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Transdermal cannabidiol and phenobarbital attenuate functional and structural alterations in pilocarpine-induced epilepsy in rats

A administração transdérmica de canabidiol e fenobarbital atenua as alterações funcionais e estruturais na epilepsia induzida por pilocarpina em ratos

El cannabidiol y el fenobarbital transdérmicos atenúan las alteraciones funcionales y estructurales en la epilepsia inducida por pilocarpina en ratón

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ABSTRACT

Objective: To evaluate the effects of transdermal administration of cannabidiol (CBD), either alone or in combination with phenobarbital, on behavioral, histopathological, and oxidative changes in an experimental model of pilocarpine-induced epilepsy. **Methods:** Seventy-two Wistar rats were divided into eight experimental groups, subjected to pilocarpine-induced epilepsy, and treated transdermally for 30 days with formulations containing CBD and/or phenobarbital. Behavioral tests were performed in an elevated plus maze and open field, along with histopathological and histomorphometric analyses of the hippocampus, as well as the evaluation of markers of oxidative stress. The nanostructured formulations were also characterized for physicochemical stability. **Results:** The groups treated with CBD showed reduced neuronal hyperexcitability, behavioral improvement, and decreased locomotor activity. Lower neuronal mortality, reduced gliosis, and partial preservation of hippocampal architecture were observed, especially in the CBD35 group. This group also demonstrated a better antioxidant response, with increased superoxide dismutase activity and partial maintenance of catalase. **Conclusion:** Transdermal administration of CBD, particularly in combination with phenobarbital, demonstrated neuroprotective and antioxidant potential in the experimental epilepsy model, proving to be a promising therapeutic strategy.

Keywords: Epilepsy, Cannabidiol, Phenobarbital.

RESUMO

Objetivo: Avaliar os efeitos da administração transdérmica de canabidiol (CBD), isolado ou associado ao fenobarbital, sobre alterações comportamentais, histopatológicas e oxidativas em modelo experimental de

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epilepsia induzida por pilocarpina. **Métodos:** Foram utilizados 72 ratos Wistar distribuídos em oito grupos experimentais, submetidos à indução de epilepsia por pilocarpina e tratados por via transdérmica durante 30 dias com formulações contendo CBD e/ou fenobarbital. Realizaram-se testes comportamentais em labirinto em cruz elevado e campo aberto, análises histopatológicas e histomorfométricas do hipocampo, além da avaliação de marcadores de estresse oxidativo. As formulações nanoestruturadas também foram caracterizadas quanto à estabilidade físico-química. **Resultados:** Os grupos tratados com CBD apresentaram redução da hiperexcitabilidade neuronal, melhora comportamental e diminuição da atividade locomotora. Observou-se menor mortalidade neuronal, redução da gliose e preservação parcial da arquitetura hipocampal, especialmente no grupo CBD35. Esse grupo também demonstrou melhor resposta antioxidante, com aumento da atividade da superóxido dismutase e manutenção parcial da catalase. **Conclusão:** A administração transdérmica de CBD, principalmente associada ao fenobarbital, apresentou potencial neuroprotetor e antioxidante no modelo experimental de epilepsia, mostrando-se uma estratégia terapêutica promissora.

Palavras-chave: Epilepsia, Canabidiol, Fenobarbital.

RESUMEN

Objetivo: Evaluar los efectos de la administración transdérmica de cannabidiol (CBD), solo o en combinación con fenobarbital, sobre los cambios conductuales, histopatológicos y oxidativos en un modelo experimental de epilepsia inducida por pilocarpina. **Métodos:** Setenta y dos ratas Wistar se dividieron en ocho grupos experimentales, se les indujo epilepsia por pilocarpina y se trataron transdérmicamente durante 30 días con formulaciones que contenían CBD y/o fenobarbital. Se realizaron pruebas de comportamiento en un laberinto elevado en cruz y en un campo abierto, junto con análisis histopatológicos e histomorfométricos del hipocampo, así como la evaluación de marcadores de estrés oxidativo. Las formulaciones nanoestructuradas también se caracterizaron por su estabilidad fisicoquímica. **Resultados:** Los grupos tratados con CBD mostraron una reducción de la hiperexcitabilidad neuronal, una mejoría conductual y una disminución de la actividad locomotora. Se observó una menor mortalidad neuronal, una reducción de la gliosis y una preservación parcial de la arquitectura del hipocampo, especialmente en el grupo CBD35. Este grupo también demostró una mejor respuesta antioxidante, con mayor actividad de la superóxido dismutasa y mantenimiento parcial de la catalasa. **Conclusión:** La administración transdérmica de CBD, particularmente en combinación con fenobarbital, demostró potencial neuroprotector y antioxidante en el modelo experimental de epilepsia, lo que la convierte en una estrategia terapéutica prometedora.

Palabras clave: Epilepsia, Cannabidiol, Fenobarbital.

INTRODUCTION

Epilepsy is a chronic neurological disease characterized by recurrent abnormal brain electrical activity, associated with hippocampal sclerosis, neuronal loss, and alterations in neurogenesis (PARENT JM and LOWENSTEIN DH, 2020; SIERRA A, et al., 2021). Following a status epilepticus, neural progenitor cells develop inadequate neural networks, contributing to the creation of hyperexcitable networks (ARSHAD MN and NAEGELE JR, 2004). These mechanisms highlight the complexity of epilepsy and the challenges associated with its management. Conventional treatment involves a variety of medications, including phenobarbital, carbamazepine, sodium valproate, and levetiracetam (TOMSON T et al., 2023; ANNE TB et al., 2025). Despite these alternatives, many individuals demonstrate resistance to medication, which requires new therapeutic tactics (GHOSH S et al., 2021). In this context, the endocannabinoid system emerges as a regulator of neuronal excitability and neuroinflammation (HILL MN et al., 2021). Cannabidiol (CBD), a phytocannabinoid from *Cannabis sativa*, has demonstrated therapeutic potential in several neurological diseases (VERHEIJEN ME, et al., 2020; CASTILLO-ARELLANO J, et al., 2023).

