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Research Article
Synergistic Attenuation of Combined CBD Oil and Low Dose Valproic Acid in PTZ-Induced Seizure and *in-silico* Analysis

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Abstract

Background and Objective: Epilepsy is one of the most prevalent Central Nervous System (CNS) disorders that affects society and the healthcare system. However, control of seizure therapeutically with the use of old-generation antiepileptic agents, such as valproic acid (VPA), comes with severe adverse effects as they all have low therapeutic index. This study aims to investigate the synergetic effect of Cannabidiol oil (CBD oil) combined with low-dose valproic acid in pentylenetetrazol (PTZ)-induced seizures in rats. **Materials and Methods:** Forty-two animals were divided into six treatment groups: PTZ only (75 mg/kg), CBD only (50 mg/kg), VPA only (100 mg/kg), protective group, CBD (50 mg/kg)+VPA (75 mg/kg) for 1 week and two lower doses of VPA (50 and 75 mg/kg), respectively with CBD (50 mg/kg). Administration of drugs was by intraperitoneal (IP) route. The procedure was repeated three times on separate days and the time of seizure onset, duration, recurrence and mortality were measured. Data were analyzed using Two-way Analysis of Variance (ANOVA) and statistical difference was taken at $p<0.05$. **Results:** The onset of seizure was significantly longer in CBD+VPA combination treatment (50+75 mg/kg) and VPA (100 mg/kg) group compared to other groups. The combination also exhibited the shortest seizure duration, compared VPA (100 mg/kg) treatment. No mortality was recorded in these groups even after 72 hrs. Molecular docking studies confirmed that CBD and VPA bind on the same GABA subsite SER 267, indicating a synergism with VPA. **Conclusion:** Overall, these findings provide a critical and innovative approach to using low-dose VPA in combination with CBD oil. Therefore, developing a combination of CBD oil and VPA could potentially decrease dose-associated side effects yet enhance the therapeutic effectiveness preferably through synergism effects.

Key words: Seizure, cannabidiol oil, valproic acid, pentylenetetrazol, γ-aminobutyric acid type AG

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epileptic seizure is a neurological disorder that affects approximately 70 million people globally and continues to be a health challenge among practitioners[1]. It has no gender nor age preferences, in addition to ethnicity as well as geographical locus[2,3]. In addition, various psychiatric comorbidity may develop in epileptic patients. Indeed, 37% of the epileptic patients received antipsychotic therapy compared to 15% in a normal person[1]. Given the high global prevalence of epilepsy, nearly half of those affected will develop drug resistance in their lifetime[4]. Moreover, traditional antiepileptic drugs (AEDs) offer the least effective option for epilepsy management, despite the recent progress in drug development. Additionally, these AEDs are used to some extent in controlling seizures due to frequent side effects that may hinder therapeutic outcomes suggesting a need for better treatment modalities[2].

Valproic acid (VPA), a well-known AED, is used effectively to treat generalized epilepsy syndromes[5]. Moreover, VPA is well suited for other types of epilepsy and seizures as well, including absence, myoclonic and partial seizures with acceptable therapeutic outcomes. Most epileptic patients receiving AEDs, including VPA, require dose incremental to effectively alleviate seizure symptoms. Unfortunately, dose increase is associated with intolerable side effects. For instance, using high doses of VPA is a risk factor for serious adverse effects such as thrombocytopenia[6,7]. Additionally, several lines of evidence have investigated using different strategies to decrease the utilization of high doses of VPA to prevent associated side effects including the induction of autism[8]. These challenges require the necessity of developing a new approach to bring about a better treatment option for epileptic seizure patients. Hence, developing a novel combinational treatment approach as a management strategy to mitigate these drawbacks associated with high doses of current AEDs is crucial.

To this end, some natural compounds and components such as flavonoids have been shown to possess antiseizure effects possibly through Gamma-Aminobutyric Acid (GABA)-Cl^- mechanism[9]. In addition, alkaloids are reportedly applied in the treatment of epileptic seizures[3]. Moreover, abundant evidence shows that Cannabidiol (CBD) oil has the most promising potential in the management of epileptic seizures, particularly drug-resistant seizures[10]. The CBD oil is phytocannabinoids derived from *Cannabis sativa* which lacks psychoactive effects and therefore has no abuse potential[11]. The CBD oil has undergone open-label intervention trials[12], randomized double-blind placebo controlled study[13]. These studies showed an improvement in childhood epilepsy[10], suggesting the crucial role of CBD in epilepsy. Additionally, it is worth noting that there were no pharmacokinetic interactions between VPA and CBD oil, suggesting that using such a combination, could be achieved. Therefore, the study aimed to investigate the synergetic effects of CBD oil combined with low-dose VPA in pentylenetetrazole (PTZ)-induced seizures in rats. Additionally, *in-silico* analysis was used to determine the interaction between CBD oil and $GABA_A$ receptors to establish pharmacodynamic synergism.

