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# Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures

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ABSTRACT

*Cannabis sativa* has been associated with contradictory effects upon seizure states despite its medicinal use by numerous epilepsy sufferers. We have recently shown that the phytocannabinoid cannabidiol (CBD) reduces seizure severity and lethality in the well-established *in vivo* model of pentylenetetrazole-induced generalised seizures, suggesting that earlier, small-scale clinical trials examining CBD effects in epileptic subjects warrant renewed attention (Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, et al. Cannabidiol displays antiepileptiform and antiseizure properties *in vitro* and *in vivo*. *J Pharmacol Exp Ther* 2010;**332**:569–77). Here, we report the effects of pure CBD (1, 10 and 100 mg/kg) in two other established rodent seizure models, the acute pilocarpine model of temporal lobe seizure and the penicillin model of partial seizure. Seizure activity was video recorded and scored offline using model-specific seizure severity scales. CBD (all doses) significantly reduced the percentage of animals experiencing the most severe pilocarpine-induced seizures. In the penicillin model, CBD (all doses) significantly increased the percentage of seizure-free animals; CBD (100 mg/kg) decreased the percentage of animals experiencing the most severe seizures, decreased median seizure severity and showed a strong trend to reduce mortality. In conclusion, these results extend the anti-convulsant profile of CBD; when combined with a reported absence of psychoactive effects, this evidence strongly supports CBD as a therapeutic candidate for a diverse range of human epilepsies.



## 1. Introduction

Approximately 50 million people worldwide are chronically affected by epilepsy, resulting in an array of health implications and socio-economic burdens.[1] Consequently, Europe alone spent an estimated €15.5 billion on epilepsy in 2004, representing a €33 cost per European inhabitant.[2] This serious neurological disorder typically manifests as recurrent, spontaneous seizures or convulsions with a possible loss of consciousness, resulting from the disturbance in the excitatory–inhibitory equilibrium of neuronal activity.[3] Currently, anti-epileptic drug (AED) treatments can induce a range of undesirable systemic and neurotoxic side effects, with idiosyncratic reactions also prevalent.[4] Furthermore, epilepsy has a significant unmet clinical need, with approximately 30% of epileptic patients experiencing intractable seizures regardless of the currently available AED treatments used.[5] Therefore, continued research to identify new, more effective and better tolerated therapeutic agents or those that have the potential to modify seizure progression is warranted.

Dating back to 4000 BC, *Cannabis sativa* has a long history of medicinal use for the treatment of a variety of disorders such as rheumatism, chronic inflammation and pain management, in addition to control of convulsions.[6] More recently, *C. sativa* has been ascribed both pro-[7] and anti-convulsant effects[8] despite numerous epilepsy sufferers continuing to use *C. sativa* medicinally for seizure control.[9,10] Since $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), the major psychoactive constituent of *C. sativa* was isolated,[11] more than 60 other phytocannabinoids (cannabis-derived components) have also been identified, isolated and shown to possess varied pharmacological activity.[12] One such phytocannabinoid is

*Abbreviations:* $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol; AED, anti-epileptic drug; CBD, cannabidiol; CCTVs, closed-circuit television cameras; CNS, central nervous system; GABA, γ-aminobutyric acid; mAChR, muscarinic acetylcholine receptor; MES, maximal electroshock; NMDA, *N*-methyl-D-aspartate; PTZ, pentylenetetrazole; TLE, temporal lobe epilepsy.

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cannabidiol (CBD), typically the second most prevalent phytocannabinoid in *C. sativa*, whose structure was first described by Mechoulam and Shvo.[13] CBD currently represents the most promising phytocannabinoid candidate for clinical utilization due to its non-psychotropic properties, low toxicity and high tolerability in humans and other animal species.[14–16]

Early preclinical work demonstrated that CBD possesses anti-convulsant properties.[17–19] In rats, CBD was an effective and relatively potent anti-convulsant in the maximal electroshock (MES) and audiogenic seizure models; findings that compared favorably with the clinically used AEDs employed in the same study.[20] In mice, CBD pretreatment prevented tonic convulsions caused by either MES seizures, γ-aminobutyric acid (GABA) antagonists or inhibitors of GABA synthesis, in addition to reliably protecting against 3-mercaptoproprionic acid-induced lethality.[15] Overall, these pre-clinical seizure studies confirmed CBD's anti-convulsant profile and is consistent with an assertion of therapeutic benefits in human epilepsies.

Interestingly and despite these promising pre-clinical results, only one clinical trial has thus far explored the potential anti-convulsant effects of CBD in humans.[14] Fifteen patients experiencing secondary generalised epilepsy with temporal lobe focus that was unresponsive to prescribed AED treatments were recruited. 50% of those patients receiving CBD in conjunction with their existing AEDs remained virtually seizure-free during the supplementation period and the remainder of this patient group exhibited a marked improvement in seizure control.[14] Surprisingly however, no further clinical trials employing CBD have been published.