Despite the growing therapeutic interest in cannabidiol, its oral administration presents important limitations, including low bioavailability due to extensive first-pass hepatic metabolism and gastrointestinal degradation

(MILLAR SA, et al., 2018). Thus, transdermal delivery systems have emerged as promising alternatives, as they can provide sustained drug release, more stable plasma concentrations, and less pharmacokinetic variability. Furthermore, since CBD is highly lipophilic, its permeation through the skin barrier can be enhanced by nanostructured systems, such as nanoemulsions, which improve the dispersion, stability, and cutaneous absorption of the drug. Recent studies have demonstrated the potential of transdermal cannabinoid formulations based on nanoemulsions to optimize systemic administration and therapeutic efficacy (MAHMOUDINOODEZH H, et al., 2022; LODZKI M, et al., 2003). Therefore, the development of transdermal nanoformulations containing CBD may represent a promising therapeutic strategy for the treatment of epilepsy. Therefore, this study aimed to investigate whether transdermal nanoemulsion formulations containing cannabidiol, alone or in combination with phenobarbital, could attenuate behavioral, histopathological, and oxidative alterations in a pilocarpine-induced epilepsy model in Wistar rats.

METHODS

Animals and ethical considerations

A total of 72 adult male Wistar rats (60-80 days old, 200-250 g) from the UFRPE animal facility were used. The animals were housed in polypropylene cages (6 animals/cage) under controlled temperature (22 ± 2 °C) with a 12-hour light/dark cycle and water and food *ad libitum.* All experimental techniques were performed in accordance with the principles of the National Council for the Control of Animal Experimentation (CONCEA) and ARRIVE, and were authorized by the Animal Experimentation Ethics Committee of UFRPE (protocol no. 8825060923).

Cannabis sp. extract and nanoemulsion preparation.

The full-spectrum oil extract containing cannabidiol (CBD) was obtained through a collaboration with the AMEC-VET medical association. Assadpour E, et al. (2023) high-energy emulsification process was used to create an oil-in-water (O/W) nanoemulsion for experimental delivery. The oil phase contained sorbitan monooleate 80 (Span 80, Sigma) mixed with the CBD-enriched extract at concentrations of 5, 10, and 20 mg/mL or corn oil. The aqueous phase included polysorbate 80 (Tween 80, Neon), distilled water, and propylparaben as a preservative. After emulsification with continuous magnetic stirring, the formulations were swirled for 20 hours to stabilize.

Characterization of the nanoemulsion

Physicochemical characteristics, such as average particle size, polydispersity index (PDI), zeta potential (ζ), and electrophoretic mobility, were evaluated. These parameters were determined using a Zetasizer Nano ZS (Malvern®) via dynamic light scattering (DLS) spectroscopy at 25 °C and a 90° angle. Samples were diluted in Type I ultrapure water to minimize turbidity, and all analyses were performed in triplicate. The formulations were developed to achieve nanoparticles smaller than 500 nanometers (nm), as these are considered the most suitable for skin permeation. The PDI was used to assess the homogeneity of the dispersion: lower values indicate a more uniform distribution. The zeta potential, in mV, was used as a measure of colloidal stability, where higher absolute values (further from zero) indicate less particle aggregation and greater formulation stability. Electrophoretic mobility (Mob, μm cm/Vs) reflects the ease with which particles move through the medium; values further from zero indicate less agglomeration and better dispersibility.

Sample characterization

The AMEC-SP (CBD) sample was characterized by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). Analyses were performed using an ACQUITY UPLC H-Class system (Waters Corporation, Milford, MA, USA) coupled to a Xevo G2-XS QTof mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) source operating in positive ion mode. Chromatographic separation was achieved using an Acquity BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters, Milford, MA, USA), maintained at 40 °C, with a flow rate of 0.4 mL/min. The mobile phase consisted of water (A) and acetonitrile (B) under a binary gradient elution program as follows: 0–7 min, 10% B; 8 min, 50% B; 9 min, 95% B; 9.1 min, 10% B; and 11 min, 10% B. Data acquisition and processing were carried out using MassLynx software (Waters Corporation).

Preparation of the transdermal gel

The cannabidiol (CBD)-rich oil was incorporated into the formulation as an oil solution, with pharmaceutical-grade corn oil, free of microbiological contaminants, as the carrier excipient. The volume added was standardized to 6.5 mL of a 1% (w/v) CBD solution per batch. Phenobarbital was incorporated at varying concentrations, corresponding to the experimental doses for each group: C35 (35 mg/kg), C25 (25 mg/kg), C15 (15 mg/kg), and C10 (10 mg/kg). The quantities were adjusted based on mass-volume equivalence, taking into account the body weight of the experimental animals and the total volume of the final formulation per unit.

Experimental Design

Animals were randomly allocated into eight experimental groups (n = 9 per group): CTL (non-induced and untreated animals); IND (pilocarpine-induced and untreated animals); FEN35 (pilocarpine-induced animals treated with phenobarbital 35 mg/kg); CBD1 (pilocarpine-induced animals treated with 1% Cannabis sp. extract containing cannabidiol); CBD35 (pilocarpine-induced animals treated with 1% CBD extract associated with phenobarbital 35 mg/kg); CBD25 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 25 mg/kg); CBD15 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 15 mg/kg); and CBD10 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 10 mg/kg).

The experimental protocol was divided into three phases. In phase 1, temporal lobe epilepsy was induced by intradermal administration of pilocarpine. Following the induction procedure, animals were allocated to their respective experimental groups. In phase 2, transdermal treatments were administered once every 24 h for 30 consecutive days after the establishment of the chronic phase of epilepsy. In phase 3, behavioral assessments were performed, followed by euthanasia and tissue collection for histopathological and oxidative stress analyses.