MATERIALS AND METHODS

Study area: This study was conducted in the Pharmacology Lab of the College of Clinical Pharmacy, King Faisal University. Animal experiment was conducted from February to May, 2022, within the specified period of Ethical approval.

Chemicals: The PTZ and VPA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The CBD oil (99.9% pure isolate) was procured from Hemp Well Limited (York, United Kingdom). The PTZ and VPA were dissolved in saline and prepared in accordance with the doses used for the present study. The injections of PTZ, CBD and VPA were freshly dissolved before administration.

Animals: Forty-two Wistar rats (4 weeks old) with an average weight of 80-100 g were used for this study. Animals were purchased from the animal house facility in the College of Clinical Pharmacy, King Faisal University. They were kept in line with the conventional protocol of natural photoperiod, consisting of 12 hrs light and 12 hrs darkness throughout the study. They were allowed constant access to food and water throughout the study.

Ethical consideration: Animal care and experimental procedures were performed in accordance with the approved guidelines of the Research Ethics Committee (with protocol ID: KFU-REC-2022-NOV-ETHICS280) at King Faisal University, Saudi Arabia.

Experimental procedure: Rats were randomly divided into 6 groups (n = 6). All injections were performed intraperitoneally. In control group 1(PTZ only): Rats in this group were administered a single dose of 75 mg/kg PTZ 30 min after the saline injection. The CBD oil only group 2: Animals were treated with 50 mg/kg CBD oil and after 30 min were treated with PTZ, doses selected according to

Devinsky *et al.*[12]. The VPA group 3: Rats were given 100 mg/kg VPA and then administered 75 mg/kg PTZ 30 min after the VPA injection. Combination groups 4 and 5 CBD oil (50 mg/kg) plus 50 and 75 mg/kg, respectively were administered after 30 min and were treated with PTZ. Pretreatment group 6: Animals were treated for 1 week with 50 mg/kg CBD oil plus 75 mg/kg VPA after which were exposed to PTZ treatment. Observations include measurement of onset, duration and recurrence of seizure were recorded. Also, mortality rates were determined in all the groups.

Crystal structures: The X-ray coordinates of Human $GABA_A$ receptor alpha1-beta2-gamma2 subtype in complex with GABA plus diazepam (PDB IDs: 6X3X) were extracted from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB)[14,15].

Protein preparation: The protein preparation workflow, available in the Maestro software (Schrodinger, LLC, 2022) was used to produce the PDB structures for docking. A pH of 7.4 was used for the preparation and minimization procedure, with ionization states being changed as needed. While non-essential water molecules were eliminated from the structures, polar hydrogens were added. Prior to docking, the receptors were adjusted in Maestro 11.8 using the OPLS3 force field. The optimization and reduction of the ligand-protein complexes were conducted in the last stage using the OPLS3 force field, with a preset RMSD value of 0.30 Å for non-hydrogen atoms[16]. The receptor grids were generated utilizing the prepared protein and the docking grids were placed centroid of residues A: ASN 26, A: MET 294, A: LEU 301 and E: SER 267. A receptor grid was established using 1.00 van der Waals radius. The van der Waals (vdW) radius is adjusted by a scaling factor and partial charges are limited to a cutoff of 0.25. The binding sites were restricted inside a 20 Å3 grid box using default settings and no limitations.

Ligand library preparation: Cannabidiol was prepared using LigPrep, a software tool available in Maestro 11.8 (LigPrep, version 11.8; Schrodinger, LLC: New York, NY, 2017). The ligand's 3D structures were designed and fine-tuned for docking by determining the most probable ionization states at a pH of 7±1 while preserving the initial ionization state. Geometry is ultimately enhanced using the OPLS3 force field. The generated conformations were used as the starting input structures for the docking procedure.

Molecular docking: The ligands were subjected to unrestricted docking experiments using the extra precision mode (XP). A partial charge cut-off of 0.15 and a van der Waals (vdw) radius scaling factor of 0.80 were the parameters used. The Glide Score, in Glide software 2.5, was used to evaluate the binding strength and prioritize ligands. The XP attitude Rank was used to determine and select the best docked pose. The compounds were extensively analyzed, considering binding scores and binding to key residues.

ADMET properties and drug-likeness predictions: The ADMET and drug-likeness properties of CBD oil were predicted by using the pkCSM web server[17]. Eight molecular descriptors were produced in order to describe the ADMET properties of CBD oil. Further, physicochemical properties, medicinal chemistry and drug-likeness properties were employed using SwissADME[18].

Statistical analysis of data: Data was presented as Mean±Standard Deviation (SD) and statistical analysis was carried out using GraphPad Prism software ver.10.2 (San Diego, California, USA). Intergroup comparisons were performed by Two-way Analysis of Variance (ANOVA) and differences between the groups were measured using Tukey's multiple comparisons test. Statistical significance was taken as $p<0.05$.

