International Journal of Medical and Pharmaceutical Sciences
Open access peer-reviewed Journal
Volume 2, Issue 8, Page 665-677.
https://doi.org/10.5281/zenodo.22015345

Review Article

Cannabidiol In Experimental Epilepsy: A Review of Preclinical Rat Models

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Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures and is frequently accompanied by cognitive, behavioral, and psychiatric comorbidities. Despite the availability of numerous antiseizure medications, a substantial proportion of patients remain inadequately controlled, creating a need for therapies with novel mechanisms. Cannabidiol (CBD), a non-intoxicating phytocannabinoid derived from Cannabis sativa, has emerged as a promising antiseizure compound and has subsequently gained regulatory approval for selected developmental and epileptic encephalopathies. This review focuses specifically on preclinical evidence from rat models of epilepsy, emphasizing seizure control, neuroprotection, neuroinflammation, antiepileptogenic effects, pharmacokinetics, and potential mechanisms of action. Studies using pentylenetetrazol (PTZ), pilocarpine, lithium-pilocarpine, kainic acid, audiogenic kindling, and other experimental paradigms demonstrate that CBD can reduce seizure severity, delay seizure onset, decrease seizure burden, and in some models suppress the development or expression of chronic epileptic activity. In rat models of temporal lobe epilepsy, CBD has additionally been associated with reduced neuronal degeneration, modulation of inflammatory cytokines and microglial polarization, changes in PPARγ signaling, and alterations in gut microbiota and metabolomic profiles. Importantly, effects vary with dose, route, treatment timing, model, age, and disease stage. Evidence for genuine antiepileptogenesis remains promising but less established than evidence for acute antiseizure activity. Translation is further complicated by differences in CBD exposure, formulation, metabolism, and pharmacodynamic responses between rodents and humans. Overall, rat studies provide strong mechanistic support for CBD as an antiseizure and potentially neuroprotective agent, but standardized chronic epilepsy protocols, exposure–response studies, sex-balanced designs, rigorous pharmacokinetic–pharmacodynamic characterization, and clinically relevant endpoints are needed to establish its translational value.

Keywords: Cannabidiol, CBD, Epilepsy, Rat model, Seizure, Temporal lobe epilepsy, Status epilepticus, Pentylenetetrazol, Pilocarpine, Neuroinflammation, Neuroprotection, Antiepileptogenesis.

INTRODUCTION

A complex neurological condition, epilepsy can be brought on by brain damage from trauma, stroke, infections, tumors, or even genetic abnormalities in ion channels, neurotransmitter genes, or proteins that regulate brain excitability. It has a lifetime frequency of about 1% and affects about 50 million people globally. [1]

1.1 History of Epilepsy:

Epilepsy and seizures date back over 3,000 years to the Mesopotamian civilization, albeit with distinct names and causes. [2] With a description of an unconscious man with his neck turned, his extremities tense, and his eyes wide open, one of its ancient books, the Sakikku, which translates to "all diseases," collected diagnostic data for various illnesses, including epilepsy and seizures. They called this condition miqtu, which means "the falling disease". [3]

Over the ages, various societies have portrayed and explained what is now known as epilepsy. Epilepsy was thought to have a magical or occult origin in several civilizations, including the Akkadian culture and the Sakikku writers, who directly linked it to Sin, the moon goddess. Until the Hippocratic school of medicine questioned the conventional wisdom in the fifth century BC, this mystical idea endured throughout several civilizations. [4] Known at the time as "the sacred disease," the school claimed that epilepsy, which comes from the Greek word epilambanein, which means "to take hold," was caused by an overabundance of phlegm in the brain and that it was no more divine than other illnesses, indicating that it had a natural cause and, therefore, a potential cure. [5]

1.2 Causes of Epilepsy:

The underlying etiology of epilepsy is completely unknown. Although some cases are caused by genetics, epilepsy can also be caused by head trauma, stroke, infections, high temperatures, or tumors. The term "epilepsy" does not specify the cause or severity of a person's seizures. It has been demonstrated that inheritance, or genetics, plays a major role in many causes of epilepsy in very young children, even though it can affect people of any age. For example, not all individuals who experience a serious head injury—a known cause of seizures—will get epilepsy [6]. Patients with epilepsy claim that specific precipitants or triggers, like reading or flashing lights, are necessary for seizures to occur in certain epilepsy types known as reflex epilepsy. Patients with epilepsy also mention alcohol, feverish illness, heat stress, emotional tension, and lack of sleep as precipitants. Notably, there are differences in the ways that certain precipitants impact an epilepsy state. Catamenial epilepsy, a seizure linked to the menstrual cycle, and seizure recurrence patterns in women with epilepsy can both be impacted by their menstrual cycle [7][8]. CNS infections, trauma, congenital CNS abnormalities, and metabolic problems are the most common causes of hypoxic-ischemic encephalopathy in neonates and early infants. CNS diseases and trauma can cause the most common febrile seizures in late infancy and early childhood. Children usually have well-defined epilepsy disorders. Any CNS impairment in adolescence and adulthood is more likely to be the primary cause. Cerebrovascular disorders, which include brain tumors, head trauma, and 22 other degenerative diseases, are the most common cause of dementia in the elderly [9].