Induction of epilepsy

The animals underwent TLE induction using a single subcutaneous dose of pilocarpine hydrochloride (250 mg/kg) in the dorsal interscapular region. After injection, behavioral patterns and general health status were observed. The animals remained in status epilepticus (SE) for at least 30 minutes. After 15 days, the animals entered the second phase (latent phase), when they did not display seizures. On day 16, the chronic phase began, characterized by recurrent spontaneous seizures of limbic origin at a constant frequency (CAVALHEIRO EA, et al., 1991).

Behavioral Tests

Elevated Plus Maze

The elevated plus maze assesses unconditioned anxiety based on natural exploration and avoidance of open, elevated areas. Over 5 minutes, we record the number of entries and time spent in the open arms, which serve as indices of anxiety (LISTER RG 1987). Animals were tested before chronic seizures began, just like the control group. Anxiety levels were assessed after treatment starts and again after 30 days of pilocarpine administration using the maze. On day 46, animals were euthanized for further analyses described below.

Open Field

The open field maze test (OFT) was conducted in a square wooden arena with a black floor. The floor was subdivided into 16 equal-sized quadrants by white lines. Each animal was placed in the center and allowed to explore for 5 minutes. Vertical exploratory activity was recorded. Time spent in the central or open area was measured. The presence or absence of absence-type epileptic seizures was also noted during observation (SEIBENHENER ML and WOOTEN MC, 2015).

Euthanasia and sample collection

At the end of the experimental period, the animals were weighed and euthanized under deep anesthesia (ketamine and xylazine, 75–90 mg/kg, intraperitoneal) (DIAS FCR, et al., 2019). For each animal, 5 mL of blood was collected in tubes without anticoagulant. The samples were centrifuged to obtain serum, which was

stored at −80 °C until biochemical analyses. The brains were carefully removed from the cranial cavity. Two fragments from zones 1 and 2 were collected, identified, and stored at −80 °C until oxidative stress analyses. The remaining brain tissue was placed in plastic containers and fixed in 10% buffered formalin for later morphometric and histopathological evaluation.

Determination of oxidative stress markers

The samples were homogenized in potassium phosphate buffer (pH 7.4, 0.2 M) containing 1 M EDTA using a homogenizer (OMNI) and centrifuged (13,800 x g at 4°C for 10 min). The supernatant was used to determine the concentrations of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), total antioxidant activity, levels of nitric oxide (NO) and malondialdehyde (MDA), and total protein concentration. The biochemical data were normalized to the total protein levels in the supernatant. The analyses were performed in duplicate. All enzymatic activities were determined in duplicate using a spectrophotometer (UV-Mini 1240, Shimadzu) or an ELISA reader (Thermo Scientific, Waltham, MA, USA).

NO production was indirectly determined through the nitrite/nitrate content using the standard Griess reaction (RICART-JANÉ D et al., 2002). CAT activity was assessed by measuring the rate of H2O2 decomposition (AEBI H, 1984), SOD activity was determined following Siddiqui IA et al. (2005), and GST activity through the formation of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate (HABIG WH, et al., 1974). The total antioxidant capacity (FRAP) was measured according to Benzie IF and Strain JJ (1996). MDA levels were measured following the protocol reported by Gutteridge and Halliwell (GUTTERIDGE JMC and HALLIWELL B, 1990). Total protein concentration was measured using the Lowry method (LOWRY OH et al. 1951) and used to standardize the stress data.

Histopathological and histomorphometric evaluation

This study used quantitative and morphological methods to assess neuronal integrity in the hippocampus. Neurons were classified as viable, dead, or vacuolated. This classification served as a parameter to analyze neurodegeneration, neuronal damage, and regenerative potential. Hippocampal tissue samples were analyzed for regional vulnerability, a widely described feature making the hippocampus highly susceptible to pathological insults (TASKER RC, 2008).

Neurons were categorized using established morphological criteria. Viable neurons had preserved cellular architecture and homogeneous cytoplasm. Dead neurons showed features such as pyknosis, karyorrhexis, or dark neurons, indicating non-apoptotic cell death, especially during chronic stress (TISHKINA AO et al., 2009). Vacuolated neurons exhibited cytoplasmic vacuoles and structural disorganization, a pattern often observed in ischemic lesions and exposure to neurotoxic agents (МАКАРЬЕВА LM, et al., 2022; ZULFIQAR NS, 2013).

Neurons were counted in predefined hippocampal fields using high-magnification microscopy. The total number and the proportion of each neuron category (viable, dead, vacuolated) were calculated and expressed as percentages. Patterns of neuronal loss related to different pathological conditions were identified. For ischemia models, a higher proportion of neurons with irreversible damage was expected (МАКАРЬЕВА LM, et al., 2022).

To contextualize the findings, neuronal data were interpreted in light of known links between hippocampal neuronal loss (HNL) and cognitive decline. Increased HNL is associated with memory impairments (AGRAWAL S, et al., 2024). Changes in adult neurogenesis were also considered, as they may precede significant cognitive impairments (KREZYMON A, et al., 2013).

Statistical Analysis

Statistical analyses were performed using normality tests between groups, with the Shapiro-Wilk test assessing normality. For cell count assessments and behavioral test times, analysis of variance between groups using ANOVA was employed. For non-parametric results where data did not show homogeneity, the Durbin-Conover test, an alternative to the Friedman test, was applied to perform multiple comparisons between groups.

