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Article

Cannabichromene from full-spectrum hemp extract exerts acute anti-seizure effects through allosteric activation of $GABA_A$ receptors

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ABSTRACT

The approval of Epidiolex, an anti-epileptic drug containing cannabidiol (CBD) as its active component, has brought hope to patients with refractory epilepsy. However, the anti-seizure effect of full-spectrum hemp extract (HE), a CBD-enriched hemp oil, remains unclear. In this study, we investigated the anti-seizure effect of HE using drug-induced seizure models. Our findings revealed that HE significantly reduced seizure susceptibility comparable to CBD at the same doses. Moreover, we explored the pharmacokinetic properties of CBD in HE and observed improved characteristics such as faster oral absorption, enhanced brain distribution, and slower elimination. We further assessed the anti-seizure effects of the other five main non-addictive components in HE. Among these components, cannabichromene (CBC) and cannabinol (CBN) showed significant anti-seizure effects. To gain insights into the mechanisms of CBC and CBN, we investigated their allosteric modulation on the $GABA_A$ receptor. Our results revealed that CBC enhanced GABA-induced currents in both Xenopus laevis oocytes and mouse primary cortical neurons. Additionally, we identified V436 in the β2 subunit of the $GABA_A$ receptor as a critical binding site for CBC. These findings provide compelling evidence for the anti-seizure activities of HE and shed light on its underlying mechanisms. Our study provides insights into the broader therapeutic potential of hemp extracts and suggests their possible development as anti-seizure treatments.

1. Introduction

Epilepsy is one of the most common brain disorders, characterized by a persistent predisposition to generate spontaneous epileptic seizures [1]. According to the World Health Organization, epilepsy accounts for approximately 1% of the global disease burden among major neuropsychiatric disorders [2]. Although antiseizure medications are the primary treatment for epilepsy, more than 30% of patients still experience inadequate seizure control with medical treatment [3]. Drug-resistant epilepsy is challenging to manage with existing drugs, often necessitating resection surgery, nerve stimulation, ketogenic diets, or other treatments. These patients also face a higher risk of persistent brain damage and other secondary consequences of epileptic seizures, which adversely affect their quality of life [4]. Therefore, the discovery of novel and effective anti-epileptic drugs remains an urgent issue.

Cannabis has been utilized for thousands of years to treat a wide range of diseases, including epilepsy and pain [5]. With over 560 known compounds in cannabis, significant progress has been made in identifying its active ingredients [6]. Recent investigations have demonstrated that cannabidiol (CBD), one of the primary non-psychoactive components of cannabis extracts, exhibits notable anti-epileptic properties [7]. A CBD preparation known as Epidiolex® has received FDA approval for the treatment of Dravet Syndrome and Lennox-Gastaut Syndrome [8]. Epileptic patients have also been using cannabis extracts with high CBD concentrations [9]. According to a meta-analysis, CBD-rich cannabis extracts are more effective in patients with refractory epilepsy compared to purified CBD [10]. However, it is disappointing to note that these cannabis extracts carry a higher risk of addiction and more adverse effects due to their psychoactive ingredient, Δ9-tetrahydrocannabinol (THC) [11].

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Hemp is a unique variety of cannabis that contains a THC concentration below 0.3%, making it distinct from other cannabis strains. Full-spectrum hemp extract (HE) typically contains around 70% CBD [12]. As a result, HE has gained popularity as a nutritional supplement among diverse populations [13]. Previous studies have indicated the potential of HE in relieving chronic pain [14] and improving cardiovascular health [15]. However, the neuroprotective properties of HE against seizures have not been extensively investigated. Therefore, our study aimed to examine the anti-seizure effect of HE using multiple drug-induced seizure models. Additionally, through electrophysiological analysis, we explored the potential role of the $GABA_A$ receptor as a target for the anti-seizure effect of HE.

2. Materials and methods

2.1. Animals and drugs

C57BL/6 mice aged 6–8 weeks and male adult Sprague-Dawley rats weighing 220–250 g were obtained from Charles River Laboratories (Beijing). The animals were housed and handled in accordance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The study protocol was thoroughly reviewed and approved by the Ethics Committee of Peking University Health Science Center to ensure ethical practices. The animals were randomly assigned to different groups and treated as per the experimental protocol. Cannabidiol (CBD) and full-spectrum hemp extract (HE) used in the study were provided by Hanyi Bio-technology Company Ltd (Beijing). The composition of HE was as follows: 71.40% CBD, 3.04% cannabidivarin (CBDV), 1.08% cannabinol (CBN), 4.62% cannabigerol (CBG), 4.40% cannabichromene (CBC), and 2.83% cannabicyclol (CBL). The THC content in HE was undetectable, measuring less than 0.3%.

2.2. PTZ/KA chemoconvulsant assay

We compared anti-seizure activities of test compounds in male C57BL/6 N mice aged 6–8 weeks. All drugs except CBD and HE were sourced from Sigma (St. Louis, MO, USA). Mice were orally administrated with carbamazepine (CBZ), CBD, or HE at the indicated concentrations, or control vehicle (MCT). After a 30-minute interval, the mice were subjected to seizure-inducing models.

For the pentylenetetrazol (PTZ) model, mice (n = 10 in each group) were subcutaneously injected with 70 mg/kg of PTZ. Following the PTZ injection, mice were caged individually, and the behaviors were recorded for 30 min, especially the first tonic-clonic seizure.

In the kainic acid (KA) model, mice (n = 7–10 in each group) were intraperitoneally injected with 30 mg/kg of kainic acid. The behaviors of the mice were recorded for 90 min, particularly the stage V status. Stage V seizure was defined as rearing and falling, according to the Racine scale [16].

The recorded data were statistically analyzed using t-test. In instances where stage V status or tonic-clonic status was not achieved, the maximum recording time was recorded as the duration.

2.3. Immunohistochemistry assay

GFAP, a biomarker of glial cells, was labeled using immunohistochemistry method. All reagents were provided by Servicebio. Paraffin sections of mouse brains were dewaxed, dipped in Citrate Antigen Retrieval Solution (pH = 6.0), heated for 10 min, and then washed three times with phosphate buffered saline (PBS). Thereafter, sections were placed in 3% H_2O_2 for 30 min, 3% bovine albumin (BSA) for 25 min. Slides were incubated with primary antibody for 12 h. Slides were then washed for 5 min in PBS and incubated for 1 hour with horseradish peroxidase (HRP) labeled secondary antibody for 50 min. After washing, the sections were then dyed with 3,3′-diaminobenzidine tetrahydrochloride (DAB) and hematoxylin solution, mounted, and examined under a microscope. The images of GFAP+ cells were acquired, labeled, and analyzed with Image J software.