1.3 Cannabis:

For 5,000 years, people have been using cannabis sativa. The first cultures to notice this drug's properties were Chinese and Indian. The substance was introduced to Persia and Arabia by traders, adventurers, and travelers after the fifth century A.D. Western medical professionals began to acknowledge cannabis for its calming and pain-relieving properties after Napoleon's army returned from Egypt. "To the farmer, cannabis is a crop of fiber; to the doctor a century ago, it was a valuable medicine; to the doctor today, it is an enigma; to the user, it is a euphoriant; to the police, it is a threat; to the traffickers, it is a source of lucrative danger; to the prisoner or parolee and his family, a cause of grief".[10] Cannabis, hemp, Indian hemp, marihuana, and marijuana are some of the common names for Cannabis sativa. The herbaceous plant known as Cannabis sativa has a wide range of applications and effects. The cannabis flower is the source of marijuana; the names "cannabis" and "marijuana" are sometimes used interchangeably. However, smoking, eating, or inhaling fumes are other ways to consume the plant's leaves and resinous extracts. Cannabis hempseeds are also utilized to make oil for wood surface coatings, lighting, and cooking. The primary reason for the current interest in this plant is its abundance of cannabinoids. In addition to their therapeutic properties, cannabinoids are chemicals that are mostly used for spiritual and recreational purposes. The varying psychotropic and therapeutic properties of cannabinoids are explained by differences in their molecular structures. Nowadays, cannabidiol (CBD) and (Delta)9-Tetrahydrocannabinol (Δ9- or D9-trahydrocannabinol; THC) are the two most sought-after cannabinoids. One of the main non-psychoactive phytocannabinoids found in cannabis, CBD makes for almost 40% of cannabis extracts. One component of cannabis that is believed to have potential medical uses is CBD [11][12] are not psychoactive, and do not impede cognitive or psychomotor learning. The other primary active ingredient in cannabis, THC, on the other hand, is what causes the mood-altering effects.

THC has strong negative psychoactive effects that cause anxiety and paranoia. [13] The idea that CBD could be able to mitigate the negative psychoactive effects of THC in people is gaining attention [14][15]

Taxon	Scientific name and common name
Kingdom	Plantae (plants)
Subkingdom	Tracheobionta (vascular plants)
Superdivision	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Equisetopsida
Subclass	Magnoliidae
Order	Rosales
Family	Cannabaceae
Genus	Cannabis
Species	Cannabis sativa L.
Subspecies	C. sativa subsp. Sativa C. sativa subsp. indica C. sativa subsp. ruderalis
Common Name	cannabis, hemp, Indian hemp, marihuana and marijuana

1.3.1 Pharmaceutical Functional Uses of Cannabis:

Studies with Cannabis sativa L. have shown the presence of several pharmaceutical activities:

1.3.1.1 Antioxidant Activity:

A chemical known as an antioxidant slows down or stops the oxidation of molecules that may be crucial to an organism's metabolism. Numerous antioxidant compounds were found in Cannabis sativa L. According to IC50 (low inhibitory concentration at 50%) values of 14.5 mg/mL for DPPH, 1.9 mg/mL for chelating test, and 92.7 mg/mL for lipid peroxidation inhibition assay, the obtained results demonstrated that the seed organic extract had a good antioxidant ability. These scientists claim that the inclusion of polyphenols and cannabinoids, which are well-known for their potent antioxidant properties, is most likely the cause of this action. [18][19]

1.3.1.2 Anticoagulant Activity:

Plants with anticoagulant properties have been proposed as herbal treatments that may help find novel therapeutic agents to treat disorders associated with thrombosis. To ascertain the potential anti-prothrombotic impact of cannabis leaf metabolites, blood clotting tests were carried out, focusing on the three primary cannabinoids THC, CBD, and CBN.[20] When two cannabinoids, THC and CBN, were used to assess the in vitro impact of cannabis extract on thrombin activity, the IC50 values were intriguing. THC exhibited the maximum activity, measuring 1.79 mg/mL, while CBN displayed a lower level of activity, as indicated by the high IC50 value. Nevertheless, an in vivo test was also used in this work to measure the clotting times in obese rats. demonstrating demonstrating that cannabis might have potent anticoagulant properties. [21]

