



Check for updates

RESEARCH ARTICLE

Assessing neuroinflammation in vitro in a microglial model: advancing cannabinoid-based therapeutic insights

[version 1; peer review: 1 approved with reservations]

Jose-Manuel Quintero ^{1,2}, Luis-Eduardo Diaz ³, Rosa Helena Bustos²,
Marta-Ximena Leon⁴

¹Doctoral Programme of Biosciences, Universidad de La Sabana, Chia, Huila, 25001, Colombia

²Department of Clinical Pharmacology, Evidence-based Therapeutics Group, Universidad de la Sabana Facultad de Medicina, Chía, Cundinamarca, 25001, Colombia

³Facultad de Ingeniería, Universidad de La Sabana, Universidad de La Sabana, Cundinamarca, Chía, 25001, Colombia

⁴Grupo Dolor y Cuidados Paliativos, Universidad de La Sabana, Chia, Cundinamarca, 25001, Colombia

V1 First published: 28 Mar 2025, 14:346
<https://doi.org/10.12688/f1000research.162356.1>

Latest published: 28 Mar 2025, 14:346
<https://doi.org/10.12688/f1000research.162356.1>

Abstract

Background

Neuroinflammation plays a critical role in the progression of neurological disorders such as Alzheimer's and Parkinson's diseases, as well as chronic pain. It is characterized by elevated production of pro-inflammatory cytokines, including IL-6, and TNF- α , by microglia, the resident immune cells of the central nervous system. Emerging research highlights the involvement of the endocannabinoid system (ECS) in neuroinflammation, proposing cannabinoids as potential therapeutic agents owing to their immunosuppressive and anti-inflammatory properties.

Methods

This study used an in vitro model, specifically the BV-2 murine microglial cell line, to assess neuroinflammation. Cell viability was measured using the MTT assay, and cytokine production was quantified using advanced nanobiosensor technologies, including Surface Plasmon Resonance (SPR). Immunocytochemistry (ICC) was used to evaluate protein expression. Microglial cells were treated with varying concentrations of dexamethasone sodium phosphate (Dex-SP), cannabidiol (CBD), a full-spectrum tetrahydrocannabinol (FSE-THC) extract (70% THC), or a combination of the two at a 1:1 ratio (FSE-THC/CBD).

Open Peer Review

Approval Status ?

1

version 1

28 Mar 2025

[view](#)

1. **Maurício dos Santos Pereira** , Broad
Institute Stanley Center for Psychiatric
Research, Cambridge, USA

Any reports and responses or comments on the article can be found at the end of the article.

Results

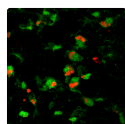
The treatments did not affect BV-2 microglial cell viability. Nitric oxide (NO) production remained unaltered following treatment with Dex-SP, CBD, FSE-THC 70%, or FSE-THC:CBD in LPS-stimulated BV-2 microglia. However, CBD and FSE-THC:CBD significantly reduced TNF- α levels, whereas Dex-SP and CBD decreased IL-6 expression. Furthermore, Dex-SP, CBD, FSE-THC (70%), and FSE-THC:CBD modulated the activation of Bcl-2, Bax, TNF- α , IFN- γ , NF- κ B, and iNOS in concentration- and treatment-specific manners.

Conclusions

Dex-SPs demonstrate acute anti-inflammatory effects in LPS-stimulated BV2 microglial cells. Specific cannabinoids at particular concentrations exhibit comparable or superior efficacy in modulating key inflammatory mediators. These findings suggest the therapeutic potential of cannabinoids in neuroinflammation, and emphasize the need for further investigation to optimize their application.

Keywords

Neuroinflammation, Cytokines, Microglia, Nanobiosensors, Cannabinoids



This article is included in the
Neuroinflammation collection.