2.4. Pharmacokinetic assay

A comparative mouse pharmacokinetic study of CBD and HE, both provided by Peking Hanyi Biotechnology Limited, was conducted using oral and intravenous administration routes. For each drug, blood samples were collected at 0, 1.5, 3, 4, 5, 6, 7, 8, 10, 12, and 16 h following oral administration (p.o.), and at 0, 3, 8, 15, 30 min, 1, 2, 3, 4, 6, and 8 h after intravenous injection (i.v.) (n = 6 mice/group). Blood was taken via ophthalmic vein with capillaries and thereafter was centrifuged for 10 min at 4000 rpm. Finally, plasma was frozen at −80 °C until analysis. 20 μL plasma sample and 60 μL internal standard solution (66.7 ng/mL, dissolved in acetonitrile) were added into 1.5 mL Eppendorf tubes. The supernatant was moved out after vortexing for 5 min and centrifuging twice at 15,000 rpm and 4 °C for 20 min.

In terms of CBD concentrations in rat brains, brains were collected at 0, 1.5, 3, 4, 6, 8, and 16 h after p.o. administration. They were homogenized (1 mL/g), and 100 μL sample was added to internal standard solution (1 ng/mL, dissolved in ethyl acetate). The supernatant was dried under nitrogen and re-dissolved in 100 μL acetonide. Then, the supernatant was moved out and mixed with 25 μL water after vortexing for 5 min and centrifuging twice at 15,000 rpm and 4 °C for 20 min.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) method was established to determine pretreated plasma and brain homogenate CBD concentrations. Gradient elution of 0.1% formic acid water and acetonitrile was used by Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) to determine CBD concentration. Diphenhydramine was used as an internal standard (IS). Mass spectrometry was in positive ion mode. Ion pairs for IS and CBD were 256.26–167.19 and 315.15–193.04, respectively. The established method was verified in terms of specificity, residual effect, linearity, precision, accuracy, matrix effect, and sample stability. Pksolver was used to analyze the biological sample concentration.

2.5. Two-electrode voltage-clamp

The cRNA transcripts encoding human GABRA1 and GABRB2 were generated by in vitro transcription using the T3 polymerase message machine kit (Thermo Fisher Scientific). Mutant cDNAs were generated by site-directed mutagenesis using a QuikChange kit according to the manufacturer's protocol. Digested stage V and VI X. laevis oocytes were injected with $GABA_A$ receptor α_1 and β_2 subunit cRNAs (~5 ng total per oocyte). Oocytes were incubated in ND96 solution at 16 °C for 1–2 days prior to two-electrode voltage-clamp (TEVC) recording.

TEVC recording was performed at room temperature with an OC-725C amplifier and pClamp10.2 software 1–2 days after cRNA injection as described in the section above. Oocytes were placed in a small-volume oocyte bath and viewed under a dissection microscope. Chemicals were all sourced from Sigma. The bath solution (ND96) consisted of the following concentrations (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, and 5 2-[4-(2-Hydroxyethyl)-1- piperazinyl] ethanesulfonic acid (HEPES) (pH 7.4). Phyto-cannabinoids were stored at −20 °C as 1 M stocks in dimethyl sulfoxide (DMSO) and diluted to working concentrations on the day of the experiment. All compounds were introduced to the recording bath via gravity perfusion at a constant flow of 1 mL/min for 2 min prior to recording. Pipettes had a resistance of 1–2 MΩ when filled with 3 M KCl. Currents were recorded at a holding voltage of −90 mV. TEVC data analysis was conducted using Clampfit10.2 and GraphPad Prism software, and the values are presented as mean ± SEM.

2.6. Primary neuron culture and recordings

Primary cortical cultures were prepared from embryos of ICR mice of either sex at embryonic day 18. The embryos were decapitated, and

the hippocampal regions were gently removed. The removed regions were then digested using 0.25% trypsin for 30 min at 37 ˚C. Following digestion, the tissue was triturated with a pipette in a plating medium, which consisted of DMEM supplemented with 10% FBS.

Dissociated neurons were plated onto poly-d-lysine coated coverslips in 35 mm dishes at a density of 1×10^6 cells per dish. After 4 h, the medium was replaced by a neurobasal medium supplemented with 2% B27 and 0.5 mmol/L GlutaMAX-I. Neurons were incubated at 37 ˚C under 5% CO_2, and half the medium was replaced every 3 days. After being cultured for 7–9 days, cortical neurons were harvested and used for electrophysiological experiments.

For whole-cell voltage-clamp recording in primary neurons, cells were held at −70 mV and recorded at room temperature using a HEKA EPC10 amplifier with PatchMaster software (HEKA Elektronik). Patch pipettes were pulled from borosilicate glass to a resistance of 4–6 MΩ when filled with an internal pipette solution composed of the following (in mM): 120 CsCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 2-[4-(2-Hydroxyethyl)-1- piperazinyl] ethanesulfonic acid (EGTA), and 10 HEPES, adjusted to pH 7.3. Cells were perfused with the external solution containing (in mM): 135 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 340 Sucrose, adjusted to pH 7.3. GABA and other compounds were applied via gravity-fed tubes for GABA current recording until the current reached a peak. Data were acquired with PatchMaster software (HEKA) and filtered at 1 kHz with an LIH 8 + 8 computer interface.

2.7. HEK293T cells culture and recordings

HEK293T cells were incubated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ˚C under 5% CO_2. Then, cells were transiently transfected with cDNA of Nav1.2, Nav1.6, and KCNT1 using Lipofectamine 2000 (Invitrogen) for 16–24 h following the manufacturer's instructions.

For recordings obtained from HEK293T cells, all whole-cell patch clamp recordings were obtained at room temperature using a HEKA EPC-10 amplifier, and the Pulse program was used for data acquisition. Electrodes were fabricated from 1.7 mm capillary glass and fire-polished to a resistance of 0.9–1.3 MΩ using a Sutter P-97 puller. To record the current of Nav1.2 and Nav1.6, the bath solution contained (in mM): 140 NaCl, 1 $MgCl_2$, 3 KCl, 1 $CaCl_2$, 10 Glucose, and 10 HEPES, adjusted to a pH of 7.3, and the pipette solution contained (in mM): 140 CsCl, 10 KCl, 20 EGTA, and 10 HEPES, adjusted to a pH of 7.3. To record current of KCNT1, the bath solution contained (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 29 Glucose, and 10 HEPES, adjusted to a pH of 7.3, and the pipette solution contained (in mM): 100 K-Gluconate, 5 NaCl, 30 KCl, 29 Glucose, 5 EGTA, and 10 HEPES, adjusted to a pH of 7.3.