RESULTS

Onset of first seizure: As shown in Fig. 1, regarding the onset of seizure induced by PTZ, the untreated control group had the fastest seizure onset. This was followed by CBD oil pretreated group for 1 week. However, there was a significant difference between ($p<0.05$) the two groups showing that CBD oil pretreated group had a delayed seizure onset. Also, administration of CBD oil with VPA (50 and 75 mg/kg) produced a marked difference ($p<0.01$) compared with the untreated control group. Interestingly, the combination group with VPA low dose of 75 mg/kg produced similar seizure onset as with 100 mg/kg VPA alone. It can also be observed that CBD oil only treatment group, had significantly increased seizure onset compared with the untreated control. However, the study revealed that treatment with CBD oil was better than pretreatment in the control of PTZ-induced seizure.

Duration of the first seizure: On the duration of seizure, as shown in Fig. 2, findings indicated that CBD oil plus 75 mg/kg VPA (lower dose) produced a better reduced duration of seizure induced by PTZ compared with a high dose of VPA (100 mg/kg) usually employed in the treatment of seizure. However, pretreatment produced the longest seizure duration

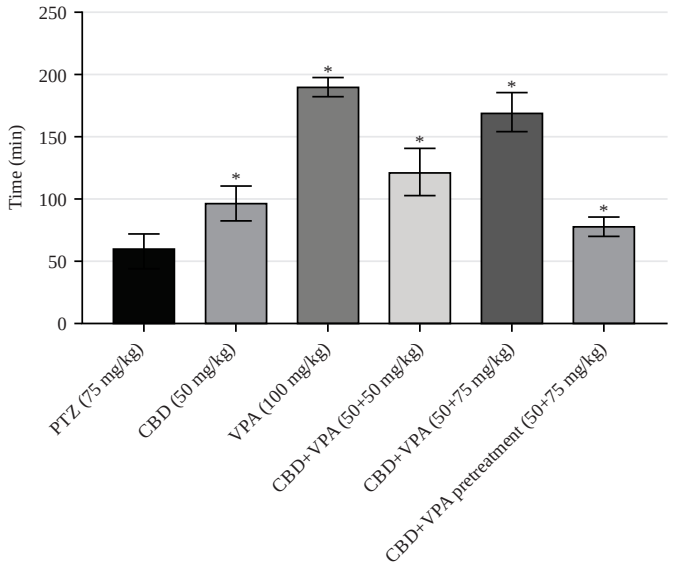

Fig. 1: Onset of the first seizure in all experimental groups

Values are represented as Mean±SD, n = 6, *Significant difference (p<0.05) between the control group and treatment groups, PTZ: Pentylenetetrazol, CBD: Cannabidiol oil and VPA: Valproic acid

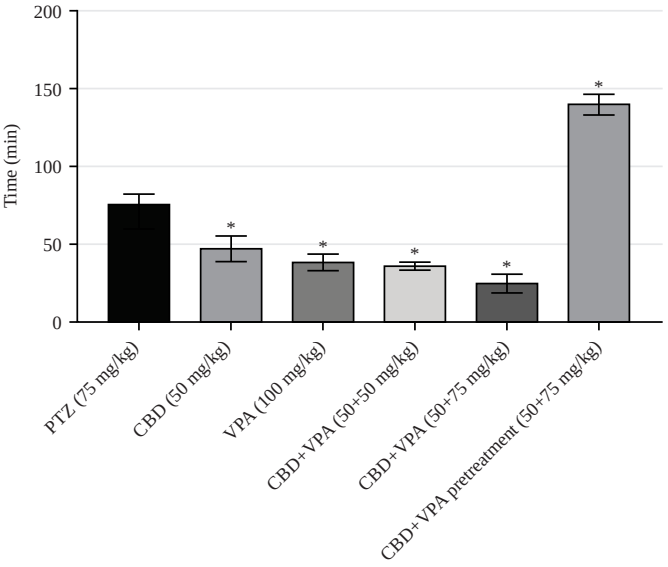

Fig. 2: Duration of seizure in the different treatment groups compared to the control

*Significant difference (p<0.05) between the control group and treatment groups, PTZ: Pentylenetetrazol, CBD: Cannabidiol oil and VPA: Valproic acid

compared with the untreated control. Although 100 mg/kg VPA caused a reduction in seizure duration, it did not abolish seizure. Results also showed that a combination of CBD oil with a low dose of 75 mg/kg VPA had better control in terms of seizure duration induced by PTZ.

Numbers of seizure episodes: In Fig. 3, the results show the number or absence of recurring seizure episodes after the initial recovery. Interestingly, CBD oil treatment alone did not show any seizure reoccurrence as it was observed with VPA 100 mg/kg. However, all the CBD oil combinations with VPA had recurrence after the initial recovery without any severity. Also, untreated control group, there were no recurrences of PTZ-induced seizures.