Corresponding author: Rosa Helena Bustos (rosabucr@unisabana.edu.co)

Author roles: **Quintero JM:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Diaz LE:** Conceptualization, Funding Acquisition, Investigation, Methodology, Supervision, Writing – Original Draft Preparation; **Bustos RH:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Leon MX:** Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: This work was financially supported by Universidad de La Sabana MED-296-2020
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2025 Quintero JM *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Quintero JM, Diaz LE, Bustos RH and Leon MX. **Assessing neuroinflammation in vitro in a microglial model: advancing cannabinoid-based therapeutic insights [version 1; peer review: 1 approved with reservations]** F1000Research 2025, **14**:346 <https://doi.org/10.12688/f1000research.162356.1>

First published: 28 Mar 2025, **14**:346 <https://doi.org/10.12688/f1000research.162356.1>

Abbreviations

AEA: Anandamide
 CB2R: Cannabinoid Receptor Type 2
 CBD: Cannabidiol
 CBG: Cannabigerol
 CMD: Carboxymethyl dextran
 CNS: Central Nervous System
 COX-2: Cyclooxygenase-2
 Dex-SP: Dexamethasone Sodium Phosphate
 DMEM: Dulbecco's Modified Eagle's Medium
 DMSO: Dimethyl Sulfoxide
 ECS: Endocannabinoid System
 EDC: 1-Ethyl-3-(3-Dimethylaminopropyl)Carbodiimide
 FBS: Fetal Bovine Serum
 FSE: Full-Spectrum Extract
 GPR: G Protein-Coupled Receptors
 IFN: Interferon Gamma
 IL: Interleukin
 LPS: Lipopolysaccharide
 NF- κ B: Nuclear Factor Kappa B Subunit 1
 NGF: Nerve Growth Factor
 NHS: Hydroxysuccinimide
 NO: Nitric Oxide
 PGE2: Prostaglandin E2
 PPAR- γ : Peroxisome Proliferator-Activated Receptor Gamma
 SD: Standard Error
 SPRi: Surface Plasmon Resonance Image
 THC: Δ^9 -Tetrahydrocannabinol
 TNF- α : Tumor Necrosis Factor-Alpha
 TRP: Transient Potential
 2-AG: 2-Arachidonoylglycerol

1. Introduction

A crucial factor in various neurological conditions, including neurodegenerative disorders like Alzheimer's and Parkinson's diseases, as well as chronic pain, is neuroinflammation. This process is marked by the release of pro-inflammatory cytokines, which significantly contribute to the development and progression of these disorders.¹

Central sensitization, a key mechanism in pain modulation, is significantly influenced by proinflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF- α) and interleukin-6 (IL-6). TNF- α markedly enhances excitatory neurotransmission in the spinal cord. Concurrently, IL-1 β and IL-6 contribute to the dysregulation of neurotransmitters in the pain-related neural pathways. This alteration in neurotransmitter balance promotes central sensitization and amplifies pain sensitivity, establishing these cytokines as crucial components in the development of pathological pain.^{2,3} Microglia, the primary immune cells of the central nervous system (CNS), play a crucial role in the development of neuropathic pain. In recent decades, substantial evidence has highlighted the capacity of microglia to induce dysfunction in the nervous system following nerve injury, thus contributing to pain regulation.⁴ These cells exert their influence by releasing cytokines such as TNF- α ,⁵ and IL-6,^{6,7} which drive the process of central sensitization, a state in which CNS neurons become hyper-responsive to stimuli. Therefore, neuroinflammation is a critical factor in the transition from acute to chronic pain.^{2,4} Cannabinoid receptor type 2 (CB2R) expressed on microglia has emerged as a promising target for therapies addressing pain related to neuroinflammatory conditions, such as chronic pain, owing to its role in mediating analgesia with minimal psychoactive effects. Modulation of CB2R has been shown to affect spinal microglial activity and offers potential for developing novel treatment strategies for neuroinflammation.⁸ Additionally, microglia, as key regulators of both the central nervous and immune systems, play a crucial role in orchestrating neuroinflammatory responses. Investigating these responses under well-controlled in vitro conditions is essential for elucidating their underlying mechanisms and exploring potential therapeutic strategies.

The endocannabinoid system (ECS) modulates neuroinflammation and may offer potential therapeutic options for the treatment of chronic pain.⁹ Two types of cannabinoid receptors have been characterized, CB1 and CB2. CB1 is expressed in neural cells and mediates the psychoactive activity of cannabinoids, whereas CB2 is abundant in the immune system

and is involved in immunomodulation.¹⁰ Cannabinoid derivatives interact with the ECS and have demonstrated immunosuppressive,¹¹ anti-inflammatory,¹² pro-apoptotic,¹³ neuroprotective¹⁴ and antitumor properties.^{13,15} These properties make them promising candidates for the treatment.¹⁶ Approximately 120 different cannabinoids have been identified in preparations from the Cannabis plant, including Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the main psychoactive constituent of cannabis, whereas CBD has no known psychoactive effects.¹⁷

