Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

An Observational Study of Adjunctive Artisanal Cannabidiol Use by Adults with Treatment Resistant Epilepsies: Behavioral and Urinalysis Data

Barbara Brett (barbara.brettgreen@csupueblo.edu)
Colorado State University - Pueblo
Matthieu Conroy
United States Army Medical Research Institute of Chemical Defense
Hardik Doshi
Cape Fear Valley Medical Center
Matthew X. Lowe
Realm of Caring
Sasha Kalcheff-Korn
Realm of Caring
Heather Jackson
Realm of Caring

Research Article

Keywords:

Posted Date: August 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3214013/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background

For approximately 30% of people with epilepsy, seizures are not well-controlled by antiepileptic drugs. This condition, called treatment resistant epilepsy (TRE), is associated with increased morbidity and mortality, and substantially impacts the quality of life of both the individual and their family. Non-responsiveness to AEDs leads many people with TRE to seek alternative therapies, such as cannabinoid-based medication, particularly cannabidiol (CBD), with or without medical or professional advice. This is due in part to widespread reporting in the media on the benefits of CBD for seizures in some forms of epilepsy.

Methods

Ten adults with TRE, opting to add CBD to their existing treatment regime, participated in this prospective, observational, longitudinal study. We hypothesized that adjunctive CBD use would positively impact participants' quality of life and psychological well-being. Participants were followed for a period of approximately six months – for approximately one month prior to the initiation of CBD use and approximately five months after. At three time points, participants provided urine samples and completed behavioral questionnaires that assessed quality of life, anxiety/depression, and adverse events.

Results

Analyses showed a statistically significant improvement in quality of life, a statistically significant decrease in anxiety symptoms, and a statistically significant decrease in the experience of adverse events over time ($p < 0.05$). Urinalysis revealed the majority of participants had no CBD/metabolites in their system at the beginning of the study, and confirmed the presence of CBD/metabolites in participants' urine after CBD was added to their treatment regime.

Conclusion

These results suggest that adjunctive use of artisanal CBD may be beneficial for the behavioral and psychological symptoms of TRE and may improve medication tolerability.

Introduction

Background on Epilepsy and Antiepileptic Drugs

Epilepsy is a heterogeneous neurological condition characterized by recurrent seizures, which are transient instances of aberrant electrical activity in the brain [1]. This disease is one of the most common

conditions affecting the brain, with approximately 50 million people diagnosed worldwide [2] . In the United States, approximately 3.4 million individuals have epilepsy according to a report from the Center for Disease Control in 2015 [3]. Epilepsy is a disease of diverse etiology, seizure patterns, and responses to treatment, and individuals with epilepsy may experience multiple types of seizures. Signs and symptoms of seizures vary, and can include behaviors such as temporary confusion, staring spells, uncontrollable jerking movements of the arms and legs, loss of consciousness, and autonomic effects such as sweating. Epilepsy has a negative impact on patients' quality of life as well as their psychological well-being.

For approximately 70% of people with epilepsy, seizures are well-controlled by antiepileptic drugs (AEDs) [4]. More than 20 AEDs utilizing a wide variety of pharmacologic mechanisms are available to treat seizures [5]. For most people, seizure freedom is achieved after the trial of two appropriate AEDs, either alone or in combination [6, 7]. However, if seizures continue, the possibility of seizure freedom declines with each successive AED treatment added [7]. Although AEDs effectively decrease seizure frequency in many people, they are associated with significant, adverse central nervous system effects, such as psychological and behavioral effects, cognitive effects, and memory problems [8-10]. Adverse effects of AEDs are a leading cause of epilepsy treatment failure [11].

For approximately 30% of people with epilepsy, seizures are not well-controlled by existing AEDs [7, 12, 13]. This condition, called treatment resistant epilepsy (TRE), is associated with severe morbidity, as evidenced by health, economic, and psychosocial problems, such as increased risk of injury, employment discrimination, and difficulties with social interactions [14-16]. People with TRE often live in fear of their next seizure, which causes them to modify their activities, undermining their self confidence and self-esteem. People with TRE are also at an increased risk of mortality due in part to sudden unexpected death in epilepsy (SUDEP) [17-20]. TRE negatively affects not only patients, but also impacts family members' psychological health and family functioning [21].

Quality of Life in Epilepsy

Impacts on quality of life (QoL) represent a significant concern for people with TRE and it is increasingly recognized as an important outcome measure in both clinical and research settings [22, 23]. Critically, TRE not only affects the quality of life of patients, but also their families [21] . Major areas of concern include emotional well-being, social function, energy/fatigue, cognitive function, seizure worry, and medication side effects [24-26]. Unfortunately, exploring the full spectrum of QoL issues is generally beyond the scope of routine medical practice [14]. Doctors typically constrain their attention to seizure control and medication side effects, which limits their capacity to help patients achieve meaningful therapeutic outcomes.

Comorbid Psychiatric Conditions in Epilepsy

In addition to poor QoL, people with epilepsy have high rates of psychiatric comorbidities, including anxiety and depression [27-30]. Using the Hospital Anxiety and Depression (HADS) scale in a community

sample of adults with epilepsy, researchers found the prevalence of anxiety, the threshold for which is (HADS>11), was 20.5% [31]. Anxiety was associated with other factors, including a current history of depression and perceived side effects of AEDs. Depressive disorders represent the most common psychiatric comorbidity in epilepsy [32]. A review and meta-analysis of 9 studies found the overall prevalence of depression in people with epilepsy was 23.1% [33]. In fact, some researchers suggest that depression may be the most powerful predictor of QoL, above seizure frequency [34, 35]. Psychological disorders have a substantial impact on individuals with TRE as they negatively affect a person's psychological well-being and make treatment more difficult due to polypharmacy and additional medication side effects.

Cannabinoid Treatment of Seizures in Epilepsy

The lack of effectiveness of AEDs for seizures has caused many people with TRE to seek alternative therapies, such as cannabinoid-based medication, with and without medical or professional advice. Public interest in the use of cannabinoids, in particular CBD, as a treatment for TRE, surged in the last decade. This was due in part to widespread media coverage of a case study which showed a dramatic decrease in seizure frequency for one child with Dravet syndrome who was treated with a high CBD/low THC cannabis plant oil extract now known as Charlotte's Web [36]. Early open-label and expanded-access studies on the adjunctive use of high CBD/low THC oral CBD extracts in people with TRE showed promising results suggesting CBD had acceptable safety and tolerability profiles and promising efficacy in reducing seizures [37, 38]. This research set the stage for the development of Epidiolex (GW Pharmaceuticals) which is now the first and only FDA approved cannabis plant-derived prescription CBD oil extract used for the treatment of seizures in epilepsy. This approval was based in part on results from randomized placebo-controlled trials which showed that adjunctive treatment with Epidiolex decreased the median seizure frequency/month in several different forms of epilepsy, including Dravet syndrome [39], Lennox-Gastaut Syndrome [40], and Tuberous Sclerosis [41]. Although most studies of Epidiolex use in TRE also reported adverse effects, such as diarrhea and somnolence, these were generally considered mild and well-tolerated [38, 40, 42]. This research suggested that Epidiolex was a safe and effective treatment for seizures in a number of different forms of epilepsy. However, Epidiolex is only available by prescription to those with a specific approved condition. It is not readily available to all people with TRE who perceive they could benefit from it due, in part, to media coverage.

Consequently, there is widespread use of widely available "artisanal" CBD products by people with TRE, despite limited research supporting their safety and efficacy [43-45]. Retrospective chart reviews of oral cannabis extract use (of a variety of types) by pediatric TRE populations of mixed etiologies have shown that parents report between 33% - 45% of patients experience a >50% reduction in seizure frequency, with some patients becoming seizure free [43, 45, 46]. Adverse effects were reported to be mild and infrequent. One prospective, observational study on the adjunctive use of CBD-enriched cannabis oil extract administered by caregivers to children with TRE for 3 months, found a significant reduction in seizures [47]. Another prospective observational study on the use of artisanal oral cannabis extracts in pediatric epilepsy found that 24% of participants had at least a 50% reduction in seizures according to

parent report, while 14% had a perceived increase and stopped treatment [48]. These researchers concluded, however, that despite a perceived benefit, the response rate was similar to the placebo rate in randomized control trials (RCTs) of new pharmaceuticals and that reported changes in seizure frequency did not correlate with cannabinoid blood levels. One recent observational study of two high CBD/low THC cannabis extract oil formulations also found no evidence of efficacy in reducing seizure frequency in people with TRE of mixed etiologies based on parent report [49]. Thus, evidence for the effectiveness of artisanal CBD formulations for reducing seizures in epilepsy is mixed.

CBD and the Endocannabinoid System

CBD is one of over 100 phytocannabinoids isolated from the cannabis plant, along with Δ9 tetra-hydrocannabinol (THC), that are being investigated for use in epilepsy and other medical conditions [50, 51]. While THC is responsible for the psychoactive effects associated with cannabis use, CBD is non-intoxicating, making it more appealing as a medication compared to THC [52]. The cannabis plant produces many additional phytocannabinoids and non-phytocannabinoid chemical constituents which have diverse biological properties [51].