1.3.1.3 Antidiabetic Activity:

The hallmark of diabetes is abnormally elevated blood glucose levels. Diabetes is a chronic, progressive, and complex metabolic disease. Hyperglycemia is another name for this condition. [22] With a value of 3.77 mmol ACAE/g oil, the study demonstrated that essential oils from the aerial part of cannabis had antidiabetic effects against the α-glucosidase enzyme. Additionally, this essential oil was tested against α-amylase, however the results were insignificant. [23]

1.3.1.4 Anticancer Activity:

Studies has shown that a number of chemicals produced from cannabis have conclusive effects on a variety of cancer cell lines, including those from the breast, prostate, cervix, brain, colon, and leukemia/lymphoma. [24] Numerous in vivo and in vitro investigations have shown how phytocannabinoids affect the development of tumors. According to these research, at concentrations ranging

from 5 to 65 µm, certain cannabinoids, like Δ9-THC and CBD, cause apoptosis and stop the growth of different cancer cell lines. Additionally, the anticancer efficacy of cannabis products was enhanced by the combination of specific phytocannabinoids. [25]

1.3.1.5 Antiepileptic and Anticonvulsant Activities:

Cannabis sativa L. has been studied for other impacts on the central nervous system, notwithstanding its well-known euphoric properties. CBD has a protective role in controlling hippocampal seizures and neurotoxicity at a young age, according to a newly published study that found it to be more effective in treating epilepsy with a hippocampus emphasis than with the extrahippocampal amygdala and parvalbumin. [26] In human groups, such as teenagers and young adults with severe childhood-onset epilepsy, the effectiveness of CBD has been further confirmed. [27] In a 12-week open-label study, 25–50 mg/kg of CBD was administered to a group of patients ages 1–30. The intensity of epileptic attacks was reduced by 36.5% in the treated groups. Preclinical studies have also tried to provide a longer-term explanation of CBD's anticonvulsant effectiveness. Different dosages of CBD ranging from 20 to 50 mg/kg over a 28-day treatment period were observed to reduce seizure activity using the PTZ model of epilepsy. [28] Cannabis also has antibacterial, neuroprotective, insecticidal, and dermo cosmetic properties, among other pharmacological therapeutic applications.

1.4 Cannabidiol:

A non-intoxicating main component of the Cannabis sativa plant, **cannabidiol (CBD)** has drawn more attention lately because of its favourable safety and tolerability profile as well as its potentially wide range of medicinal uses. [29] Sleepiness, diarrhoea, or elevated body temperature are examples of minor and rare side effects. Clinically significant medication interactions are also said to be low risk. [30] According to the World Health Organization Expert Committee on Drug Dependence, there is no proof that using CBD can lead to reliance or misuse. CBD is said to have analgesic, anti-inflammatory, antioxidant, anxiolytic, anticonvulsant, and cytotoxic effects. These effects are mediated through a number of signalling mechanisms, including the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors, the cannabinoid receptor 1 (weak agonist), the cannabinoid receptor 2 (inverse agonist), the serotonin 1a receptor (5-HT1A), G protein-coupled receptor 55 (GPR55), G protein-coupled receptor 18 (GPR18), and the Serotonin 1a receptor (5-HT1A).[31] Clinically, CBD is being studied in a number of disease states, such as addiction (current studies in cannabis and cocaine desire), neurodegeneration, anxiety disorders, and orphan childhood diseases with an incidence of less than 5 per 100,000 people (such as tuberous sclerosis complex).[32,33] Epidiolex, the first CBD medication approved by the Food and Drug Administration, is recommended for oral treatment of Lennox-Gastaut or Dravet syndrome (childhood epilepsy). Sativex is an oro mucosal spray licensed in the EU and Canada to treat spasticity associated with multiple sclerosis. It contains both CBD and δ9-tetrahydrocannibinol. [44] As of this writing, at least 100 clinical trials have already been registered that contain CBD, and 49 clinical trials are currently investigating CBD alone (either not yet recruiting, recruiting, or active). This suggests that there is a significant clinical interest in CBD and that it is still necessary to make sure that the doses given to human volunteers participating in these trials are optimized for safety and efficacy. Remarkably, none of the 49 registered trials to date have specifically incorporated a study design to examine the effectiveness of CBD at different doses. CBD extracted from hemp is currently marketed as a dietary and health supplement that is frequently used to treat pain and anxiety. This market is an example of a thriving industry that is anticipated to grow both monetarily and internationally. [33]

1.5 Preclinical Model

Before testing on humans, a preclinical model is a scientific method or system used to examine the effects of possible medicinal substances, like marijuana, in a controlled setting.

