigation, Writing – original draft, Writing – review & editing. **Supratim Choudhuri:** Conceptualization, Investigation, Writing – original draft. **Patrick Cournoyer:** Conceptualization, Writing – review & editing, Project administration. **Jason Downey:** Writing – review & editing. **Kristi Muldoon Jacobs:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Recommended articles

Data availability

No data was used for the research described in the article.

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