Both THC and CBD are widely utilized for therapeutic applications,¹⁸ as they reduce cytokine production in human immune cells while inhibiting both T cell proliferation and effector functions.¹⁹ When exposed to proinflammatory stimuli, microglia treated with THC or CBD exhibit decreased secretion of cytokines, chemokines, and neurotrophic factors.²⁰ Nevertheless, the precise molecular pathways mediating these cannabinoid effects remain incompletely understood.²¹ The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR- γ) is a key intracellular target that mediates cannabinoid-associated immunosuppression unrelated to the cannabinoid CB1 and CB2 receptors.²² Other targets have also been suggested, including G protein-coupled receptors (GPR)55 and GPR18 as well as transient potential (TRP) channels.²³

The use of in vitro microglial models, including BV2 cells, facilitates systematic study of neuroinflammation in controlled and reproducible environments. These models enable the manipulation of experimental conditions, permitting the meticulous examination of cellular responses to diverse stimuli. However, accurate quantification of subtle shifts in cytokine secretion and other inflammatory markers within microglial models has historically presented challenges. Conventional techniques are often intricate and time intensive, potentially interrupting the temporal intricacies of the inflammatory process.^{24,25} In the context of understanding neuroinflammation and its modulation, microglia exhibit a functional dichotomy, with their phenotype having the potential to either support neuronal maintenance or contribute to neuronal harm through low-grade inflammation and reduced neurotrophic support,²⁶ the application of cannabinoids adds a compelling dimension.^{8,27,28} Active compounds from cannabis have demonstrated anti-inflammatory potential, stimulating interest in their effects on microglial responses.^{29–31}

This study aimed to evaluate the changes in the production of neuroinflammatory mediators in an in vitro model of microglial cells exposed to different concentrations of natural cannabinoids. A Surface Plasmon Resonance imaging (SPRi) nanobiosensor was used for the real-time evaluation of neuroinflammation biomarkers, while immunocytochemistry (ICC) was employed to assess the expression of proteins related to neuroinflammation. This article highlights the role of these techniques in advancing basic research and in developing potential therapeutic approaches for neuroinflammation-associated diseases.

2. Methods

2.1. Microglia cell culture

The BV-2 murine microglial cell line (Accegen-ABC-TC212S) were cultured according to a modified protocol³² at 37 °C in a humidified atmosphere of 95% air and 5% CO₂ in high-glucose Dulbecco's modified Eagle's medium (DMEM, Lonza-12-604F) supplemented with 10% fetal bovine serum (FBS, PANBIOTECH-P30-1402). BV-2 microglial cells were used at passage numbers ranging from P3 to P5 to ensure phenotypic stability and responsiveness to the inflammatory stimuli. Cells were regularly monitored under a microscope to confirm morphological consistency and absence of spontaneous activation, which could indicate phenotypic drift at higher passages.

2.2. Culture media preparation and neuroinflammatory changes

Plating medium was prepared according to a previously established protocol.³³ In summary, BV-2 cells were initially seeded in T75 flasks and maintained in DMEM supplemented with 10% fetal bovine serum (FBS). To prepare BV-2 conditioned medium, microglial cells were cultured in complete growth medium until reaching 70–80% confluence. Subsequently, the cells were transferred to a 12-well plate and incubated overnight before treatment with the indicated compounds. The treatments included Dexamethasone Sodium Phosphate (Dex-SP, Vitalis-0012145) at 4 μ M and cannabis-derived products supplied by Clever Leaves in accordance with state regulations. These derivatives were (CBD-23050412) at 5 and 10 μ M, full-spectrum extract (FSE-THC-PE-21-78) (THC 70%, CBD <6%) at 5 and 10 μ M, or a combination of FSE-THC and CBD at 5 and 10 μ M. The cannabinoids were solubilized in ethanol to ensure proper dissolution before being introduced into the culture medium. All treatment and control conditions (with or without LPS) contained ethanol at a final concentration of 100 mM (equivalent to 460 mg/dL or 0.46%). To control potential vehicle effects, all experimental groups, including the untreated controls, received the same ethanol concentration.³⁴

Cells were exposed to these treatments for 2 h in a volume of 0.5 mL, followed by the addition of lipopolysaccharide (LPS, Sigma Aldrich-L2630-10MG) at 100 ng/mL for an additional 4 h. Exposure to LPS for 4 hours in BV-2 cells is a widely utilized protocol as it facilitates the induction of a robust and reproducible inflammatory response, minimizes

cytotoxic effects, and enables the investigation of early mechanisms of neuroinflammation.^{35,36} This methodology aligns with established scientific literature and provides an optimal balance between the induction of inflammation and preservation of cellular viability.^{37,38} After 6 h, aliquots of the medium were collected and stored at 4 °C until further analysis. The cells were fixed with paraformaldehyde for subsequent cytochemical analysis.