The therapeutic potential of the phytocannabinoids attracted renewed interest following the discovery and characterisation of the endocannabinoid signaling system that comprises the G protein-coupled cannabinoid $CB_1$ and $CB_2$ receptors, a family of endogenous cannabinoid receptor ligands and several enzymes involved in their metabolism and degradation.[21] Whilst a number of phytocannabinoid actions are mediated via $CB_1$ and/or $CB_2$ receptors,[12,22] including the now well-known CB1 receptor-mediated modulation of epileptiform and seizure activity,[23,24] CBD exhibits negligible affinity for either $CB_1$ and/or $CB_2$ receptors.[12,25,26] Consequently, it is likely that the anti-convulsant effects of CBD described above arise via cannabinoid receptor-independent mechanisms.[22,27,28]

Recently, we have shown that CBD inhibits epileptiform activity *in vitro* and reduces seizure severity and lethality in the pentylenetetrazole (PTZ) model of generalised seizures *in vivo*, strongly supporting reconsideration of the use of CBD in the treatment of human epilepsies.[28] However, in order to strengthen earlier findings and inform appropriate human study design, assessment of the anti-convulsant potential of CBD against untested seizure phenotypes *in vivo* is required.

In this present study, we have investigated whether CBD exerts anti-convulsant effects in the acute pilocarpine-induced model of temporal lobe seizure and the penicillin-induced model of partial seizure. Furthermore, an accelerating rotarod investigation was undertaken to assess the effects of CBD on rodent motor function, providing complementary evidence of CBD's lack of toxicity.

## 2. Materials and methods

### 2.1. Animals

Adult male Wistar Kyoto rats (Harlan, Bicester, UK) were used in both seizure models and the rotarod test described below (acute pilocarpine model of temporal lobe seizure: >P21, 70–110 g; penicillin model of partial seizure: >P40, 250–300 g; accelerating rotarod test for motor function: >P21, 70–110 g). Animals were housed at room temperature on a 12:12-h day/night cycle (lights on at 0800) and given *ad libitum* access to food and water. On days prior to seizure induction, animals were habituated to handling, the test environment and experimental procedures (including, in the penicillin model only, manipulation of the cannula stylet to maintain patency for drug infusion). As these are acute models of seizure, habituation to sham injection protocols was not conducted. All experiments were carried out in accordance with UK Home Office regulations (Animals (Scientific Procedures) Act, 1986).

### 2.2. CBD administration

CBD penetrates the blood–brain barrier such that 120 mg/kg delivered intraperitoneally in Wistar Kyoto rats provides $C_{max}$ = 6.8 μg/g at $T_{max}$ = 120 min and, at the same dosage, no major toxicity, genotoxicity, or mutagenicity has been observed (personal communication with GW Pharmaceuticals Ltd; Study Report UNA-REP-02). Prior to seizure or rotarod protocols, animals received (i.p.) 1, 10 or 100 mg/kg CBD for all seizure experiments or 50, 100 or 200 mg/kg CBD for the accelerating rotarod experiment (GW Pharmaceuticals, Porton Down, Wiltshire, UK). The vehicle employed was a 1:1:18 solution of ethanol, Cremophor (Sigma–Aldrich, Poole, UK) and 0.9% (w/v) NaCl. In each experiment, a group of animals that received volume-matched doses of vehicle alone served as a negative control.

### 2.3. Acute pilocarpine in vivo seizure model

Pilocarpine is a muscarinic acetylcholine receptor agonist that, following systemic administration, causes localised seizure foci in the limbic system consistent with temporal lobe seizures[29] ($n \geq 14$ for each group). 15 min after CBD or vehicle administration, animals were injected with the muscarinic receptor antagonist methylscopolamine (Sigma–Aldrich, Poole, UK; 1 mg/kg; i.p.) to minimise peripheral pilocarpine-induced side-effects. 45 min later, pilocarpine (Sigma–Aldrich, Poole, UK; 380 mg/kg; i.p.) was administered to induce seizures and animal behaviour was monitored for a further 60 min. On completion of the experimental procedure animals were euthanized by $CO_2$ inhalation.

### 2.4. Penicillin in vivo seizure model

Penicillin selectively antagonises $GABA_A$-receptor mediated inhibitory postsynaptic potentials in the central nervous system (CNS).[30,31] Surgical implantations of cannulae were required to enable the focal administration of penicillin G potassium salt (penicillin; Sigma–Aldrich, Poole, UK) directly into the cerebral ventricles to induce partial seizures.[32] Prior to surgery, animals received buprenorphine hydrochloride (Reckitt Benckiser Healthcare (UK) Ltd., Hull, UK; 1 mg/kg; s.c.), chlorpromazine hydrochloride (Sanofi Aventis, Guildford, UK; 0.12 mg/kg; i.m.) and ketamine hydrochloride (Fort Dodge Animal Health Ltd., Southampton, UK; 1 mg/kg, s.c.) anaesthesia before being placed in a stereotaxic frame (ASI Instruments Inc., MI, USA). After cranial midline incision, each animal was implanted with a 26-gauge cannula with stainless steel guide (Bilaney Consultants Ltd., Kent, UK) using flat-skull stereotaxic technique into the right lateral cerebral ventricle. In all experiments, bregma was used as a reference point and implantation co-ordinates were taken from the atlas of Paxinos and Watson[33] (lateromedial: −0.1 cm; anteroposterior: +0.16 cm; dorsoventral: −0.4 cm). After fixation to the skull with three stainless steel screws (1 mm diameter; Bilaney Consultants Ltd., Kent, UK) and dental cement (Stoelting Inc., IL, US), each cannula was sealed with a stylet to maintain patency. Post-operatively and following examination, buprenorphine hydrochloride (1 mg/kg,

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s.c.) and 0.9% (w/v) NaCl (1 ml, s.c.) were administered as required. Animals were housed individually and allowed at least one week to recover from surgery.