Both CBD and THC interact with the endocannabinoid system (ECS), which is a cell signaling system involved in the regulation of many important physiologic functions, including nervous, emotional, metabolic, digestive and immune [53]. The ECS is also involved in the regulation of neurotransmission in the central nervous system [54]. Moreover, studies have identified possible ECS deficits in epilepsy [5], making the ECS a novel therapeutic target [55]. The endocannabinoid system is typically defined as a system of 1. Two G protein-coupled receptors for THC – cannabinoid receptor type 1 and cannabinoid receptor type 2 (CB1 and CB2); 2. Their two most studied endogenous ligands – N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG); and, 3. The enzymes responsible for their synthesis [52]. However, this system also includes additional receptors to which cannabinoids bind and additional metabolic enzymes, among other elements [52]. Compared to THC, CBD has a relatively low affinity for the main endocannabinoid receptors, where THC exerts its most meaningful anticonvulsant effects [56]. Though the exact mechanism for the anticonvulsant action of CBD remains to be elucidated [57], it may be that these effects are mediated in part through CBDs' actions on receptors other than CB1 and CB2 as CBD interacts with many other "non-cannabinoid" receptors, including TRPV1, PPARG, voltage-gated calcium and sodium channels, GPR55, and the $5HT_{1A}$ and $5HT_{2A}$ serotonin receptors, affecting a wide variety of biological processes [57]. Given the plethora of receptors and channels that CBD interacts with, CBD is currently being investigated for additional therapeutic benefits beyond decreasing seizure frequency.

Cannabinoid Effects on Quality of Life in Epilepsy

While a number of studies have reported the positive effects of CBD (Epidiolex and artisanal) on seizure frequency and other seizure-related variables, fewer studies have examined the effects of CBD on QoL. In

one of few studies to specifically address the effects of CBD on QoL in adults with TRE, Epidiolex was given in an open-label format [22]. Data were collected at baseline and after one year of treatment, or at study exit. Mean QOLIE-89 total scores for 53 participants showed a statistically significant improvement in QoL over time ($p = 0.004$). Statistically significant improvements were also found in self or caregiver reports of seizure frequency, seizure severity, mood, and adverse events. Multiple regression showed QOLIE-89 improvements were associated with improvements in mood, but not seizure frequency, seizure severity, or adverse events. A recent observational study comparing the effects of artisanal CBD use in people with epilepsy to controls also found improved QoL, as well as lower psychiatric symptom severity, and improved sleep [44]. Significantly better AED tolerability and decreased prescription drug use was also reported. A systematic review of controlled and observational studies further supported a relationship between adjunctive CBD use and improved QoL [58]. Although the evidence above suggests that both Epidiolex and artisanal CBD may improve QoL, one prospective observational study of artisanal CBD use in a pediatric epilepsy population did not show QoL improvement [48]. And, another prospective open label study of Epidiolex in 41 children with TRE concluded while CBD is an efficacious anti-seizure drug for Dravet syndrome and Lennox-Gastaut syndrome, it did not improve patient QOL [59]. This was hypothesized to be due to all of the patients in the study having profound intellectual disabilities.

Cannabinoid Effects on Psychiatric Comorbidities in Epilepsy

A growing body of research points to the potential of CBD as a therapy for anxiety and depression [60-62]. One review examining CBD use for anxiety disorders found support from both preclinical and clinical studies for its' potential efficacy for multiple forms of anxiety [62]. In a neuroimaging study, CBD use reduced anxiety in adults with generalized social anxiety relative to placebo due to its effects on limbic and paralimbic brain areas [63]. In addition, CBD has also been shown to reduce public speaking anxiety in healthy volunteers [64]. Evidence for CBD's antidepressant actions in humans is limited [62]. A recent online survey of CBD users found mood-improving effects of CBD for a number of medical conditions [65]. An observational study further found CBD users endorsed less anxiety and depression symptoms on the HADS compared to controls [66]. Although research is lacking in clinical populations, [67], preliminary studies support the potential efficacy of CBD as an anxiolytic, antidepressant, and antipsychotic [68].

The ultimate goal of epilepsy treatment is to abolish seizures, minimize side effects, and improve quality of life [69]. Therefore, the goal of this study was to determine if independent adjunctive use of artisanal CBD was beneficial for adults with TRE. We hypothesized that participants treating themselves with CBD would report an improvement in quality of life and anxiety/depression symptoms. We also hypothesized that CBD use would be associated with limited adverse effects.

Materials and Methods

Participants

All procedures were approved by the Colorado State University-Pueblo Institutional Review Board for human subjects research. All participants provided voluntary informed consent. A community sample of adults with TRE was recruited to participate in this study. Twenty-one participants enrolled in the study and ten participants completed it. Participants included 5 males and 5 females ranging in age from 18 – 64 years (average age = 34.6±12.78SD). See Table 1 for demographic and clinical information.

[Insert Table 1: Demographics; Legend: Basic demographic and clinical characteristics of the participants are shown.]

Table 1

Male (%)	5 (50%)
Female (%)	5 (50%)
Mean age (range)	34.6 (18 – 64)
Hispanic	3 (30%)
African American	3 (30%)
Caucasian	4 (40%)
Focal or Multifocal Epilepsy (%)	3 (30%)
Generalized Epilepsy (%)	6 (60%)
Absence	1 (10%)
AEDs (%): baseline	
Phenytoin	1 (1%)
Clobazam	2 (20%)
Lacosamide	3 (30%)
Levetiracetam	4 (40%)
Carbamezepine	2 (20%)
Brivaracetam	2 (10%)
Zonasimide	1 (10%)
Lamotrigine	2 (20%)
Topiramate	1 (10%)
Valproate	1 (10%)
Vagal Nerve Stimulator	2 (20%)

Inclusion criteria were: male or female; 18 years of age or older; resident of Colorado or state with cannabinoids legalized for epilepsy; documentation of a diagnosis of TRE as evidenced by medical records, and/or the following clinical feature - failure to control seizures after trial of two anticonvulsant medications at therapeutic levels; verbal report of baseline seizure frequency of at least 4 seizures/28 days; and, 1– 5 antiepileptic medications at stable doses for 1 month prior to enrollment. Exclusion criteria were: verbal report of cannabinoid use within the last 30 days; and, epilepsies associated with neurodegenerative diseases and/or inborn errors of metabolism.

Procedures

Participants were followed for a period of approximately six months. Participation began approximately 30 days prior to the initiation of cannabinoid use and continued for approximately five months after. This time-frame was selected so that a pre-CBD baseline could be established and the presence of cannabinoids/metabolites and any sustained effects on behavior could be tracked over an extended period of time. A timeline for the study is provided in Figure 1.

[Insert Figure 1_Timeline: Timeline; Legend: The arrow in the center of the figure represents the six month time course of the study. Below the arrow, the three time points for data collection are displayed in boxes: TIME 1, TIME 2, TIME 3. Above the arrow, the timing of the initiation of CBD is displayed along with the ~timing of data collection relative to the initiation of CBD use. The information collected at each time point is indicated in the lowest boxes.]

Instruments

Behavioral Questionnaires

1. The Quality of Life in Epilepsy 31-P [70, 71].

The Patient-Weighted Quality of Life in Epilepsy Inventory (QOLIE-31-P) is version 2 of the original QOLIE-31. This instrument included seven multi-item subscales that assessed: emotional well-being, social functioning, energy/fatigue, cognitive functioning, seizure worry, medication effects, and overall quality of life. The QOLIE-31-P added one new item to each subscale asking about distress, defined as bothersomeness for the respondent. Items were measured on 4- to 6- point Likert scales, with a maximum total score of 100. Higher values indicated better quality of life. The validity and reliability of the original QOLIE-31 were demonstrated [72]. No information about the reliability and validity of the QOLIE 31-P was found. Data collected were analyzed using “Distress” as a separate subscale.

2. The Hospital Anxiety and Depression Scale (HADS; [73])

The HADS is a self-administered scale used to assess the presence and severity of anxiety and depression symptoms. The HADS consisted of 14 items that were scored on a 4-point severity scale ranging from 0 to 3, with higher scores indicating greater severity. There were two subscales (anxiety and depression), with seven items related to anxiety and seven items related to depression. Subscale scores

ranged from 0 to 21. The HADS authors suggested that a score of 0 to 7 for either the anxiety or depression subscale may be regarded as being in the normal range, a score of 8 to 10 suggests the presence of the respective state, and a score of 11 or higher indicates the probable presence of clinically significant anxiety or depression. The HADS depression subscale is recognized as a valid and reliable measure of depressive symptoms in patients with epilepsy [73]. No information about the validity of the HADS anxiety sub-scale in patients with epilepsy was identified.

3. The Liverpool Adverse Events Profile (LAEP; [74]).

The LAEP is a 19-item self-report questionnaire that is typically used to screen for adverse effects of antiepileptic drugs. It's scored on a four-point Likert scale. Higher scores indicated more side effects. The validity and reliability of the LAEP have been verified [74-76].

Cannabidiol Use

Because this was an observational study and not a clinical trial, no CBD or cannabinoids were provided to the participants by the researchers. Products were obtained by families following state regulations. Participants were strongly encouraged to discuss participation in this study with their physician, and they were informed about potential drug interactions between CBD and AEDs [77]. Participants planning to add CBD to their treatment regime were encouraged to seek guidance about cannabinoid use from the Realm of Caring Foundation. Realm of Caring Foundation is a 501(c)(3)-non-profit organization that provides support services to people interested in the medicinal use of cannabinoids. Six of 10 participants reported using Charlotte's Web Advanced Formula Hemp Oil Extract-only on a daily basis, beginning at a dose of approximately 0.25 mL 2x/day (= approximately 25 mg cannabinoids/CBD/day), and increasing over time, depending on their experience of benefits vs. side effects. One participant was using Epidiolex (10 mL/day). Three participants reported using a different CBD product or a variety of products including CBD.

