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(54) **USE OF ONE OR A COMBINATION OF PHYTO-CANNABINOIDS IN THE TREATMENT OF EPILEPSY**

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CPC ***A61K 31/05*** (2013.01); ***A61K 31/352*** (2013.01)

(57) **ABSTRACT**

This invention relates to the use of one or more cannabinoids in the treatment of epilepsy and more particularly to the use of one or a combination of cannabinoids in the treatment of generalized or partial seizure. In one embodiment it relates to the use of the cannabinoid THCV, as a pure or isolated compound, or as a plant extract in which significant amounts of any THC naturally present has been selectively removed. In another embodiment the phytocannabinoid is CBD.

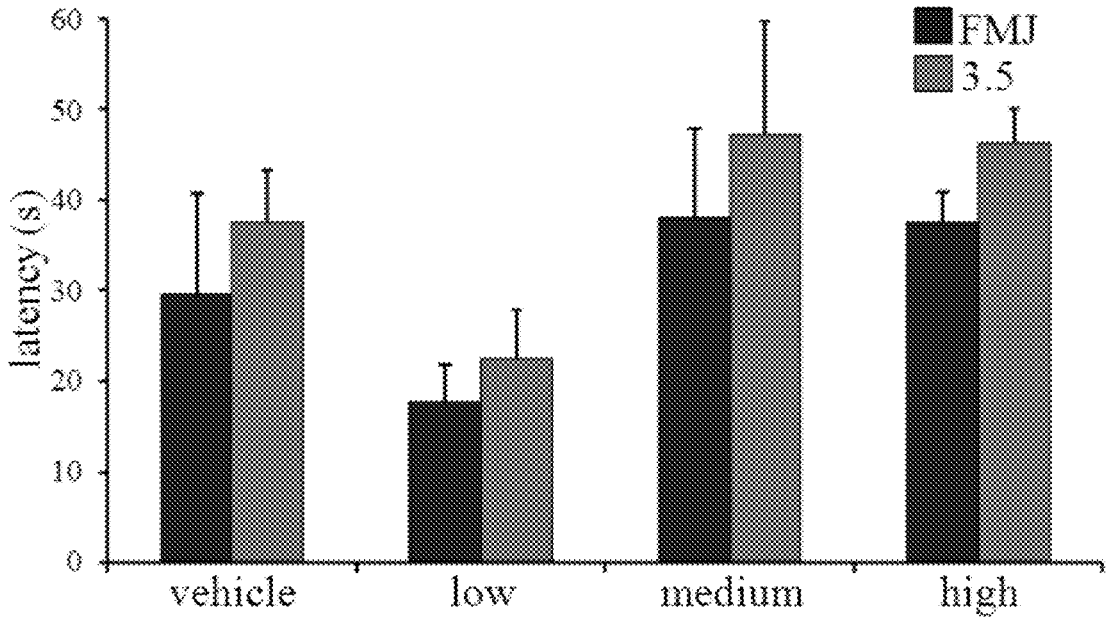


Figure 1

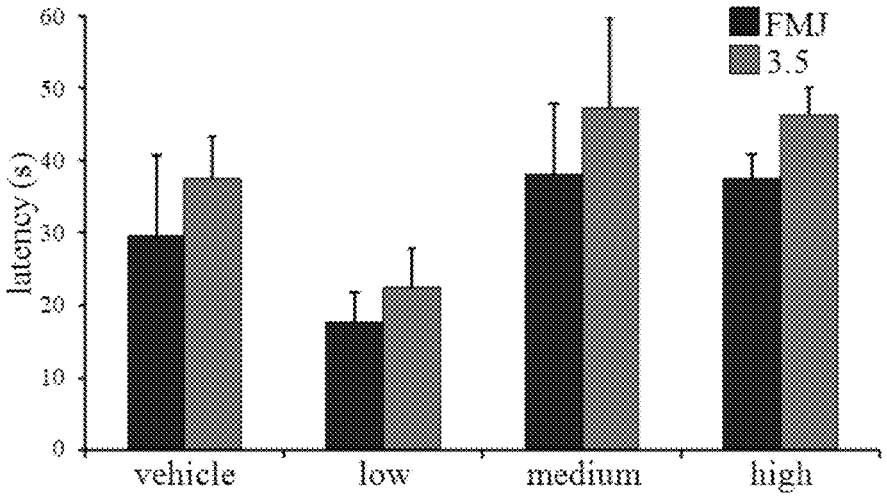


Figure 2