One hour after CBD administration, 150 IU penicillin was infused into the right lateral ventricle in 1.5 μl 0.9% (w/v) NaCl to induce partial seizures ($n = 12$ for each group). Intracerebroventricular infusions were made by attaching the implanted cannula (Bilaney Consultants Ltd., Kent, UK) to a 10 μl Hamilton syringe (Fisher Scientific, Loughborough, UK; infusion rate 1.5 μl/min) via a polyethylene tube (Bilaney Consultants Ltd., Kent, UK). Animal behaviour was then monitored for 120 min after penicillin administration. On completion of the experimental procedure, animals were euthanized by $CO_2$ inhalation before immediate infusion of 2 μl pontamine sky blue (3% in $dH_2O$; Sigma–Aldrich, Poole, UK) into the right lateral ventricle for later verification of cannulae positions (conducted blind with respect to seizure scoring results).

### 2.5. Seizure analysis

An observational system utilising closed-circuit television cameras (CCTVs)[34] was used to monitor the behaviour of up to five animals simultaneously and was started prior to CBD administration. Input from CCTVs was managed on a PC and recorded by Zoneminder (v1.2.3; Triornis Ltd., Bristol, UK) software before post-processing to yield complete videos for each animal. Videos of seizure behaviour were scored offline according to modified seizure severity scales appropriate for the acute pilocarpine[35] (Table 1) and penicillin models (Table 2; adapted from Bostanci and Bagirici[32]) using Observer Video-Pro software (Noldus, Wageningen, The Netherlands). Specific markers of seizure behaviour and development were assessed and compared between vehicle control and CBD groups. The percentage of animals that developed the two most severe seizure states was noted for each seizure model (see Tables 1 and 2). In addition, the mean number of incidences of each state that occurred within the total recording period was calculated. Finally, the median severity, the percentage of animals that remained seizure-free (severity score = 0), and the percentage mortality in each group was determined for each seizure model.

### 2.6. Accelerating rotarod test for motor function

An accelerating rotarod apparatus (Panlab/Harvard Apparatus, Holliston, USA) was used to assess CBD effects on rodent motor function with the order of drug administration randomised using a standard Latin square design. Each animal received either CBD (50, 100 or 200 mg/kg) or vehicle on a given experimental day ($n = 12$ for each group) with a two day rest period between successive treatments. 60 min after CBD or vehicle administration, animals were placed on the rotarod that linearly increased rotation speed from 4 to 40 rpm during a 300 s period. An accelerating protocol was employed to eliminate the need for habituation to the rotarod (based upon Baytan et al.[36]). Latency to fall from the rotarod in seconds was compared between vehicle control and CBD groups to assess motor function. Each animal undertook three accelerating rotarod runs per experimental day and was permitted a 5 min recovery between each run to avoid any fatigue-induced decline in motor performance.

**Table 1**
Severity scoring scale for acute pilocarpine-induced temporal lobe seizures.[35]

| Seizure score | Behavioural expression | Righting reflex |
|---|---|---|
| *Acute pilocarpine-induced temporal lobe seizures* | | |
| 0 | No change in behaviour | Preserved |
| 1 | Mouth clonus | Preserved |
| 2 | Unilateral forelimb clonus | Preserved |
| 3 | Bilateral forelimb clonus | Preserved |
| 4 | Bilateral forelimb clonus with rearing and falling | Preserved |
| 5 | Tonic–clonic seizure | Lost |

**Table 2**
Modified severity scoring scale for penicillin-induced partial seizures adapted from Bostanci and Bagirici.[32]

| Seizure score | Behavioural expression | Righting reflex |
|---|---|---|
| *Penicillin-induced partial seizures* | | |
| 0 | No change in behaviour | Preserved |
| 1 | Wild running/leaping | Preserved |
| 2 | Myoclonic phase | Preserved |
| 3 | Unilateral forelimb clonus | Preserved |
| 4 | Bilateral forelimb clonus | Preserved |
| 5 | Tonic–clonic seizure with postural control retained | Preserved |
| 6 | Tonic–clonic seizure without postural control | Lost |

### 2.7. Statistical analysis

All statistical procedures were performed using SPSS 15.0.0 software (SPSS Inc., Chicago, IL, USA). Differences between groups for the mean number of occurrences of each seizure state and median seizure severity values were assessed using one-way analysis of variance (ANOVA) with a post-hoc Tukey test. Differences between groups for the percentage of animals that remained seizure-free, percentage of animals that developed the most severe seizure states and percentage mortality were assessed using a nonparametric binomial test. Differences between groups for motor function were assessed using a two-way ANOVA with 'latency' and 'run' as factors. In all cases, $P \leq 0.05$ was considered significant.