Cannabinoid Analysis

Urine samples were packed in ice and shipped overnight to the company iC42. Participants' urine was batch processed for the presence of cannabinoids at the end of the study. The purpose of testing participants' urine was to collect preliminary information about the levels of cannabinoids and metabolites present in participants' urine over the course of the study. The assay (#2) developed by iC42 simultaneously tested for the following: Δ9-tetrahydrocannabinol (THC), 11-hydroxy-Δ9-tetrahydrocannabinol (11OH-THC), 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid glucuronide (THC-Gluc), Cannabidiol (CBD), 6a-hydroxy cannabidiol (6a-OH-CBD), 6b-hydroxy cannabidiol (6b-OH-CBD), 7-hydroxy cannabidiol (7OH-CBD), 7-cannabidiol-9-carboxylic acid (7-CBD-COOH), Cannabidiol-9-carboxylic acid glucuronide (CBD-Gluc), Cannabichromene (CBC), Cannabinol (CBN), Cannabigerol (CBG), Cannabidivarin (CBDV), and Δ9-tetrahydrocannabivarin (THCV). See Anderson [78] for details about the LC-MS-MS methodology used to process the samples.

Statistical Analyses

This study used a within-subjects research design. Repeated measures ANOVAs were run using IBM SPSS Statistics 26 to analyze behavioral questionnaire data that was collected at three different time points (TIME 1, TIME 2, and TIME 3). For each a priori analysis, the p value was set to p = 0.05 and confidence intervals were 95%. Bonferroni corrections were applied to post hoc tests to correct for the inflation of Type 1 error. Greenhouse-Geisser corrections were utilized for violations of sphericity.

Results

Behavioral Data

Main Effect of Time on the QOLIE-31-P

Descriptive statistics for the QOLIE-31-P are shown in Figure 2.

[Insert Figure 2_QOLIE Descriptive Statistics: QOLIE Descriptive Statistics; Legend: Figure 2 (top) shows the mean and standard deviation for the total score on the QOLIE-31-P for each of the three time points during which data was collected. Means and standard deviations for each of the QOLIE-31-P subscales are also shown. The chart below plots the total score means for each of the three time points, along with 95 % Confidence Intervals.]

The mean QOLIE-31-P TOTAL SCORE at enrollment (TIME 1) was 37.51±17.47 SD, compared to 52.96±19.52 SD at TIME 2, and 63.84±23.04 SD at the end of the study (TIME 3), representing an average increase in QoL scores of 26.33 points over time. Higher scores were indicative of improved QoL. QOLIE-31-P SUBSCALE means also increased over time.

Inferential statistics for the QOLIE-31-P and its subscales are shown in Figure 3.

[Insert Figure 3_QOLIE-31-P Inferential Statistics: QOLIE-31-P Inferential Statistics; Legend: The results of a one-way repeated measures ANOVA on the QOLIE TOTAL SCORE with a factor of TIME (3 levels) are shown, as are the results for Bonferroni-corrected pairwise comparisons. Results for post-hoc one-way repeated measures ANOVAs on the SUSCALES of the QOLIE-31-P are also shown along with the results of Bonferroni-corrected pairwise comparisons.]

A one-way repeated measures ANOVA, with a factor of TIME (3 levels = TIME 1, 2, 3), found a significant main effect of TIME on the QOLIE TOTAL SCORE (2 df, F = 8.042, p = 0.003). This indicates that participants' TOTAL SCORES increased over TIME. Partial Eta squared was 0.472, which was indicative of a large effect. Pairwise comparisons showed TIME 1 was significantly different from TIME 3 (p = 0.024), but not TIME 2 (p = 0.221). TIME 2 was also not significantly different from TIME 3 (p = 0.124). Therefore, the increase measured in TOTAL SCORE over TIME was largely due to the increase in scores between TIME 1 and TIME 3.

Post hoc one-way repeated measures ANOVAs showed a main effect of TIME on almost all of the QOLIE-31-P SUBSCALES, including: distress (2df, F = 8.687, p = 0.010), cognition (2df, F = 6.046, p = 0.010), seizure worry (2df, F = 8.070, p = 0.014), overall quality of life (2df, F = 5.328, p = 0.015), mood (2df, F = 4.65, p = 0.024), daily activities (2df, F = 4.459, p = 0.027), and energy (2df, F = 3.851, p = 0.041) (Figure 3). Partial Eta's on significant tests ranged from 0.300 to 0.491, and were indicative of large effects. Two subscales required Greenhouse-Geisser corrections due to sphericity violations (distress and seizure worry). No main effect of TIME was found for medication effects (2df, F = 0.992, p = 0.396). Pairwise comparisons showed TIME 1 was significantly different from TIME 3 for the following subscales: distress (p = 0.036), cognition (p = 0.032), seizure worry (p = 0.041), and daily activities (p = 0.032). No other post hoc tests were statistically significant. While many of the subscale scores increased over time, those that contributed most to the statistically significant difference in TOTAL SCORE found between TIME1 and TIME 3 were: distress, cognition, seizure worry, and daily activities.

Main Effect of Time on the HADS

Descriptive and inferential statistics for the HADS are shown in Figures 4a and 4b, respectively.

[Insert Figure 4_HADS: Hospital Anxiety and Depression Scale; Legend: Figure 4 (top) shows the means and standard deviations for both the anxiety and depression subscales of the HADS for each TIMES 1, 2 and 3. Figure 4 (bottom) shows the results of a two way repeated measures ANOVA on data from the HADS with factors of TIME and SUBSCALE, along with the results of Bonferroni-corrected pairwise comparisons. Figure 4 also shows the results of post-hoc one-way repeated measures ANOVA on each of the two separate SUBSCALES, along with the results of Bonferroni-corrected pairwise comparisons.]

The mean value for anxiety symptoms on the HADS anxiety SUBSCALE at TIME 1 was 11.3±3.92 SD. The HADS anxiety subscale scores decreased from 11.3±3.92 SD at TIME 1 to 5.3±3.97 SD at TIME 3, representing an improvement, on average, of 6 points. Levels of depression symptoms at TIME 1 were 7.3±5.01SD. The HADS depression subscale scores decreased from 7.3±5.01 SD at TIME 1 to 3.9±2.85 SD at TIME 3, representing an average improvement of 3.4 points. Mean anxiety and depression scores both decreased over TIME.

HADS data was initially analyzed using a two-way repeated measures ANOVA, with factors of TIME (3 levels = TIME 1, 2, 3) and SUBSCALE (2 levels = anxiety, depression). A main effect of TIME was found (2 df, F = 5.616, p = 0.013). Partial Eta squared was 0.384. A main effect of SUBSCALE was almost significant (p = 0.054). Partial eta was 0.354. The interaction of TIME and SUBSCALE was not significant (p = 0.280). Pairwise comparisons showed TIME 1 was significantly different from TIME 3 (p = 0.013), but not TIME 2 (p = 0.461). Time 2 was not significantly different from TIME 3 (p = 0.407). These results indicated that one subscale was likely primarily responsible for the differences observed in HADS scores between TIME 1 and TIME 3.

HADS data was further analyzed using two separate one-way repeated measures ANOVAs in order to independently assess the effects of TIME on anxiety vs. depression. A main effect of TIME on anxiety

was found (2 df, F = 5.718, p = 0.012). Partial Eta squared was 0.388. Post hoc pairwise comparisons showed TIME 1 was significantly different from TIME 3 (p = 0.021) but not TIME 2 (p = 0.251). Time 2 was not significantly different from TIME 3 (p = 0.513). No main effect was found for TIME on depression (2 df, F = 2.859, p = 0.083). Although estimated marginal means confirmed a decreasing trend in depression scores over time, this data indicated that anxiety changed more over TIME than Depression.

Main Effect of Time on the LAEP

LAEP items reported as “always” or “sometimes” a problem at TIMES 1, 2, and 3 are shown in Figure 5.

[Insert Figure 5_LAEP: Liverpool Adverse Events Profile; Legend: This figure shows the frequency of participant endorsement of items on the LAEP at TIMES 1, 2, and 3.]

Some of the most frequently reported problems were: memory problems, difficulty concentrating, and nervousness/agitation. Most problems appeared to decline over the course of the study.

LAEP global scores were calculated by summing all responses to LAEP items that indicated the symptom was “always” or “sometimes” a problem. The mean global scores for the LAEP decreased from 52.1± 8.71 SD at TIME 1 to 38.5±10.80 SD at TIME 3, indicative of an average improvement of 13.6 points.

LAEP global scores were subsequently analyzed using a one way repeated measures ANOVA, with a factor of TIME (3 levels). A highly significant main effect of TIME was found (2df, F= 13.936, p = 0.000). Partial Eta was 0.608, which indicated a very large effect. Estimated marginal means confirmed a decrease in global LAEP scores over TIME. Pairwise comparisons showed TIME 1 was significantly different from TIME 2 (p = 0.007) and TIME 3 (p = 0.005). TIME 2 did not differ from TIME 3 (p = 1.000). This means that there was a significant decrease in adverse medication effects within the first 2.5 months that continued over the course of the study, albeit at a slower rate.

Urinalysis Data:

Cannabinoids and metabolites found in participants’ urine included: THC, 11-OH-THC, THCCOOH, THCCOOH-Gluc, THC-Gluc, CBD, 6a-OH-CBD, 6b-OH-CBD, 7-OH-CBD, 7-CBD-COOH, CBD-Gluc. The only metabolites tested for and not found in any of the urine samples were: CBC, CBN, CBG, THCV, and CBDV.

The primary metabolites for CBD excretion detected in participants’ urine were CBDCOOH and CBD-Gluc (See Figure 6).

[Insert Figure 6_Urinalysis: Urinalysis; Legend: Figure 6 (top) shows the levels of THC and CBD metabolites (THC-COOH, THCCOOH-gluc, CBD-COOH and CBD-gluc) present in participants urine in ng/mL during each of the three time points when data was collected. A key below shows the lower limits of quantification (LLOQ) and upper limits of quantification (ULOQ) for each of the cannabinoids/metabolites tested.]