2.3. MTT assay

BV-2 cell viability was evaluated using an MTT reduction assay (Life Technologies-1721505).³⁹ The cells (1×10^4 cells/well) were seeded in 96-well plates and incubated for 24 h. The cells were then treated for 6 h with Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC (70%) (5 and 10 μ M), or a combination of FSE-THC and CBD (5 and 10 μ M). After treatment, 100 μ L MTT solution (5 mg/mL) was added to each well and incubated for 4 h. Subsequently, 50 μ L of dimethyl sulfoxide (DMSO) was added, and absorbance was measured for each well was recorded at 570 nm wavelength using a Bio-Rad Model 630 microplate reader (Bio-Rad Laboratories, Richmond, CA, USA).

2.4. Nitric oxide quantification

In 96-well plates, cells were treated with stimuli, and the production of nitric oxide (NO) was evaluated by measuring NO levels in the medium using the Griess reaction (R&D Systems-KGE0001) following a previously described method.⁴⁰ The process involved combining 50 μ L of sample aliquots with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, and 2% phosphoric acid) in a 96-well plate. A microplate reader was used to measure absorbance at 550 nm. To determine NO₂ concentrations, NaNO₂ was used as the standard.

2.5. Immunohistochemistry

The cells were fixed with 4% (v/v) formaldehyde in PBS for 20 minutes at room temperature, washed twice with PBS, and stored at 4°C. Endogenous peroxidase activity was inhibited and a blocking solution prepared with PBS supplemented with 5% BSA was used to minimize non-specific binding. Samples were then incubated overnight at 4°C with primary antibodies (Invitrogen), including Bcl-2 (MAB8272, 1:200), Bax (AF820, 1:200), TNF- α (52B83, 1:200), IFN- γ (JM10-10, 1:200), RelA/NF- κ B p65 (MAB5078, 1:200), and iNOS (NB300-605, 1:200), and then washed with PBS. VisUCyte™ HRP Polymer was applied for 30 min at room temperature, and after additional PBS washes, DAB substrate was added, followed by incubation for 5 min. The reaction was terminated by rinsing it with distilled water. Staining intensity was evaluated under a light microscope, with a minimum of four representative images captured per sample and analyzed using ImageJ 1.54d software. The selected images represented homogeneous areas free of artifacts within each sample, ensuring comparability across experimental groups. Four representative fields were chosen per sample, covering different regions of interest while avoiding overlap to reflect intramural variability. The images were processed in ImageJ 1.54d, converted to grayscale, and analyzed using the auto-threshold tool to identify and quantify the positive areas corresponding to DAB staining. The quantified parameters included the total positive area (in pixels) and the mean pixel intensity within these areas, which were used to calculate the values presented in Table 1.

The selection of the time point for analysis was based on prior literature demonstrating that the key endpoints of interest undergo significant changes within this timeframe under similar experimental conditions.^{41–43} Furthermore, preliminary experiments conducted in our laboratory confirmed that these endpoints were both detectable and biologically relevant, supporting our decision to perform analyses at this specific time point. In models of injury and neuroinflammation, microglia detect damage-associated or pathogen-associated signals and become activated within 30 min to a few hours, further justifying the chosen timeframe for analysis. While some studies have reported the induction of these endpoints at later time points,⁴⁴ previous findings indicate that relevant molecular and cellular changes occur within the selected timeframe, ensuring the validity of our approach. Although full time-course optimization was not performed in this study, our experimental design was guided by well-established literature and prior observations in our laboratory.