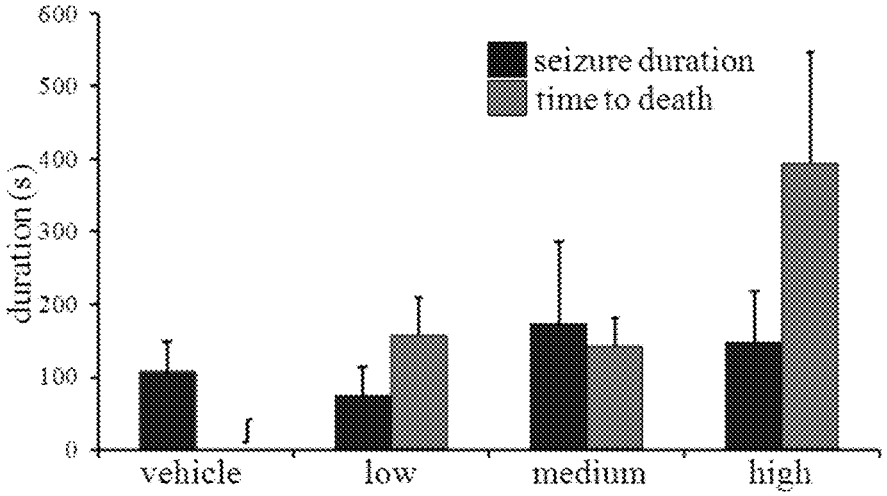


Figure 3

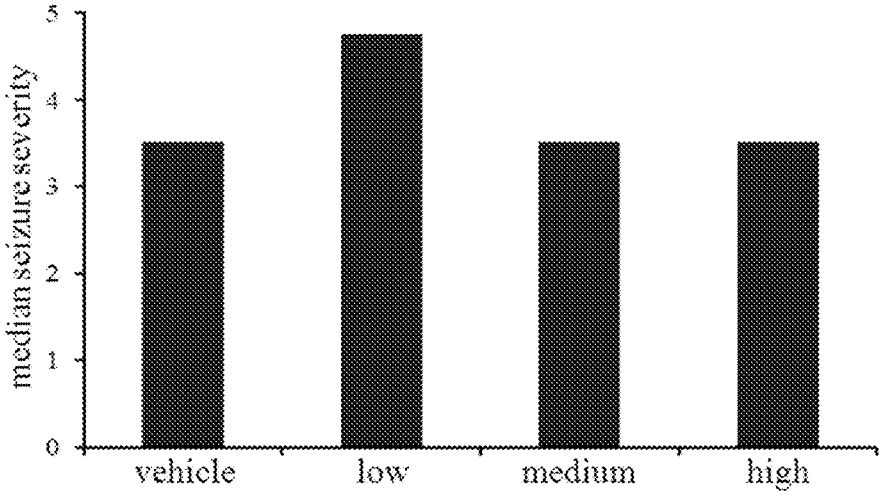


Figure 4

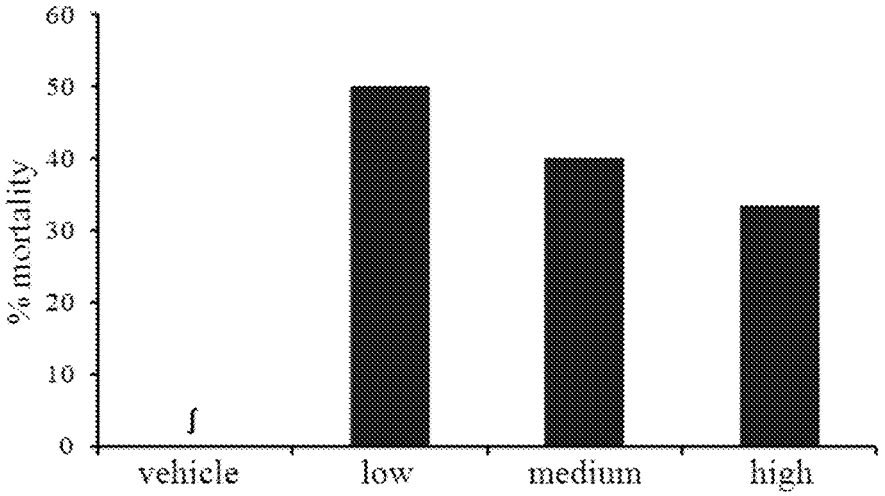


Figure 5

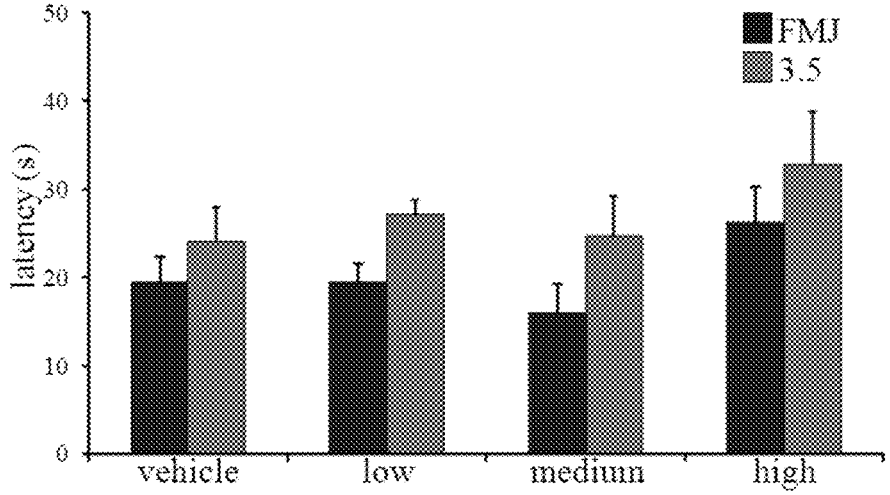


Figure 6

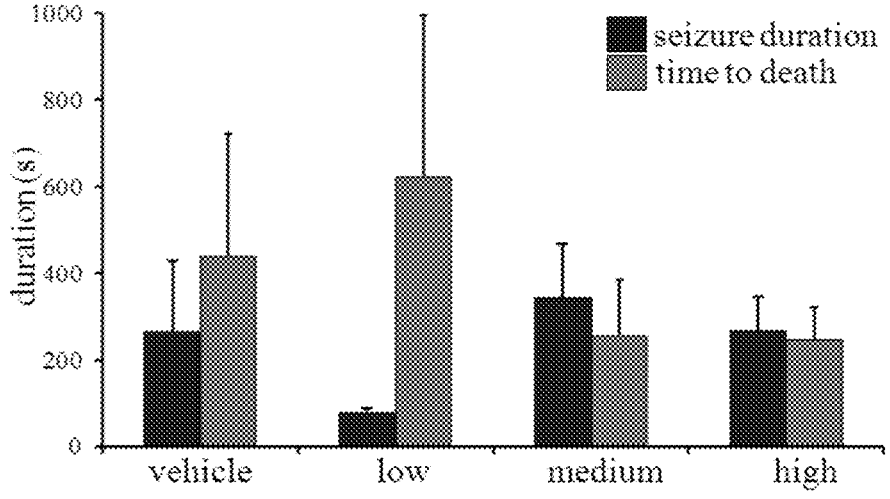


Figure 7

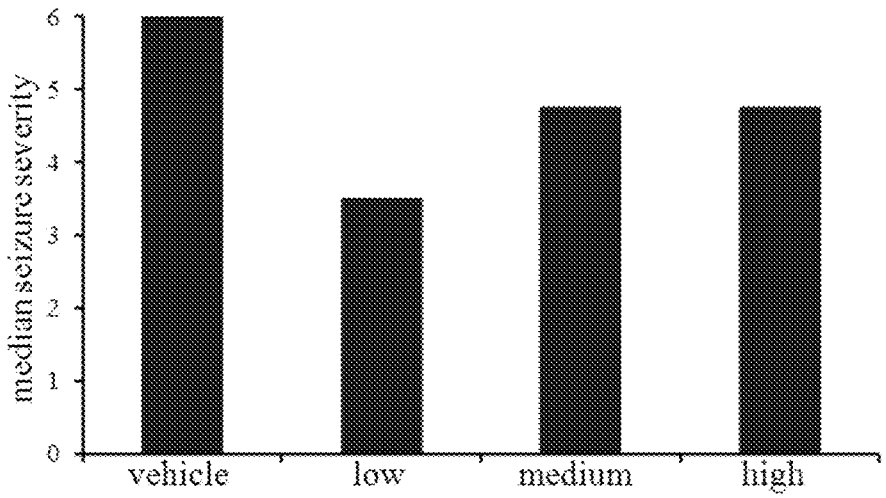
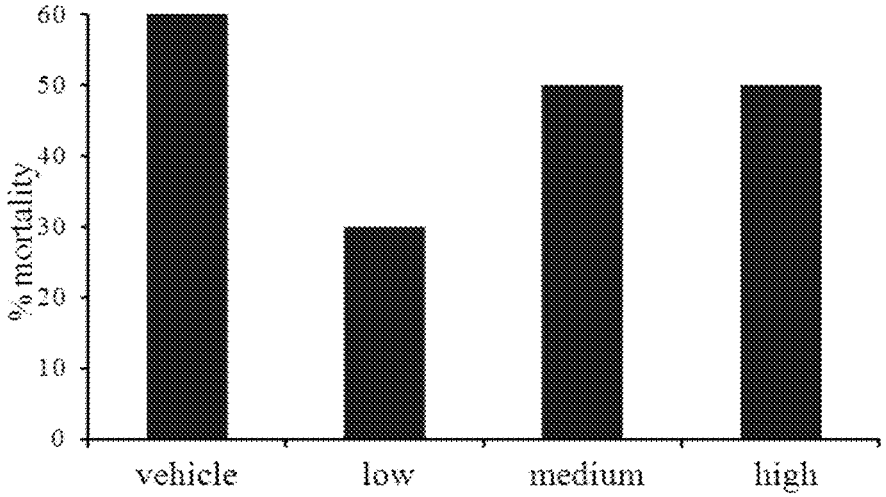


Figure 8



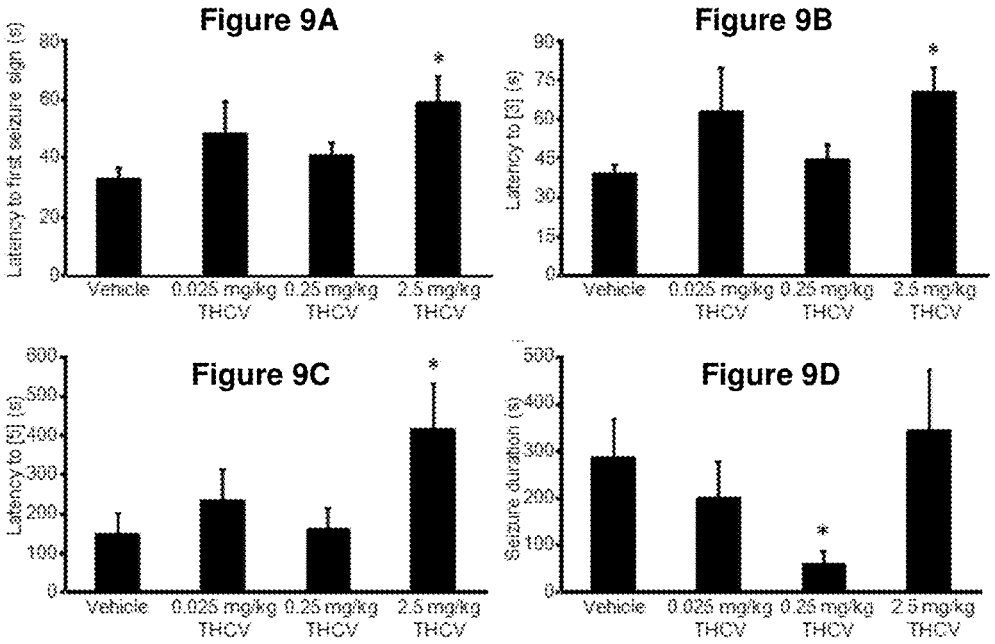


Figure 9A
Latency to first seizure sign (s)
0
20
40
60
80
*
Vehicle
0.025 mg/kg THCV
0.25 mg/kg THCV
2.5 mg/kg THCV
Figure 9B
Latency to [5] (s)
0
15
30
45
60
75
90
*
Vehicle
0.025 mg/kg THCV
0.25 mg/kg THCV
2.5 mg/kg THCV
Figure 9C
Latency to [5] (s)
0
100
200
300
400
500
600
*
Vehicle
0.025 mg/kg THCV
0.25 mg/kg THCV
2.5 mg/kg THCV
Figure 9D
Seizure duration (s)
0
100
200
300
400
500
*
Vehicle
0.025 mg/kg THCV
0.25 mg/kg THCV
2.5 mg/kg THCV