The median concentration of CBDCOOH at TIME 1 was 0.00 ng/mL; the mean was 3.11 ng/mL; the range was (0.00 – 24.84 ng/mL). The median concentration of CBDCOOH at TIME 2 was 1.39 ng/mL; the mean was 22.09 ng/mL; the range was (0.00 – 157.18 ng/mL). The median concentration of CBDCOOH at TIME 3 was 4.184 ng/mL; the mean was 24.76 ng/mL; the range was (0.00 -126.82 ng/mL).

The median CBD-Gluc concentrations detected in participants' urine at TIME 1 was 0.00 ng/mL; the mean was 7.25 ng/mL; the range was (0.00 – 32.52 ng/mL). The median CBD-Gluc concentration at TIME 2 was 162.52 ng/mL; the mean was 180.20 ng/mL; the range was (24.192 – 473.70 ng/mL). The median CBD-Gluc concentration at TIME 3 was 77.16 ng/mL; the mean was 173.55 ng/mL; the range was (7.75 – 547.27 ng/mL).

The primary metabolites for THC excretion detected in participant’s urine were THCCOOH and THCCOOH-Gluc. The median THCCOOH concentration detected at TIME 1 was 0.25 ng/ml; the mean was 2.20 ng/mL; the range was (0.00 – 9.11 ng/mL). The median THCCOOH concentration detected at TIME 2 was 0.00 ng/mL; the mean was 12.70 ng/mL; the range was (0.00 – 106.54 ng/mL). The median THCCOOH concentration detected at TIME 3 was 0.94 ng/mL; the mean was 26.38 ng/mL; the range was (0.00 – 248.75 ng/mL).

The median THCCOOH-Gluc concentration detected at TIME 1 was 0.00 ng/mL; the mean was 153.36 ng/mL; the range was (0 – 1196.96 ng/mL). The median THCCOOH-Gluc concentration detected at TIME 2 was 18.07 ng/mL; the mean was 635.46 ng/mL; the range was (0 – 5478.00 ng/mL). The median THCCOOH-gluc concentration detected at TIME 3 was 9.766 ng/ml; the mean was 1309.71 ng/mL; the range was (0 -12529.83 ng/mL).

Levels of metabolites varied widely from below the lower limits of quantification (LLOQ) to above the upper limits of quantification (ULOQ) and differed across participants for different metabolites. The ULOQ was exceeded in 9/30 samples across five different people. Five of thirty possible urine samples were missing or not tested.

All ten participants reported no cannabinoid use in the last 30 days. However, five of eight participants tested were positive for cannabinoids/metabolites at TIME 1. Two of these five participants tested positive for both THC/metabolites and CBD/metabolites (#’s 6, 8). Three of these five participants tested positive for THC/metabolites only (#’s 2, 3, 6). One of these five participants reported a substantial history of smoking cannabis (# 3), but no cannabis or CBD use within the thirty days prior to enrolling in the study. One of these participants (# 6) reported some previous CBD use, but not within the thirty days prior to enrolling in the study. One participant (# 8) reported previous CBD use, but not within the ninety days prior to enrolling in the study. Two of these five participants reported no history of use, but reported exposure to second hand cannabis smoke (# 2, 7).

Discussion

The results of this prospective, observational, longitudinal study on artisanal CBD use revealed that CBD use is associated with a statistically significant increase in QoL over time. These findings support our primary hypothesis that chronic adjunctive use of artisanal CBD was beneficial for adults with TRE. Critically, these resultsare consistent with previous studies showing improved QoL associated with the use of either Epidiolex or artisanal CBD in adults and children with TRE [44, 58, 79]. Although QoL is increasingly recognized as an important measure of treatment outcome [23, 79], determining its clinical significance still represents a challenge. While the present study used the QOLIE-31-P, the amount of change found to be clinically relevant for the QOLIE-31 (on which the QOLIE-31-P is based) was an 11.8 point change [80]. The mean increase in QoL scores from TIME 1 to TIME 3 was 26.33 points. This suggests that the improvement observed in QoL, associated with the adjunctive use of CBD, was not only statistically significant, but also clinically relevant.

While a statistically significant and clinically relevant level of improvement in the QOLIE-31-P TOTAL SCORE was found between TIME 1 and TIME 3, a clinically relevant level of change = 15.45 points was also found between TIME 1 and TIME 2 [80]. In addition, the level of change between TIME 2 and TIME 3 was = 10.88 points, which was just below clinical relevance. This suggests that the addition of CBD to an existing treatment regime had a clinically relevant effect on QoL afterapproximately two and one-half months of treatment, and that QoL improvement was sustained and reached a statistically significant level with an additional two and one-half months of CBD treatment. In addition, almost all of the QOLIE-31-P SUBSCALES showed improvement over time and specific SUBSCALES showed statistically significant levels of improvement. Subscales with statistically significant levels of improvement included: distress, seizure worry, cognition, and daily activities.As there is an urgent need to improve QoL in people with TRE, these results provide promising insights into the therapeutic potential of CBD. Although this data suggests chronic adjunctive use of artisanal CBD may improve QOL for people with TRE of mixed etiologies, more controlled research is needed to determine the optimal CBD product choice, phytocannabinoids ratio, dosage, and route of administration.

Supporting our secondary hypothesis, we found a significant improvement in mood and psychological well-being in the form of a statistically significant decrease in anxiety and a decreasing trend in depression symptoms that was associated with CBD use. This finding was supported by a growing body of evidence from both human and animal research studies pointing to the anxiolytic and antidepressant potential of CBD [60–62, 64, 67].The majority of participants in this study exhibited the probable presence of a clinically significant level of anxiety on the HADS at TIME 1. This level steadily decreased over the course of the study to a level considered to be in the normal range at TIME 3 [73]. At TIME 2, the mean HADS score was still indicative of the presence of anxiety, albeit at a lower level. This suggests that adjunctive CBD use beyond TIME 2 may be associated with continued improvements in anxiety. Participants also presented with the possible presence of depression at enrollment. However, the level of depression was just barely above the threshold [73]. This was inconsistent with previous reports of depression being the most prevalent comorbid condition in TRE [33]. Although depression symptoms decreased over the course of the study to within a normal level, the decrease in depressive symptoms did not reach statistical significance.

Evidence from studies examining CBD mechanisms of action further supports our findings of decreased anxiety and depression associated with CBD use. While CBD has a relatively low affinity for the main CB1 and CB2 cannabinoid receptors, it has a good affinity for serotonin 5HT-1a receptors, where it acts as a receptor agonist [56, 57]. The 5HT-1a receptor is an established anxiolytic target [62]. Because anxiety and depression are considered major problems for people with TRE [31], decreasing symptoms of these conditions was considered a meaningful therapeutic outcome. Additionally, it is possible that some of the improvement we found in QoL may be attributed to the effect CBD had on anxiety and depression, as previous studies have found QoL was predicted by mood [38, 79]. Decreasing anxiety, for example, could reasonably decrease distress and seizure worry, leading to improved cognition and more comfort with daily activities. Improving symptoms of anxiety and depression are important therapeutic outcomes in TRE. Symptoms of anxiety and depression should be more widely assessed and treated in TRE.

Interestingly, we also found a statistically significant decrease in reported adverse events over time that was associated with CBD use. This result was consistent with a previous study, which found improvement in an adverse events profile from enrollment to follow up with Epidiolex treatment [22]. It was also consistent with an observational study of artisanal CBD users where participants reported significantly better epilepsy medication tolerability compared to controls [66]. One possible explanation for these findings was that after adding CBD to their existing treatment regime, participants were better able to tolerate their regular medication side effects and CBD use did not cause any additional adverse events. Improving medication tolerance and decreasing side effects is an important goal of TRE treatment.

The urinalysis performed in this study was exploratory and provided only a few time points of cannabinoid levels over the course of the study for each participant. However, the data confirmed that the majority of participants enrolled in this study added an amount of artisanal CBD to their treatment regime that was measurable in their urine at TIME 2 and TIME 3. Median levels of the primary CBD metabolites CBDCOOH and CBDgluc at TIME 1 were 0.00 ng/mL, indicating that the majority of participants tested had no CBD in their system at the beginning of the study. Median CBD/metabolites increased to levels well above the LLOQ at TIME 2 and TIME 3, indicating that the majority of participants had a measurable amount of CBD/metabolites in their urine at these times. While an increase in the median level of CBDCOOH was observed between TIME 2 and TIME 3, a decrease was observed in CBDgluc. These types of changes could have occurred for a whole variety of reasons, for example, decreased compliance with medication protocols, differences in the time-frames between last cannabinoid use and when the samples were collected, or, how well the samples shipped.

The median level of THC/metabolites at TIME 1 were very low but not zero. Median levels of the primary THC metabolites THCCOOH and THCCOOHgluc were 0.25 ng/mL and 0.0 ng/mL, respectively at TIME 1, both of which were below the LLOQ. However, finding a measurable median level of THCCOOH indicates that some participants had some level of THC/metabolites in their system at the beginning of the study. Although participants reported not having used any cannabinoids in the last 30 days, they were not required to be naïve. Because pharmacokinetic processes are dynamic, and may change over time, they

may be affected by the frequency and magnitude of drug use [81]. How long cannabis stays in your system varies depending on many different factors including frequency of use, potency, consumption method, body composition, nature of drug test, etc. [82].