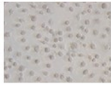
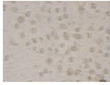
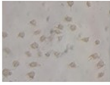
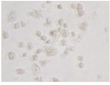
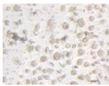
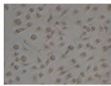
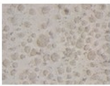
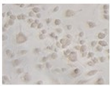
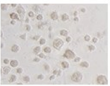
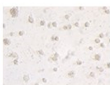
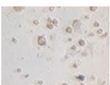
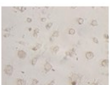
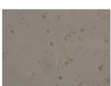
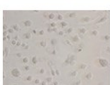
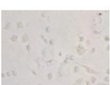
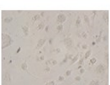
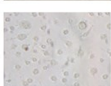
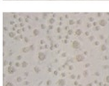
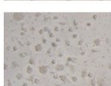
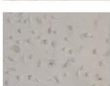
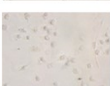
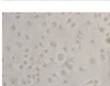
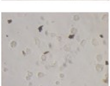
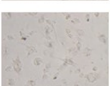
2.6. Biosensor system

Cytokine levels were measured using a nanobiosensor-based detection system that allows real-time, label-free quantification with high specificity and minimal sample volume requirements. This technology was selected over conventional methods, such as ELISA, because of its ability to capture dynamic cytokine secretion patterns and its faster processing time, reducing the need for extensive sample handling.⁴⁵ The nanobiosensor was calibrated using standard cytokine solutions within the expected physiological range for our experimental conditions to ensure the accurate detection of relevant concentrations. Measurements were performed according to the manufacturer's specifications, with controls included to verify assay performance.

Real-time biomarker quantification was achieved using an OpenPlex system equipped with SPRI technology and a microfluidic setup, which was maintained at 25 °C for all the experiments. This setup facilitated direct interactions between recombinant proteins and monoclonal antibodies against TNF- α (Invitrogen-AMC3012), and IL-6, which were

Table 1. ICC Analysis of Apoptotic and Inflammatory Marker Expression in BV-2 Microglial Cells with Illustrative Images.

	Dex-SP (%)	CBD		FSE-THC 70%		FSE-THC:CBD	
		(%)		(%)		(%)	
	4μM	5μM	10μM	5μM	10μM	5μM	10μM
Bcl-2	***-22,66	***-18,57	***-19,28	***-14,42	-24,64	-7,24	-16,54
Bax	-16,71	-12,43	-12,80	-8,00	***-22,66	-8,00	**0,84
TNF-α	***-21,39	***-18,69	-3,98	**11,36	***-23,07	-9,21	-8,80
IFN-γ	-4,95	***-2,60	**5,40	3,36	***-21,43	-0,21	0,86
NF-κB	-14,43	1,78	2,43	***8,37	2,91	14,07	***7,56
iNOS	1,97	-1,07	***21,43	***9,90	-1,62	6,06	***9,02

	Control	Control+LPS	Dex-SP+LPS 4μM	CBD+LPS		FSE-THC+LPS		FSE-THC:CBD+LPS	
				5μM	10μM	5μM	10μM	5μM	10μM
Bcl-2									
Bax									
TNF-α									
IFN-γ									
NF-κB									
iNOS									

ICC analysis of the differences in inflammatory mediator expression between LPS exposure and treatment. Data are expressed as the percentage change in apoptotic (Bcl-2, Bax) and inflammatory (TNF-α, IFN-γ, NF-κB, iNOS) marker expression following treatment with Dex-SP (4 μM), CBD (5 and 10 μM), FSE-THC 70% (5 and 10 μM), and FSE-THC:CBD (5 and 10 μM) in BV-2 microglial cells. Negative values indicate a reduction, whereas positive values indicate an increase relative to the LPS-treated control. Data are presented as mean ± SD. Representative immunocytochemistry (ICC) images illustrating these differences are provided in the supplementary figures.

*** $p < 0.01$.

** $p < 0.05$.

sourced from Abcam and Thermo Fisher Scientific (Waltham, MA, USA), respectively, along with other essential reagents from XanTec Bioanalytics (Düsseldorf, Germany), GE Healthcare (Marlborough, MA 01752 USA), and Sigma-Aldrich (Taufkirchen Germany). The detection method was based on changes in the refractive index as the analytes were bound to their respective ligands,⁴⁶ quantified in real time, and represented graphically in sensorgrams. Data were analyzed using OriginPro 8.5 (Northampton, MA 01060 USA) (A free trial version of Origin/OriginPro is available at <https://www.originlab.com/demodownload.aspx>), Excel (LibreOffice Calc is an open-access software that provides functionalities similar to Excel. It is available at <https://www.libreoffice.org/download/download/>), and Python, with carefully controlled parameters such as flow rate and protein concentrations, and standard curves were established using serial antibody dilutions ranging from 1000–20,000 ng/mL. To ensure specificity and repeatability, the culture samples were thawed, diluted, and injected in triplicate, with real-time monitoring of dissociation and a post-analysis regeneration step using a regeneration solution (glycine, pH 2.0) to maintain the integrity of the sensor chips.