Figure 10A

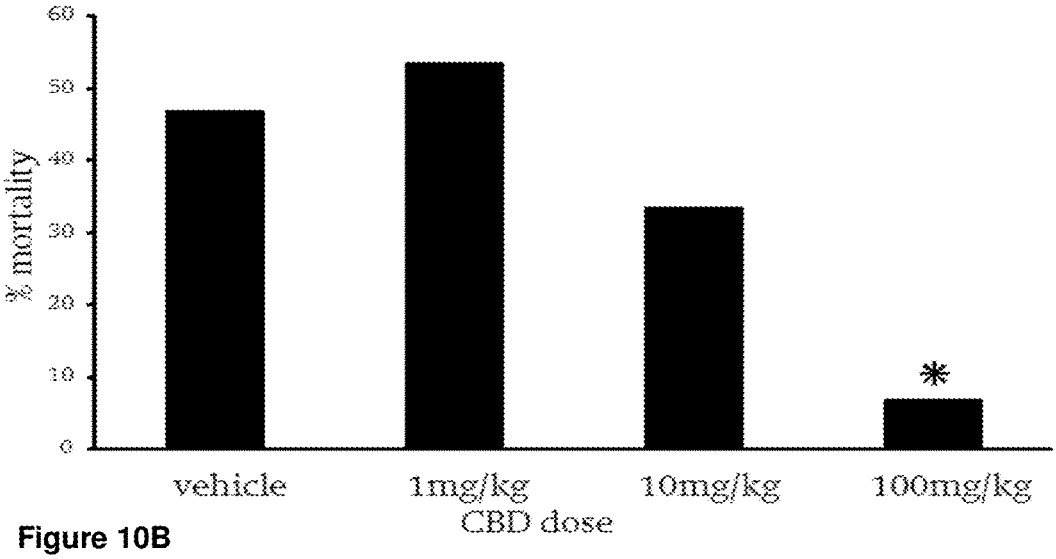
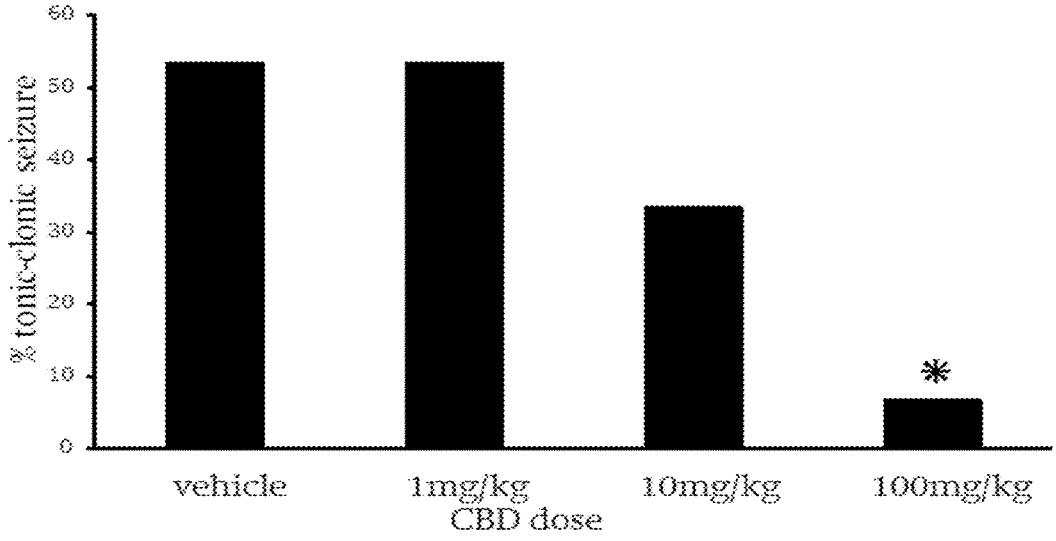


Figure 10B



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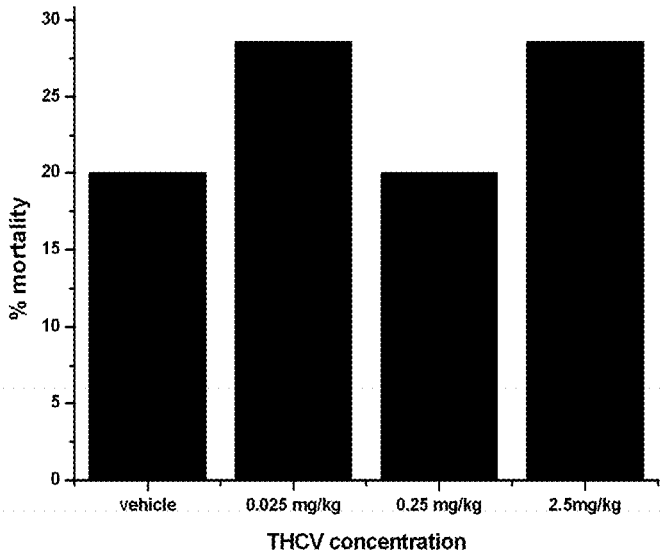


Figure 12

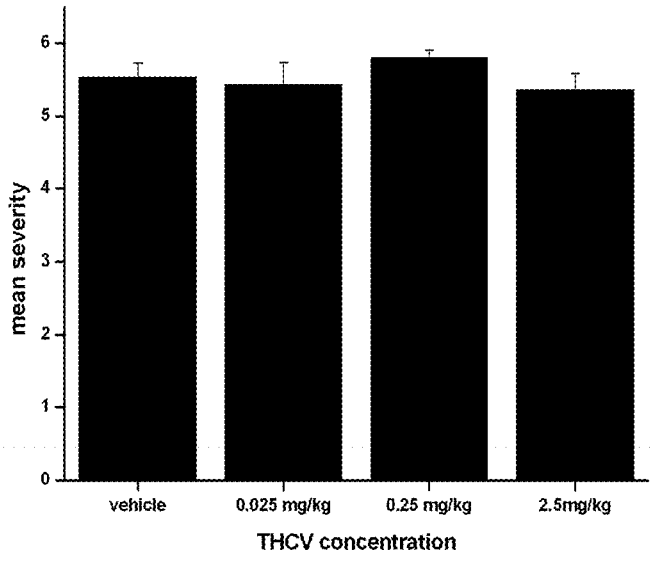


Figure 13A

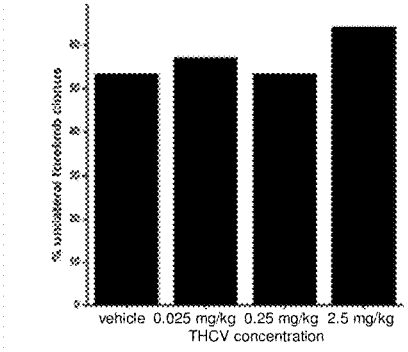


Figure 13B

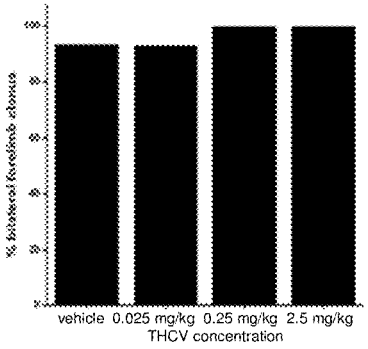


Figure 13C

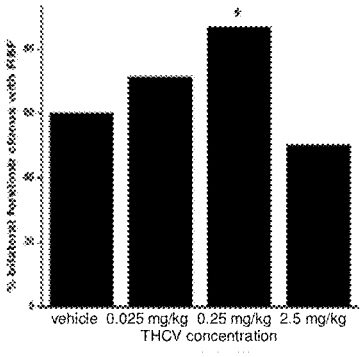
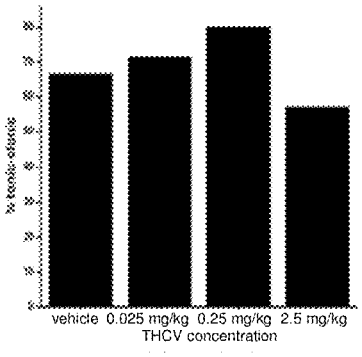


Figure 13D



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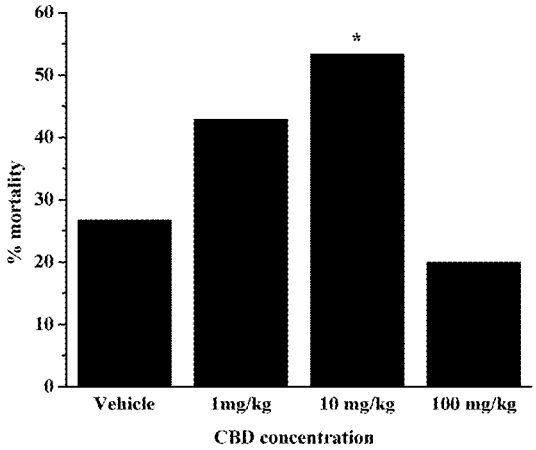

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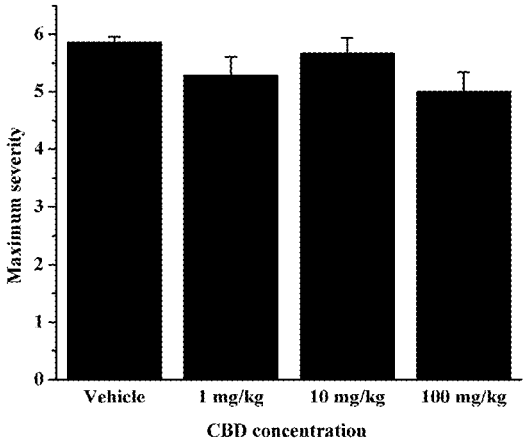