Median THC/metabolite levels increased to levels above the LLOQ for TIMES 2 and 3, indicating the majority of participants had a measurable level of THC/metabolites in their system at these times. Results of the urinalysis are consistent with the majority of participants reporting using the whole spectrum product - Charlotte's Web. We highly recommend incorporating urine and/or blood testing of cannabinoid levels in future studies of chronic artisanal CBD use so that researchers can begin to define therapeutic cannabinoid metabolite levels.

Strengths and Limitations

This study had a number of strengths and weaknesses. One strength of this study was the use of a within subjects research design. This allowed participants to serve as their own controls and limited the number of participants required to reach statistical significance assuming a moderate effect. This study also had the benefit of being longitudinal, which allowed us to track sustained effects of CBD on participants' behavior. A further strength was that, through urinalysis, we were able to confirm that participants had added CBD to their treatment regime.

One limitation of this study was that the results were based on data from only 10 participants. Although the longitudinal nature of the study was positive, the length of enrollment made it difficult for some participants to complete the study.

Another limitation was that because this study was observational and not interventional, participants chose their own cannabinoid products and controlled their own dosing and drug administration. While most participants (60%) chose to use Charlotte's Web Advanced Formula Hemp Oil Extract, their dosing varied, and some participants chose to use other products. Although this could potentially impact the generalization of this study, research shows that participants prefer to have control over their own treatment [83]. A sense of control is considered integral to a patient's perception of their health and well-being. Presumably, participants in this study managed their CBD dosing in a manner that felt most therapeutic for them personally, and this was reflected in their improved perception of QoL, improved psychological well-being, and diminished experience of adverse effects.

Since this was not a RCT, this study lacked control for the placebo effect. The role of the placebo effect is particularly important to acknowledge as it is known to be particularly strong in epilepsy drug studies [84], and in studies of cannabinoid use in epilepsy [45, 48]. Moreover, widespread media coverage of CBD effectiveness at reducing seizures, together with the belief that natural products may be safer and more effective than traditional pharmaceuticals, could have led to selection bias. We cannot exclude the possibility that the placebo effect and selection bias may be partially responsible for the findings of this study. Although observational studies have some inherent limitations which constrain their ability to define causality, their strengths include that they reflect daily practice more closely than RCTs in terms of

both the heterogeneous nature of the populations that are included in research and the treatment that is received [85].

A further limitation of this study was that participants were not required to be cannabis naïve. This likely affected the urinalysis results at TIME 1. Moreover, urinalysis was performed at the end of the study and was not used as an exclusion criteria. In general, real-time testing of CBD/metabolites was not readily available at the time the study was conducted.

Conclusion

Our results suggest that widely available artisanal CBD formulations may be useful for the treatment of anxiety and depression, and for improving QoL in adults with TRE of varying etiologies. Use of CBD may also decrease the experience of AED-related side effects, improving medication tolerability.

Abbreviations

AED = Antiepileptic drugs

Anandamide = N-arachidonoylethanolamine

ANOVA = Analysis of variance

CB1 = Cannabinoid receptor type 1

CB2 = Cannabinoid receptor type 2

df = Degrees of freedom

ECS = Endocannabinoid system

F = Fisher statistic

LAEP = Liverpool adverse events profile

LLOQ = Lower limits of quantification

mg = milligram

mL = milliliter

n.d. = No date

ng = Nanogram

p = Probability value

RCT = Randomized control trial

SD = Standard deviation

SUDEP = Sudden unexplained death in epilepsy

THC = Delta-9-tetrahydro cannabinol

TRE = Treatment resistant epilepsy

TRPV1 = Transient receptor potential cation channel subfamily V member 1

PPARG = Peroxisome proliferator- activated receptor gamma

GPR55 = G-protein coupled receptor 55

ULOQ= Upper limits of quantification

QoL = Quality of life

QOLIE-31-P = Quality of life in epilepsy – 31-patient weighted

2-AG = 2-arachidonoylglycerol

5-HT-1a = 5-hydroxytryptamine 1a receptor

5-HT-2a = 5-hydroxytryptamine 2a receptor

HADS = Hospital anxiety and depression scale

THC = Δ9-tetrahydrocannabinol

11OH-THC = 11-hydroxy-Δ9-tetrahydrocannabinol

THC-COOH = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid

THC-Gluc = 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid glucuronide

CBD = Cannabidiol

6a-OH-CBD = 6a-hydroxy cannabidiol

6b-OH-CBD = 6b-hydroxy cannabidiol

7OH-CBD = 7-hydroxy cannabidiol

7-CBD-COOH = 7-cannabidiol-9-carboxylic acid

CBD-Gluc = Cannabidiol-9-carboxylic acid glucuronide

CBC = Cannabichromene

CBN = Cannabinol

CBG = Cannabigerol

CBDV = Cannabidivarin

THCV = Δ9-tetrahydrocannabivarin

Declarations

Ethical Approval and Consent to participate

This research was performed in accordance with the Declaration of Helsinki and was approved by the Colorado State University-Pueblo Institutional Review Board for Human Subjects Research. All participants provided written informed consent.

Consent for publication

N.A.

Availability of supporting data

The dataset used and analyzed for the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funding by grants from the Pueblo County Commissioners and the Institute of Cannabis Research at Colorado State University–Pueblo.

Authors' contributions

B.B. is the primary author, responsible for all aspects of this study from conceptualization and design to data acquisition, data analysis, data interpretation and writing the manuscript; M.C. participated in data acquisition, data analysis, and data interpretation; H.D contributed to data interpretation and substantial manuscript revisions; M.X.L contributed to data interpretation and substantial manuscript revisions, S.K-K contributed to data interpretation and substantial manuscript revisions, and H.J. contributed to study conceptualization, study design, data interpretation, and substantial manuscript revisions.

Acknowledgements

The authors would like to thank the funding agencies that supported this work as well as all of the people who participated and their families.

References

1. Stroke, N.I.N.D.S. The Epilepsies and Seizures: Hope and Help through Research. 2015.
2. Organization, T.W.H. (n.d.).
3. Prevention, C.D.C. Epilepsy 2020.
4. Sander, J.W., *Some aspects of prognosis in the epilepsies: a review.* Epilepsia, 1993. **34**(6): p. 1007-16.
5. Friedman, D. and O. Devinsky, *Cannabinoids in the Treatment of Epilepsy.* N Engl J Med, 2015. **373**(11): p. 1048-58.
6. Kwan, P. and M.J. Brodie, *Epilepsy after the first drug fails: substitution or add-on?* Seizure, 2000. **9**(7): p. 464-8.
7. Brodie, M.J., et al., *Patterns of treatment response in newly diagnosed epilepsy.* Neurology, 2012. **78**(20): p. 1548-54.
8. Uijl, S.G., et al., *A cross-sectional study of subjective complaints in patients with epilepsy who seem to be well-controlled with anti-epileptic drugs.* Seizure, 2006. **15**(4): p. 242-8.
9. Carpay, J.A., A.P. Aldenkamp, and C.A. van Donselaar, *Complaints associated with the use of antiepileptic drugs: results from a community-based study.* Seizure, 2005. **14**(3): p. 198-206.
10. Loring, D.W., S. Marino, and K.J. Meador, *Neuropsychological and behavioral effects of antiepilepsy drugs.* Neuropsychol Rev, 2007. **17**(4): p. 413-25.
11. Perucca, P. and F.G. Gilliam, *Adverse effects of antiepileptic drugs.* Lancet Neurol, 2012. **11**(9): p. 792-802.
12. Kwan, P. and M.J. Brodie, *Early identification of refractory epilepsy.* N Engl J Med, 2000. **342**(5): p. 314-9.
13. Kwan, P., et al., *Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies.* Epilepsia, 2010. **51**(6): p. 1069-77.
14. Devinsky, O. and J.K. Penry, *Quality of life in epilepsy: the clinician's view.* Epilepsia, 1993. **34 Suppl 4**: p. S4-7.
15. Suurmeijer, T.P., M.F. Reuvekamp, and B.P. Aldenkamp, *Social functioning, psychological functioning, and quality of life in epilepsy.* Epilepsia, 2001. **42**(9): p. 1160-8.
16. Szaflarski, M., et al., *Quality of life in medication-resistant epilepsy: the effects of patient's age, age at seizure onset, and disease duration.* Epilepsy Behav, 2006. **8**(3): p. 547-51.
17. Sillanpaa, M. and S. Shinnar, *SUDEP and other causes of mortality in childhood-onset epilepsy.* Epilepsy Behav, 2013. **28**(2): p. 249-55.