## 3. Results

### 3.1. Acute pilocarpine model of temporal lobe seizure

In the acute pilocarpine model, 1 and 100 mg/kg CBD had no effect on percentage mortality when compared to vehicle ($P > 0.1$), although 10 mg/kg CBD significantly increased percentage mortality ($n = 15, P \leq 0.05$). However, when the severity of pilocarpine-induced seizures is considered, CBD had neither a pro- nor anti-convulsant effect as all animal groups reached a median severity score of 5 ($F_{3,56} = 1.902$, $P = 0.140$). Furthermore, no CBD doses had an effect on the percentage of animals that remained seizure-free, with all animals experiencing a pilocarpine-induced seizure event during the experiment.

All doses of CBD (1, 10 and 100 mg/kg) significantly reduced the percentage of animals manifesting with bilateral forelimb clonus with rearing and falling (seizure score of 4; Table 1); a reduction from 71% (vehicle-dosed animals) to 43% following 1 mg/kg CBD ($n = 14$, $P \leq 0.05$), 47% following 10 mg/kg CBD ($n = 15$, $P \leq 0.05$), and 29% following 100 mg/kg CBD ($n = 14$, $P \leq 0.01$; Fig. 1a). However, despite the significant reduction in the number of animals exhibiting this seizure state, no significant CBD effect upon the mean number of occurrences for each animal of this state was seen at any dose ($F_{3,56} = 0.575$, $P = 0.634$; Fig. 1b). Thus, although CBD significantly decreased the percentage of animals exhibiting bilateral forelimb clonus with rearing and falling, it did not significantly decrease the number of occurrences.

Analysis of tonic–clonic seizure events revealed a significant decrease in the percentage of animals that developed this most severe state (seizure score 5; Table 1), which was reduced from 86%

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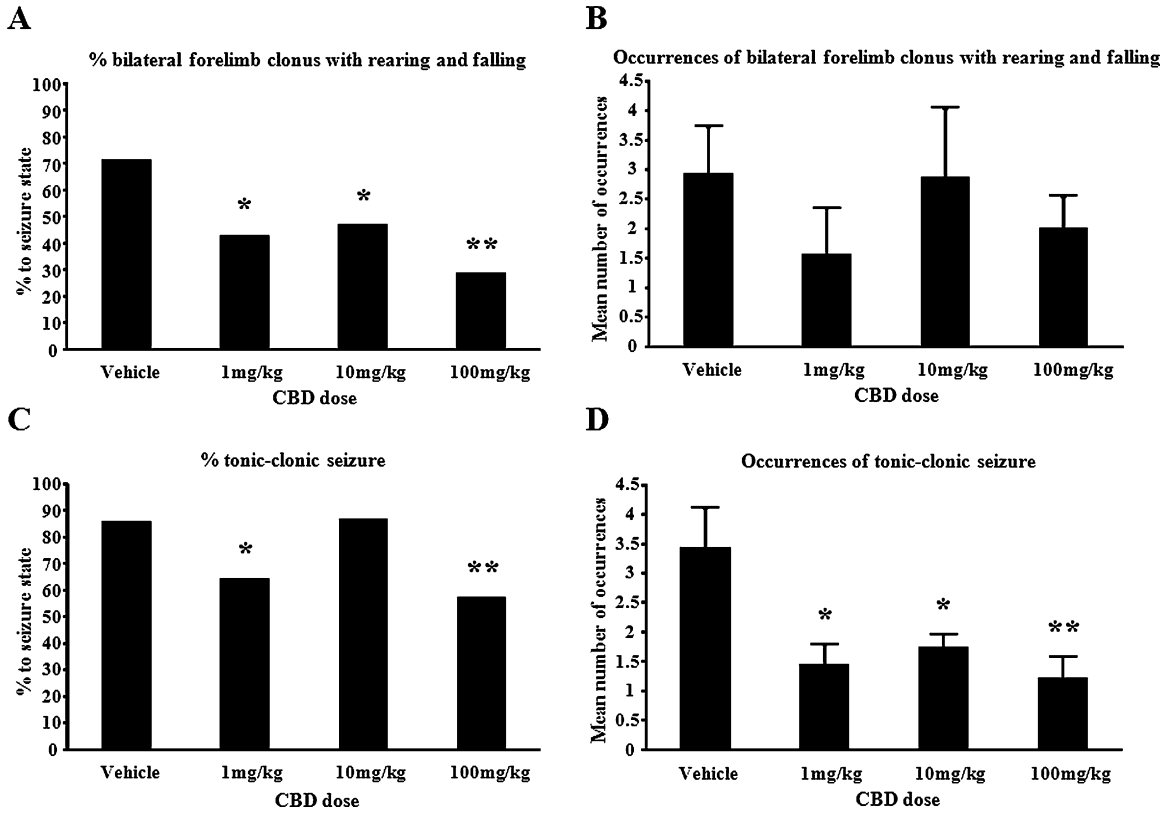