Figure 16A

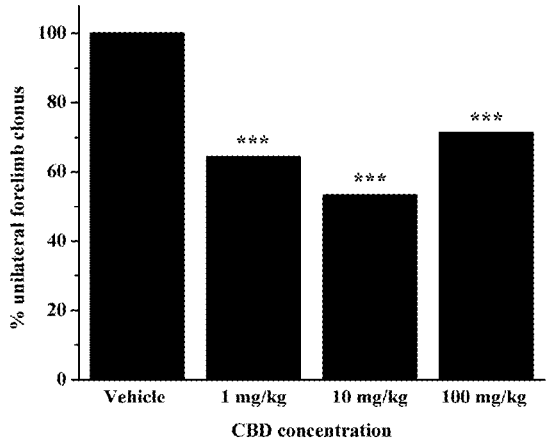


Figure 16B

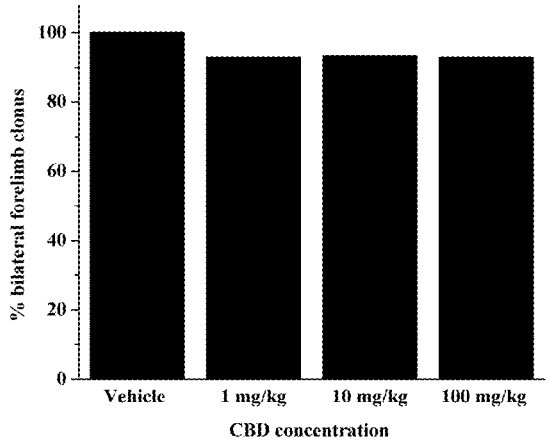


Figure 16C

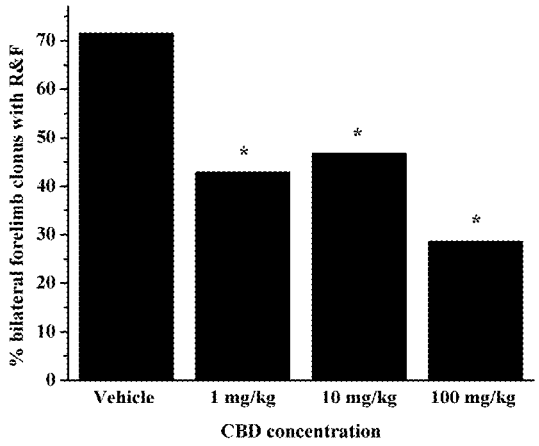


Figure 16D

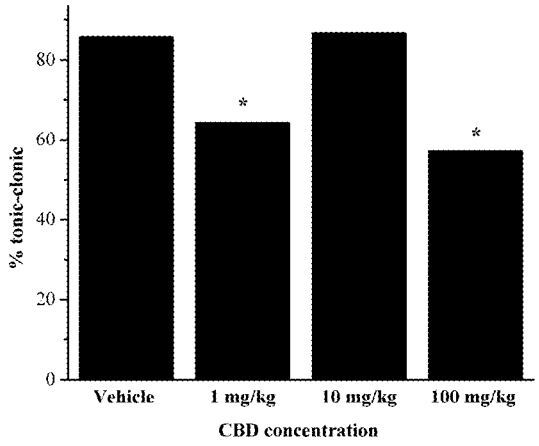
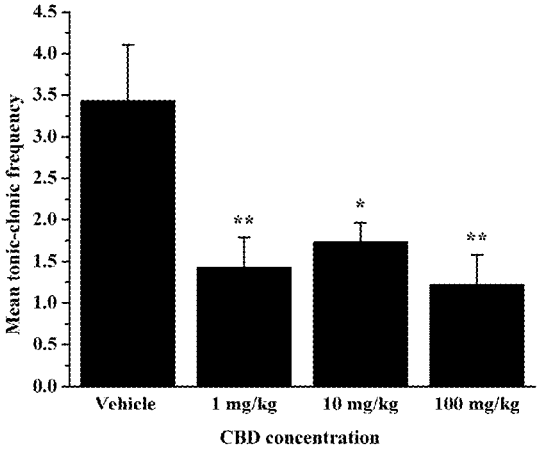


Figure 17



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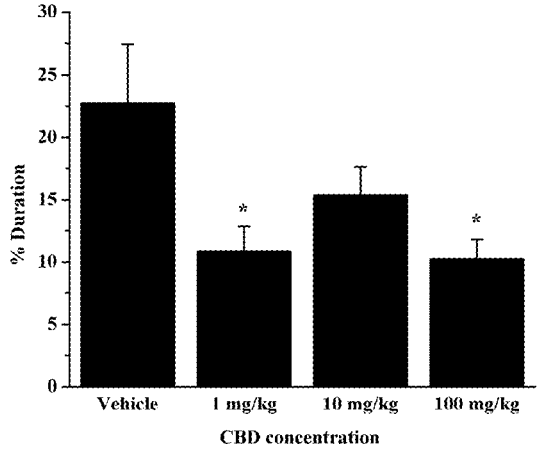


Figure 19A

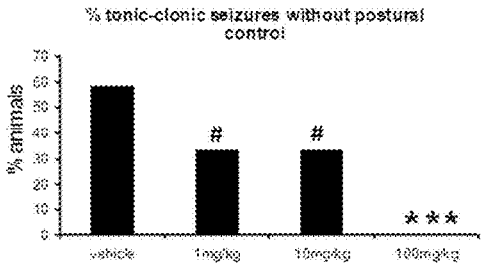


Figure 19B

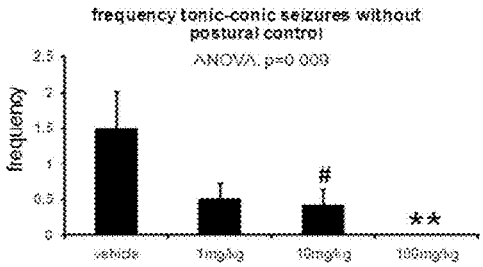


Figure 20A

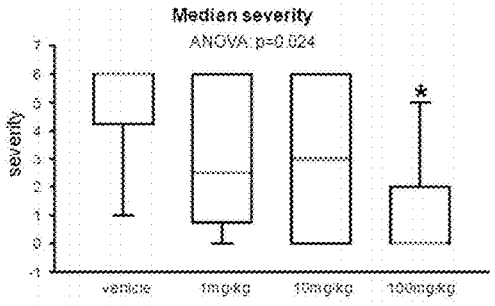


Figure 20B

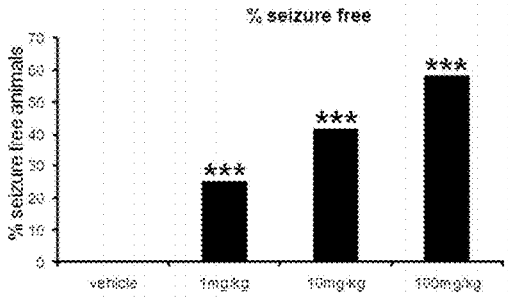
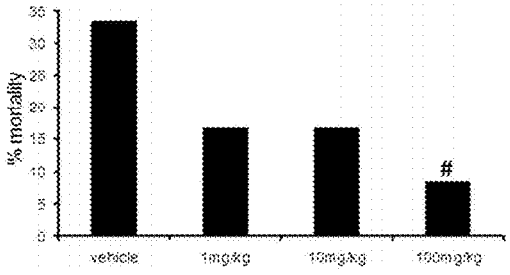


Figure 20C



USE OF ONE OR A COMBINATION OF PHYTO-CANNABINOIDS IN THE TREATMENT OF EPILEPSY

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 14/579,061, filed Dec. 22, 2014, which is a continuation of U.S. patent application Ser. No. 13/380,305, now U.S. Pat. No. 9,066,920, filed Mar. 19, 2012, which is a national stage filing under 35 U.S.C. §371 of international application PCT/GB2010/051066, filed Jun. 29, 2010, which was published under PCT Article 21(2) in English, the entire contents of which are incorporated herein by reference.

FIELD

[0002] This invention relates to the use of one or a combination of phyto-cannabinoids in the treatment of epilepsy and more particularly to the use of tetrahydrocannabivarin (THCV) in the treatment of generalized seizure and/or cannabidiol (CBD) in generalized seizure and/or partial seizures (in contrast to temporal lobe seizures).

BACKGROUND

[0003] Epilepsy is a chronic neurological disorder presenting a wide spectrum of diseases that affects approximately 50 million people worldwide (Sander, 2003). Advances in the understanding of the body's internal 'endocannabinoid' system have lead to the suggestion that some cannabis-based medicines may have the potential to treat this disorder of hyperexcitability in the central nervous system (Mackie, 2006, Wingerchuk, 2004, Alger, 2006).

[0004] Cannabis has been ascribed both pro-convulsant (Brust et al., 1992) and anti-convulsant effects. Therefore, it remains to be determined whether cannabinoids represent a yet to be unmasked therapeutic anticonvulsant or, conversely, a potential risk factor to recreational and medicinal users of cannabis (Ferdinand et al., 2005).

[0005] In 1975 Consroe et al. described the case of young man whose standard treatment (phenobarbital and phenytoin), didn't control his seizures. When he began to smoke cannabis socially he had no seizures. However when he took only cannabis the seizures returned. They concluded that 'marihuana may possess an anti-convulsant effect in human epilepsy'.

[0006] A study by Ng (1990) involved a larger population of 308 epileptic patients who had been admitted to hospital after their first seizure. They were compared to a control population of 294 patients who had not had seizures, and it was found that using cannabis seemed to reduce the likelihood of having a seizure. However this study was criticized in an Institute of Medicine report (1999) which claimed it was 'weak', as 'the study did not include measures of health status prior to hospital admissions and differences in their health status might have influenced their drug use' rather than the other way round.

[0007] In WO02/064109 reference is made to the anti epileptic effects of the cannabinoid cannabidiol (CBD).

[0008] WO 2006/054057 makes reference to the potential use of THCV to treat epilepsy amongst a range of diseases.

[0009] WO2009/007697 discloses formulations containing THCV and CBD.

[0010] Three controlled trials have investigated the anti-epilepsy potential of cannabidiol. In each, cannabidiol was given in oral form to sufferers of generalised grand mail or focal seizures.

[0011] Cunha et al (1980) reported a study on 16 grand mal patients who were not doing well on conventional medication. They received their regular medication and either 200-300 mg of cannabidiol or a placebo. Of the patients who received CBD, 3 showed complete improvement, 2 partial, 2 minor, while 1 remained unchanged. The only unwanted effect was mild sedation. Of the patients who received the placebo, 1 improved and 7 remained unchanged.