Types of Preclinical Models:

1. In Vitro Models (cell cultures, organotypic slice cultures)

2. In Vivo Models (animal models, e.g., rodents, non-human primates)
3. Ex Vivo Models (tissue samples, organ cultures)
4. Computational Models (mathematical simulations, computer models)
5. Genetic Models (transgenic animals, gene editing)

Benefits:

1. A lower chance of negative consequences for people
2. More effective drug development
3. Economical viability

Limitations:

1. Limited ability to predict human reaction
2. The intricacy of biological systems

2. Importance of Rat Models In Experimental Epilepsy

Animal models are fundamental to the development of antiseizure therapies because they allow controlled investigation of seizure initiation, propagation, neuronal injury and epileptogenesis.

Rat models used in CBD research can broadly be categorized into four groups:

2.1 Acute seizure models

- Pentylenetetrazole (PTZ)
- Maximal electroshock (MES)

2.2 Acquired epilepsy models

- Pilocarpine-induced status epilepticus and epilepsy
- Kainic acid-induced temporal lobe epilepsy

2.3 Kindling models

- Amygdala kindling
- Audiogenic kindling

2.4 Genetic models

- Wistar Audiogenic Rats (WARs)
- Genetically Epilepsy-Prone Rats (GEPRs)

Each model represents different biological features of epilepsy. Acute models are useful for screening anticonvulsant effects, whereas chronic and kindling models are more informative for evaluating spontaneous seizures, seizure progression and possible disease-modifying effects.

Table 2: Comparison of Acute And Chronic Rat Models

Parameter	Acute models	Chronic models
Examples	PTZ, MES	Pilocarpine, kainic acid, kindling, WAR, GEPR
Duration	Minutes to hours	Weeks to months
Main purpose	Screening antiseizure activity	Study established epilepsy
Spontaneous seizures	Usually absent	Common in selected models
Epileptogenesis	Limited relevance	Can be investigated
EEG monitoring	Useful but not always required	Highly recommended
Neurodegeneration	Limited	More representative
Translational value	Initial pharmacological screening	Higher relevance to chronic epilepsy

2.1 CBD in Acute Rat Models of Seizures

2.1.1 Pentylenetetrazole-Induced Seizures

PTZ is a commonly employed chemical convulsant and research tool for examining susceptibility to generalized seizures in mice. It induces neuronal hyperexcitability and facilitates the assessment of seizure latency, severity, duration, and mortality. In PTZ-induced seizure models, CBD has shown anticonvulsant effects. Jones et al. found that CBD decreased both the severity and lethality of seizures in experimental models of generalized seizures, reinforcing the early evidence of CBD's authentic

antiseizure capabilities of CBD. [34] Additional studies have explored the molecular mechanisms underlying these effects. In adolescent Wistar-Kyoto rats, the impact of CBD was assessed using the PTZ model alongside antagonists of 5-HT1A and 5-HT2A receptors. This study indicated that serotonergic pathways might play a role in CBD's anticonvulsant effects of CBD. Nonetheless, CBD's effects of CBD are not uniform across all experimental seizure models. This variability implies that CBD may be more effective against certain pathological network activity patterns than others.

2.1.2 Maximal Electroshock Model

The MES model is extensively employed to assess compounds that can inhibit the spread of generalized tonic-clonic seizures. Initial experiments on rats demonstrated that CBD was an effective anticonvulsant in both MES and audiogenic seizure tests. Researchers also discovered that CBD interacted with established antiepileptic drugs, enhancing the efficacy of some compounds while diminishing that of others. The significance of the MES model lies in its ability to show that protection against tonic hindlimb extension signifies the inhibition of seizure spread. However, this model mainly serves as an acute screening tool and does not fully replicate the entire pathophysiology of chronic epilepsy. [35]