Mortality: Results as presented in Fig. 4, show a mortality rate after seizure occurrence with the highest mortality of 83% in the group of untreated control (PTZ only). The CBD oil only

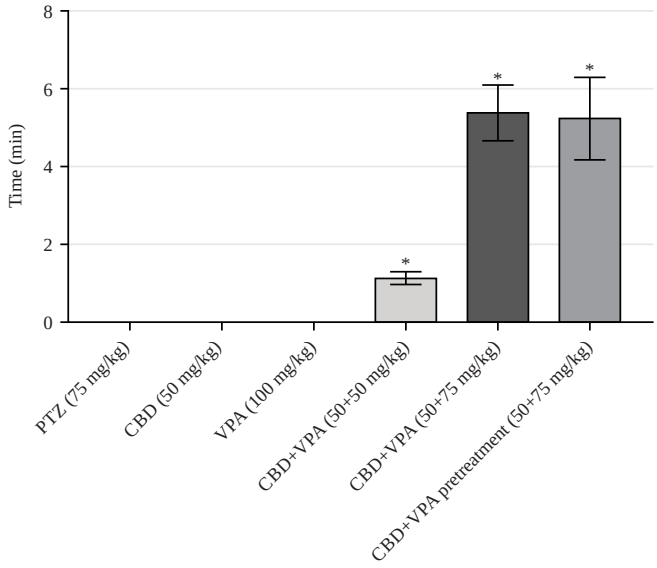

Fig. 3: Number of recurrences of seizure episodes after initial recovery from seizure in the different treatment groups compared to the control

*Significant difference ($p<0.01$) between the control group and treatment groups, PTZ: Pentylenetetrazol, CBD: Cannabidiol oil and VPA: Valproic acid

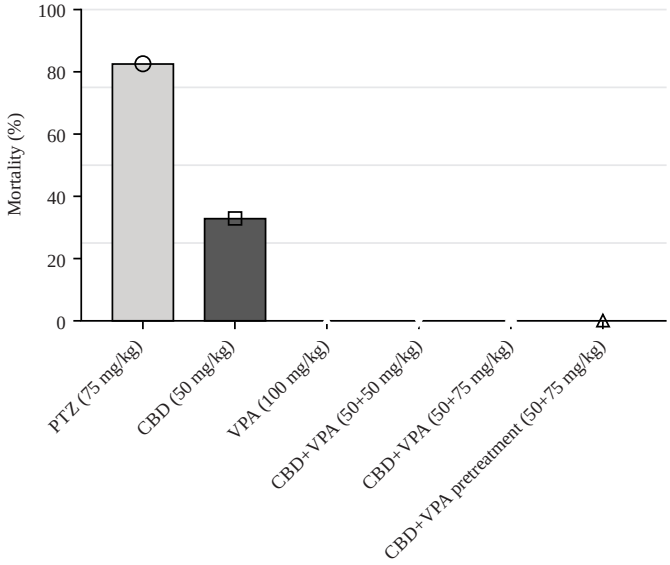

Fig. 4: Mortality percentage after seizure induction without and with treatment in the different treatment groups compared to the control

PTZ: Pentylenetetrazol, CBD: Cannabidiol oil and VPA: Valproic acid

group had a mortality rate of 33%. Whereas there was no mortality with VPA only and all CBD oil combinations with VPA, which suggests good protection. It shows again that CBD oil combination with low dose VPA appears to have a good potential in the control of PTZ-induced seizure in rats.

***In silico* studies:** The results showed that CBD can bind the three subsites and modulate the activity of benzodiazepines and other allosteric modulators, however, with different binding affinities (Fig. 5).

The CBD docked at the A: ASN 265 subsite and established several interactions with residues such as HID 267, THR 266 and THR 268, among others. The HID 267 was found crucial in this binding pattern by establishing hydrogen bonding and π-π stacking with CBD. The ASN 265 is not involved in direct interaction; however, it is located at a

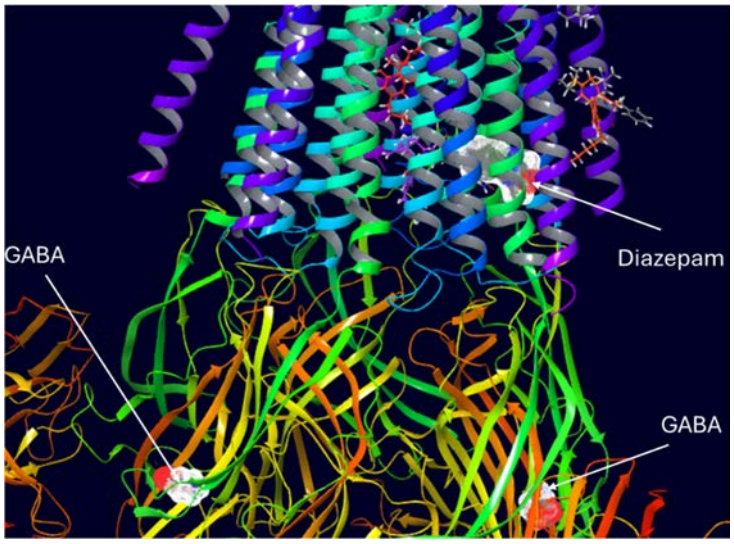

Fig. 5: Ribbon representation of $GABA_A$ receptor

Key residues are shown in ball and stick representation and with co-crystallized GABA and diazepam (Space-filling representation) and docked cannabidiol (ball and stick representation) at the three key subsites; ASN 265 carbon in magenta, SER 267 carbon in red and MET 294 carbon in brown