[0012] Ames (1986) reported a less successful study in which 12 epileptic patients were given 200-300 mg of cannabidiol per day, in addition to standard antiepileptic drugs. There seemed to be no significant improvement in seizure frequency.

[0013] Trembly et al (1990) reports an open trial with a single patient who was given 900-1200 mg of cannabidiol a day for 10 months. This trial showed seizure frequency was markedly reduced in the patient.

[0014] It is perhaps significant that some 20 years since these trials there has been no further development. This could be down to a number of factors including a general prejudice against medicines based on cannabis. It is also possible that the dose levels used in the trials were not optimal and the applicant has determined that cannabinoids may produce bell shaped dose response curves.

[0015] In addition to the disclosures suggesting CBD may be beneficial there is a report (Davis & Ramsey) of tetrahydrocanibinol (THC) being administered to 5 institutionalized children who were not responding to their standard treatment (phenobarbital and phenoytin). One became entirely free of seizures, one became almost completely free of seizures, and the other three did no worse than before.

[0016] However, there are more than forty recognisable types of epileptic syndrome partly due to seizure susceptibility varying from patient to patient (McCormick and Contreras, 2001, Lutz, 2004) and a challenge is finding drugs effective against these differing types.

[0017] Neuronal activity is a prerequisite for proper brain function. However, disturbing the excitatory—inhibitory equilibrium of neuronal activity may induce epileptic seizures. These epileptic seizures can be grouped into two basic categories: partial and generalised seizures. Partial seizures originate in specific brain regions and remain localised—most commonly the temporal lobes (containing the hippocampus), whereas generalised seizures appear in the entire forebrain as a secondary generalisation of a partial seizure (McCormick and Contreras, 2001, Lutz, 2004). This concept of partial and generalised seizure classification did not become common practice until the International League Against Epilepsy published a classification scheme of epileptic seizures in 1969 (Merlis, 1970, Gastaut, 1970, Dreifuss et al., 1981).

[0018] The International League Against Epilepsy further classified partial seizures, separating them into simple and complex, depending on the presence or the impairment of a consciousness state (Dreifuss et al., 1981).

[0019] The league also categorized generalised seizures into numerous clinical seizure types, some examples of which are outlined below:

[0020] Absence seizures occur frequently, having a sudden onset and interruption of ongoing activities. Additionally, speech is slowed or impeded with seizures lasting only a few seconds (Dreifuss et al., 1981).

[0021] Tonic-clonic seizures, often known as "grand mal", are the most frequently encountered of the generalised seizures (Dreifuss et al., 1981). This generalised seizure type has two stages: tonic muscle contractions which then give way to a clonic stage of convulsive movements. The patient remains unconscious throughout the seizure and for a variable period of time afterwards.

[0022] Atonic seizures, known as "drop attacks", are the result of sudden loss of muscle tone to either a specific muscle, muscle group or all muscles in the body (Dreifuss et al., 1981).

[0023] The onset of epileptic seizures can be life threatening with sufferers also experiencing long-term health implications (Lutz, 2004). These implications may take many forms:

[0024] mental health problems (e.g. prevention of normal glutamatergic synapse development in childhood);

[0025] cognitive deficits (e.g. diminishing ability of neuronal circuits in the hippocampus to learn and store memories);

[0026] morphological changes (e.g. selective loss of neurons in CA1 and CA3 regions of hippocampus in patients presenting mesial temporal lobe epilepsy as a result of excitotoxicity) (Swann, 2004, Avoli et al., 2005)

[0027] It is noteworthy that epilepsy also greatly affects the lifestyle of the sufferer—potentially living in fear of consequential injury (e.g. head injury) resulting from a grand mal seizure or the inability to perform daily tasks or the inability to drive a car unless having had a lengthy seizure-free period (Fisher et al., 2000).

[0028] Three well-established and extensively used in vivo models of acute seizure, which mimic the neuronal activity and consequent physical symptoms that manifest in a seizure suffered by someone with epilepsy, are:

[0029] pentylenetetrazole-induced model of generalised seizures (Obay et al., 2007, Rauca et al., 2004);

[0030] pilocarpine-induced model of temporal lobe (i.e. hippocampus) seizures (Pereira et al., 2007); and

[0031] penicillin-induced model of partial seizures (Bostanci and Bagirici, 2006).

These provide a range of seizure and epilepsy models, essential for therapeutic research in humans.

[0032] It is an object of the present invention to identify phyto-cannabinoids or combinations of phyto-cannabinoids which have use in treating specific forms of seizure associated with epilepsy.

[0033] It is another object of the present invention to determine dose ranges which are likely to prove effective and identify combinations of cannabinoids (as might be present in different cannabis chemotypes or varieties) which are likely to prove more beneficial due to likely differences in their mechanisms of action.

BRIEF SUMMARY OF THE DISCLOSURE

[0034] In accordance with a first aspect of the present invention there is provided one or a plurality of phytocannabinoids selected from the group consisting of tetrahydrocannabivarin (THCV) and cannabidiol (CBD) for use in the treatment of generalised seizures and/or partial seizure.

[0035] Preferably the medicament is to treat clonic and/or tonic seizures.

[0036] The preferred daily dose of THCV is at least 1.5 mg, more preferably at least 5 mg through 10 mg to 15 mg or more.

[0037] Preferably the THCV is used in combination with at least a second, therapeutically effective cannabinoid, preferably CBD.

[0038] The CBD is preferably present in an amount which will provide a daily dose of at least 400 mg, more preferably at least 600 mg and as much as 800 mg or more, but preferably less than 1200 mg.

[0039] The cannabinoids may be present as pure or isolated cannabinoids or in the form of plant extracts. Where a plant extract is used it is preferable that the THC content is less than 5% by weight of the total cannabinoids, more preferably less than 4% through 3%, 2% and 1%. THC can be selectively removed from extracts using techniques such as chromatography.

[0040] The invention also extends to using a phyto-cannabinoid in the manufacture of a medicament to treat a specific form of epilepsy.

[0041] In accordance with a second aspect of the present invention there is provided a composition for use in treating generalised seizures and/or partial seizure comprising THCV and or CBD

[0042] The composition preferably takes the form of a plant extract containing one or more phytocannabinoids and one or more excipients.

[0043] In accordance with a third aspect of the present invention there is provided THCV and/or CBD for use in the manufacture of a medicament for use in the treatment of generalised seizures and/or partial seizure.

[0044] In accordance with a forth aspect of the present invention there is provided a method of treating generalised seizures and/or partial seizure comprising administering to a patient a medicament comprising an effective amount of THCV and/or CBD.

[0045] The combined use is predicated on apparent different mechanisms of action given the different results observed in different animal models and at different doses.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which

[0047] FIG. **1** shows latencies to initial and later seizure severities. The mean latencies to first myoclonic jerk (FMJ) and scores of 3.5 are shown ±S.E.M. for vehicle or for low, medium or high doses of THCV BDS and 70 mg/kg PTZ. n=8-10;

[0048] FIG. **2** shows seizure duration and time to death. The mean durations of seizures in animals that survived, and the time from first seizure sign to death in those that died, are shown ±S.E.M. for vehicle or for low, medium or high doses of THCV BDS and 70 mg/kg PTZ. n=3-10 dependent on proportions of animals that died within experimental groups. ∫=vehicle group had no deaths and so no value is shown here;

[0049] FIG. **3** shows median severity scores. Median severity scores for groups of animals treated with vehicle or with low, medium or high doses of THCV BDS prior to 70 mg/kg PTZ. n=10 for all groups;

[0050] FIG. **4** shows mortality rates. Mortality rates expressed as percentages for animals treated with vehicle or with low, medium or high doses of THCV BDS and 70 mg/kg PTZ. n=10 for all groups. ∫=vehicle group had no deaths, therefore no value is shown;

[0051] FIG. **5** shows latencies to initial and later seizure severities. The mean latencies to first myoclonic jerk (FMJ) and scores of 3.5 are shown ±S.E.M. for vehicle or for low, medium or high doses of THCV BDS and 80 mg/kg PTZ. n=7-10;

[0052] FIG. **6** shows seizure duration and time to death. The mean durations of seizures in animals that survived, and the time from first seizure sign to death in those that died, are shown ±S.E.M. for vehicle or for low, medium or high doses of THCV BDS and 80 mg/kg PTZ. n=3-7 dependent on proportions of animals that died within experimental groups;

[0053] FIG. **7** shows median severity scores. Median severity scores for groups of animals treated with vehicle or with low, medium or high doses of THCV BDS prior to 80 mg/kg PTZ. n=10 for all groups;

[0054] FIG. **8** shows mortality rates. Mortality rates expressed as percentages for animals treated with vehicle or with low, medium or high doses of THCV BDS and 80 mg/kg PTZ. n=10 for all groups;

[0055] FIGS. **9**A-**9**D show PTZ-induced seizure development and duration with pure THCV. FIGS. **9**A, **9**B and **9**C show the mean latency (s) from injection of 80 mg/kg PTZ to: first sign of seizure (FIG. **9**A); development of myoclonic seizures (FIG. **9**B) and full tonic-clonic seizures (FIG. **9**C) for vehicle and THCV-dosed groups. n=5-16 depending on incidence of each marker within a specific group). (FIG. **9**D) shows the mean duration of seizures (s) in animals that survived post-seizure. All values ±S.E.M., * indicates significant difference from vehicle group (P<0.05; Mann-Whitney U test);

[0056] FIGS. **10**A-**10**B show the effect of CBD on PTZ-induced seizures. FIG. **10**A: % mortality experienced as a result of IP injection of 80 mg/kg PTZ in vehicle and CBD-dosed (1, 10,100 mg/kg CBD) animals (n=15 for all groups). FIG. **10**B: % of vehicle- and CBD-dosed (1, 10,100 mg/kg CBD) animals that experienced tonic-clonic seizures as a result of IP injection of 80 mg/kg PTZ. * indicates significant result (p<0.01);

[0057] FIG. **11** shows the lack of effect of THCV on the percentage mortality. Significance assessed using a binomial test by comparison with control and significance accepted at P<0.05. No significant difference vs control was found at any dose;

[0058] FIG. **12** shows the lack of effect of THCV on the mean maximum seizure severity. Significance was assessed by 1-way ANOVA with Tukey's post-hoc test; P>0.5 for all comparisons vs control;

[0059] FIGS. **13**A-**13**D shows the percentage of animals in each group that reached specified seizure states when treated with THCV. Significant differences vs control were assessed using a binomial test. P≤0.05.