3. Results

3.1. Full-spectrum hemp extract exhibits anti-seizure properties in mouse epilepsy models

We conducted experiments to evaluate the anti-seizure properties of HE using two drug-induced seizure models: pentylenetetrazol (PTZ) model and kainic acid (KA) model, which have been widely used to mimic clinical symptoms of temporal lobe epilepsy [16].

In our experimental design, mice were pretreated with intraperitoneal injections of 50 mg/kg, 100 mg/kg, or 200 mg/kg HE for 7 consecutive days (Fig. 1a). Following the pretreatment period, the mice were susceptible to acute seizures after PTZ or KA injection. In the positive control groups, pretreatment with 120 mg/kg carbamazepine (CBZ) or 200 mg/kg CBD significantly delayed seizure latency in both PTZ and KA seizure models (Fig. 1b, 1d). In the PTZ model, administration of 200 mg/kg of HE significantly prolonged the latency of the first tonic-clonic seizure and reduced the incidence of the seizure onset (Fig. 1b, 1c; Table S1). Similarly, in the KA model, 200 mg/kg HE significantly delayed the onset of stage V seizure and decreased the incidence of stage V seizure (Fig. 1d, 1e; Table S2).

Previous studies have reported that epileptic seizures will lead to astrogliosis [17]. To further investigate the neuroprotective effect of HE, we assessed the degree of astrogliosis in the mouse hippocampus using glial fibrillary acidic protein (GFAP) as a biomarker for astrocytes [18]. Injection of kainic acid resulted in a significant increase in GFAP-positive cells (Fig. 1f, 1g). As expected, the proliferation of astrocytes was significantly reduced in the pretreatment groups with CBZ (120 mg/kg), CBD (200 mg/kg), and HE (200 mg/kg) (Fig. 1f, 1g). These findings suggest that HE exhibits similar anti-seizure activity to CBD at the same dose.

3.2. HE shows higher bioavailability of CBD in rats

To compare the pharmacokinetic properties of CBD and HE, we developed a new LC-MS/MS method to measure CBD levels in the plasma and brains of rats (Figs. 2a and S1). Then we used the established method to measure CBD concentrations after acute intragastric or intravenous administration of CBD and HE at the same CBD dose of 15 mg/kg.

Intravenous injections of HE or CBD have no difference in CBD pharmacokinetics (Fig. 2b). However, after intragastric administration, the area under plasma concentration-time curves from 0 to ∞ ($AUC_{0-\infty}$) and peak plasma concentration (C_{max}) of the HE group was larger than CBD group (Fig. 2c), indicating better oral absorption of HE. Consequently, the absolute bioavailability (calculated as the $AUC_{0-\infty}$ ratio of gavage to intravenous administration) of HE (2.67%) was higher than that of CBD (1.86%) (Fig. 2b, 2c). Additional pharmacokinetic parameters are provided in Table 1. Moreover, the distribution of CBD in the brain is also higher in the HE group (Fig. 2d; Table 2). These findings suggest that other components present in HE may aid in the absorption of CBD, prolong the elimination process, enhance distribution in brain tissue, and improve its absolute bioavailability in SD rats.

3.3. Cannabichromene and cannabinol exhibit anti-seizure activity on PTZ-induced seizure in mice

HE, despite containing only 70% CBD, exhibits similar anti-seizure effects to CBD, indicating that other components in HE may also possess similar therapeutic properties. To investigate this further, we examined the therapeutic effect of other phyto-cannabinoids against PTZ-induced seizures (Fig. 3a). Before PTZ injection, 100 mg/kg phyto-cannabinoids were administered orally for 7 consecutive days. The results show that CBD, CBC, and CBN significantly delayed the onset of the first tonic-clonic seizure and reduced the incidence of seizures, while other components of HE did not display similar effects (Fig. 3b, 3c). These findings suggest that CBC and CBN, present in HE, may contribute to its anti-seizure efficacy.

3.4. Cannabinoids allosterically modulate $GABA_A$ receptor by increasing its current

$GABA_A$ receptor ($GABA_AR$) plays an important role in seizures and status epilepticus [19], and various drugs have been developed to target $GABA_AR$ for the treatment of epilepsy [20–22]. In order to investigate the potential anti-seizure mechanism of HE, we conducted electrophysiological experiment to evaluate the potential effect of cannabinoids on recombinant $GABA_AR$. We established the $\alpha_1\beta_2$ $GABA_AR$ expression system on Xenopus oocytes. As anticipated, the cannabinoids, particularly CBC, enhanced $GABA_AR$ current (Fig. 4a, 4b). Given the observed anti-seizure effect of CBC in the PTZ model, we selected CBC for further study. Using the same expression system, we examined the dose-response relationship of CBC on $GABA_AR$ (Fig. 4c) and determined the EC_{50} value of CBC to be 10.83 ± 2.06 μM.

To further investigate the interaction between CBC and $GABA_AR$, we examined the impact of specific amino acid mutations on CBC bind-