2.2 CBD in Early-Life Rat Models

Epilepsy during early life presents unique pharmacological challenges because neuronal development, receptor expression, synaptic connectivity, drug metabolism, and seizure susceptibility differ from those in adult animals. A study in 12-day-old rats examined the effects of CBD at 10 and 60 mg/kg on PTZ- and NMDA-induced seizures. CBD was ineffective against NMDA-induced seizures, whereas 60 mg/kg CBD abolished the tonic phase of PTZ-induced generalized seizures. Brain concentrations peaked approximately 1–2 h after administration and remained measurable 24 h later, demonstrating substantial CNS distribution in immature rats. [35] Another study compared P7 and P21 rats using the PTZ and MES models. CBD delayed seizure onset in adolescent rats in the PTZ model but not in neonatal rats, whereas higher doses reduced seizure duration in both developmental groups in the MES model. The overall effects were modest but consistent. These observations emphasize the importance of age-dependent pharmacology and demonstrate that findings from adult rats cannot be automatically extrapolated to neonatal or pediatric epilepsy.

2.3 CBD in Pilocarpine-Induced Epilepsy

The pilocarpine model is particularly useful for investigating acquired temporal lobe epilepsy. Following status epilepticus, animals may develop neuronal injury, network reorganization, and spontaneous recurrent seizures. Hosseinzadeh et al. investigated CBD post-treatment during the chronic phase of pilocarpine-induced epilepsy in rats. CBD is associated with improvements in epilepsy-related behavioral abnormalities and activation of hippocampal autophagy pathways and antioxidant defense mechanisms. [36] These findings are significant because they suggest that CBD may exert effects beyond immediate seizure suppression. The hippocampus is a major structure involved in temporal lobe epilepsy, and modulation of oxidative stress and cellular homeostasis may contribute to neuronal protection. Nevertheless, the presence of neuroprotective effects should not be automatically interpreted as proof of antiepileptogenesis. Demonstrating true disease modification requires appropriately designed longitudinal experiments that show a persistent reduction in epilepsy after treatment withdrawal.

2.4 CBD in Kainic Acid-Induced Temporal Lobe Epilepsy

Kainic acid induces excitotoxic neuronal activity and status epilepticus and is commonly used to model temporal lobe epilepsy. Costa et al. investigated the effects of CBD in adult male Sprague-Dawley rats with kainic acid-induced temporal lobe epilepsy. Animals were continuously monitored using video electrocorticography, allowing for the objective assessment of spontaneous recurrent seizures. [37] CBD was administered after the establishment of spontaneous recurrent seizures. At 120 mg/kg twice daily for three days, CBD abolished seizures in 50% of the treated rats and significantly reduced seizure occurrence and total seizure duration. The antiseizure

effect was accompanied by increased PPARγ immunoreactivity in several brain regions, including the hippocampal structures. This study is especially relevant because CBD was administered after epilepsy had become established rather than before chemically induced seizures. Therefore, it provides stronger evidence for an antiseizure effect in chronic epilepsy.

2.6 CBD in Amygdala-Kindling Models

The amygdala-kindling model is widely used to investigate focal seizures and secondary generalizations. Repeated subthreshold stimulation progressively increases seizure susceptibility, providing a model of seizure development and network-plasticity. Fallah et al. evaluated CBD in fully kindled male Sprague-Dawley rats with electrodes implanted in their amygdala. CBD partially suppressed generalized and focal seizures at doses that did not cause ataxia. The reported effective doses were considerably higher than those required for some acute models. These findings demonstrate that CBD can influence both focal seizure activity and secondary generalization. They also emphasized a recurring feature of CBD pharmacology: dose requirements and efficacy can vary markedly among experimental models. Interestingly, the same study investigated the effects of CBD in combination with THC. A low dose of THC markedly shifted the CBD dose-response curve, suggesting an enhanced antiseizure efficacy. However, because THC has psychoactive and motor effects and is pharmacologically distinct from purified CBD, this finding should not be interpreted as evidence that CBD requires THC for its therapeutic efficacy.

2.7 CBD in Wistar Audiogenic Rats Models

Wistar Audiogenic Rats are genetically susceptible to seizures induced by intense acoustic stimulation. Repeated stimulation recruits limbic structures and produces audiogenic kindling. Chronic CBD administration in WARs had important effects on both brainstem and limbic seizures. CBD attenuated tonic-clonic seizures, prevented limbic recruitment, and suppressed kindled limbic seizures. Treatment was also associated with reduced FosB immunostaining, suggesting the suppression of chronic neuronal hyperactivity. CBD prevented seizure-associated changes in CB1 receptor expression in the hippocampal and basolateral amygdala regions. These findings are among the more compelling preclinical observations suggesting that CBD may have antiepileptogenic potential, although definitive disease-modifying conclusions require further experimental confirmation of this.