[0060] FIG. **14** shows the effect of CBD on the percentage mortality. Significance assessed by binomial test; * shows a significant increase in mortality (P<0.05). Note that this effect only appeared at 10 mg/kg and was lost at 100 mg/kg, suggestive of a biphasic effect;

[0061] FIG. **15** shows the mean maximum seizure severity. Significance was assessed by 1-way ANOVA with Tukey's post-hoc test; P>0.5 for all comparisons vs control;

[0062] FIGS. **16**A-**16**D describes the percentage of animals in each group that reached specified seizure states. Significant differences vs control were assessed using a binomial test. P≤0.05 (*); P≤0.001 (***);

[0063] FIG. **17** shows the effects of CBD upon mean tonic-clonic frequency. Significance was assessed using a one way ANOVA with Tukey's post-hoc test. P≤0.05 (*); P≤0.01 (**);

[0064] FIG. **18** describes the percentage duration of time spent in a tonic-clonic state compared to the total duration of the seizure period. Significance was assessed using a one way ANOVA with Tukey's post-hoc test. P≤0.05 (*);

[0065] FIGS. **19**A-**19**B describes the effects of CBD upon tonic-clonic seizures without postural control. FIG. **19**A: % of animals that experienced tonic-clonic seizures without postural control. FIG. **19**B: frequency with which animals displayed tonic-clonic seizures without postural control in the two hour recording period (or until death). FIG. **19**A: binomial statistical test; FIG. **19**B: one-way ANOVA followed by Tukey test. **, *** and # indicate p≤0.01, 0.001 and 0.1 respectively; and

[0066] FIGS. **20**A-**20**C describes the effect of CBD on penicillin-induced seizure severity and mortality. FIG. **20**A: Median severity of seizures (grey line), also shown is the 25th and 75th percentiles (black horizontal lines) and the maximum and minimum values (upward and downward error bars respectively). FIG. **20**B: Percentage of animals that remained seizure free throughout. FIG. **20**C: Percentage mortality. FIG. **20**A: one-way ANOVA followed by Tukey test. FIGS. **20**B&C: binomial statistical test; *, *** and # indicate p≤0.05, 0.001 and 0.1.

DETAILED DESCRIPTION

PTZ Model—Examples 1-3

General Methodology for PTZ Model

Animals

[0067] Male Wistar rats (P24-29; 75-110 g) were used to assess the effects of the cannabinoids: THCV (BDS and pure) and CBD on the PTZ model of generalised seizures. Animals were habituated to the test environment, cages, injection protocol and handling prior to experimentation. Animals were housed in a room at 21° C. on a 12 hour light: dark cycle (lights on 0900) in 50% humidity, with free access to food and water.

Experimental Setup

[0068] Five 6 L Perspex tanks with lids were placed on a single bench with dividers between them. Closed-circuit television (CCTV) cameras were mounted onto the dividers to observe rat behaviour. Sony Topica CCD cameras (Bluecherry, USA) were linked via BNC cables to a low-noise PC via Brooktree digital capture cards (Bluecherry, USA). Zoneminder (http://www.zoneminder.com) software was used to monitor rats, start and end recordings and manage video files. In-house Linux scripts were used to encode video files into a suitable format for further offline analysis using The Observer (Noldus Technologies).

Dose

[0069] A range of doses of PTZ (50-100 mg/kg body weight) were used to determine the best dose for induction of seizures (see below). As a result, doses of 70 and 80 mg/kg injected intra-peritoneally (IP; stock solution 50 mg/ml in 0.9% saline) were used to screen the cannabinoids.

Experimental Protocols

[0070] On the day of testing, animals received an IP injection with either the cannabinoids (low, medium or high dose) or a matched volume of the cannabinoids vehicle (1:1:18 ethanol:Cremophor: 0.9% w/v NaCl solution), which served as the negative control group. Animals were then observed for 30 mins, after which time they received an IP injection of 70 or 80 mg/kg PTZ. Negative vehicle controls were performed in parallel with cannabinoid-dosed subjects. After receiving a dose of PTZ, animals were observed and videoed to determine the severity of seizure and latency to several seizure behaviour types (see in vivo analysis, below). Animals were filmed for half an hour after last sign of seizure, and then returned to their cage.

In Vivo Analysis

[0071] Animals were observed during experimental procedures, but all analysis was performed offline on recorded video files using The Observer behavioural analysis software (Noldus, Netherlands). A seizure severity scoring system was used to determine the levels of seizure experienced by subjects (Pohl & Mares, 1987). All signs of seizure were detailed for all animals.

TABLE 1

Seizure severity scoring scale, adapted from Pohl & Mares, 1987.

Seizure score	Behavioural expression	Righting reflex
0	No changes to behaviour	Preserved
0.5	Abnormal behaviour (sniffing, excessive washing, orientation)	Preserved
1	Isolated myoclonic jerks	Preserved
2	Atypical clonic seizure	Preserved
3	Fully developed bilateral forelimb clonus	Preserved
3.5	Forelimb clonus with tonic component and body twist	Preserved
4	Tonic-clonic seizure with suppressed tonic phase	Lost
5	Fully developed tonic-clonic seizure	Lost
6	Death	

Latency from Injection of PTZ to Specific Indicators of Seizure Development:

[0072] The latency (in s) from injection of PTZ to first myoclonic jerk (FMJ; score of 1), and to the animal attaining "forelimb clonus with tonic component and body twist" (score of 3.5) were recorded. FMJ is an indicator of the onset of seizure activity, whilst >90% of animals developed scores of 3.5, and so is a good marker of the development of more severe seizures. Data are presented as the mean±S.E.M. within an experimental group.

Maximum Seizure Severity:

[0073] This is given as the median value for each experimental group based on the scoring scale below.

% Mortality:

[0074] The percentage of animals within an experimental group that died as a result of PTZ-induced seizures. Note that the majority of animals that developed tonic-clonic seizures (scores of 4 and 5) in the THCV (BDS) study died as a result, and that a score of 6 (death) automatically denotes that the animal also experienced tonic-clonic seizures.

Seizure Duration:

[0075] The time (in seconds) from the first sign of seizure (typically FMJ) to either the last sign of seizure or, in the case of subjects that died, the time of death—separated into animals that survived and those that did not. This is given as the mean±S.E.M. for each experimental group.

Statistics:

[0076] Differences in latencies and durations were assessed by one-way analysis of variance (ANOVA) with post-hoc Tukey's test. $P \leq 0.05$ was considered significant.

Example 1—THCV (BDS)

[0077] The THCV BDS comprised a whole extract of a chemovar in which THCV was the predominant cannabinoid. (i.e. it was the major cannabinoid present in the extract, 80% by weight of the total cannabinoid content). THC was the second most prevalent cannabinoid, and was present in significant amounts. (i.e. it comprised greater than 10% by weight of the total cannabinoid content, being present at about 16%), and there were a number of minor cannabinoids identified, each comprising less than 2% by weight of the total cannabinoid content as measured by HPLC analysis. The ratio of THCV to THC in this extract is about 5:1.

[0078] In fact the THCV content was 67.5% by weight of the extract and the THC content was 13.6% by weight of the extract, with the other identified cannabinoids in total comprising about 3% by weight of the extract, the remaining 16% comprising non-cannabinoids.

PTZ Pilot Study

[0079] Seizures induced by a range of PTZ concentrations (50-100 mg/kg; the range present in the literature) in rats were investigated to determine an optimal dose prior to the investigation of the cannabinoid effect. PTZ doses of:

[0080] 50 mg/kg and 60 mg/kg induced very little seizure-like activity (n=4);

[0081] 70 mg/kg typically induced clonic seizures (score of 3.5; 8 of 13 subjects);

[0082] 80 mg/kg regularly induced tonic-clonic seizures (scores of 4 and 5; 6 of 10 subjects).

[0083] Additionally, it was found that repeated dosing with PTZ resulted in increased sensitivity over time; therefore no experiments were performed on animals that had already received a dose of PTZ.

[0084] The effect of THCV BDS on PTZ-induced seizures was first assessed against a PTZ dose of 70 mg/kg. As described below, this yielded a vehicle control group that did not typically experience severe seizure scores. Therefore THCV BDS was also screened against an 80 mg/kg dose of PTZ. It was felt that the increased seizure severity experi-

enced by vehicle control animals exposed to 80 mg/kg PTZ was a more appropriate test of potential anti-convulsant activity.