**Fig. 1.** Cannabidiol (CBD) attenuates acute pilocarpine-induced temporal lobe seizures. Percentage reaching: (A) bilateral forelimb clonus with rearing and falling seizures, (C) tonic–clonic seizures. Mean number of occurrences: (B) bilateral forelimb clonus with rearing and falling seizures, (D) tonic–clonic seizures. Each data set $n \geq 14$ animals. Statistical testing was performed using either a binomial test (panels A and C) or one-way ANOVA with post-hoc Tukey test (panels B and D). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

in vehicle-dosed animals to 64% following 1 mg/kg CBD treatment ($n = 14$, $P \leq 0.05$) and 57% following 100 mg/kg CBD treatment ($n = 14$, $P \leq 0.01$). However, no reduction in the percentage of animals that developed tonic–clonic seizures was seen following 10 mg/kg CBD treatment (87%; $P > 0.1$; Fig. 1c). CBD significantly reduced the occurrence of tonic–clonic seizure state seizures at all doses ($F_{3,56} = 5.306$, $P = 0.003$). For individual CBD doses, occurrence was decreased from $3.4 \pm 0.7$ in vehicle-dosed animals to $1.4 \pm 0.4$ following 1 mg/kg CBD treatment ($n = 14$, $P \leq 0.05$), $1.7 \pm 0.2$ following 10 mg/kg CBD treatment ($n = 15$, $P \leq 0.05$) and $1.2 \pm 0.4$ following 100 mg/kg CBD treatment ($n = 14$, $P \leq 0.01$; Fig. 1d). Thus, administration of 1 mg/kg and 100 mg/kg CBD significantly reduced the percentage of animals exhibiting tonic–clonic seizures, whilst CBD administration at all doses significantly reduced the mean number of occurrences of those animals reaching this state.

It is clear that in some of our measures, administration of CBD failed to produce dose-dependent effects. In general, both 1 mg/kg and 100 mg/kg CBD produced significant anti-convulsant effects whilst 10 mg/kg CBD often produced little or no beneficial actions. The lack of dose-dependency in this model will be considered in the discussion.

### 3.2. Penicillin model of partial seizure

CBD (100 mg/kg) produced a reduction in the mortality of animals exhibiting penicillin-induced partial seizures which was just below significance levels when compared to vehicle-dosed animals ($P = 0.057$; Fig. 2a). An analysis of seizure severity in penicillin-treated animals revealed that vehicle-dosed animals reached a median seizure severity score of 6 (tonic–clonic seizure without postural control; the most severe on the scoring scale; Table 2) (Fig. 2b). In contrast, CBD treatment significantly reduced seizure severity ($F_{3,47} = 3.469$, $P = 0.024$) such that animals that received 100 mg/kg CBD exhibited a median seizure severity score of 0 (no change in behaviour; $n = 12$, $P \leq 0.05$; Fig. 2b); although 1 and 10 mg/kg CBD doses had no effect upon seizure severity ($n = 12$; $P > 0.1$). Furthermore, all CBD doses (1, 10 and 100 mg/kg) significantly increased the percentage of animals that remained seizure-free for the duration of the experiment when compared to vehicle-dosed animals ($P \leq 0.001$; all doses; Fig. 2c). This reduction in seizure severity was associated with a marked decrease in the percentage of animals that developed tonic–clonic seizures whilst retaining postural control (seizure score 5, Table 2), which was significantly reduced from 58% in vehicle-dosed animals to 25% following 100 mg/kg CBD ($n = 12$, $P \leq 0.05$; Fig. 3a). However, no significant CBD effect upon this measure was observed at lower doses of 1 mg/kg and 10 mg/kg CBD. Moreover, despite the reduction in the number of animals exhibiting tonic–clonic seizures with retained postural control, CBD did not affect the mean number of occurrences of this state ($F_{3,47} = 1.012$, $P = 0.397$) (Fig. 3b). Therefore, despite the percentage of animals exhibiting this state decreasing, those that reached this state exhibited the same number of occurrences as vehicle-dosed animals.

Investigation of tonic–clonic seizures without postural control (seizure score 6, Table 2) revealed a marked decrease in the percentage of animals that developed this most severe state which was reduced from 58% in vehicle-dosed animals to 33% following both a 1 mg/kg CBD and 10 mg/kg CBD ($n = 12$ animals per group, $P \leq 0.1$) and, more significantly, was completely abolished following 100 mg/kg CBD ($n = 12$, $P \leq 0.001$; Fig. 3c). CBD also reduced the mean number of occurrences of a tonic–clonic seizure without postural control

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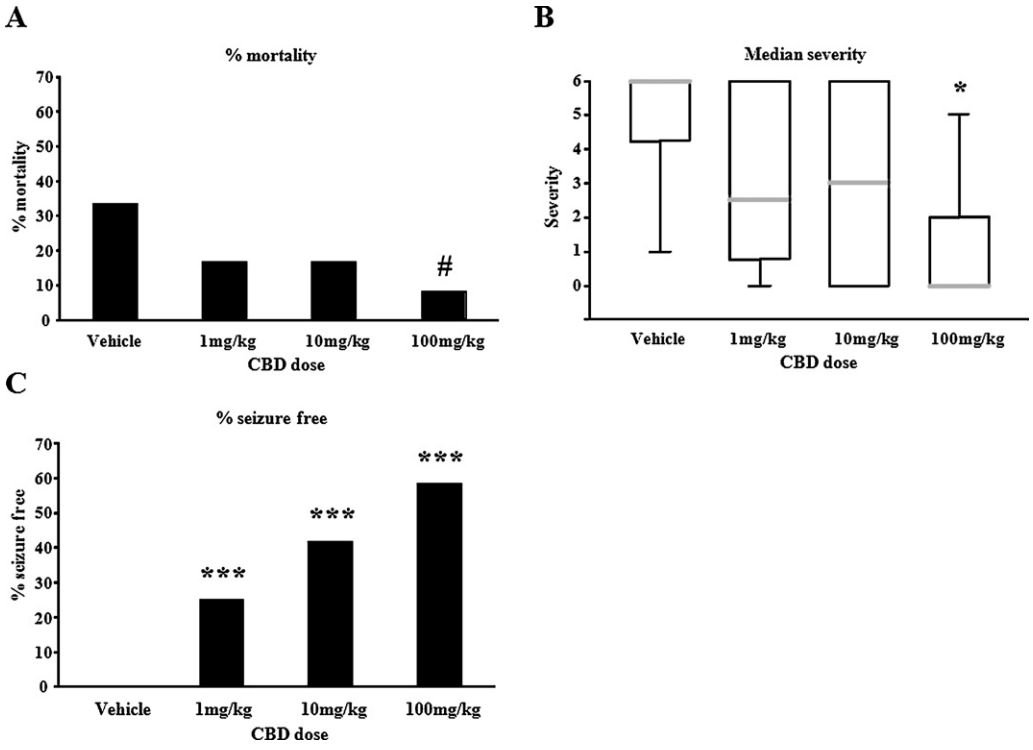