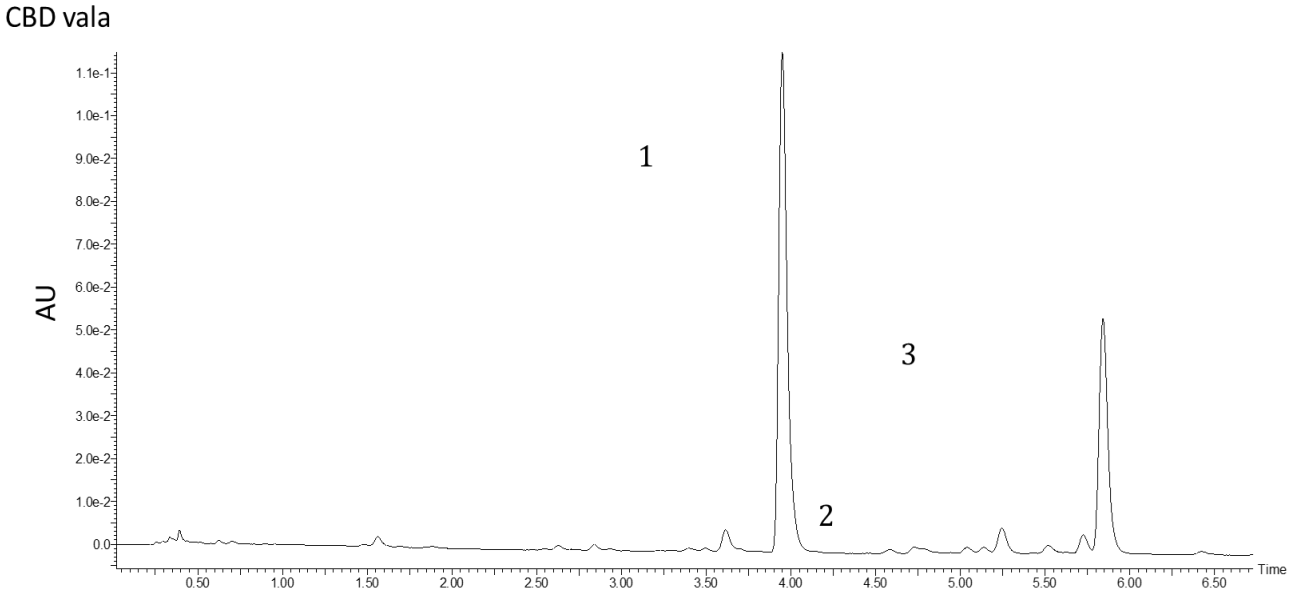
RESULTS AND DISCUSSION

Physicochemical characterization of the gel and its formulations

Table S1 lists the values obtained for the characteristics of the nanoemulsion, showing that the formulations were in the nanometric range, with values below 500 nm. The Polydispersity Index was less than 0.8 in most groups. This indicates the homogeneity of the applied product. The Zeta potential (mV), with values below -40 mV in some groups, indicates more stable molecules. The results among the products suggest more promising formulations for long-term therapy. These parameters are important because they guarantee colloidal stability, skin permeation, and bioavailability of the drug. These factors are essential for nanostructured transdermal systems (MCCLEMENTS DJ, 2012).

In the CBD extract obtained from AMEC, cannabidiol (CBD, **1**) was identified as the predominant constituent, followed by lower amounts of tetrahydrocannabinol (THC, **2**) and cannabichromene (CBC, **3**). The cannabinoids were identified by comparing their retention times with those of authentic standards, together with high-resolution mass spectrometry analysis using UPLC-DAD-qTOF-MS. All compounds exhibited the same protonated molecular ion peak in positive ionization mode at *m/z* 315.2323 $[M+H]^+$, which is characteristic of the isomeric nature of these cannabinoids. The UPLC-DAD chromatogram obtained for the extract is presented in **Figure 1**.

Figure 1 - UPLC-DAD chromatogram (280 nm) of the CBD extract from AMEC.



Source: Pedrosa MB, et al., 2026.

Behavioral tests

Elevated plus maze

The elevated plus maze assessment after 45 days of treatment revealed differences in exploratory behavior among the experimental groups (**Table S2**). The induced and untreated (IND) group made more open arm entries than the control (CTL) group, suggesting increased excitability. Among CBD-treated groups, entry frequencies were lowest in CBD1 and CBD15 and highest in CBD25 and CBD35. In comparing CBD35 and FEN35, both had the same number of animals entering open arms, but FEN35 had a higher total entry frequency.

Significant differences in CTL, CBD35, and FEN35 indicate treatment-related behavioral changes after the experimental period. Results showed that epilepsy induction led to anxiety and neuronal hyperexcitability, evidenced by fewer and shorter entries into open arms after status epilepticus. After treatment, low-CBD groups behaved similarly to controls, suggesting a possible anxiolytic effect. The CBD35 group had fewer

entries than the FEN35 group, suggesting reduced hyperactivity and better behavioral regulation. These findings support studies showing CBD modulates excitability and anxiety by interacting with the endocannabinoid system and serotonergic receptors (BLESSING EM, et al., 2015; IBEAS BIH C, et al., 2015).

Open Field Maze

In the open field test, both induced and untreated animals exhibited increased locomotor activity, suggesting greater neural excitability and psychomotor agitation. Among the treated groups, only the FEN35 group maintained high crossing rates, while those treated with CBD showed a reduction in behavioral hyperactivity, except in the CBD25 group.

Analysis of thigmotaxis and passages through the central region showed no significant differences between the groups. During the test, episodes consistent with absence seizures were also observed, characterized by immobility and lack of response to external stimuli. The results of the open field test suggest that induced epilepsy increases neuronal excitability and locomotor alterations, characteristics frequently associated with hippocampal and limbic dysfunction.

The groups treated with CBD showed a reduction in hyperactivity at most concentrations, indicating a possible modulating effect on neural activity. Cannabidiol has been indicated as a reducer of neuronal excitability and a modulator of circuits involved in epileptogenesis and anxiety, through mechanisms related to the endocannabinoid system and serotonergic neurotransmission (IBEAS BIH C, et al., 2015; BLESSING EM, et al., 2015).