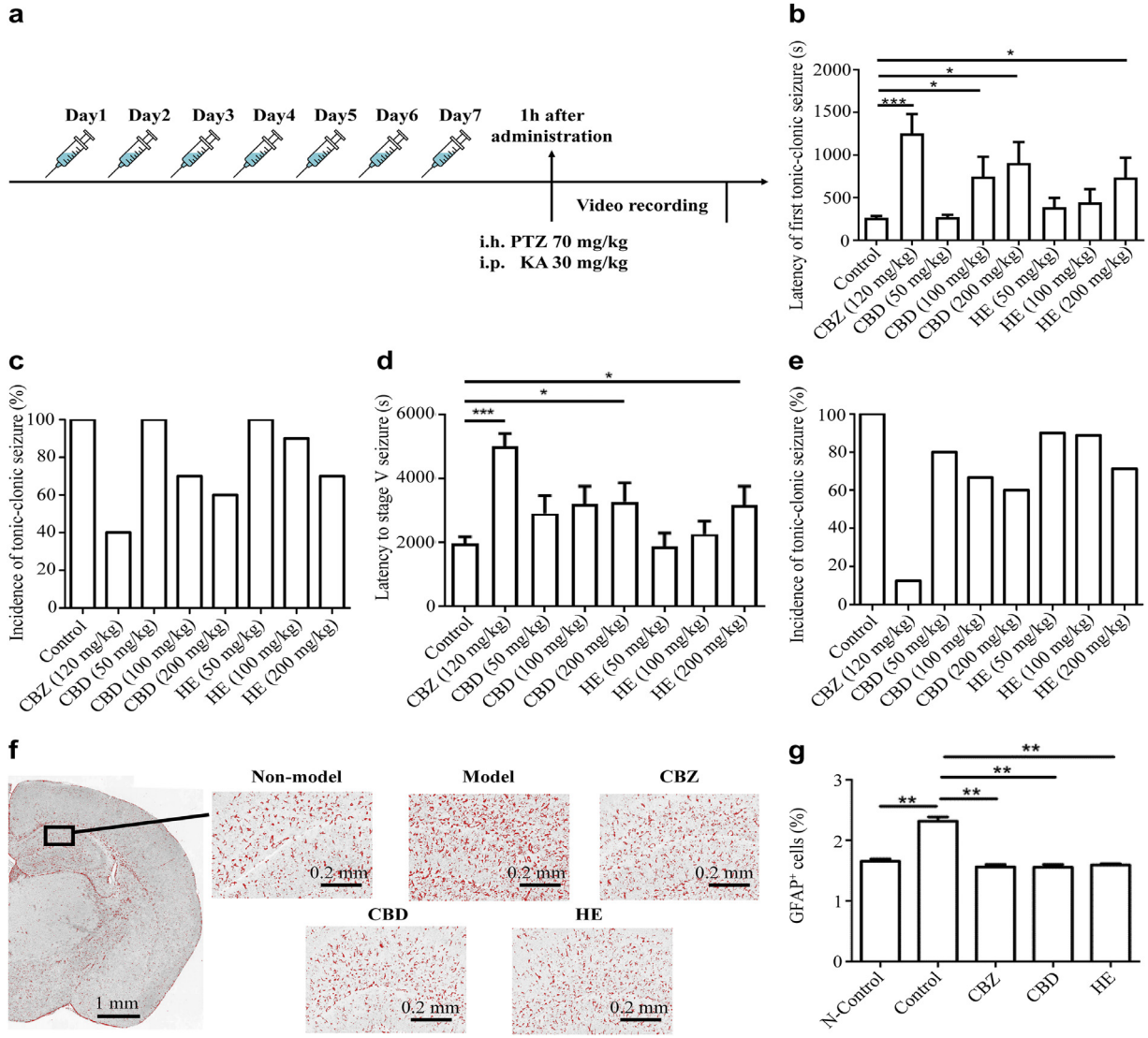

Fig. 1. The anti-seizure effects of HE on drug-induced seizure in mice. (a) A schematic diagram showing the dosing and modeling processes of PTZ/KA induced acute epilepsy mouse models. (b) Latency of first tonic-clonic seizure in PTZ-induced mouse model (mean ± SEM, n = 10, * P < 0.05, *** P < 0.001, t-test). (c) Incidence of tonic-clonic seizure in PTZ-induced mouse models (n = 7–10). (d) Latency to stage V seizure in KA-induced mouse model (mean ± SEM, n = 7–10, * P < 0.05, *** P < 0.001, t-test). (e) Incidence of stage V seizure in KA-induced model (n = 10). (f) Immunohistochemistry images showing the expression of GFAP in mice hippocampus. GFAP+ cells are marked in red. (g) Statistical result of GFAP+ cell rate in each group. (mean ± SEM, n = 4, * P < 0.05, ** P < 0.01, *** P < 0.001, t-test).

Table 1
Pharmacokinetic parameters after drug administration (n = 6).

Parameters	Unit	p.o.		i.v.	
		CBD	HE	CBD	HE
$T_{1/2}$	min	180.50 ± 69.48	267.92 ± 98.75	229.59 ± 73.82	185.30 ± 53.77
T_{max}	min	210 ± 32.86	190 ± 24.49	NA	NA
C_{max}	ng/mL	103.65 ± 104.66	129.25 ± 101.57	105,137.96 ± 39,285.55	146,050.19 ± 35,419.82
AUC_{0-t}	ng/mL·min	24,701.78 ± 17,208.96	36,947.37 ± 27,175.90	1,178,969 ± 497,526.85	1,327,188.70 ± 535,373.30
$AUC_{0-\infty}$	ng/mL·min	25,881.28 ± 17,511.48	40,158.64 ± 27,171.22*	1,390,348.45 ± 633,014.49	1,517,895.63 ± 722,079.52
Vd	L/kg	197.20 ± 91.89	274.69 ± 231.90	3.87 ± 1.38	2.84 ± 0.58
Cl	L/kg·min	0.92 ± 0.71	0.63 ± 0.48	0.0123 ± 0.00484	0.0118 ± 0.00551
F	%	1.86	2.67	100	100

Note: CBD, cannabidiol; HE, full spectrum hemp extract; $T_{1/2}$, half lifetime; T_{max}, peak time; C_{max}, peak concentration; AUC_{0-t}, the area under curve from 0 to t; $AUC_{0-\infty}$, the area under curve from 0 to ∞; Vd, apparent volume of distribution; Cl, clearance; F, absolute bioavailability; NA, not applicable. The results are expressed as the mean ± SEM, except for absolute bioavailability. The P value was calculated in comparison to the CBD group, *P < 0.05, t-test.