18. Racoosin, J.A., et al., *Mortality in antiepileptic drug development programs.* Neurology, 2001. **56**(4): p. 514-9.
19. Ryvlin, P., M. Cucherat, and S. Rheims, *Risk of sudden unexpected death in epilepsy in patients given adjunctive antiepileptic treatment for refractory seizures: a meta-analysis of placebo-controlled randomised trials.* Lancet Neurol, 2011. **10**(11): p. 961-8.
20. Devinsky, O., et al., *Recognizing and preventing epilepsy-related mortality: A call for action.* Neurology, 2016. **86**(8): p. 779-86.
21. Lv, R., et al., *Depression, anxiety and quality of life in parents of children with epilepsy.* Acta Neurol Scand, 2009. **120**(5): p. 335-41.
22. Gaston, T., et al., *Quality of life in adults enrolled in an open-label study of cannabidiol (CBD) for treatment-resistant epilepsy.* Epilepsy Behav, 2019. **95**: p. 10-17.
23. Miguel, R.S., et al., *Measuring health-related quality of life in drug clinical trials: is it given due importance?* Pharmacy World & Science, 2008. **30**(2): p. 154-160.
24. Cramer, J.A., et al., *The influence of comorbid depression on seizure severity.* Epilepsia, 2003. **44**(12): p. 1578-84.
25. Borghs, S., C. de la Loge, and J.A. Cramer, *Defining minimally important change in QOLIE-31 scores: estimates from three placebo-controlled lacosamide trials in patients with partial-onset seizures.* Epilepsy Behav, 2012. **23**(3): p. 230-4.
26. Devinsky, O., et al., *Development of the quality of life in epilepsy inventory.* Epilepsia, 1995. **36**(11): p. 1089-104.
27. Marsh, L. and V. Rao, *Psychiatric complications in patients with epilepsy: a review.* Epilepsy Res, 2002. **49**(1): p. 11-33.
28. Jackson, M.J. and D. Turkington, *Depression and anxiety in epilepsy.* J Neurol Neurosurg Psychiatry, 2005. **76 Suppl 1**: p. i45-47.
29. Lee, S.A., et al., *Factors contributing to anxiety and depressive symptoms in adults with new-onset epilepsy.* Epilepsy Behav, 2018. **88**: p. 325-331.
30. Tellez-Zenteno, J.F., et al., *Psychiatric comorbidity in epilepsy: a population-based analysis.* Epilepsia, 2007. **48**(12): p. 2336-44.
31. Mensah, S.A., et al., *A community study of the presence of anxiety disorder in people with epilepsy.* Epilepsy Behav, 2007. **11**(1): p. 118-24.
32. Kanner, A.M. and S. Palac, *Depression in epilepsy: a common but often unrecognized comorbid malady.* Epilepsy Behav, 2000. **1**(1): p. 37-51.
33. Fiest, K.M., et al., *Depression in epilepsy: a systematic review and meta-analysis.* Neurology, 2013. **80**(6): p. 590-9.
34. Boylan, L.S., et al., *Depression but not seizure frequency predicts quality of life in treatment-resistant epilepsy.* Neurology, 2004. **62**(2): p. 258-61.

35. Loring, D.W., K.J. Meador, and G.P. Lee, *Determinants of quality of life in epilepsy.* Epilepsy Behav, 2004. **5**(6): p. 976-80.
36. Maa, E. and P. Figi, *The case for medical marijuana in epilepsy.* Epilepsia, 2014. **55**(6): p. 783-6.
37. Devinsky, O., et al., *Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial.* Lancet Neurol, 2016. **15**(3): p. 270-8.
38. Szaflarski, J.P., et al., *Long-term safety and treatment effects of cannabidiol in children and adults with treatment-resistant epilepsies: Expanded access program results.* Epilepsia, 2018. **59**(8): p. 1540-1548.
39. Devinsky, O., J.H. Cross, and S. Wright, *Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome.* N Engl J Med, 2017. **377**(7): p. 699-700.
40. Thiele, E.A., et al., *Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial.* Lancet, 2018. **391**(10125): p. 1085-1096.
41. Thiele, E.A., et al., *Add-on Cannabidiol Treatment for Drug-Resistant Seizures in Tuberous Sclerosis Complex: A Placebo-Controlled Randomized Clinical Trial.* JAMA Neurol, 2021. **78**(3): p. 285-292.
42. Pauli, C.S., et al., *Cannabidiol Drugs Clinical Trial Outcomes and Adverse Effects.* Front Pharmacol, 2020. **11**: p. 63.
43. Sulak, D., R. Saneto, and B. Goldstein, *The current status of artisanal cannabis for the treatment of epilepsy in the United States.* Epilepsy Behav, 2017. **70**(Pt B): p. 328-333.
44. Strickland, J.C., et al., *Cross-sectional and longitudinal evaluation of cannabidiol (CBD) product use and health among people with epilepsy.* Epilepsy Behav, 2021. **122**: p. 108205.
45. Press, C.A., K.G. Knupp, and K.E. Chapman, *Parental reporting of response to oral cannabis extracts for treatment of refractory epilepsy.* Epilepsy Behav, 2015. **45**: p. 49-52.
46. Porcari, G.S., et al., *Efficacy of artisanal preparations of cannabidiol for the treatment of epilepsy: Practical experiences in a tertiary medical center.* Epilepsy Behav, 2018. **80**: p. 240-246.
47. Hausman-Kedem, M., S. Menascu, and U. Kramer, *Efficacy of CBD-enriched medical cannabis for treatment of refractory epilepsy in children and adolescents - An observational, longitudinal study.* Brain Dev, 2018. **40**(7): p. 544-551.
48. Knupp, K.G., et al., *Prospective evaluation of oral cannabis extracts in children with epilepsy.* Seizure, 2019. **72**: p. 23-27.
49. Devinsky, O., et al., *Observational study of medical marijuana as a treatment for treatment-resistant epilepsies.* Ann Clin Transl Neurol, 2022. **9**(4): p. 497-505.
50. Devinsky, O., et al., *Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders.* Epilepsia, 2014. **55**(6): p. 791-802.
51. ElSohly, M. and W. Gul, *Constituents of Cannabis sativa.* Handbook of cannabis, 2014. **3**: p. 1093.
52. Di Marzo, V. and F. Piscitelli, *The Endocannabinoid System and its Modulation by Phytocannabinoids.* Neurotherapeutics, 2015. **12**(4): p. 692-8.

53. Marsicano, G. and B. Lutz, *Neuromodulatory functions of the endocannabinoid system.* Journal of endocrinological investigation, 2006. **29**(3): p. 27.
54. Szabo, B. and E. Schlicker, *Effects of cannabinoids on neurotransmission.* Handb Exp Pharmacol, 2005(168): p. 327-65.
55. Pacher, P., S. Bátkai, and G. Kunos, *The endocannabinoid system as an emerging target of pharmacotherapy.* Pharmacological reviews, 2006. **58**(3): p. 389-462.
56. Pertwee, R., *The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin.* British journal of pharmacology, 2008. **153**(2): p. 199-215.
57. Gaston, T.E. and D. Friedman, *Pharmacology of cannabinoids in the treatment of epilepsy.* Epilepsy Behav, 2017. **70**(Pt B): p. 313-318.
58. Stockings, E., et al., *Evidence for cannabis and cannabinoids for epilepsy: a systematic review of controlled and observational evidence.* J Neurol Neurosurg Psychiatry, 2018. **89**(7): p. 741-753.
59. Kim, S.H., et al., *Effects of Cannabidiol on Adaptive Behavior and Quality of Life in Pediatric Patients With Treatment-Resistant Epilepsy.* J Clin Neurol, 2022. **18**.
60. Crippa, J.A., et al., *Translational Investigation of the Therapeutic Potential of Cannabidiol (CBD): Toward a New Age.* Front Immunol, 2018. **9**: p. 2009.
61. Garcia-Gutierrez, M.S., et al., *Cannabidiol: A Potential New Alternative for the Treatment of Anxiety, Depression, and Psychotic Disorders.* Biomolecules, 2020. **10**(11).
62. Blessing, E.M., et al., *Cannabidiol as a Potential Treatment for Anxiety Disorders.* Neurotherapeutics, 2015. **12**(4): p. 825-36.
63. Crippa, J.A., et al., *Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report.* J Psychopharmacol, 2011. **25**(1): p. 121-30.
64. Zuardi, A.W., et al., *Human experimental anxiety: actual public speaking induces more intense physiological responses than simulated public speaking.* Braz J Psychiatry, 2013. **35**(3): p. 248-53.
65. Corroon, J. and J.A. Phillips, *A Cross-Sectional Study of Cannabidiol Users.* Cannabis Cannabinoid Res, 2018. **3**(1): p. 152-161.
66. Strickland, J.C., et al., *Cross-sectional and longitudinal evaluation of cannabidiol (CBD) product use and health among people with epilepsy.* Epilepsy & Behavior, 2021. **122**: p. 108205.
67. Shannon, S., et al., *Cannabidiol in Anxiety and Sleep: A Large Case Series.* Perm J, 2019. **23**: p. 18-041.
68. García-Gutiérrez, M.S., et al., *Cannabidiol: a potential new alternative for the treatment of anxiety, depression, and psychotic disorders.* Biomolecules, 2020. **10**(11): p. 1575.
69. Detyniecki, K. and L.J. Hirsch, *Cannabidiol for epilepsy: trying to see through the haze.* The Lancet Neurology, 2016. **15**(3): p. 235-237.
70. Cramer, J.A., G. Van Hammée, and N.S. Group, *Maintenance of improvement in health-related quality of life during long-term treatment with levetiracetam.* Epilepsy & Behavior, 2003. **4**(2): p. 118-123.