Effect of THCV BDS on Moderately Severe (70 mg/kg) PTZ-Induced Seizures

[0085] Three doses of THCV BDS were assessed against a concentration of PTZ known to induce moderate seizures in rats (70 mg/kg; see pilot, above). The low, medium and high doses of THCV BDS used were 0.37, 3.70 and 37.04 mg/kg, and yielded actual THCV doses of 0.25, 2.5 and 25 mg/kg respectively. These doses were matched by THCV content to those being used for screening pure THCV against PTZ-induced seizures.

[0086] THCV BDS did not have any significant effects on latency to first myoclonic jerk or on latency to attaining a severity score of 3.5 on the seizure severity scale (FIG. **1**). It should be noted that although values for both these variables were higher for animals treated with medium and high dose THCV BDS compared to control, this failed to reach significance (P>0.05). Similarly, no significant impact on duration of seizure was seen (FIG. **2**).

[0087] The effects of THCV BDS on seizure severity (FIG. **3**) and mortality (FIG. **4**) in animals that received doses of 70 mg/kg PTZ did not conform to a simple pattern. No animal injected with vehicle-alone exceeded the median severity score of 3.5 for that group, and no animals died (n=10).

[0088] In contrast, 70 mg/kg PTZ induced severe tonic-clonic seizures and death in 50% of animals injected with a low dose of THCV BDS, demonstrating a median severity score of 4.75. This increase in severity was not significant. However, animals injected with medium and high doses of THCV BDS exhibited a lower median severity score and lower mortality rates than those exposed to low doses (FIGS. **3** & **4**). Medium and high dose mortality rates were higher than that of the vehicle group, but not significantly so (P>0.05; FIG. **4**). However, median severity scores were the same between medium & high doses (FIG. **3**). This pattern of results suggested that a further set of experiments, in which THCV BDS was screened against a dose of PTZ which would induce severe seizures in control (vehicle-treated) animals, was required.

Effect of THCV BDS on Severe (80 mg/kg) PTZ-Induced Seizures

[0089] The effects of the same three doses of THCV BDS on seizures induced by 80 mg/kg PTZ were assessed. It is worth noting that 80 mg/kg induced significantly more severe seizures than 70 mg/kg in vehicle control groups (P=0.009), with median seizure severity scores of 6 and 3.5 respectively. THCV BDS did not have a significant effect on latencies to FMJ or a severity score of 3.5 (FIG. **5**). Similarly, no effect was observed on seizure durations (FIG. **6**).

[0090] Low dose THCV BDS decreased both seizure severity (FIG. **7**) and mortality (FIG. **8**) in animals that received doses of 80 mg/kg PTZ. Animals that received low THCV BDS had a lower median severity score (3.5 compared to 6) than vehicle controls. However, this difference was not significant (P>0.5). The low THCV BDS dose group also had a mortality rate half that of the vehicle control group (30% vs 60%).

[0091] Groups treated with medium and high doses of THCV BDS had a lower seizure severity score of 4.75 (P>0.5 vs control), and a lower mortality rate of 50%, compared to 6 and 60% respectively.

In Vivo Summary and Conclusion

[0092] Screening of THCV BDS in the PTZ model did not appear to have any significant anti- or pro-convulsant effects on either moderate or severe PTZ-induced seizures. However, a trend towards lower severity and mortality was seen in animals that received a low dose of THCV BDS prior to induction of severe (80 mg/kg PTZ) seizures, compared to vehicle controls.

[0093] It is possible that this effect is masked at higher doses of THCV BDS by higher levels of other cannabinoid constituents (such as THC) present in the non-THCV content of the THCV BDS. Higher doses of THCV BDS will contain increasing doses of non-THCV content, such as THC, which may oppose any potential positive effects of THCV.

Example 2—THCV (Pure)

Effect of Pure THCV Against PTZ-Induced Seizures

[0094] Low (0.025 mg/kg), medium (0.25 mg/kg) and high (2.5 mg/kg) doses of pure THCV were assessed for their effects on PTZ-induced seizures. It is worth noting at this point, for comparisons to Example 1 (THCV BDS), that differing doses of pure THCV were used compared to THCV BDS. See Table 2 below.

TABLE 2

Comparison of THCV BDS and pure THCV doses used in PTZ model

Test CB	"low" dose (mg/kg)	"medium" dose (mg/kg)	"high" dose (mg/kg)
THCV BDS	0.25	2.5	25
Pure THCV	0.025	0.25	2.5

Values given are for effective THCV content of doses (therefore actual doses of THCV BDS are approx 1.5 times larger).

[0095] 80 mg/kg PTZ successfully induced seizures of varying severities in animals from all 4 experimental groups (n=16 per group). PTZ-induced seizures led to the death of 44% of animals that received vehicle alone. Groups that received low, medium and high THCV all exhibited lower mortality rates of 41%, 33% and 38% respectively; however these values were not significantly different from that of the vehicle group (p>0.05, binomial test).

[0096] The mean values for latency to first seizure sign, and to scores of [3] and [5] on the seizure scoring scale used, as well as the duration of seizure for surviving animals, are described in FIGS. **9**A-D.

[0097] It can be seen that seizures started later, as shown by increased latency to first manifestation of seizure-like behaviour (FIG. **9**A) in animals that received THCV compared to vehicle controls.

[0098] The delay of onset was significant at the highest dose of THCV (p=0.02). A similar pattern was seen for latencies to scores of [3] and [5] (FIGS. **9**B and **9**C) with all

THCV doses exhibiting increased latencies, reaching a significant level at the highest dose of THCV (p=0.017 and 0.013 for [3] and [5] respectively).

[0099] It was also observed that duration of PTZ-induced seizures in animals that survived the experimental period were significantly shorter after administration of the medium dose of THCV compared to vehicle controls (FIG. **9**D; p=0.03).

Table 3 below displays the values for median seizure severity in each experimental group.

TABLE 3

Seizure severity and incidence

	Vehicle	0.025 mg/kg THCV	0.25 mg/kg THCV	2.5 mg/kg THCV
Median severity	4.25	3.5	3.5	3.5
% no seizure	12.5	5.9	33.3*	18.8

[0100] The median maximum severities and % of animals that did not experience any signs of seizure for each experimental group are given (n=16 for each value). * indicates significant difference from vehicle group (binomial significance test, P<0.05).

[0101] Vehicle control animals exhibited a median seizure severity of 4.25, whereas all groups which received THCV had a median severity score of 3.5. This decrease was not significantly different.

[0102] 12.5% vehicle control animals displayed no indicators of seizure, suggesting these animals did not develop seizures after PTZ administration. A significantly higher number of animals (33.3%) displayed no signs of seizure in the group that received 0.25 mg/kg (Table 3; p=0.031). This data suggests that the medium dose of 0.25 mg/kg THCV protected against the development of seizures.

In Vivo Summary and Conclusion

[0103] The effects of the high dose of THCV on latency values suggest that THCV can delay both onset and seizure development, whilst the significant effects of the medium dose on the incidence of seizure at medium (0.25 mg/kg) THCV doses suggest a significant anticonvulsive action on PTZ-induced seizures.

Example 3—CBD (Pure)

[0104] In addition to THCV, CBD was also screened in the PTZ model. The results strongly indicate that CBD (at levels of 100 mg/kg) in this model is anti-convulsant as it significantly decreased the mortality rate and incidence of the most severe seizures compared to vehicle control animals.

Effect of Pure CBD Against PTZ-Induced Seizures

[0105] Pure CBD was injected intra-peritoneally (IP) in the standard vehicle (1:1:18 ethanol: Cremophor: 0.9% w/v NaCl) at doses of 1, 10 and 100 mg/kg alongside animals that received vehicle alone at a matched volume (n=15 for each group). 60 minutes later PTZ (80 mg/kg, IP) was administered.

[0106] 46.7% of control animals that received vehicle alone died within 30 minutes of PTZ administration (FIG. **10**). In contrast only 6.7% (only 1 of 15) of animals that received 100 mg/kg CBD died, a marked reduction that proved to be significant (p<0.001).

[0107] Additionally only 6.7% of animals that received 100 mg/kg CBD experienced the most severe seizures (score of 5) in comparison to 53.3% of vehicle control animals, a decrease that was also significant (p<0.001; FIG. **10** in vivo).

[0108] In contrast to pure THCV, no significant increases in latency of seizure development were observed. However, the marked and significant reductions indicate a striking anti-convulsant effect on PTZ-induced seizures.

[0109] Screening and analysis of pure CBD in the PTZ model at high dose (100 mg/kg) of CBD on mortality levels and incidence of the most severe seizures suggests that CBD can attenuate the severity of PTZ-induced seizures

Pilocarpine Model—Examples 4 and 5

Example 4—Pure THCV

Effect of Pure THCV Against Pilocarpine-Induced Seizures

[0110] Pure THCV was injected intra-peritoneally (IP) in the standard vehicle (1:1:18 ethanol:Cremophor:0.9% $^{w}/_{v}$ NaCl) at doses of 0.025, 0.25 and 2.5 mg/kg alongside animals that received vehicle alone at a matched volume (n≥14 for each group). 15 minutes later methylscopolamine (1 mg/kg; to reduce peripheral muscarinic effects of pilocarpine) was administered followed, 45 minutes later by pilocarpine (380 mg/kg, IP) administration.