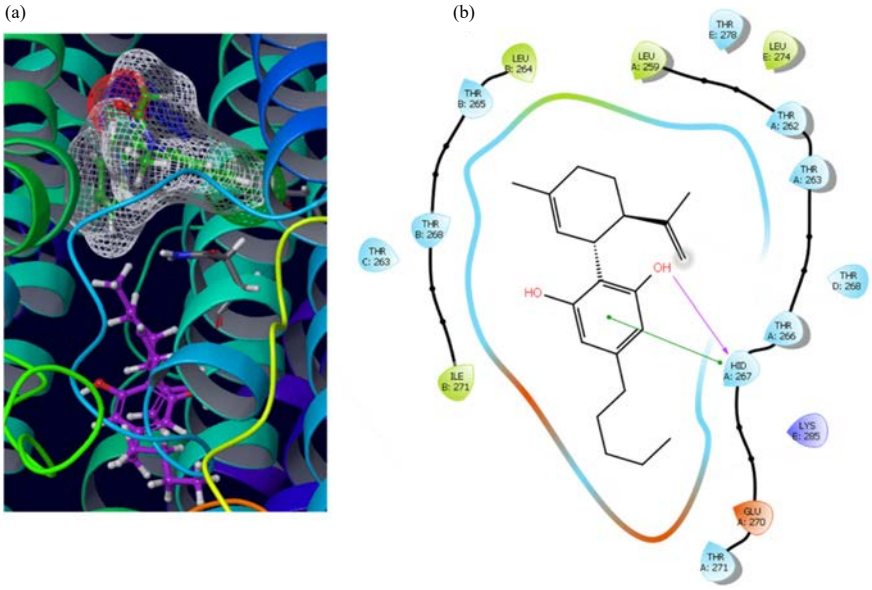

Fig. 6(a-b): Binding interactions of $GABA_A$ receptor, subsite A: ASN 265 with cannabidiol, (a) 3D representation and (b) 2D representation

Cannabidiol in a ball and stick representation in magenta, key residues in a ball and stick representation with carbons in gray and diazepam in space filling and ball and stick representation in green

perfect distance for hydrogen bonding through a water molecule (Fig. 6a-b).

In the E: SER 267 subsite, CBD established various interactions with VAL 260, THR 256, LEU 253 and ILE 255. No direct interaction was noted with the SER 267; however, as previously discussed in the ASN 265 subsite, there is potential hydrogen bonding through a water molecule (Fig. 7a-b).

The binding of CBD in A: MET 294 subsite showed interactions with residues such as VAL 290, PHE 291, MET 296 and GLY 297 (Fig. 8a-b). This subsite is more spacious than the other two subsites and would allow for more conformational flexibility and binding of the flexible molecule.

The XP scores showed that CBD binds the SER 267 subsite with higher affinity compared to the other two subsites (Table 1).

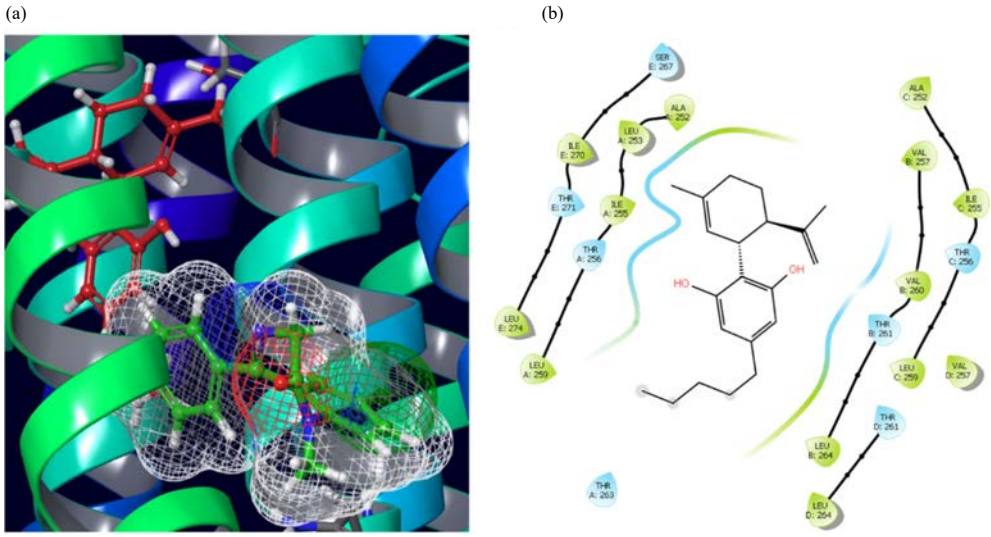

Fig. 7(a-b): Binding interactions of $GABA_A$ receptor, subsite E: SER 267 with cannabidiol, (a) 3D representation and (b) 2D representation