In the SPR assays in this study, immobilization of TNF-α and IL-6 monoclonal antibodies was critical, requiring careful consideration of ligand properties, such as isoelectric point, molecular weight, and amino acid sequence, to optimize conditions for attachment to the carboxymethyl dextran (CMD) 200 sensor surface.^{47,48} Amine-based immobilization, utilizing an N-hydroxysuccinimide (NHS)/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) mixture to activate a

carboxymethyl-dextran layer on the sensor chip, enables a strong covalent bond between the sensor surface and the protein, ensuring high sensitivity and specificity. After activation, the recombinant proteins were injected until the desired immobilization level was reached, with excess active sites blocked using ethanolamine. Regeneration of the surface, which is crucial for reusing the sensor chip, involves injecting a 10 mM glycine hydrochloride solution with 0.00005% sodium dodecyl sulfate (SDS) at a flow rate of 60 μ L/min to ensure over 80% recovery, maintain surface integrity, and allow for consistent, repeatable measurements.^{49–51}

Cytokine levels were assessed by measuring the levels of IL-6 and TNF- α , two well-established pro-inflammatory mediators in microglial activation and neuroinflammation. While IL-1 β is also a key inflammatory cytokine, the present study focused on IL-6 and TNF- α because of their rapid and robust induction in response to inflammatory stimuli, making them reliable indicators of early microglial activation in this model.^{52,53}

2.7. Statistical analysis

The findings are displayed as average values accompanied by standard deviations (SD), derived from three separate trials. The Shapiro-Wilk test was used to evaluate normality. The majority of groups satisfied the criteria for normal distribution ($p > 0.05$), one of the experimental groups did not ($p < 0.05$). Given this, along with the heterogeneity of variances across groups and the presence of nonlinear relationships, non-parametric statistical tests were employed. Comparisons were performed between all experimental groups (Dex-SP, CBD, FSE-THC 70%, and FSE-THC:CBD) and the control (with and without LPS stimulation), as well as between treatment groups. The Kruskal–Wallis test was used to assess the overall group differences. Subsequently, Dunn’s post hoc test with Bonferroni correction was implemented for pairwise comparisons, adjusting p-values to mitigate the Type I error risk. Statistical significance was set at $p < 0.05$. All analyses were conducted using Python (version 3.12) with the SciPy (version 1.11.2) and Scikit-Posthoc (version 0.7.0) libraries.