Histomorphometry

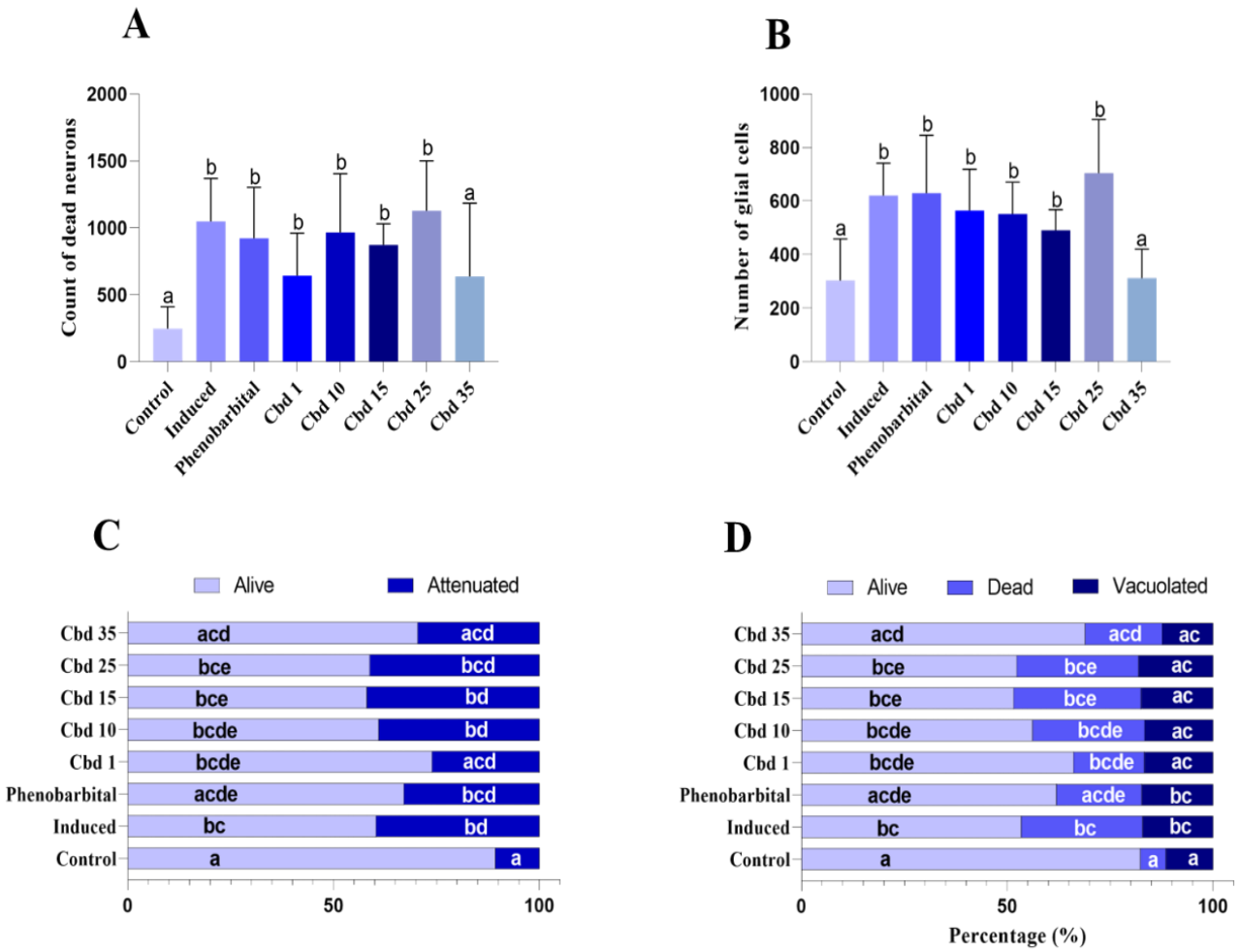
Histomorphometric analysis of the hippocampus revealed a significant increase in neuronal death in the induced and untreated groups compared to the control group (**Figure 2A**). The CBD1 and CBD35 groups showed fewer dead neurons, indicating a neuroprotective effect, whereas the CBD25 group exhibited greater neuronal damage. Experimental studies show that CBD reduces neuronal damage and preserves hippocampal architecture in models of temporal lobe epilepsy (Do VAL-DA SILVA RA, et al., 2017; CAMPOS AC, et al., 2016).

Regarding glial cells, the induced group showed a significant increase compared with the control group, whereas the CBD35 group exhibited values closer to those of the CTL group, with a significant difference (**Figure 2B**). Studies demonstrate that CBD reduces microglial activation and the release of pro-inflammatory mediators, thereby contributing to neuronal protection and slowing the progression of epileptogenesis (IBEAS BIH C, et al., 2015; CAMPOS AC, et al., 2016).

Analysis of the percentages of live, vacuolated, and dead neurons revealed differences among the experimental groups, with a reduction in atrophied neurons observed in the treated groups (**Figure 2C** and **2D**).

No significant differences in the number of vacuolated cells were observed among the groups, except in the FEN35 group, which showed a significant increase. This observed increase suggests the persistence of cellular changes associated with neural stress even after treatment (WEN X and MA H, 2025). The reduction in atrophied neurons in the treated groups reinforces the neuroprotective potential of the formulations, indicating partial preservation of neuronal viability in the hippocampus.

Figure 2 - A - Count of death neurons.



The graph correlates groups with their mean number of dead neurons throughout the hippocampus, including standard deviations.

B - Glial cell count. Graph showing differences between group means.

C - Comparison of percentage means across groups: viable neurons (blue) versus dead and vacuolated neurons affected by induced TLE, as well as treatment groups showing greater protective effects.