Cannabidiol in a ball and stick representation in red, key residues in a ball and stick representation with carbons in gray and diazepam in space filling and ball and stick representation in green

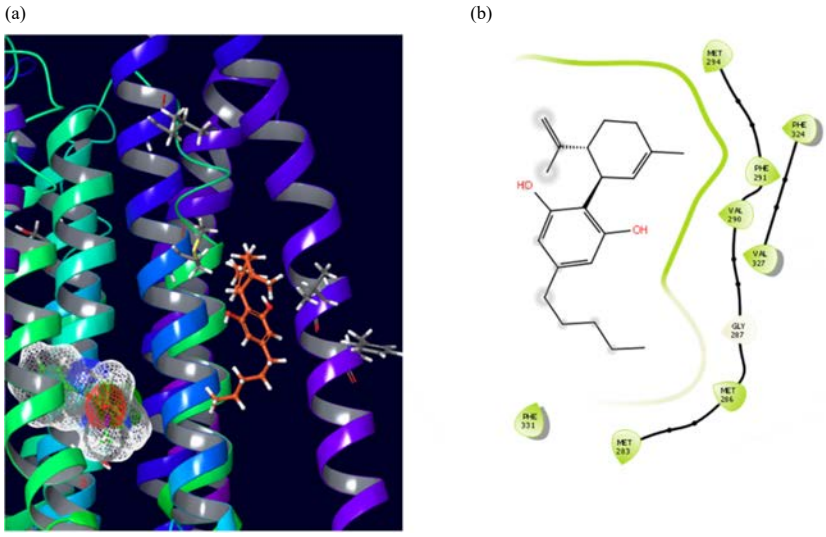

Fig. 8(a-b): Binding interactions of $GABA_A$ receptor (PDB ID: 6X3X), subsite A: MET 294 with cannabidiol, (a) 3D representation and (b) 2D representation

Cannabidiol in a ball and stick representation in brown, key residues in a ball and stick representation with carbons in gray and diazepam in space filling and ball and stick representation in green

Table 1: XP scores of cannabidiol docked at the three putative subsites in $GABA_A$ receptor (PDB ID: 6X3X)

Subsite	XP score
A: ASN 265	-3.9
A: MET 294	-3.6
E: SER 267	-5.9

ADMET and drug-likeness: The molecular descriptors were used to investigate the absorption mechanisms and possible routes of administration to estimate bioavailability, water solubility, Caco-2 and human intestinal absorption. Table 2 categorically stated that the oral absorption of CBD oil is favorable. Furthermore, the assessment of ADMET profiles and the drug-like profile of CBD oil was also analyzed. Medicinal chemistry evaluation prediction revealed the

Table 2: ADMET profiling of CBD oil

ADMET parameter	CBD oil	ADMET parameter	CBD oil
Absorption		Metabolism	
Water solubility (log mol/L)	-6.07	CYP2D6 substrate (Yes/No)	Yes
Caco-2 permeability (log Papp in 10-6 cm/sec)	1.50	CYP3A4 substrate (Yes/No)	Yes
Intestinal absorption (human) (Absorbed %)	90.34	CYP1A2 inhibitor (Yes/No)	No
P-glycoprotein substrate (Yes/No)	No	CYP2C19 inhibitor (Yes/No)	Yes
Distribution		CYP2C9 inhibitor (Yes/No)	Yes
BBB permeability (log BB)	0.24	CYP2D6 inhibitor (Yes/No)	Yes
CNS permeability (log PS)	-1.45	CYP3A4 inhibitor (Yes/No)	Yes
Excretion		Toxicity	
Total clearance (log mL/min/kg)	1.12	AMES*toxicity (Yes/No)	No
Renal OCT2 substrate (Yes/No)	No	Max. tolerated dose (human) (log mg/kg/day)	1.173
		hERG I inhibitor (Yes/No)	No
		Hepatotoxicity (Yes/No)	No

*AMES: Ames test. A biological test used to evaluate the mutagenic potential of chemical compounds. It is named after its developer, Dr. Bruce Ames, the test helps in predicting whether a substance may cause genetic mutations, which are usually associated with carcinogenicity

Table 3: Physicochemical properties, drug-likeness and medicinal chemistry prediction of CBD oil

Molecule properties	CBD oil	Molecule properties	CBD oil
Physicochemical properties		Drug-likeness	
Molecular weight	314.46	Lipinski alert	Pass: 1 violation
LogP	5.80	Ghose	No: 1 violation
#Acceptors	2	Veber	Pass
#Donors	3	Egan	Pass
#Heavy atoms	23	Muegge	Pass
#Aromatic heavy atoms	6	Bioavailability score	0.55
Fraction Csp3	0.52	Medicinal chemistry	-
#Rotatable bonds	6	PAINS	Pass
Molar refractivity	99.85	Brenk	1 alert, isolated alkene
TPSA# ($Å^2$)	40.46	Synthetic accessibility	4.05

#TPSA: Topological polar surface area. It is a physicochemical property that measures the surface area of a molecule occupied by polar atoms such as oxygen and nitrogen, along with their attached hydrogen atoms

physicochemical properties and drug-likeness, of CBD oil as shown in Table 3.