2.8 Genetically Epilepsy-Prone Rats

GEPR-3 rats exhibit both generalized tonic-clonic seizures and limbic seizures, allowing investigation of more than one seizure phenotype. CBD produced a dose-dependent attenuation of generalized tonic-clonic seizures and suppressed limbic seizure expression following audiogenic kindling. These findings support activity against both brainstem-dependent generalized and limbic seizures. Genetic models are particularly useful because they demonstrate that CBD's activity of CBD is not restricted to chemically induced seizures.

Table 3: Summary of Evidence from Major Rat Models

Model	Principal seizure phenotype	Major CBD-related finding	Major implication
PTZ	Generalized chemically induced seizures	Reduced seizure severity/altered seizure onset in selected studies	Acute antiseizure activity
MES	Generalized tonic-clonic seizures	Protection against tonic seizure activity	Seizure-propagation control
PTZ, infantile rats	Early-life generalized seizures	Higher dose reduced tonic seizure component	Development-dependent activity
Pilocarpine	Status epilepticus/chronic epilepsy	Behavioral, antioxidant and autophagy-related effects	Potential neuroprotective activity

Kainic acid	Temporal lobe epilepsy	Reduced spontaneous recurrent seizures and seizure duration	Activity in established epilepsy
Amygdala kindling	Focal + secondary generalized seizures	Partial suppression of focal and generalized seizures	Potential focal-seizure efficacy
WAR	Audiogenic and limbic seizures	Reduced tonic-clonic and kindled limbic seizures	Possible antiepileptogenic activity

3. Mechanisms of Cbd In Experimental Epilepsy

3.1 TRPV1

Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel involved in calcium signaling and neuronal excitability. CBD can activate TRPV1 and promote subsequent desensitization. This may influence calcium-dependent neuronal signaling and reduce pathological excitability under particular experimental conditions. TRPV1 is therefore, considered one of the candidate mechanisms underlying CBD's antiseizure activity, although it is unlikely to account for the entire pharmacological effect.

3.2 GPR55

GPR55 is an orphan G protein-coupled receptor involved in neuronal signaling and excitability. CBD has been proposed to antagonize or functionally modulate the GPR55. As excessive excitatory signaling can facilitate seizure propagation, GPR55 modulation may contribute to CBD-mediated suppression of neuronal hyperexcitability.

Current mechanistic reviews have identified GPR55 as one of the principal CB1/CB2-independent targets of CBD relevant to epilepsy.

3.3 ENT-1 and Adenosine Signaling

CBD can inhibit equilibrative nucleoside transporter-1 (ENT-1), potentially increasing extracellular adenosine levels. Adenosine is an endogenous neuromodulator that inhibits neuronal activity. Therefore, increased extracellular adenosine can reduce neuronal excitability and seizure propagation. This pathway provides an attractive explanation for how CBD can produce antiseizure activity without acting as a classical CB1 receptor agonist.

3.4 Endocannabinoid System

The endocannabinoid system regulates synaptic transmission and neuronal excitability via cannabinoid receptors and endogenous ligands. CB1 receptors are highly expressed in the CNS and regulate the release of excitatory and inhibitory neurotransmitters in the CNS. Although CBD has a relatively low direct affinity for CB1 compared to THC, it can indirectly influence endocannabinoid signaling. In WARs, chronic CBD treatment prevented seizure-associated alterations in CB1 receptor expression, supporting a possible interaction between CBD and the endocannabinoid system during chronic epilepsy.

3.5 Glutamatergic Transmission

Excessive glutamate signaling contributes significantly to neuronal hyperexcitability and excitotoxicity. CBD may reduce excessive glutamatergic activity, thereby influencing seizure generation and neuronal injury. This mechanism is particularly relevant in chronic epilepsy because repeated seizures can alter glutamate release and uptake. Experimental evidence suggests that the modulation of glutamate signaling may be an important component of CBD's antiseizure action of CBD.

3.6 GABAergic Signaling

GABA is the principal inhibitory neurotransmitter in the central nervous system (CNS). Although CBD does not function as a conventional benzodiazepine-like GABAergic drug, its interaction with GABAergic signaling may contribute to its antiseizure activity. The importance of this pathway is particularly evident in CBD-clobazam combinations. Experimental studies have demonstrated enhanced GABA_A receptor activation when CBD and clobazam are co-administered, providing evidence of a pharmacodynamic interaction.