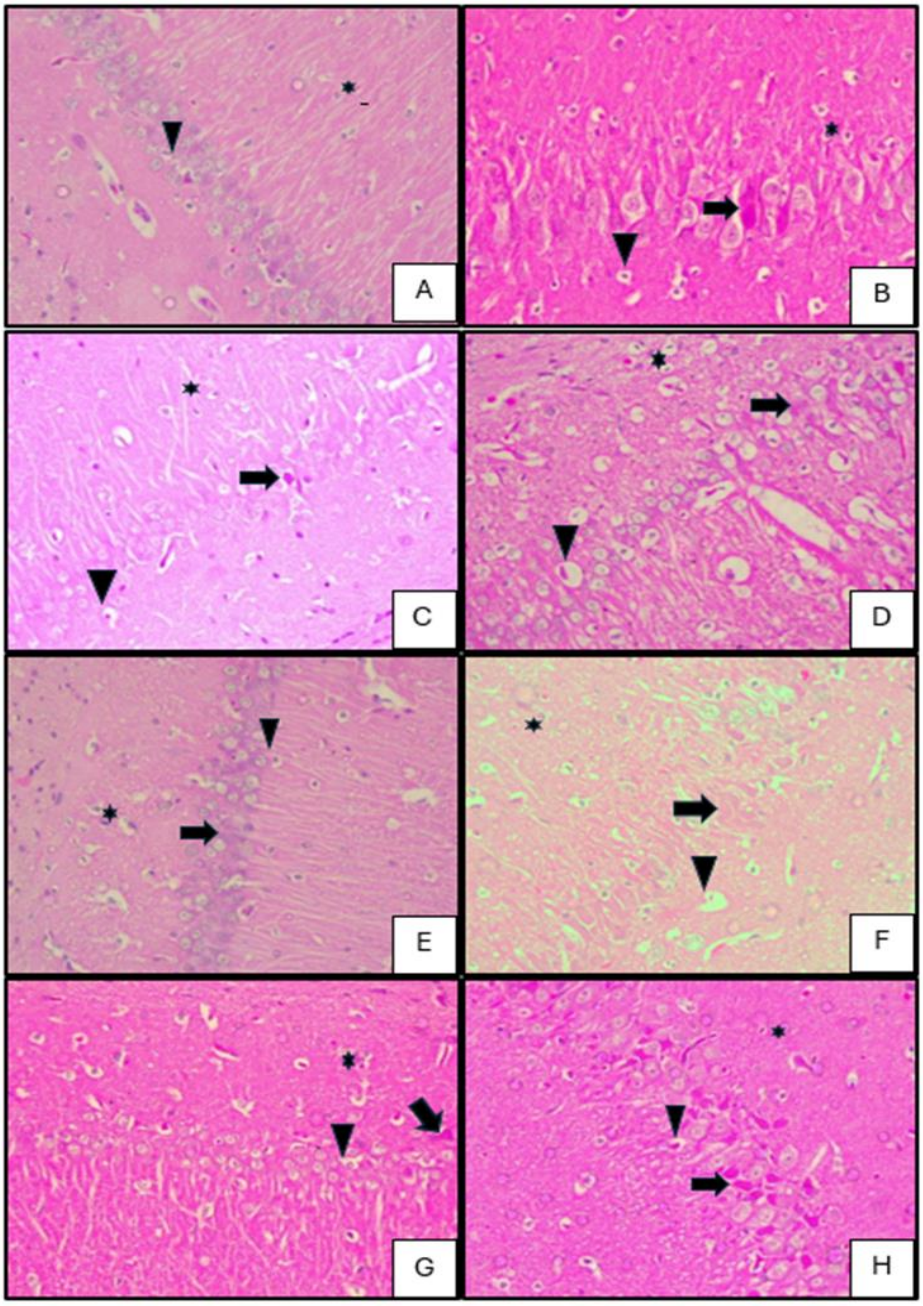
D - Hippocampal neuron percentages. CTL (non-induced and untreated animals); IND (pilocarpine-induced and untreated animals); FEN35 (pilocarpine-induced animals treated with phenobarbital 35 mg/kg); CBD1 (pilocarpine-induced animals treated with 1% Cannabis sp. extract containing cannabidiol); CBD35 (pilocarpine-induced animals treated with 1% CBD extract associated with phenobarbital 35 mg/kg); CBD25 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 25 mg/kg); CBD15 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 15 mg/kg); and CBD10 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 10 mg/kg).

Data are expressed as mean ± standard deviation (SD). Statistical differences between groups were considered significant at $p < 0.05$.

Source: Pedrosa MB, et al., 2026.

Histopathological analyses revealed areas of edema, vacuolization, increased glial cells, and neuronal loss in the CA2 region of the hippocampus (**Figure 3**).