Results

[0111] No significant effect of THCV at any dose was observed upon latency to the onset of seizure (P>0.5 for all doses vs control; 1-way ANOVA with Tukey's post-hoc test). No significant change in percentage mortality vs control was seen for any THCV dose (FIG. **11**).

[0112] In addition THCV had no effect upon the mean maximum severity of seizure reached per animal group (FIG. **12**).

[0113] The percentage of animals in each group that reach a particular seizure state (unilateral forelimb clonus, bilateral forelimb clonus, bilateral forelimb clonus with rearing and falling and tonic-clonic) was also assessed (FIG. **13**A-D).

[0114] THCV caused no significant changes in the percentage of animals showing unilateral forelimb clonus, bilateral forelimb clonus or tonic-clonic seizures at any dose. Interestingly, 0.25 mg/kg THCV caused a significant increase in the percentage of animals showing bilateral forelimb clonus with rearing and falling although this effect was not seen at any other dose.

Example 5—Pure CBD

Effect of Pure CBD Against Pilocarpine-Induced Seizures

[0115] Pure CBD was injected intra-peritoneally (IP) in the standard vehicle (1:1:18 ethanol:Cremophor0.9% $^{w}/_{v}$ NaCl) at doses of 1, 10 and 100 mg/kg alongside animals that received vehicle alone at a matched volume (n≥14 for each group). 15 minutes later methylscopolamine (1 mg/kg;

to reduce peripheral muscarinic effects of pilocarpine) was administered followed, 45 minutes later by pilocarpine (380 mg/kg, IP) administration.

Results

[0116] No significant effect of CBD at any dose was observed upon latency to the onset of seizure (P>0.5 for all doses vs control; 1-way ANOVA with Tukey's post-hoc test). A significant increase in percentage mortality vs control was seen for the 10 mg/kg CBD dose as shown in FIG. **14**.

[0117] FIG. **15** details that CBD had no effect upon the mean maximum severity of seizure reached per animal group.

[0118] FIGS. **16** A-D detail the percentage of animals in each group that reached particular seizure states (unilateral forelimb clonus, bilateral forelimb clonus, bilateral forelimb clonus with rearing and falling and tonic-clonic).

[0119] CBD caused significant decreases in the percentage of animals showing unilateral forelimb clonus at CBD doses >1 mg/kg, Interestingly, although no significant differences in the percentage of animals exhibiting bilateral forelimb clonus were found, the percentage of animals manifesting with bilateral forelimb clonus with rearing and falling were significantly reduces at all CBD doses >1 mg/kg. The percentage of animals exhibiting tonic-clonic seizures was significantly reduced at CBD doses of 1 mg/kg and 100 mg/kg but not 10 mg/kg (c.f. FIG. **14**).

[0120] The effects of CBD upon tonic-clonic seizure events by examining the mean frequency of tonic-clonic events as is shown in FIG. **17**. CBD caused a significant reduction in mean tonic-clonic frequency at all doses tested. CBD effects upon the mean frequency of all other seizure scores were also assessed in the same way but no significant differences vs control were found (P>0.5 for all).

[0121] The percentage duration of time spent in a tonic-clonic state compared to the total duration of the seizure period was examined (FIG. **18**). CBD significantly reduced the percentage duration at doses of 1 mg/kg and 100 mg/kg but not 10 mg/kg.

Example 6

Penicillin Model—Example 6 (Only)

Example 6—Pure CBD

Effects of Pure CBD on Penicillin-Induced Seizures

[0122] CBD (1, 10 and 100 mg/kg) or CBD vehicle (1:1:18 ethanol:Cremophor: 0.9% $^{w}/_{v}$ NaCl) was administered i.p. to adult male Wistar rats (>250 g). One week prior to this, animals had been surgically implanted with a cannula into the right lateral ventricle under anaesthesia. One hour after CBD administration, 150 IU penicillin was infused into the right lateral ventricle in 1.5 μl saline solution over one minute and seizure behaviour video recorded for two hours.

[0123] Following detailed examination of animal responses to penicillin alone (using data obtained from vehicle control groups, a finalised seizure scoring scale for penicillin-induced partial seizures has been derived. The following scoring system which was derived from several existing and published scoring systems for this model, will therefore be used for analysis of drug effects upon such seizures.

Seizure Scoring Scale for Penicillin-Induced Partial Seizures.

[0124]

0	Latent period
1	Wild running/leaping
2	Myoclonic phase
3	Unilateral forelimb clonus
4	Bilateral forelimb clonus
5	Tonic-clonic seizure with postural control retained
6	Tonic-clonic seizure without postural control

[0125] Seven of the twelve vehicle-treated animals developed the most severe seizures (tonic-clonic seizures without postural control; FIG. **19** A), whereas, administration of 100 mg/kg CBD completely prevented development of these seizures in a significant manner (p=0.001). Near-significant decreases in development of these seizures were observed in animals treated with 1 and 10 mg/kg CBD (FIG. **16**A, p=0.076 for both). The frequency with which animals experienced the most severe seizures was also significantly affected (ANOVA, p=0.009; FIG. **19** B), with a significant decrease compared to the vehicle group at 100 mg/kg CBD (p=0.006) and a near-significant effect at 10 mg/kg (p=0.071).

[0126] The effect of CBD treatment on seizure severity and animal mortality is described in FIG. **20** A-C. A dose of 100 mg/kg CBD significantly reduced the median severity of penicillin-induced seizures compared to vehicle-treated animals (ANOVA p=0.024; difference between vehicle and 100 mg/kg CBD p=0.012; FIG. **20** A). Interestingly, all doses of CBD (1, 10 and 100 mg/kg) significantly increased the proportion of animals that remained seizure-free (p<0.001 for all doses; FIG. **20** B). Finally, 100 mg/kg had a near-significant effect on mortality compared to vehicle (p=0.057).

OVERALL CONCLUSION

[0127] From these studies it would appear that both THCV (pure) and CBD (pure) show promise as an anti-epileptic for generalized seizure, particularly clonic/tonic seizure. The data generated for a THCV rich extract, containing other cannabinoids including significant amounts of THC, suggest that the THC may be countering the effect of the THCV and that a cannabinoid extract which contains THCV as a major or predominant cannabinoid, but which also contains minimal, or substantially no, THC would be desirable for treating epilepsy.

[0128] Furthermore the results with pure CBD suggest that an extract containing significant amounts of both THCV and CBD, but again, minimal or substantially no THC may provide an optimum combination. Accordingly it may prove desirable to prepare a THCV predominant extract in which THC is selectively, and substantially, removed (to levels of less than a few percent). This could be mixed with a CBD rich extract (which contains much lower levels of THC) in which CBD is the major and predominant cannabinoid (also with low levels of THC) to produce an extract with clearly defined, and significant levels of both THCV and CBD, but with insignificant levels of THC. Such an extract may contain other cannabinoids and the non-cannabinoid components which result from extraction, by for example, car-

bon dioxide as disclosed in WO04/016277, which components may support an "entourage" effect in the endocannabinoid system.

[0129] On dosage, a rat/human conversion factor (×6) suggests a CBD daily dose of at least 600 mg (and optionally between 400 mg and 800 mg) and for THCV at least 1.5 mg (medium) to preferably at least 15 mg (high).

[0130] Where a phytocannabinoid extract is to be used, an extract with low or negligible levels of THC and therapeutically effective levels of THCV and/or CBD is desired.

[0131] The data described in the Examples above clearly show that although CBD shows some anti-convulsant properties in all of the three models tested, it would appear best in treating generalized or partial seizures. In contrast THCV was only effective in the PTZ-model. This finding suggests that the two cannabinoids may have different mechanisms of action and that the combination may provide for more general treatments. In this regard THCV appears selective for generalized seizures, more particularly tonic-clonic seizures and CBD appears to be most effective in generalized and partial seizures.

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1.-10. (canceled)

11. A method of treating complex partial seizure that remains localized comprising administering cannabidiol (CBD) to a patient wherein the CBD is present in an amount which provides a daily dose of at least 400 mg.

12. The method of claim **11**, wherein CBD is present in an amount which provides a daily dose of from 400 to 800 mg.

13. The method of claim **11**, wherein the CBD is used in combination with tetrahydrocannabivarin (THCV).

14. The method of claim **13**, wherein the THCV is present in an amount which provides a daily dose of at least 1.5 mg.

15. he method of claim **14**, wherein the THCV is present in an amount which provides a daily dose of at least 15 mg.

16. The method of claim **11**, wherein the CBD is present as a plant extract.

17. The method of claim **16**, wherein the plant extract comprises less than 5% by weight of tetrahydrocannabinol (THC) as a percentage of any cannabinoids present in the plant extract.

18. The method of claim **17**, wherein the plant extract comprises less than 1% by weight of tetrahydrocannabinol (THC) as a percentage of any cannabinoids present in the plant extract.

19. The method of claim **11**, wherein the CBD is present as a pure or isolated cannabinoid.

20. The method of claim **13**, wherein the CBD in combination with THCV is present as a plant extract.

21. The method of claim **20**, wherein the plant extract comprises less than 5% by weight of tetrahydrocannabinol (THC) as a percentage of any cannabinoids present in the plant extract.

22. The method of claim **21**, wherein the plant extract comprises less than 1% by weight of tetrahydrocannabinol (THC) as a percentage of any cannabinoids present in the plant extract.

23. The method of claim **13**, wherein the CBD in combination with THCV is present as a pure or isolated cannabinoid.

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