**Fig. 2.** Cannabidiol (CBD) attenuates penicillin-induced partial seizures. (A) Percentage mortality. (B) Median seizure severity. Grey lines show median severity, black boxes show 25th and 75th percentiles and error bars indicate 10th and 90th percentiles. (C) Percentage of seizure-free animals. Each data set $n = 12$ animals. Statistical testing was performed using either a binomial test (panels A and C) or one-way ANOVA with post-hoc Tukey test (panel B). $^{\#}P \leq 0.1$, $^{*}P \leq 0.05$, $^{***}P \leq 0.001$.

developing ($F_{3,47} = 4.366$, $P = 0.009$) from $1.5 \pm 0.5$ in vehicle-dosed animals to $0.4 \pm 0.2$ following 10 mg/kg CBD ($n = 12$, $P \leq 0.1$) and was completely abolished following 100 mg/kg CBD ($0 \pm 0$, $n = 12$, $P \leq 0.01$; Fig. 3d).

### 3.3. Accelerating rotarod test for motor function

In the accelerating rotarod test, CBD (50, 100 or 200 mg/kg) had no effect on the latency to fall when compared to vehicle-dosed

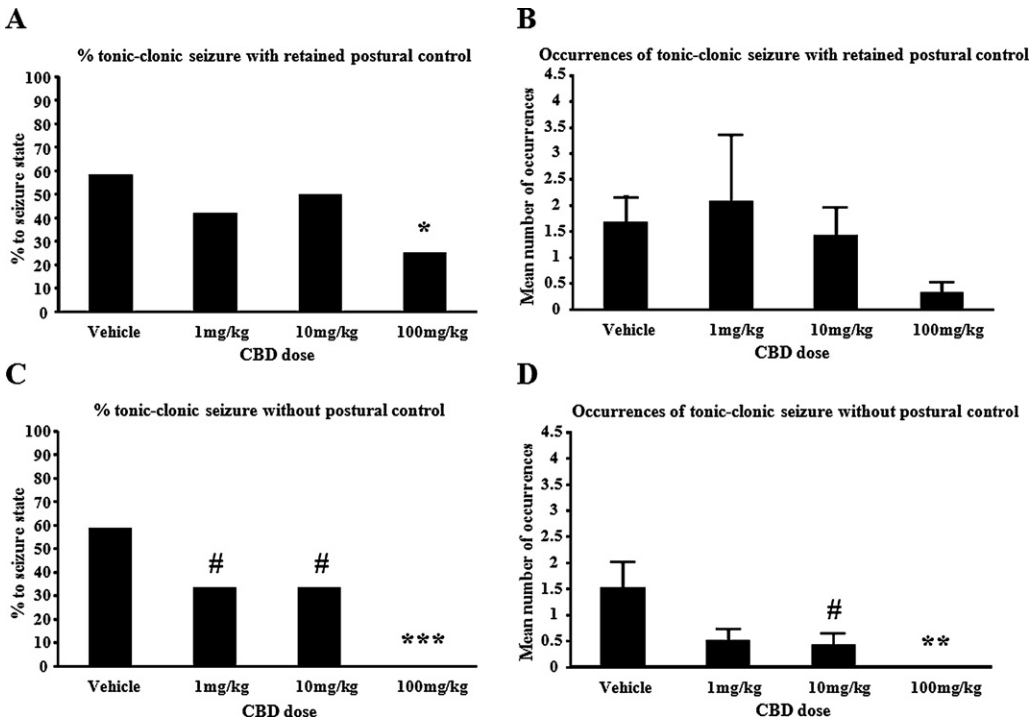


**Fig. 3.** Effects of cannabidiol (CBD) on penicillin-induced partial tonic–clonic seizures. Percentage reaching: (A) tonic–clonic seizures with retained postural control, (C) tonic–clonic seizures without postural control. Mean number of occurrences: (B) tonic–clonic seizures with retained postural control, (D) tonic–clonic seizures without postural control. Each data set $n = 12$ animals. Statistical testing was performed using either a binomial test (panels A and C) or one-way ANOVA with post-hoc Tukey test (panels B and D). $^{\#}P \leq 0.1$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