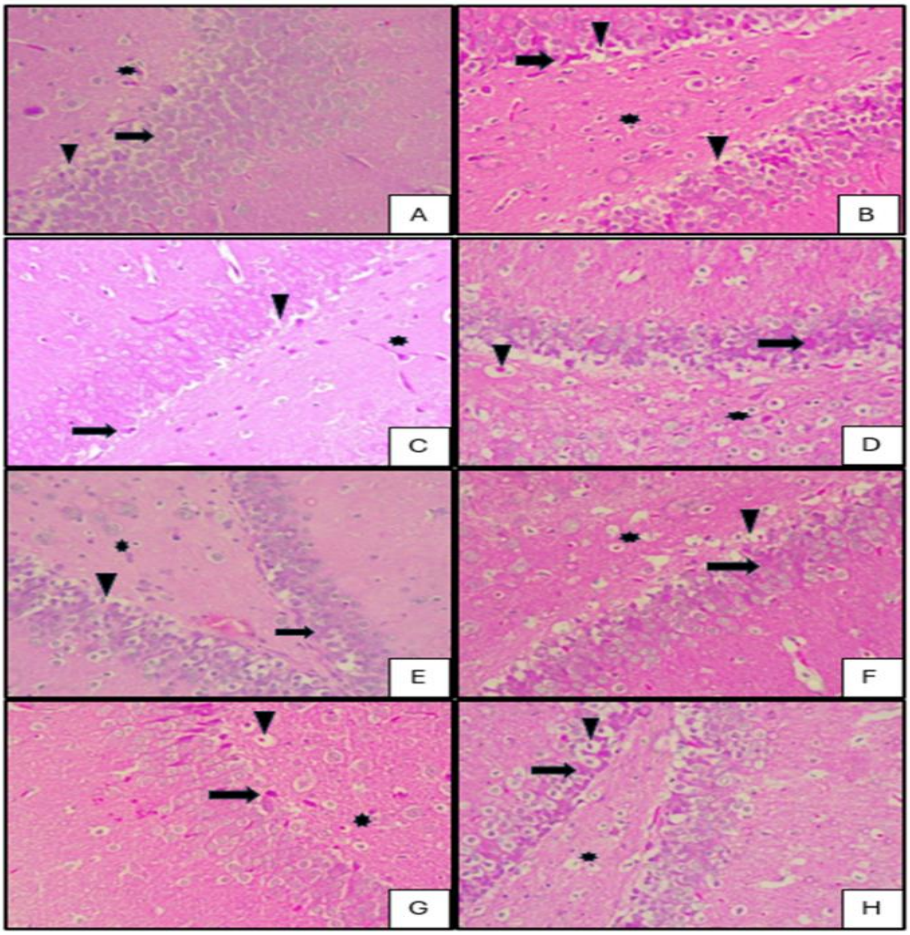
Figure 3 - Photomicrograph of the CA2 region of the hippocampus (H&E, 400×).



Asterisk: glial cells; **arrow:** dead neurons; **arrowhead:** vacuolated neurons. Group descriptions: **A:** Control (CTL) – predominance of viable neurons, very few dead neurons, presence of glial cells. **B:** IND – presence of vacuolated cells, many dead neurons, increased glial population. **C:** CBD1 – fewer dead neurons, large glial population. **D:** CBD10 – many dead neurons, vacuolization, glial cells. **E:** CBD15 – many dead neurons, vacuolization, glial cells. **F:** CBD25 – many dead neurons, vacuolization, glial cells. **G:** CBD35 – fewer dead neurons, reduced vacuolization, lower glial population. **H:** FEN35 – many dead neurons, vacuolization, glial cells. CTL (non-induced and untreated animals); IND (pilocarpine-induced and untreated animals); FEN35 (pilocarpine-induced animals treated with phenobarbital 35 mg/kg); CBD1 (pilocarpine-induced animals treated with 1% Cannabis sp. extract containing cannabidiol); CBD35 (pilocarpine-induced animals treated with 1% CBD extract associated with phenobarbital 35 mg/kg); CBD25 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 25 mg/kg); CBD15 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 15 mg/kg); and CBD10 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 10 mg/kg).
Source: Pedrosa MB, et al., 2026.

In the dentate gyrus, an increase in dead neurons and a reduction in neural plasticity were observed in animals with induced epilepsy, suggesting impaired neurogenesis (**Figure 4**). The observed histopathological changes confirm this structural impairment of the hippocampus, characterized by edema, vacuolization, gliosis, and neuronal loss. These alterations are directly related to excitotoxicity and the neuroinflammatory process caused by status epilepticus. The reduction in neural plasticity in the dentate gyrus is a phenomenon frequently associated with aberrant reorganization of neuronal circuits and the maintenance of epileptiform hyperexcitability (PARENT JM and LOWENSTEIN DH, 2002; SIERRA A, et al., 2010). Studies also demonstrate that modulation of the endocannabinoid system mediated by CBD can contribute to neuronal preservation and reduction of neuroinflammation in experimental models of epilepsy (CAMPOS AC, et al., 2016).

Figure 4 - Photomicrograph of the dentate gyrus (H&E, 400×).