71. Borghs, S., C. de la Loge, and J.A. Cramer, *Defining minimally important change in QOLIE-31 scores: estimates from three placebo-controlled lacosamide trials in patients with partial-onset seizures.* Epilepsy & Behavior, 2012. **23**(3): p. 230-234.
72. Cramer, J.A., et al., *Development and cross-cultural translations of a 31-item quality of life in epilepsy inventory.* Epilepsia, 1998. **39**(1): p. 81-8.
73. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. **67**(6): p. 361-70.
74. Baker, G.A., et al., *The initial development of a health-related quality of life model as an outcome measure in epilepsy.* Epilepsy Res, 1993. **16**(1): p. 65-81.
75. Baker, G.A., et al., *Development of a novel scale to assess life fulfillment as part of the further refinement of a quality-of-life model for epilepsy.* Epilepsia, 1994. **35**(3): p. 591-6.
76. Baker, G.A., *Health-related quality-of-life issues: optimizing patient outcomes.* Neurology, 1995. **45**(3 Suppl 2): p. S29-34.
77. Gaston, T.E., et al., *Interactions between cannabidiol and commonly used antiepileptic drugs.* Epilepsia, 2017. **58**(9): p. 1586-1592.
78. Andersson, M., et al., *Simultaneous quantification of 11 cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry using WAX-S tips.* Analytical and bioanalytical chemistry, 2016. **408**(23): p. 6461-6471.
79. Gaston, T.E., et al., *Long-term safety and efficacy of highly purified cannabidiol for treatment refractory epilepsy.* Epilepsy Behav, 2021. **117**: p. 107862.
80. Wiebe, S., et al., *Clinically important change in quality of life in epilepsy.* J Neurol Neurosurg Psychiatry, 2002. **73**(2): p. 116-20.
81. Lowe, R.H., et al., *Extended urinary Δ9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure.* Drug and alcohol dependence, 2009. **105**(1-2): p. 24-32.
82. Huestis, M.A., et al., *Free and Glucuronide Urine Cannabinoids after Controlled Smoked, Vaporized and Oral Cannabis Administration in Frequent and Occasional Cannabis Users.* J Anal Toxicol, 2020. **44**(7): p. 651-660.
83. Gloss, D. and B. Vickrey, *Cannabinoids for epilepsy.* Cochrane database of systematic reviews, 2014(3).
84. Goldenholz, D.M. and S.R. Goldenholz, *Response to placebo in clinical epilepsy trials—old ideas and new insights.* Epilepsy research, 2016. **122**: p. 15-25.
85. Yang, W., et al., *Observational studies: going beyond the boundaries of randomized controlled trials.* Diabetes research and clinical practice, 2010. **88**: p. S3-S9.

Figures

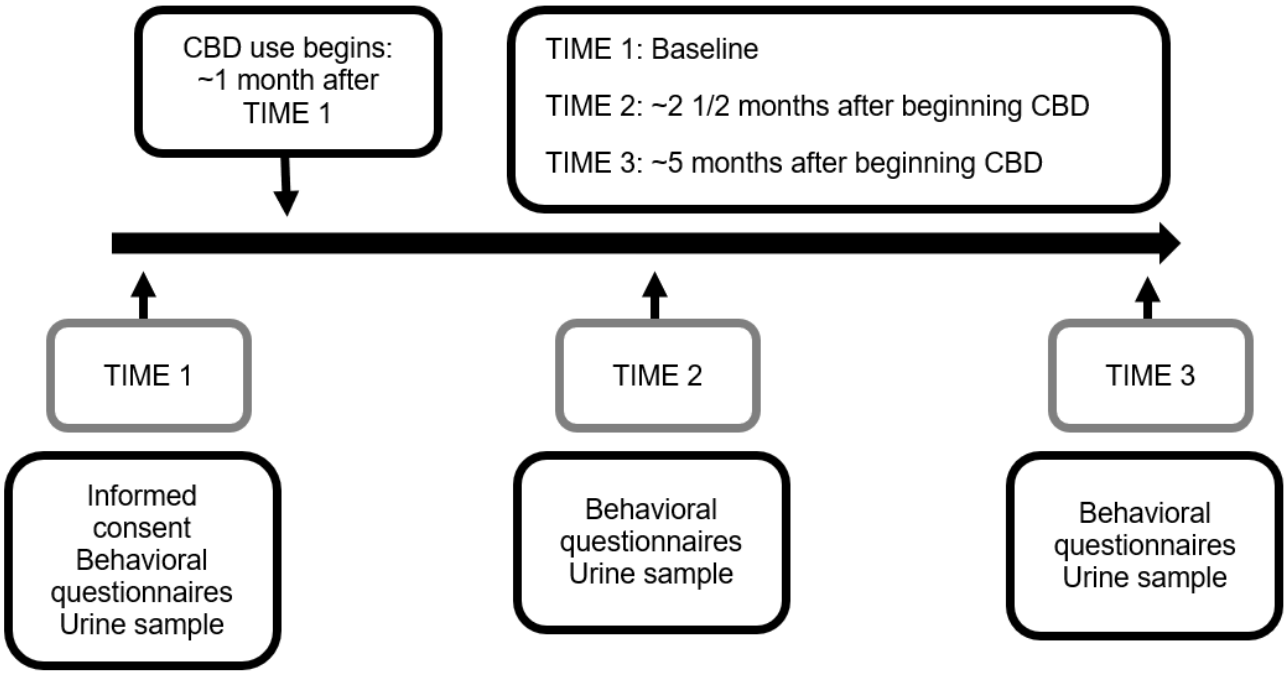

Figure 1

Timeline; Legend: The arrow in the center of the figure represents the six month time course of the study. Below the arrow, the three time points for data collection are displayed in boxes: TIME 1, TIME 2, TIME 3. Above the arrow, the timing of the initiation of CBD is displayed along with the ~timing of data collection relative to the initiation of CBD use. The information collected at each time point is indicated in the lowest boxes.]

QOLIE-31-P (Using "Distress" as a separate subscale)	TIME 1 (Mean and SD)	TIME 2 (Mean and SD)	TIME 3 (Mean and SD)
Total Score	37.51±17.47	52.96±19.52	63.84±23.04
Subscale A (Energy)	33.50±32.41	53.50±35.12	56.00±33.23
Subscale B (Mood)	48.40±24.98	61.60±26.81	72.80±25.77
Subscale C (Daily Activities)	36.56±32.15	52.88±24.65	68.98±31.16
Subscale D (Cognition)	31.08±25.54	43.55±29.82	58.93±28.92
Subscale E (Medication Effects; n = 8)	33.32±21.41	33.31±26.57	46.52±33.36
Subscale F (Seizure Worry)	35.80±30.93	56.73±31.91	63.00±35.66
Subscale G (Overall Quality of Life)	45.25±20.73	60.50±21.56	71.50±25.77
Subscale (Distress)	37.93±28.90	61.90±23.42	71.97±25.80

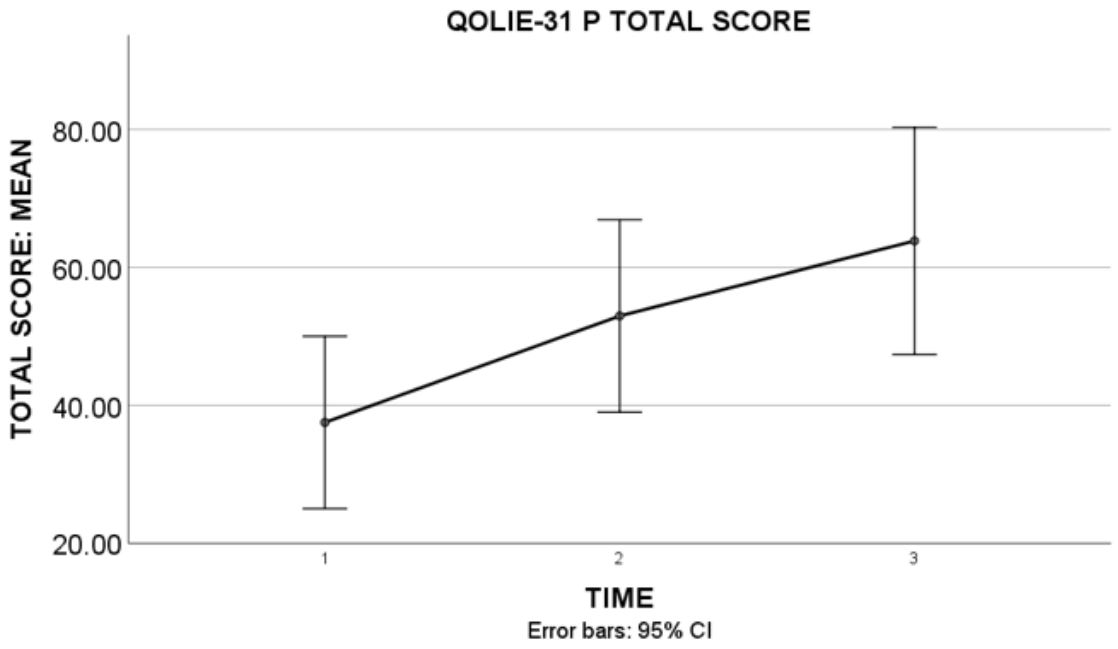

Figure 2

QOLIE Descriptive Statistics: QOLIE Descriptive Statistics; Legend: Figure 2 (top) shows the mean and standard deviation for the total score on the QOLIE-31-P for each of the three time points during which data was collected. Means and standard deviations for each of the QOLIE-31-P subscales are also shown. The chart below plots the total score means for each of the three time points, along with 95 % Confidence Intervals.

QOLIE-31-P (Using "Distress" as a separate subscale)	F statistic	P value	Partial Eta squared	Pairwise comparisons w/Bonferroni correction (T = Time 1, 2, 3) (Sigdif = Statistically significant difference) NS = No statistically significant difference
Total Score	8.042	0.003*	0.472	T1 Sigdiff T3 (p = 0.024)
Subscale A (Energy)	3.851	0.041*	0.300	NS
Subscale B (Mood)	4.650	0.024*	0.341	NS
Subscale C (Daily Activities)	4.459	0.027*	0.331	T1 Sigdif T3 (p = 0.032)
Subscale D (Cognition)	6.046	0.010*	0.402	T1 Sigdif T3 (p = 0.032)
Subscale E (Medication Effects; n = 8)	0.992	0.396	0.124	N.A.
Subscale F (Seizure Worry)	8.070	0.003 (G-G = 0.014*)	0.473	T1 Sigdif T3 (p = 0.041)
Subscale G (Overall Quality of Life)	5.328	0.015*	0.372	NS
Subscale (Distress)	8.687	0.002 (G-G = 0.010*)	0.491	T1 Sigdif T3 (p = 0.036
* = p < 0.05; G-G = Greenhouse-Geisser correction for Mauchly's Test of Sphericity violation				

Figure 3

QOLIE-31-P Inferential Statistics: QOLIE-31-P Inferential Statistics; Legend: The results of a one-way repeated measures ANOVA on the QOLIE TOTAL SCORE with a factor of TIME (3 levels) are shown, as are the results for Bonferroni-corrected pairwise comparisons. Results for post-hoc one-way repeated measures ANOVAs on the SUSCALES of the QOLIE-31-P are also shown along with the results of Bonferroni-corrected pairwise comparisons.