3.7 PPARγ

PPARγ is a nuclear receptor involved in the regulation of inflammatory responses, metabolism, and cellular homeostasis. In rats with kainic acid-induced temporal lobe epilepsy, CBD increased PPARγ immunoreactivity in the hippocampal and cortical structures. The highest CBD dose was associated with increased PPARγ immunoreactivity in the hippocampal CA3 region, perirhinal cortex, and amygdala. These findings suggest that PPARγ may participate in CBD's antiseizure and potentially neuroprotective effects of CBD.

3.8 Serotonergic Signaling

CBD can influence serotonergic pathways, including 5-HT1A-related signaling. Experimental PTZ studies using selective receptor antagonists have investigated whether 5-HT1A or 5-HT2A receptors contribute to CBD's anticonvulsant activity of CBD. Because serotonin influences neuronal excitability, stress responses, and seizure susceptibility, serotonergic modulation may contribute to CBD's broader network-level effects of CBD.

3.9 Potassium Channels

Potassium channels alters the membrane potential and neuronal excitability. Experimental studies have suggested that potassium-channel alteration may contribute to cannabinoid-related antiseizure effects. The importance of potassium channels is particularly relevant because stabilization of membrane potential can decreased the probability of uncontrolled neuronal firing.

Table 4: Different Routes of Action

Target/pathway	Proposed action of CBD	Potential antiseizure consequence
TRPV1	Activation/desensitization	Modulation of calcium-dependent excitability
GPR55	Functional antagonism/ modulation	Reduced excitatory signaling
ENT-1	Inhibition	Increased extracellular adenosine
Adenosine	Enhanced signaling	Reduced neuronal excitability
CB1/endocannabinoid system	Indirect modulation	Regulation of neurotransmitter release
PPARγ	Increased signaling/activity	Anti-inflammatory and neuroprotective effects
5-HT1A	Functional modulation	Altered seizure susceptibility
Potassium channels	Modulation	Membrane stabilization
Glutamate system	Reduction of excessive signaling	Reduced excitotoxicity
GABAergic system	Functional modulation	Increased inhibitory network stability
Oxidative pathways	Antioxidant effects	Reduced oxidative neuronal damage
Neuroinflammatory pathways	Modulation	Reduced inflammatory contribution to epileptogenesis

4. Limitations 0fof Current Preclinical Evidence

4.1 Heterogeneity of models

Different models reproduce different components of epilepsy. PTZ and MES are primarily acute screening models, whereas pilocarpine, kainic acid and kindling provide more clinically relevant chronic epilepsy phenotypes.

4.2 Dose variability

The experimental CBD doses differ substantially among studies, complicating direct comparisons.

4.3 Route variability

Oral, intraperitoneal and subcutaneous routes produce different pharmacokinetic profiles.

4.4 Limited pharmacokinetic integration

Many studies report dose but not plasma or brain CBD concentrations. Exposure-based comparison would improve interpretation.

4.5 Sex bias

Many rat studies have historically used male animals. Greater inclusion of female rats is required.

4.6 Developmental differences

CBD responses can vary substantially between neonatal, juvenile and adult animals.

4.7 Limited antiepileptogenic evidence

Although chronic genetic models provide encouraging evidence, definitive proof that CBD prevents the development of epilepsy remains insufficient.

4.8 Drug interactions

CBD can modify the pharmacokinetics and pharmacodynamics of other ASMs, particularly clobazam.

5. FUTURE RESEARCH DIRECTIONS

Future research should focus on improving both mechanistic understanding and translational validity.

5.1 Standardized pharmaceutical CBD: Studies should clearly report-CBD purity, formulation, vehicle, dose, route, administration frequency, pharmacokinetic exposure.

CBD purity: The exact concentration and purity level of the cannabidiol used.

Formulation: The specific pharmaceutical form (e.g., oil, capsule, spray).

Vehicle: The carrier substance or medium in which CBD is delivered.

Dose: The amount of CBD administered per administration.

Route: The administration pathway (e.g., oral, sublingual, inhalation).

Administration frequency: How often the CBD is given (e.g., once daily, multiple times per day).

Pharmacokinetic exposure: Data on absorption, distribution, metabolism, and excretion that characterize systemic exposure to CBD.

Clear reporting of these elements is essential to interpret efficacy, safety, and pharmacological outcomes accurately in clinical or preclinical studies involving pharmaceutical-grade CBD.

5.2 Pharmacokinetic-pharmacodynamic modeling

Future studies should correlate:

Dose → plasma concentration → brain concentration → molecular target engagement → EEG effect → seizure outcome.