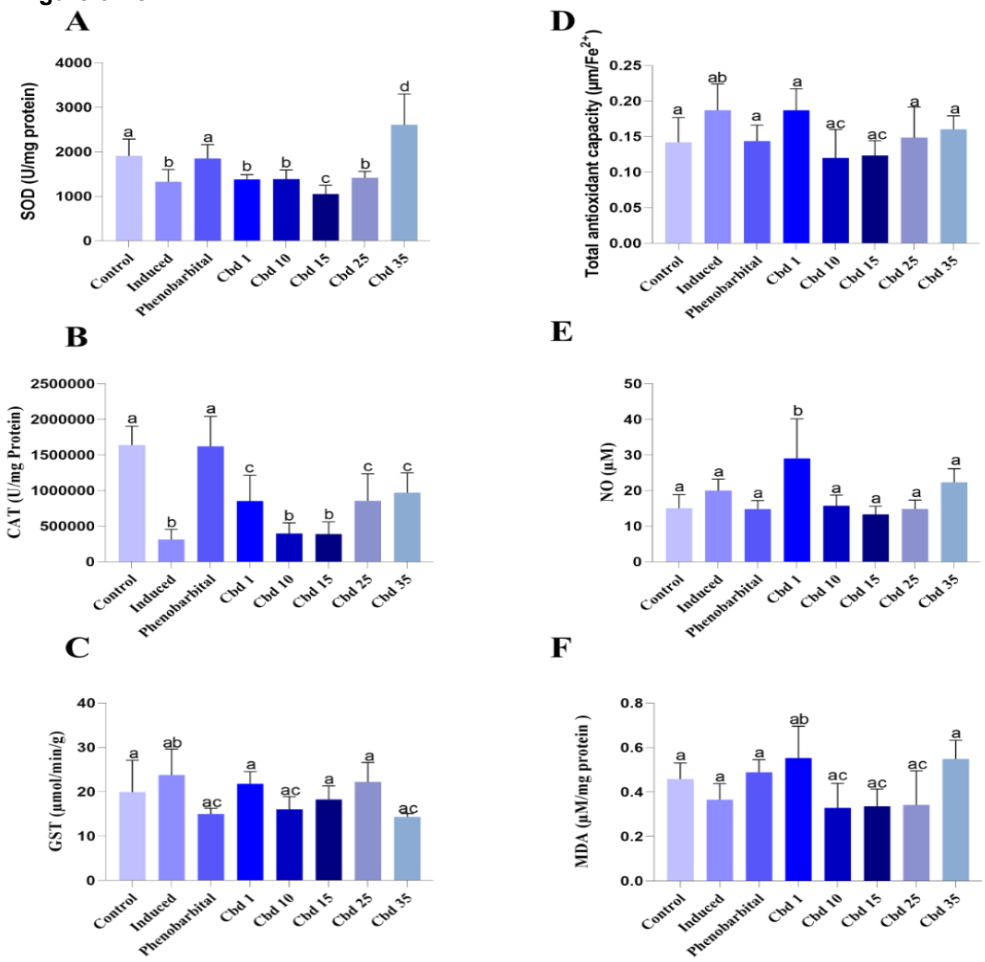


Asterisk: glial cells; **arrow:** dead neurons; **arrowhead:** vacuolated neurons. Group descriptions: **A:** Control (CTL) – predominance of viable neurons, very few dead neurons, presence of glial cells. **B:** IND – presence of vacuolated cells, many dead neurons, increased glial population. **C:** CBD1 – fewer dead neurons, large glial population. **D:** CBD10 – many dead neurons, vacuolization, glial cells. **E:** CBD15 – many dead neurons, vacuolization, glial cells. **F:** CBD25 – many dead neurons, vacuolization, glial cells. **G:** CBD35 – fewer dead neurons, reduced vacuolization, lower glial population. **H:** FEN35 – many dead neurons, vacuolization, glial cells. CTL (non-induced and untreated animals); IND (pilocarpine-induced and untreated animals); FEN35 (pilocarpine-induced animals treated with phenobarbital 35 mg/kg); CBD1 (pilocarpine-induced animals treated with 1% Cannabis sp. extract containing cannabidiol); CBD35 (pilocarpine-induced animals treated with 1% CBD extract associated with phenobarbital 35 mg/kg); CBD25 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 25 mg/kg); CBD15 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 15 mg/kg); and CBD10 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 10 mg/kg).
Source: Pedrosa MB, et al., 2026.

Oxidative Stress

In nerve tissue, superoxide dismutase (SOD) activity was reduced in the induced group. The groups treated with CBD showed even lower values, except the CBD35 group, which demonstrated a significant increase (**Figure 5A**). Catalase (CAT) activity decreased in all groups except the FEN35 group. Although not returning to normal, the CBD1, CBD25, and CBD35 groups showed higher CAT levels than the other groups (**Figure 5B**). Glutathione transferase activity changed only in the group treated with isolated CBD (**Figure 5C**). Total antioxidant capacity remained high only in the CBD1 group. The other treated groups showed reduced values in this parameter (**Figure 5D**). Regarding nitric oxide (NO), only the CBD1 group showed a marked increase in concentration. Other groups had values similar to those of the control group (**Figure 5E**). Malondialdehyde (MDA) levels did not differ significantly among the groups (**Figure 5F**). The CBD1, CBD35, and FEN35 groups had higher MDA concentrations per tissue, while the induced group showed a reduction (**Figure 5b**).

Figure 5 - Oxidative stress markers in nervous tissue.



The graphs represent the levels of antioxidant enzymes and lipid peroxidation markers evaluated in the nervous tissue of the experimental groups. Panels show the activities/concentrations of oxidative status biomarkers, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA), Nitric Oxid (NO), and Total antioxidant capacity (FRAP). CTL (non-induced and untreated animals); IND (pilocarpine-induced and untreated animals); FEN35 (pilocarpine-induced animals treated with phenobarbital 35 mg/kg); CBD1 (pilocarpine-induced animals treated with 1% Cannabis sp. extract containing cannabidiol); CBD35 (pilocarpine-induced animals treated with 1% CBD extract associated with phenobarbital 35 mg/kg); CBD25 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 25 mg/kg); CBD15 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 15 mg/kg); and CBD10 (pilocarpine-induced animals treated with 1% CBD extract + phenobarbital 10 mg/kg). Data are expressed as mean ± standard deviation (SD). Statistical differences between groups were considered significant at $p < 0.05$. **Source:** Pedrosa MB, et al., 2026.

The reduction in SOD and CAT activity in the induced group suggests a response from the organism to combat a possible oxidative imbalance, probably through increased production of reactive oxygen species. The groups treated with CBD showed distinct responses. The CBD35 group showed an increase in SOD activity and partial maintenance of CAT activity. This suggests a more efficient antioxidant and neuroprotective effect at this concentration. CBD acts as an antioxidant, reducing oxidative stress by neutralizing free radicals and preserving cellular antioxidant systems (HAMPSON AJ, et al., 1998; CAMPOS AC, et al., 2016). The increase in NO in the CBD1 group may be associated with the anti-inflammatory and vascular modulating effects of cannabidiol, although elevated NO levels may also contribute to neuroinflammatory processes. However, with unchanged MDA levels, the treatments may have been effective in controlling lipid peroxidation. Overall, CBD35 suggests partial antioxidant protection.

CONCLUSION

We concluded that transdermal administration of cannabidiol, alone or in combination with phenobarbital, was able to attenuate behavioral, histopathological, and oxidative alterations in animals subjected to pilocarpine-induced epilepsy. The nanostructured formulations presented physicochemical characteristics compatible with stability and potential for transdermal application. The CBD35 group showed better neuroprotective effects, including reduced neuronal mortality, reduced glial activation, and partial improvement in antioxidant markers. Thus, the combination of CBD and phenobarbital demonstrates promising therapeutic potential in experimental epilepsy. Further studies are needed to elucidate their mechanisms of action and evaluate long-term safety and efficacy.

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