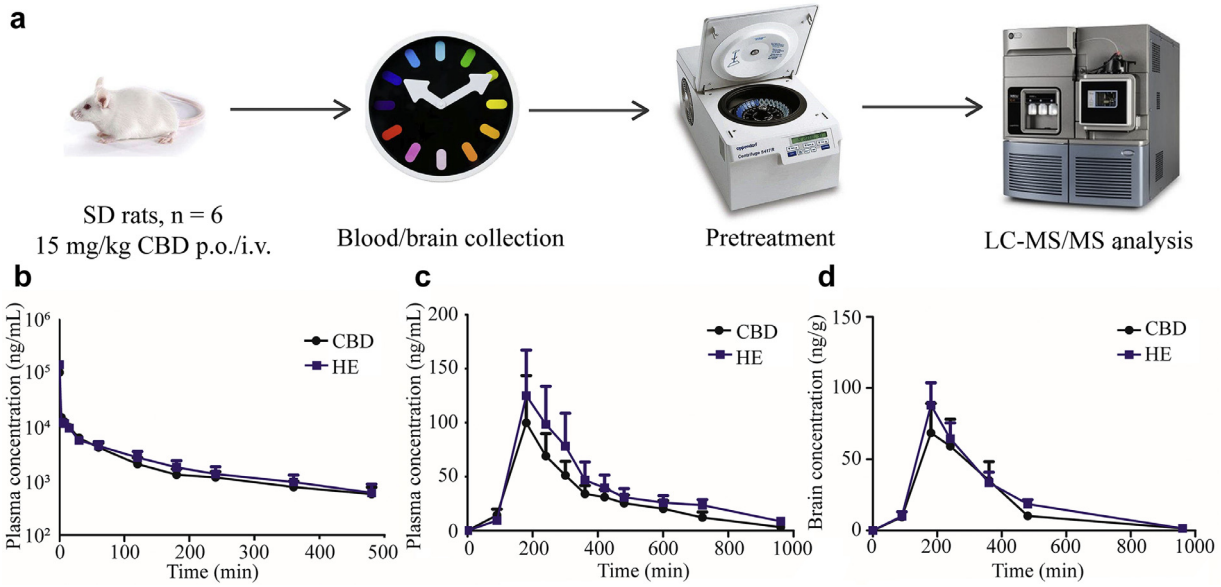

Fig. 2. HE shows higher bioavailability of CBD in rats. (a) A workflow diagram of pharmacokinetic study. (b) Time-mean plasma concentration curves after i.v. administration of CBD and HE from 0 to 8 h; (c) Time-mean plasma concentration curves after p.o. administration of CBD and HE from 0 to 16 h; (d) Time-mean brain concentration curves after p.o. administration of CBD and HE from 0 to 16 h.

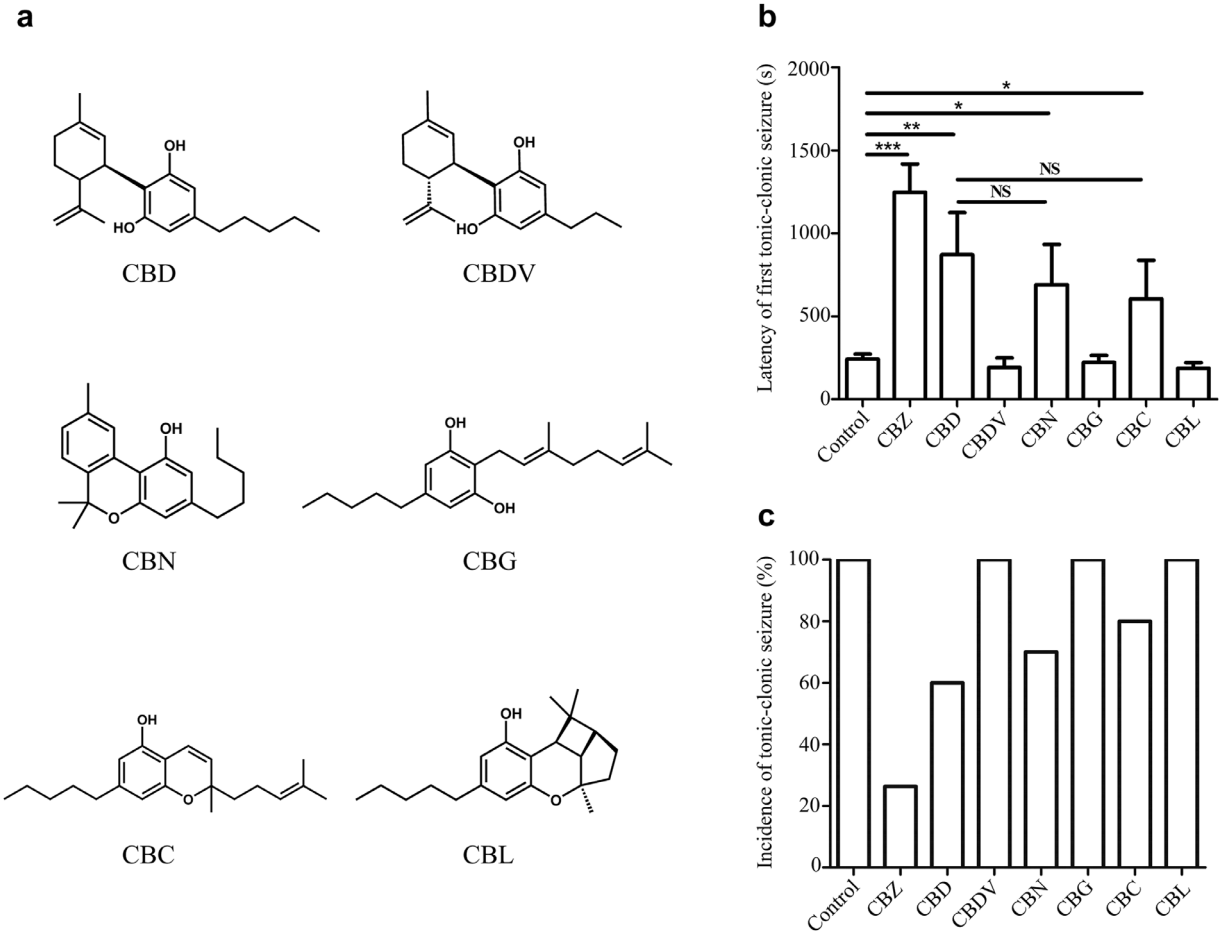

Fig. 3. CBC and CBN show anti-seizure effects in PTZ induced seizure model. (a) Structures of phyto-cannabinoids involved in HE. CBDV, cannabidivarin; CBN, cannabinol; CBG, cannabigerol; CBC, cannabichromene; CBL, cannabicyclol. (b) Latency of first tonic-clonic seizure in PTZ-induced epilepsy mouse model (mean ± SEM, $n = 10$, NS: no significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, t-test). CBZ was used as a positive drug. (c) Incidence of tonic-clonic seizure ($n = 10$ mice per group).