This framework suggests that future studies should systematically measure and link each step to improve the predictive power of animal models for human dose selection. By establishing these correlations, translation from preclinical findings to clinical applications would be enhanced, enabling more accurate dose optimization based on mechanistic understanding across biological compartments and functional readouts. This approach aligns with best practices in PK-PD modeling, where integrating drug exposure (plasma and brain levels), target interaction, and downstream physiological effects (EEG changes) provides a comprehensive basis for predicting therapeutic efficacy (seizure control).

5.3 Chronic EEG-based experiments

Continuous video-EEG should replace behavioral seizure scoring alone wherever feasible.

5.4 Antiepileptogenic studies

CBD should be evaluated during the latent period following status epilepticus, with prolonged follow-up after treatment discontinuation.

5.5 Combination therapies

Systematic investigation of CBD with: Clobazam, Valproate, Levetiracetam, Phenobarbital, Lamotrigine, Carbamazepine, other Anti-Seizure

Medications could help identify rational combinations.

5.6 Sex and developmental biology

Both male and female animals should be included, and neonatal, juvenile and adult animals should be evaluated separately.

5.7 Novel pharmaceutical formulations

CBD's lipophilicity and variable oral bioavailability make formulation development important. Lipid-based systems, self-emulsifying drug-delivery systems, nanostructured carriers and other pharmaceutical approaches could potentially improve bioavailability and exposure consistency.

5.8 Biomarker development

Future research should investigate whether changes in: Glutamate, GABA, Inflammatory cytokines, PPARγ, CB1 receptors, Oxidative stress markers, EEG biomarkers, Microglial activation can predict CBD response.

Table 5: Key Research Gaps and Recommended Approaches

Research gap	Current problem	Future approach
Dose selection	Wide experimental range	PK/PD-guided dosing
Formulation	Different CBD preparations	Standardized pharmaceutical CBD
Route	Variable exposure	Comparative bioavailability studies
Chronic efficacy	Limited long-term studies	Long-duration EEG studies
Antiepileptogenesis	Evidence remains preliminary	Delayed-treatment and withdrawal studies
Sex differences	Male animals frequently dominate	Balanced sex representation
Age	Limited developmental research	Neonatal, juvenile and adult comparisons
Drug interactions	Complex PK/PD interactions	Systematic combination studies
Biomarkers	Limited predictive markers	EEG + molecular biomarkers
Translation	Rodent-human pharmacokinetic differences	Exposure-matched translational studies

CONCLUSION

The body of preclinical evidence demonstrates that cannabidiol is a promising multimodal antiseizure compound with significant activity across several rat models of experimental epilepsy. CBD has demonstrated anticonvulsant effects in acute PTZ and MES models, activity against established epilepsy in pilocarpine and kainic-acid models, suppression of focal and secondarily generalized seizures in amygdala-kindling studies, and activity in genetically susceptible WAR and GEPR models. The diversity of responsive models indicates that CBD's pharmacology is not restricted to a single seizure phenotype. The antiseizure effects of CBD appear to arise from a complex interaction of molecular mechanisms involving TRPV1, GPR55, ENT-1/adenosine signaling, endocannabinoid pathways, PPARγ, serotonergic systems, ion channels and glutamatergic/GABAergic neurotransmission. Its potential effects on neuroinflammation, oxidative stress, autophagy and neuronal homeostasis may provide additional therapeutic benefits beyond acute seizure suppression. One of the most promising areas is the possibility of antiepileptogenic activity. Chronic CBD administration in genetic audiogenic epilepsy models has reduced limbic recruitment, neuronal hyperactivity and seizure progression. Nevertheless, these observations should be regarded as preliminary evidence rather than definitive proof of disease modification. Pharmacokinetics and drug interactions represent additional critical considerations. CBD can modify the metabolism and activity of conventional ASMs, particularly clobazam, and its variable oral bioavailability complicates direct translation of experimental doses. Accordingly, future investigations should integrate pharmacokinetic exposure, EEG-based seizure monitoring, molecular biomarkers and long-term treatment designs. Overall, preclinical rat studies provide a strong scientific foundation for continued investigation of CBD in epilepsy. The next generation of research should move from simple acute seizure screening toward standardized chronic models, exposure-response

analysis, sex- and age-specific studies, rational combination therapy and rigorous assessment of antiepileptogenic potential. Such work may facilitate the development of optimized CBD-based therapeutic strategies for drug-resistant and other difficult-to-treat epilepsies.

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Cite: Aphroz Ansari*, Pushpa Prasad Gupta, Ramsheela, Masoom Rahbar, Cannabidiol In Experimental Epilepsy: A Review of Preclinical Rat Models, Int. J. Med. Pharm. Sci., 2026, 2 (8), 665-677. https://doi.org/10.5281/zenodo.22015345