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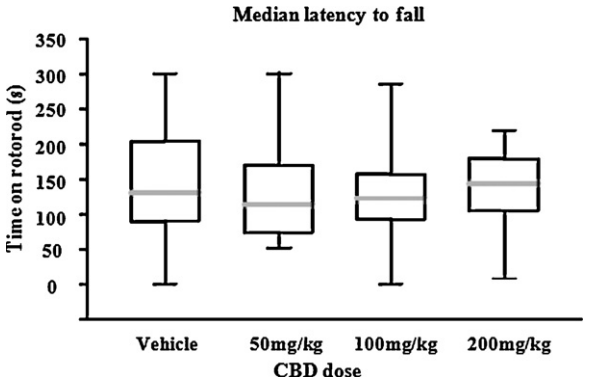


**Fig. 4.** Box plot showing the latency to fall for vehicle and CBD (50, 100 and 200 mg/kg) treated animals on the accelerating rotarod test for motor function. Grey lines show median latency to fall from the rotarod, black boxes show 25th and 75th percentiles and error bars indicate 10th and 90th percentiles. Each data set $n = 12$ animals. Statistical testing was performed using a two-way ANOVA, revealing no significant differences in motor function.

animals ($F_{3,99} = 0.568$, $P = 0.637$; Fig. 4). Furthermore, neither significant differences between runs were found ($F_{2,33} = 1.378$, $P = 0.266$) nor was any significant interaction between drug and run found ($F_{6,99} = 0.284$, $P = 0.943$). These data indicate that CBD had no adverse effect on motor function that may compromise data collected in this study.

## 4. Discussion

In the present study, we examined the anti-convulsant potential of CBD, the most prevalent non-psychoactive phytocannabinoid found in *C. sativa*, in models of temporal lobe and partial seizures. In the acute pilocarpine model, CBD showed modest anti-convulsant effects, significantly lowering the incidence of the most severe seizures. However, these findings were not reflected in effects on mortality and severity. In contrast, strong anti-convulsant CBD effects were seen in the penicillin model of partial seizure. CBD reduced mortality and had significant anti-convulsant effects by reducing median seizure severity, increasing the percentage of animals that remained seizure-free, decreasing the percentage of animals experiencing both tonic–clonic seizure states and decreasing the mean number of occurrences of tonic–clonic seizures without postural control. Furthermore, the accelerating rotarod test revealed that CBD exerted no adverse effects on motor function. Thus, here we demonstrate CBD's potential as a novel AED in temporal lobe and partial seizures, complementing previous research in other *in vivo* animal models.[15,20,28]

### 4.1. Acute pilocarpine model of temporal lobe seizure

In the temporal lobe model, acute pilocarpine-induced seizure development is thought to be dependent on the activation of the muscarinic acetylcholine receptor (mAChR) M1 subtype since $M1R^{-/-}$ mice do not develop seizures in response to pilocarpine.[37] However, once initiated, seizure maintenance is dependent on *N*-methyl-D-aspartate (NMDA) receptor activation with centrally acting muscarinic antagonists (e.g. atropine) failing to abolish pilocarpine-induced seizures.[38] Although CBD did not reduce mortality in the acute pilocarpine model, this could be a consequence of the well-reported high mortality rate associated with this model[39–42] that is attributed to an increased likelihood of respiratory failure associated with severe tonic–clonic seizure states compared to other models. Any respiratory depression could obscure or confound potentially mitigating drug effects upon mortality. Conversely, the lack of CBD effects upon mortality, median seizure severity and percentage of animals that remained seizure-free could also have occurred as a result of CBD specifically lacking activity against M1 mAChR-mediated seizure initiation. Since CBD significantly reduced the number of occurrences of tonic–clonic seizures but did not affect seizure initiation, it is plausible that CBD's anti-convulsant effects mitigate against NMDAR-mediated maintenance, but not mAChR-mediated initiation of acute pilocarpine-induced seizures.

In models of temporal lobe epilepsy (TLE), manifestation of the more severe seizure states (e.g. tonic–clonic seizures) is correlated with the eventual development of spontaneous recurrent excitation.[43] Consequently, the CBD-induced reduction in the percentage of animals developing such severe seizure states suggests that further investigation of CBD effects in the chronic pilocarpine model of spontaneously recurrent seizures is warranted. Moreover, the lack of an apparent dose dependent CBD effect in this model may represent either an idiosyncratic and model-specific difference or a pertinent issue for further investigation during clinical development. Consequently, an assessment of CBD against spontaneous recurrent and secondarily generalised seizures modelled on TLE may prove worthwhile since Cunha and colleagues[14] reported beneficial CBD effects in human epileptic patients displaying secondary generalised epilepsy with a temporal lobe focus. CBD could also hold additional benefits for TLE patients that exhibit a high comorbid association with depression as a result of decreased serotonergic function.[44,45] CBD has shown agonistic properties at 5-$HT_{1A}$ receptors *in vitro* resulting in increased serotonergic function, albeit at concentrations of $>10$ μM.[46] Furthermore, anecdotal reports suggest *C. sativa* can alleviate symptoms of depression in humans.[47] Therefore, CBD could potentially have beneficial effects upon the depression associated with TLE.