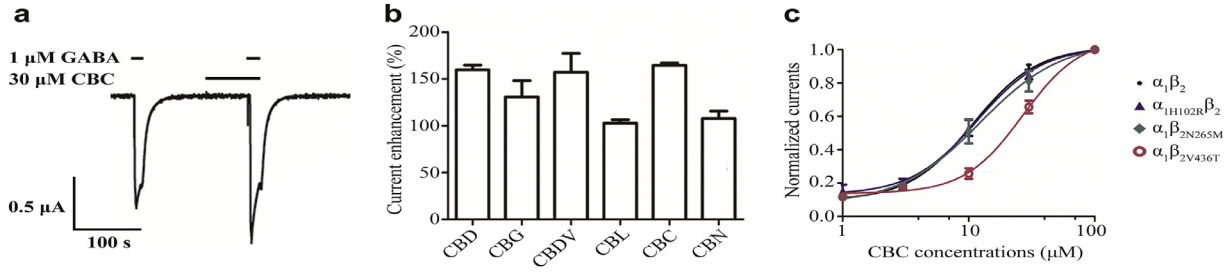

Fig. 4. Cannabichromene (CBC) allosterically enhances GABAAR current in Xenopus oocytes. (a) Representative trace of 30 μM CBC's positive allosteric modulation on GABAAR current. (b) Current enhancement of 30 μM natural cannabinoids to 1 μM GABA-induced currents (n = 5 per group). (c) Dose-effect curves of CBC on wild-type and mutant $GABA_A$Rs.

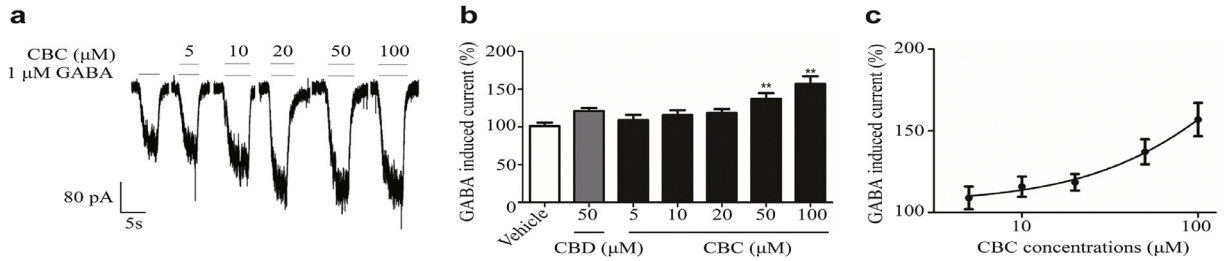

Fig. 5. CBC allosterically enhances GABA currents in primary cerebral cortical neurons. (a) Representative trace of CBC's effects on 1 μM GABA-induced current. (b) CBC exhibits enhancing effect on GABA-induced current in a dose-dependent manner (n = 5 per group). (**P < 0.01, t-test). (c) Dose-effect curve of CBC on GABA-induced currents in primary cortical neurons.

Table 2
Brain distribution parameters after p.o. administration (n = 6).

Parameters	Unit	CBD	HE
$T_{1/2}$	min	141.73 ± 58.01	133.42 ± 33.28
Tmax	min	190.00 ± 24.49	190.00 ± 24.49
Cmax	ng/g	71.64 ± 49.44	88.11 ± 38.05
AUC_{0-t}	ng/g·min	17,268.72 ± 11,356.83	23,369.50 ± 7094.25
$AUC_{0-\infty}$	ng/g·min	18,878.74 ± 12,144.83	24,751.16 ± 7201.84*
Cl	g/g·min	0.0010 ± 0.0005	0.0008 ± 0.0005

Note: CBD, cannabidiol; HE, full spectrum hemp extract; $T_{1/2}$, half lifetime; T_{max}, peak time; C_{max}, peak concentration; AUC_{0-t}, the area under curve from 0 to t; $AUC_{0-\infty}$, the area under curve from 0 to ∞; Cl, clearance. The results are expressed as the mean ± SEM. The P value was calculated in comparison to the CBD group, *P < 0.05, t-test.

ing. We generated three $GABA_A$R mutants: $\alpha_{1H102R}\beta_2$ (benzodiazepines binding site [23]), $\alpha_1\beta_{2N265M}$ (etomidate and propofol binding site [24]), and $\alpha_1\beta_{2V436T}$ (CBD binding site [7]). These mutants, along with the wild-type $GABA_A$R, were expressed in Xenopus oocytes, and the effects of CBC on their currents were evaluated. No significant difference was observed between wild-type $GABA_A$R and $\alpha_1H_{102R}\beta_2$ or $\alpha_1\beta_{2N265M}$ mutants (Fig. 4c). However, the $\alpha_1\beta_{2V436T}$ mutation caused a significant rightward shift in the dose-effect curve of CBC, indicating a decrease in CBC efficacy (Fig. 4c). These findings suggest that CBC and CBD bind to the same pocket on $GABA_A$R, and the amino acid residue V436 on β_2 subunit is important for their binding.

Furthermore, we sought to validate the impact of CBC on $GABA_A$R in mammalian neurons. Primary cortical neurons were derived from ICR mice embryos at embryonic day 18 and cultured for 7 to 9 days in vitro. Electrophysiological experiments were performed thereafter. The result demonstrated that CBC dose-dependently enhanced the GABA-induced current, and no significant difference was observed between CBD and CBC groups (Fig. 5b). These findings provide further evidence supporting the positive allosteric modulation effect of CBC on $GABA_A$ receptor.

In order to assess the target selectivity of CBC, whole-cell patch clamp recording on HEK293T cells was performed to evaluate CBC's effect on KCNT1, Nav1.2, and Nav1.6, which are known as epilepsy-related ion channels [25]. We measured the currents of these ion channels before and after CBC application. As a result, bath application of 20 μM CBC did not have any significant effect on these ion channels (Fig. 6). These results suggest that the $GABA_A$ receptor is a potential target for the anti-epileptic effects of CBC.

4. Discussion

The investigation of cannabinoids for their potential anti-epileptic properties has gained significant attention, particularly after the approval of Epidiolex for the treatment of Dravet Syndrome and Lennox-Gastaut Syndrome [26]. CBD, in particular, has been extensively studied from its therapeutic effects to its mechanisms of anti-convulsant action [27,28]. However, there is a lack of comprehensive research on the anti-seizure activity of HE, which contains CBD along with other components. In this study, we conducted a comparison between HE and CBD regarding their anti-seizure effects in vivo. Despite HE containing only 70% CBD, we observed similar anti-convulsant effects of HE and CBD at the same dose (Fig. 1). We suspected CBD may act synergistically with other minor components in HE, leading to an enhanced anti-seizure effect.