The table shows detailed ADMET parameters of CBD oil, including absorption metrics, distribution characteristics, metabolic interactions with CYP enzymes, excretion and toxicity assessments.

DISCUSSION

The main findings of this study are that combinational treatment of CBD oil and a low dose of VPA significantly delayed seizure onset, decreased the duration of seizure attacks and provided appreciable changes in the recurrence of the seizures without significant severity. Moreover, CBD oil and VPA completely prevent mortality in treated animals. *In silico* studies, it was demonstrated that CBD oil binds to three subunits at the $GABA_A$ receptor preferably by hydrogen-bond and it exhibited favorable pharmacokinetics parameters and strong oral bioavailability. Collectively, these results suggested that CBD oil could potentially enhance VPA activity through synergism opening the possibilities for new formulations with low doses of VPA. To the best of our knowledge, this is the first report of its kind exploring CBD oil with a low dose of VPA.

The CBD oil has been shown to have anticonvulsant properties[19], especially in children. Studies have indicated that CBD oil, apart from affecting T-type Ca^{2+} channels involved in neuronal excitation, affects $GABA_A$ receptors which influence calmness[20]. Therefore, for safety in the management of seizures to be realized, a lower dose representing a subtherapeutic dose in combination with CBD oil could provide a safe choice. Bearing in mind that seizure treatment is a long-time course. In addition, accumulated evidence shows that CBD oil is safe according to Iffland and Grotenhermen[21]. In the present study, the use of CBD oil significantly decreased PTZ-induced seizure onset. In line with present report, studies showed that the use of 50 mg/kg of CBD oil reduced PTZ-induced seizures[22].

The findings of the present study further showed that CBD oil has the potential to control seizures alone. Apart from delaying the onset of seizure, the duration of seizure was also significantly reduced as well and no recurrence of seizure

after recovery was reported. However, mortality during the experiment was very low compared with untreated control. In agreement with the present study results, several evidences reported that mortality increased after the consumption of CBD in epilepsy patients and animal models of epilepsy[11,12], yet the precise association is still not fully understood.

In the present study, it was found that 100 mg/kg VPA markedly increased the seizure onset, reduced the duration of seizure and had no mortality. These findings agreed with the work reported by Lotfy *et al.*[23], where they demonstrated the reduction in PTZ-induced convulsions in rats with the administration of VPA. Furthermore, the present study also showed that a combination of CBD oil, with either 50 or 75 mg/kg VPA, produced a similar increase in seizure onset and reduction in seizure duration. However, combination with 75 mg/kg VPA, showed a better seizure reduction profile in terms of seizure duration compared with all other treatment groups, including VPA alone. Although recurrence was observed in all CBD oil combination groups, no mortality was observed even after 72 hrs.

This study has shown the effectiveness of CBD oil in controlling seizures induced by PTZ. Hence with its good safety profile, the usual adverse effect observed with the use of AEDs' combination might be mitigated because reports have shown that the combination of AEDs enhanced their adverse effect profile[24]. To this end, several studies have emerged, reporting the search for alternatives, particularly, natural products having the potential to control seizures with no or low side effects[25,26]. This notion emerged as CBD oil is used in different disorders/diseases prompting its application in epilepsy-related disorders. More importantly, evidence indicated that approximately 90% of seizures were reduced by CBD oil in epileptic patients, displaying different good efficacies in different seizure types[27]. It, therefore, indicates that CBD oil has the potential to reduce seizures with a good safety profile. The study findings indicated that combination with a lower dose of VPA produced a profoundly delayed onset of the first seizure comparable with 100 mg/kg with very much reduced duration. However, it is worth mentioning that this combination was given for a short period of time and therefore we have not observed any appreciable outcome compared with the treatment dose. Therefore, using it as a preventive measure was not effective, although, no mortality was recorded as well. Further, the present study offers insight into the possible synergistic action of both CBD and VPA affecting the GABA system which ultimately protects against PTZ-induced seizure in rats[28,29]. Additionally, evidence suggests that CBD oil is highly lipophilic and can therefore distribute rapidly in the Central Nervous System (CNS) as well as other body organs[30].