### 4.2. Penicillin model of partial seizure

In the penicillin model of partial seizure, penicillin administration into a cerebral ventricle leads to the local suppression of GABA-mediated inhibitory neurotransmission (overall causing disinhibition of the local circuitry) and, consequentially, partial seizures. In the present study, we have shown that CBD exerts clear anti-convulsant effects with significant reductions in median seizure severity, significant increases in the percentage of animals that remained seizure-free, and a clear trend to reduce mortality. The anti-convulsant effects in this model are comparable to data previously reported, showing CBD (100 mg/kg) to be effective in the PTZ model of generalised seizure.[28] Previously Consroe and colleagues using several other seizure models found CBD to be exerting an anti-convulsant effect via the disinhibition of GABA, resulting in a proposed GABA-related mechanism of action.[15] Therefore, CBD could also be exerting its beneficial effects here in the penicillin model through the same GABA related mechanism of action. However, this provides only an indirect assessment, so further investigation is therefore required before definitive mechanistic conclusions regarding CBD's anti-convulsant effects can be drawn.

### 4.3. Accelerating rotarod test for motor function

Although CBD has previously been reported to be devoid of motor side-effects,[14–16] we investigated CBD effects upon performance in the accelerating rotarod test across a dose range that included those used in the present seizure experiments. We demonstrate that CBD had no effect on motor function at doses up to 200 mg/kg. In contrast, all AEDs licensed for clinical use in the

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UK cause significant motor side-effects emphasising the advantage of CBD as a potential clinical anti-convulsant.[48]

### 4.4. Mechanisms

The specific cellular mechanisms underlying lethality in both the pilocarpine and penicillin models are unknown, so we are presently unable to rationalise the observed CBD-induced reduction in mortality in the penicillin model of seizure only. However, CBD has a significant anti-convulsant effect by reducing the percentage of animals developing the most severe tonic–clonic seizure states in both models employed. Therefore, in these models, CBD may act preferentially to reduce seizure spread irrespective of its focal origin in the brain.[15] Moreover, if CBD is indeed preventing seizure spread, this is unlikely to also affect normal biophysical signal propagation, as demonstrated in the $Mg^{2+}$-free and 4-aminopyridine *in vitro* hippocampal slice models[28] and further supports the positive side-effect profile of CBD[14–16] which is not shared by most clinically used AEDs.[4]

Consistent with our previous findings,[28] CBD appears to hold the greatest potential for the treatment of partial and generalised seizures, rather than temporal lobe seizures. It is already well known that CBD has only very low affinity for both endogenous $CB_1$ and $CB_2$ receptors[12,25,26] and is therefore likely to be exerting its anti-convulsant activity via cannabinoid receptor-independent mechanisms.[22,27,28]

Thus far, CBD has shown a poly-pharmacological profile, potentially modulating neuronal hyperexcitability via a number of different mechanisms (see also Jones et al.[28]). In this regard, proposed mechanisms include: (1) the bidirectional regulation of $Ca^{2+}$ homeostasis via the mitochondrial $Na^+/Ca^{2+}$-exchanger to either elevate or decrease cytosolic $Ca^{2+}$ levels, dependent on whether the neuron is under normal physiological or a highly-excitable state;[49] (2) agonistic properties at 5-$HT_{1A}$ receptors,[46,50–52] with receptor activation eliciting membrane hyperpolarising responses, consistent with an inhibitory role in seizure generation;[45,53] (3) enhancing endogenous adenosine levels in the CNS by reducing adenosine reuptake,[54,55] thereby increasing inhibitory adenosinergic tone to aid seizure suppression. Moreover, numerous additional cellular and molecular CBD effects and mechanisms of action have also been proposed, but are less likely to be related to CBD's anti-convulsant profile but, for example, have pharmacological relevance in pain, inflammation and cancer (reviewed by Izzo et al.[56]). In summary, CBD's anti-convulsant effects may not be due to be one specific mechanism of action but the result of numerous cannabinoid receptor-independent mechanisms. The understanding of these mechanisms of action will be critical to improve CBD's efficacy, safety profile and to enhance drug combination strategies for this potential anti-convulsant in the future.

### 4.5. Therapeutic potential

We propose that CBD exerts a cumulative anti-convulsant effect; this may be achieved by a poly-pharmacological profile, with CBD simultaneously modulating a number of endogenous systems to attenuate and/or prevent epileptic neuronal hyperexcitability. Importantly, despite numerous potential targets, CBD has an excellent side-effect profile, as revealed in this investigation and others.[14–16] Moreover, CBD may have attractive synergistic or additive effects when co-administered with currently prescribed AEDs, noting that it is compulsory for new therapeutic agents to firstly be co-administered with currently available AEDs. Therefore, adjunctive CBD treatment may potentially have beneficial effects as an anti-convulsant, whilst attenuating the known undesirable side-effects of current AED treatments.

## 5. Conclusions

Overall, we demonstrate the anti-convulsant actions of CBD for the first time in the acute pilocarpine and penicillin models of temporal lobe and partial seizures respectively. These results clearly extend previously published data from other *in vivo* models which point to CBD being of potential therapeutic use (alone or as an adjunct) in the treatment of epilepsies. Future research is now required to investigate the potential synergistic or additive effects of CBD on AEDs and to establish in greater detail this phytocannabinoid's exact anti-convulsant mechanism(s) of action.

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