To provide relevant evidence, we conducted the pharmacokinetic study and HE single component analysis accordingly. The findings revealed better absorption and slower elimination of CBD when administered in the form of HE (Fig. 2). This suggests that other cannabinoids present in HE may impact the pharmacokinetic properties of CBD. CBD is primarily metabolized by enzymes of the cytochrome (CYP) complex, and cannabinoids have proven to be potent inhibitors of these enzymes [29]. This inhibition could result in a slower metabolic process for CBD. Additionally, CBN and other cannabinoids have been found to stimulate P-gp ATPase [30]. Since P-gp plays a crucial role in the transport of various drugs, the interaction of other cannabinoids in HE with P-gp could impact the absorption and disposition of CBD.

Furthermore, other cannabinoids, especially CBC and CBN, showed significant anti-convulsant activities as well (Fig. 3). This aligns with previous research indicating that CBC exhibits anti-seizure effects in a

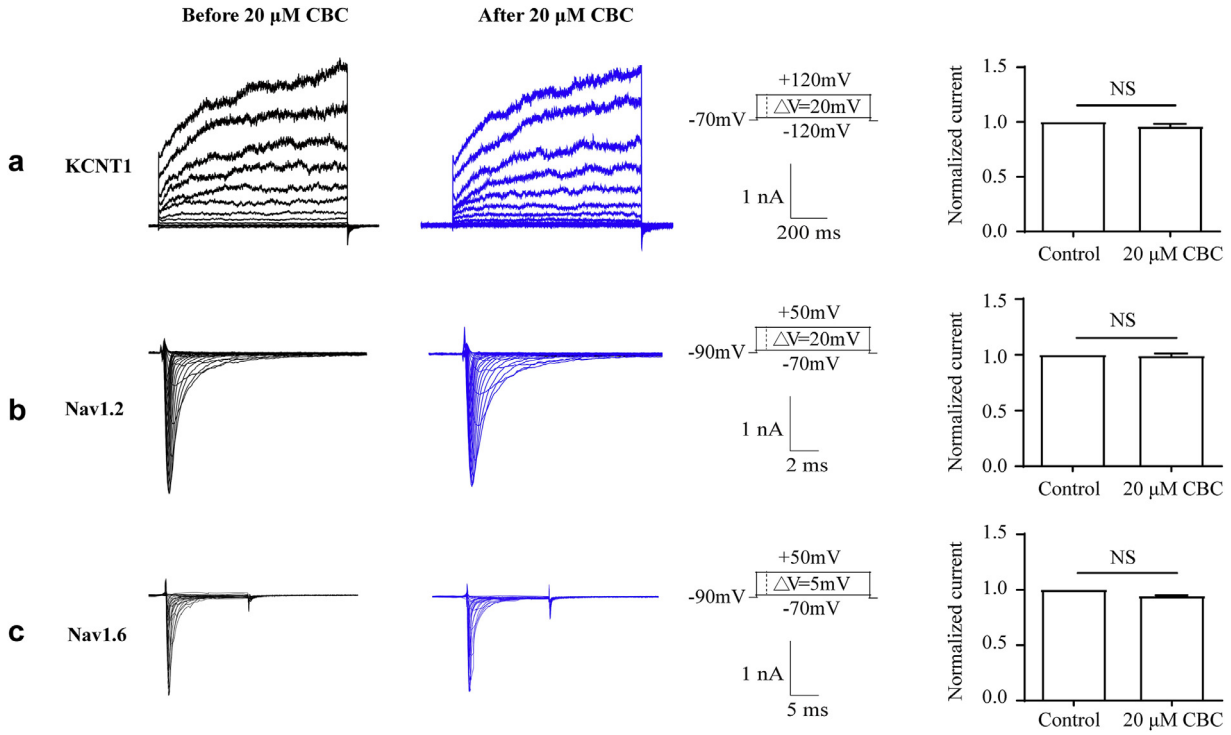

Fig. 6. CBC has no influence on the currents of KCNT1, Nav1.2, and Nav1.6. Representative traces and statistical results of (a) KCNT1, (b) Nav1.2, (c) Nav1.6 currents before and after 20 μM CBC perfusion (n = 5 per group, t-test). Currents were recorded following the corresponding voltage protocols on the right. Traces before and after CBC administration are indicated in black and blue respectively.

mouse model of Dravet syndrome [31]. Therefore, the favorable pharmacokinetic properties of CBD in the HE, together with other cannabinoids with anti-convulsant activities, may contribute to the potency of the HE in treating epilepsy.

Considering that $GABA_A$ receptor is an important therapeutic target for conventional anti-seizure drugs, we assessed the effect of different components on this receptor. Our findings revealed that various cannabinoids, especially CBC exhibited positive allosteric modulating effects on $GABA_A$ receptor (Figs. 4, 5). Unlike traditional anti-seizure drugs (α_{1H102} for benzodiazepines and β_{2N265M} for etomidate), the critical amino acid residue involved in the interaction with CBC is supposed to be β_{2V436T}, which is consistent with the binding site for CBD. This suggests a shared mechanism of action between CBC and CBD on the $GABA_A$ receptor.

Although $GABA_A$ receptor can be modulated by CBC, the precise mechanism responsible for the anti-seizure effect of CBC and other cannabinoids has yet to be fully understood. We found that CBN, another component of the hemp extract, did not significantly modulate $GABA_A$ receptors (Fig 4b), suggesting the involvement of alternative targets. CBN has been identified to interact with the cannabinoid type 1 receptor (CB1) [32]. CB1 receptors are abundantly expressed in the central nervous system, which suggests that CBN's anti-seizure effect may be attributed to its modulation of neurotransmitter release. Furthermore, CBN's ability to reduce seizures could potentially involve modulation of transient receptor potential (TRP) cation channels. Studies have shown that CBC, CBN, and other cannabinoids can modulate various TRP channels, including TRPA1, TRPM8, and TRPV1–4 [33], which may contribute to their effects on seizures. Further research is needed to fully uncover the specific mechanisms underlying the anti-seizure effects of cannabinoids.

In consideration of CBC's selective enhancing effect on the $GABA_A$ receptor and its non-psychoactive nature, the clinical applications of CBC are likely go beyond epilepsy treatment. It may also have potential uses in the areas of analgesia, anxiolysis, and sedative-hypnotic therapy.

5. Conclusion

HE exhibits dose-dependent anti-epileptic activity by reducing seizure severity and delaying seizure onset in drug-induced seizure models. The neuroprotective effect of HE against epilepsy is further supported by immunohistochemical study. In comparison to purified CBD, HE has faster CBD absorption, slower elimination, and higher oral bioavailability. To our surprise, the natural cannabinoids CBN and CBC have been identified to have anti-seizure properties in the PTZ mouse model. Electrophysiology studies reveal that CBC exhibits an allosteric enhancement on the $GABA_A$ receptor, indicating the anti-seizure activity of HE may involve GABAergic transmission. However, further studies are still required to elucidate the mechanism underlying CBC's anti-seizure effect.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fmre.2023.05.023.