Molecular docking analysis showed a profound interaction between CBD oil and $GABA_A$ receptors. The analysis indicates that this could be one of the mechanisms of action of CBD oil as well as VPA. Previous studies have identified a putative binding site for Cannabidiol (CBD) and 2-arachidonoyl glycerol (2-AG) between the M3 and M4 transmembrane domains of the β2 subunit α1 containing $GABA_A$ receptors[31,32]. The amino acid residues (AARs) mediating the selectivity of 2-AG for the β2 subunits were also described in another research and corresponded to β2MET 294 and β2LEU 301 in transmembrane region M3 and β2VAL 436 and β2PHE 439 in M4[32]. It was hypothesized that this site is likely important for the gating of the receptor and that other nearby amino acid residues in the M3 and M4 transmembrane domains may be involved in the binding of CBD and 2-AG. Additional research revealed that neither a valine nor a threonine at position 436 is directly contributing to the binding of either agent[28].

Furthermore, a mutation investigation has demonstrated that the M2 transmembrane domain-more especially, serine 267-is implicated in the effects of CBD[33]. The analogous amino acid in the $GABA_A$ receptor subunits ASP 265 and ASP 265 is present in the β2 and β3 subunit, whereas SER 65 is present in the β1 subunit. When altered to a serine in β2 or β3 subunit, the homologous amino acid in the $GABA_A$ receptor subunit was found to decrease responses to a range of structurally unrelated GABAA allosteric modulators, such as alcohol and general anesthetics[34].

In this research, the binding of CBD was tested *in silico* to the putative subsites of $GABA_A$ receptor, namely A: ASP 265, A: MET 294 and E: SER 267. The results of the docking studies showed that cannabidiol has good affinity to the studied three subsites and would allosterically potentiate the action of diazepam and other allosteric modulators that bind the benzodiazepines site. The results also showed that the binding to subsite E: SER is the most energetically preferred based on the binding scores, however, the relative vastness of subsite A: MET 294 would be also considered in binding a flexible molecule such as cannabidiol, a concept that needs further investigations. Therefore, this could be a synergistic combination as both compounds act at different $GABA_A$ systems to enhance GABA transmission. Hence reducing the excitation of neuronal cells seen during epileptic seizures.

Poor pharmacokinetics in drug development leads to considerable drug wastage and hence is costly. The ADMET-Absorption, Distribution, Metabolism, Excretion and Toxicity are critical pharmacokinetic properties that a drug has to possess during its discovery and development. A promising drug candidate should display its efficacy towards its target, retaining an optimal ADMET profile at their respective

therapeutic concentrations[35]. An extensive pharmacokinetic analysis was conducted on CBD oil. In this context, ADMET profiling showed that CBD oil possesses favorable pharmacokinetic characteristics. It demonstrated excellent intestinal absorption and is predicted to cross the blood-brain barrier (BBB), allowing for substantial distribution within the Central Nervous System (CNS)-a beneficial property for treating CNS-related conditions. Metabolism analysis indicated that certain compounds in CBD oil inhibit CYP450 enzymes, suggesting a heightened potential for drug-drug interactions and bioaccumulation. Enzymes like CYP2D6 and CYP3A4 were involved in its metabolism. According to excretion data, including total clearance and renal OCT2 profiles, CBD oil displayed effective elimination from the body without acting as a substrate for renal OCT2.

The standard drug-like criteria, including some outstanding rules such as Lipinski's[36] rule of five, Ghose[37], Veber[38], Egan[39] and Muegge[40] rules were met by the ADME properties of CBD oil. Therefore, Lipinski's rule of five and other criteria confirmed that CBD oil generally adheres to established drug-like profiles. Indicating that CBD is more likely to exhibit favorable pharmacokinetics and potentially strong oral bioavailability. Medicinal chemistry assessments indicated good synthetic accessibility does not raise any PAINS alert[41]. Hence, no significant alerts for pans assay interference structures (PAINS), an observation further supporting its affinity for the $GABA_A$ receptor. While the present study focused on investigating the combinational approach of CBD oil and VPA mainly, further studies incorporating CBD oil pharmacodynamic properties especially the effects on mortality would be an addition to the field of epilepsy.

CONCLUSION

In summary, the presented data support the notion that CBD oil has the potential to protect against PTZ-induced seizure in rats. Moreover, the study showed that CBD oil can allosterically activate the $GABA_A$ receptor as evidenced by computational studies. Therefore, it is hypothesized that the synergism effects were the possible mechanism for the anti-seizure activity. Given this strong evidence, therefore, it is anticipated that the study could advance the development of new formulations that provide better therapeutic outcomes.

SIGNIFICANCE STATEMENT

Epilepsy is a prevalent Central Nervous System (CNS) disorder treated with conventional antiepileptic agents such as valproic acid (VPA) that exhibits a low therapeutic index. The presented study describes the combination of CBD-oil with low dose VPA in PTZ-induced seizures in rats. Results showed that the CBD plus VPA (50+75 mg/kg) group had significantly longer seizure onset and shorter duration compared to VPA alone group. No mortality was observed in the combination groups, unlike the high mortality in the untreated group. Findings from the docking study support the hypothesis of synergistic effect through $GABA_A$ receptor binding. Therefore, a combination of CBD oil and low dose VPA could be clinically useful in reducing adverse effects encountered with therapeutic doses in long-term treatment of epilepsy.

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