	Mean	Std. Deviation	N
HADS_T1 Anx	11.3000	3.91720	10
HADS T1 Dep	7.3000	5.01221	10
HADS_T2 Anx	8.3000	6.07454	10
HADS_T2 Dep	5.5000	4.81318	10
HADS T3 Anx	5.3000	3.97352	10
HADS T3 Dep	3.9000	2.84605	10

HADS (2 Way Repeated Measures ANOVA with factors of TIME (3 levels) and SUBSCALE (2 levels)	F statistic	P value	Partial Eta squared	Pairwise comparisons w/Bonferroni correction (T = Time 1, 2, 3) (Sigdif = Statistically significant difference) NS = No statistically significant difference
TIME	5.616	0.013*	0.384	T1 sigdif T3 (p = 0.013
ANX_DEP	4.930	0.054	0.354	NS
TIME*ANX_DEP	1.368	0.280	0.132	NS

HADS (1 Way Repeated Measures ANOVA with factors of TIME (3 levels) and ANXIETY	F statistic	P value	Partial Eta squared	Pairwise comparisons w/Bonferroni correction (T = Time 1, 2, 3) (Sigdif = Statistically significant difference) NS = No statistically significant difference
TIME	5.718	0.012*	0.388	T1 sigdif T3 (p = 0.021

HADS (1 Way Repeated Measures ANOVA with factors of TIME (3 levels) and DEPRESSION	F statistic	P value	Partial Eta squared	Pairwise comparisons w/Bonferroni correction (T = Time 1, 2, 3) (Sigdif = Statistically significant difference) NS = No statistically significant difference
TIME	2.859	0.083	0.241	T1 sigdif T3 (p = 0.013

Figure 4

HADS: Hospital Anxiety and Depression Scale; Legend: Figure 4 (top) shows the means and standard deviations for both the anxiety and depression subscales of the HADS for each TIMES 1, 2 and 3. Figure 4 (bottom) shows the results of a two way repeated measures ANOVA on data from the HADS with factors of TIME and SUBSCALE, along with the results of Bonferroni-corrected pairwise comparisons.

Figure 4 also shows the results of post-hoc one-way repeated measures ANOVA on each of the two separate SUBSCALES, along with the results of Bonferroni-corrected pairwise comparisons.

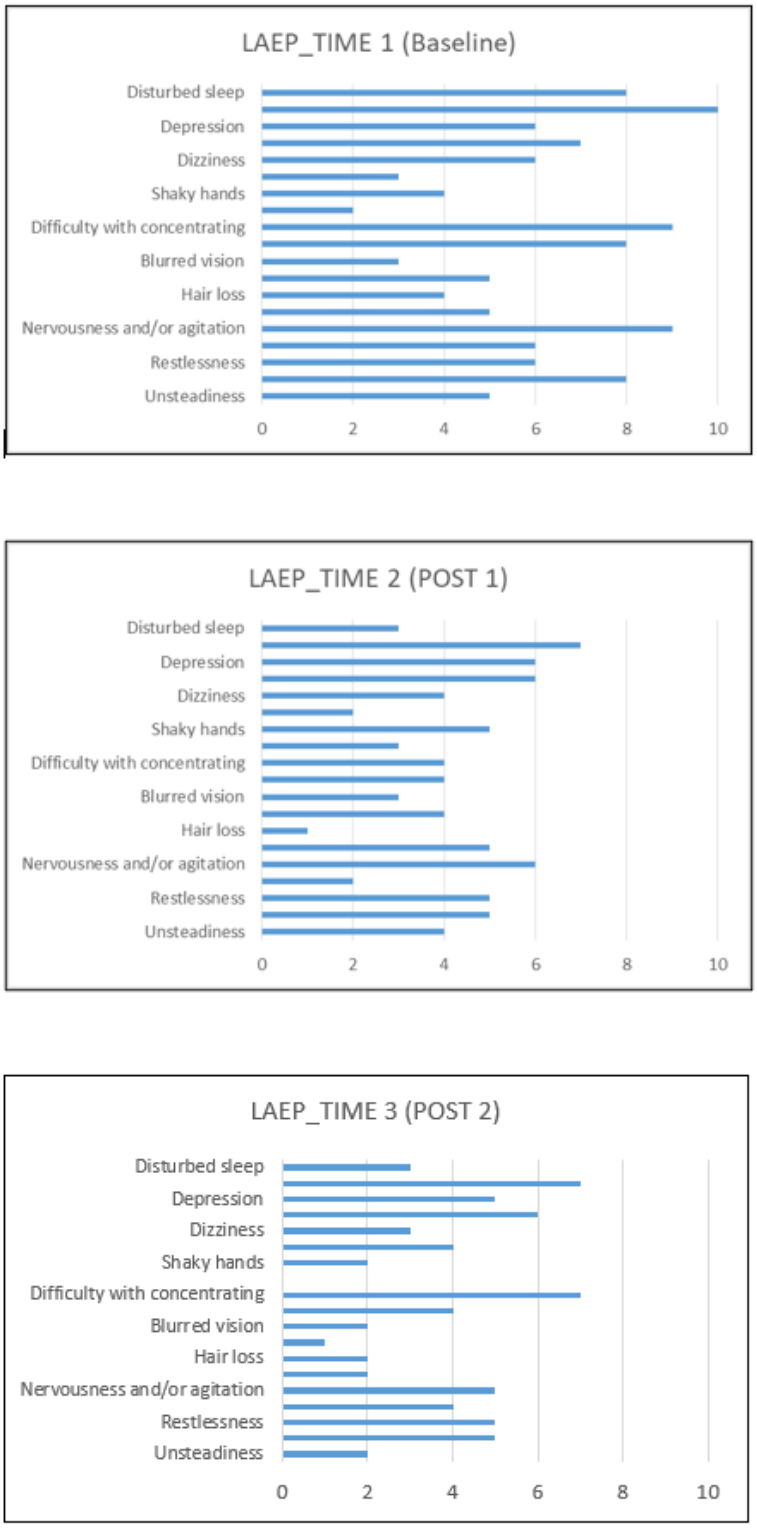

Figure 5

LAEP: Liverpool Adverse Events Profile; Legend: This figure shows the frequency of participant endorsement of items on the LAEP at TIMES 1, 2, and 3.

Subject	THCCOOH TIME 1	THCCOOH TIME 2	THCCOOH TIME 3	THCCOOH-Gluc TIME 1	THCCOOH-Gluc TIME 2	THCCOOH-Gluc TIME 3	CBD COOH TIME 1	CBD COOH TIME 2	CBD COOH TIME 3	CBD-Gluc TIME 1	CBD-Gluc TIME 2	CBD-Gluc TIME 3
1	0	0	0	0	0	0	0	0	0	0	59.096	7.75
2	0	0	0	8.583	19.293	0	0	1.386	0	0	47.801	54.585
3	9.105	106.543	248.75	1196.975	5477.995	12529.83	0	0	0	0	225.416	77.162
4	0	0	0	0	12.786	0	0	2.126	16.661	0	141.879	547.273
5	0	0	0	0	9.258	9.49	0	N.A.	N.A.	0	N.A.	N.A.
6	0.504	1.138	0.653	21.335	18.068	10.062	0	1.052	0.509	25.473	24.192	11.28
7	0.69	0	1.222	0	45.686	68.091	0	1.388	4.184	0	286.315	8.093
8	7.323	N.A.	3.781	0	N.A.	58.53	24.843	N.A.	8.86	32.52	N.A.	151.172
9	N.A.	2.241	2.56	N.A.	136.017	421.067	N.A.	13.598	65.819	N.A.	183.169	216.251
10	N.A.	4.337	6.869	N.A.	0	0	N.A.	157.175	126.819	N.A.	473.697	488.381
Median	0.252	0	0.9375	0	18.068	9.776	0	1.387	4.184	0	162.524	77.162
Mean	2.203	12.695	26.384	153.362	635.455	1309.707	3.105	22.091	24.761	7.249	180.195	173.549

N.A. = Missing or not analyzed with assay #2

Working range		
Cannabinoid	LLOQ	ULOQ
	[ng/mL]	[ng/mL]
THC	0.78	400
11OH-THC	1.56	400
THC-COOH	0.39	400
THCCOOH-gluc	7.8	2000
THC-Gluc	1.56	200
CBD	0.78	400
6α-OH-CBD	1.56	400
6β-OH-CBD	1.56	400
7-OH-CBD	1.56	400
CBD-COOH	0.39	400
CBD-Gluc	1.56	200
CBC	1.56	400
CBN	1.56	400
CBG	0.78	400
THCV	0.78	400
CBDV	1.56	400

Figure 6

Urinalysis: Urinalysis; Legend: Figure 6 (top) shows the levels of THC and CBD metabolites (THC-COOH, THCCOOH-gluc, CBD-COOH and CBD-gluc) present in participants urine in ng/mL during each of the three time points when data was collected. A key below shows the lower limits of quantification (LLOQ) and upper limits of quantification (ULOQ) for each of the cannabinoids/metabolites tested.