References

[1] R.S. Fisher, C. Acevedo, A. Arzimanoglou, et al., ILAE official report: A practical clinical definition of epilepsy, Epilepsia 55 (2014) 475–482.

[2] A. Ali, Global health: Epilepsy, Semin. Neurol. 38 (2018) 191–199.
[3] J. Sheng, S. Liu, H. Qin, et al., Drug-resistant epilepsy and surgery, Curr. Neuropharmacol. 16 (2018) 17–28.
[4] J.Y. Yoo, F. Panov, Identification and treatment of drug-resistant epilepsy, Continuum 25 (2019) 362–380.
[5] K. Cohen, A. Weizman, A. Weinstein, Positive and negative effects of cannabis and cannabinoids on health, Clin. Pharmacol. Ther. 105 (2019) 1139–1147.
[6] Z. Breijyeh, B. Jubeh, S.A. Bufo, et al., Cannabis: A toxin-producing plant with potential therapeutic uses, Toxins 13 (2021) 117.
[7] T. Bakas, P.S. van Nieuwenhuijzen, S.O. Devenish, et al., The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors, Pharmacol. Res. 119 (2017) 358–370.
[8] D.A. Moss, M.K. Hawks, M.J. Snyder, et al., Integrative medicine: Cannabis and cannabis-related drugs, FP. Essent. 505 (2021) 28–34.
[9] G.S. Wang, D.W.A. Bourne, J. Klawitter, et al., Disposition of oral cannabidiol-rich cannabis extracts in children with epilepsy, Clin. Pharmacokinet. 59 (2020) 1005–1012.
[10] F.A. Pamplona, L.R. da Silva, A.C. Coan, Potential clinical benefits of CBD-rich cannabis extracts over purified CBD in treatment-resistant epilepsy: Observational data meta-analysis, Front. Neurol. 9 (2018) 759.
[11] M. Sorkhou, R.H. Bedder, T.P. George, The behavioral sequelae of cannabis use in healthy people: A systematic review, Front. Psychiatry 12 (2021) 630247.
[12] X. Tan, S.B. Wang, W.X. Sun, et al., Preparation method of industrial hemp extract, 2019. 201910670464.6.
[13] C. Schluttenhofer, L. Yuan, Challenges towards revitalizing hemp: A multifaceted crop, Trends. Plant Sci. 22 (2017) 917–929.
[14] A. Capano, R. Weaver, E. Burkman, Evaluation of the effects of CBD hemp extract on opioid use and quality of life indicators in chronic pain patients: A prospective cohort study, Postgrad. Med. 132 (2020) 56–61.
[15] D. Rodriguez-Leyva, G.N. Pierce, The cardiac and haemostatic effects of dietary hempseed, Nutr. Metab. 7 (2010) 32.
[16] M. Lévesque, M. Avoli, The kainic acid model of temporal lobe epilepsy, Neurosci. Biobehav. Rev. 37 (2013) 2887–2899.
[17] M.V. Sofroniew, H.V. Vinters, Astrocytes: Biology and pathology, Acta Neuropathol. 119 (2010) 7–35.
[18] H. Baba, K. Nakahira, N. Morita, et al., GFAP gene expression during development of astrocyte, Dev. Neurosci. 19 (1997) 49–57.
[19] M.J. Jembrek, J. Vlainic, G.A.B.A. Receptors, Pharmacological potential and pitfalls, Curr. Pharm. Des. 21 (2015) 4943–4959.
[20] A.R. Brooks-Kayal, M.D. Shumate, H. Jin, et al., Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy, Nat. Med. 4 (1998) 1166–1172.
[21] G.J. Sills, M.A. Rogawski, Mechanisms of action of currently used antiseizure drugs, Neuropharmacology 168 (2020) 107966.
[22] A. Ghit, D. Assal, A.S. Al-Shami, et al., $GABA_A$ receptors: Structure, function, pharmacology, and related disorders, J. Genet. Eng. Biotechnol. 19 (2021) 123.
[23] D.B. Pritchett, H. Sontheimer, B.D. Shivers, et al., Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology, Nature 338 (1989) 582–585.
[24] J.J. Kim, A. Gharpure, J. Teng, et al., Shared structural mechanisms of general anaesthetics and benzodiazepines, Nature 585 (2020) 303–308.
[25] J. Oyrer, S. Maljevic, I.E. Scheffer, et al., Ion channels in genetic epilepsy: From genes and mechanisms to disease-targeted therapies, Pharmacol. Rev. 70 (2018) 142–173.
[26] T.E. Gaston, D. Friedman, Pharmacology of cannabinoids in the treatment of epilepsy, Epilepsy Behav. 70 (2017) 313–318.
[27] V. Golub, D.S. Reddy, Cannabidiol therapy for refractory epilepsy and seizure disorders, Adv. Exp. Med. Biol. 1264 (2021) 93–110.
[28] S. Lattanzi, E. Trinka, E. Russo, et al., Cannabidiol as adjunctive treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome, Drugs Today 55 (2019) 177–196.
[29] F. Grotenhermen, Pharmacokinetics and pharmacodynamics of cannabinoids, Clin. Pharmacokinet. 42 (2003) 327–360.
[30] H.-J. Zhu, J.-S. Wang, J.S. Markowitz, et al., Characterization of P-glycoprotein inhibition by major cannabinoids from marijuana, J. Pharmacol. Exp. Ther. 317 (2006) 850 –7.
[31] L.L. Anderson, A. Ametovski, J.Lin Luo, et al., Cannabichromene, related phytocannabinoids, and 5-fluoro-cannabichromene have anticonvulsant properties in a mouse model of dravet syndrome, ACS Chem. Neurosci. 12 (2021) 330–339.
[32] M.H. Rhee, Z. Vogel, J. Barg, et al., Cannabinol derivatives: Binding to cannabinoid receptors and inhibition of adenylylcyclase, J. Med. Chem. 40 (1997) 3228–3233.
[33] L. De Petrocellis, A. Ligresti, A.S. Moriello, et al., Effects of cannabinoids and cannabinoid-enriched cannabis extracts on TRP channels and endocannabinoid metabolic enzymes, Br. J. Pharmacol. 163 (2011) 1479–1494.

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