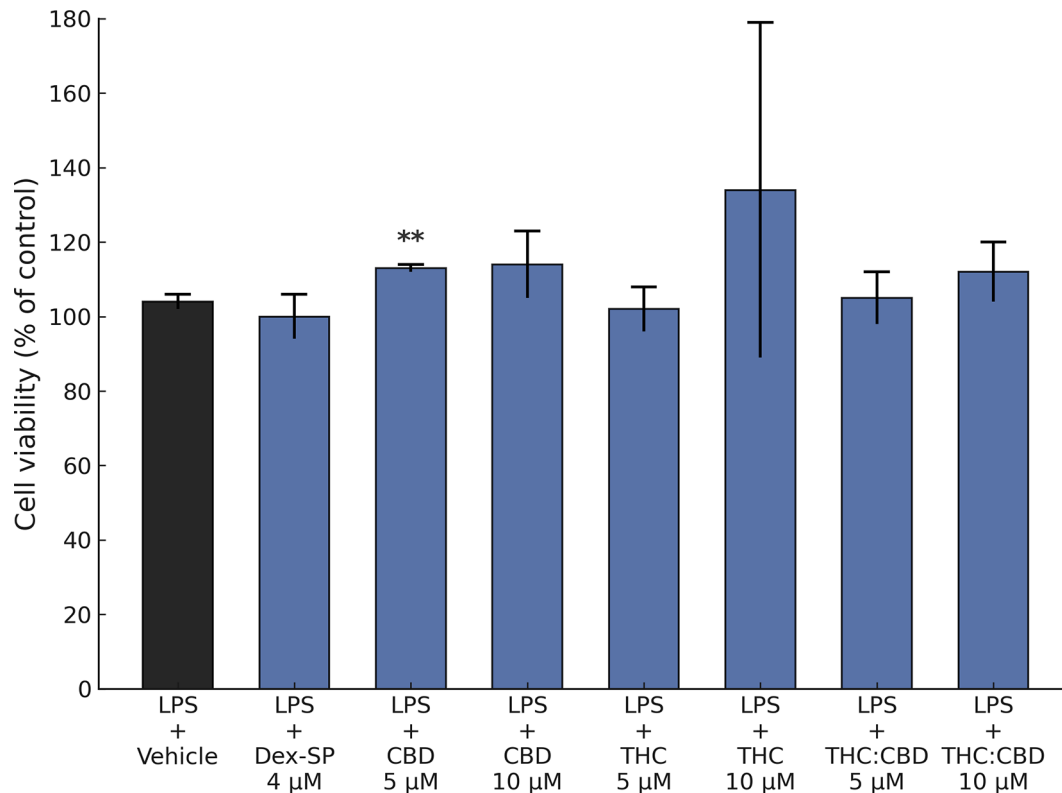


Figure 1. Effects of Dex-SP, CBD, and FSE-THC on BV-2 Microglial Cell Viability via MTT Assay. The MTT assay was used to evaluate BV-2 microglial cell viability after 2 h of LPS incubation and 4 h of exposure to various treatments. These treatments included Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M), and a combination of THC and CBD (50% FSE-THC, standardized to 70% THC, and CBD, 5 and 10 μ M). The results indicated that none of the administered treatments had a significant effect on cell viability. The findings were based on three separate experiments, with each condition having six technical replicates. The results were based on three independent experiments, and each condition was tested in six technical replicates. Data are presented as mean $\pm$ SD, !!! $p < 0.05$ versus vehicle, ** $p < 0.05$ versus LPS + Vehicle, *** $p < 0.01$ versus LPS + Vehicle.

3. Results

3.1. Effects of DEX-SP, CBD, FSE-THC 70%, and FSE-THC:CBD on BV2 cell viability

In the study of cytotoxic effects on BV2 microglial cells, cell viability was evaluated using the MTT assay. After a 2 h incubation with LPS, pretreatment with Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M), and FSE-THC:CBD (5 and 10 μ M), followed by stimulation with LPS 100 ng/ml for 4 h, did not reduce the viability of the BV-2 microglia (Figure 1).

3.2. The Dex-SP, CBD, FSE-THC 70% and FSE-THC:CBD Do Not Reduced Nitric Oxide (NO) Production in LPS-Stimulated BV-2 Microglia

Studies assessing the impact of Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M), and FSE-THC:CBD (5 and 10 μ M) on nitric oxide (NO) release showed that when BV-2 microglia were exposed to LPS (100 ng/mL) for 4 h, there was no increase in NO production compared to unstimulated cells ($p > 0.05$). (Figure 2).

3.3. CBD and FSE-THC:CBD reduced TNF- α production, whereas Dex-SP and CBD decreased IL-6 levels

The LPS-induced (100 ng/mL) increase in TNF- α release by BV-2 microglia was significantly attenuated by CBD (5 and 10 μ M) and FSE-THC:CBD (5 and 10 μ M) ($p < 0.001$). In contrast, Dex-SP (4 μ M) and FSE-THC 70% (5 and 10 μ M) did not elicit a significant reduction (Figure 3A). Similarly, the LPS-induced increase in IL-6 production was significantly diminished by Dex-SP (4 μ M) ($p < 0.05$) and CBD 10 μ M ($p < 0.01$), whereas other cannabinoids did not induce a significant effect (Figure 3B).

3.4. Dex-SP, CBD, FSE-THC (70%), and FSE-THC:CBD differentially attenuated Bcl-2, Bax, TNF- α , IFN- γ , NF- κ B, and iNOS activation in LPS-stimulated BV-2 Microglia in a Concentration-Dependent Manner

The results of the immunocytochemistry (ICC) experiments were used to evaluate the effects of Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M) and FSE-THC:CBD (5 and 10 μ M). There was a significant reduction in

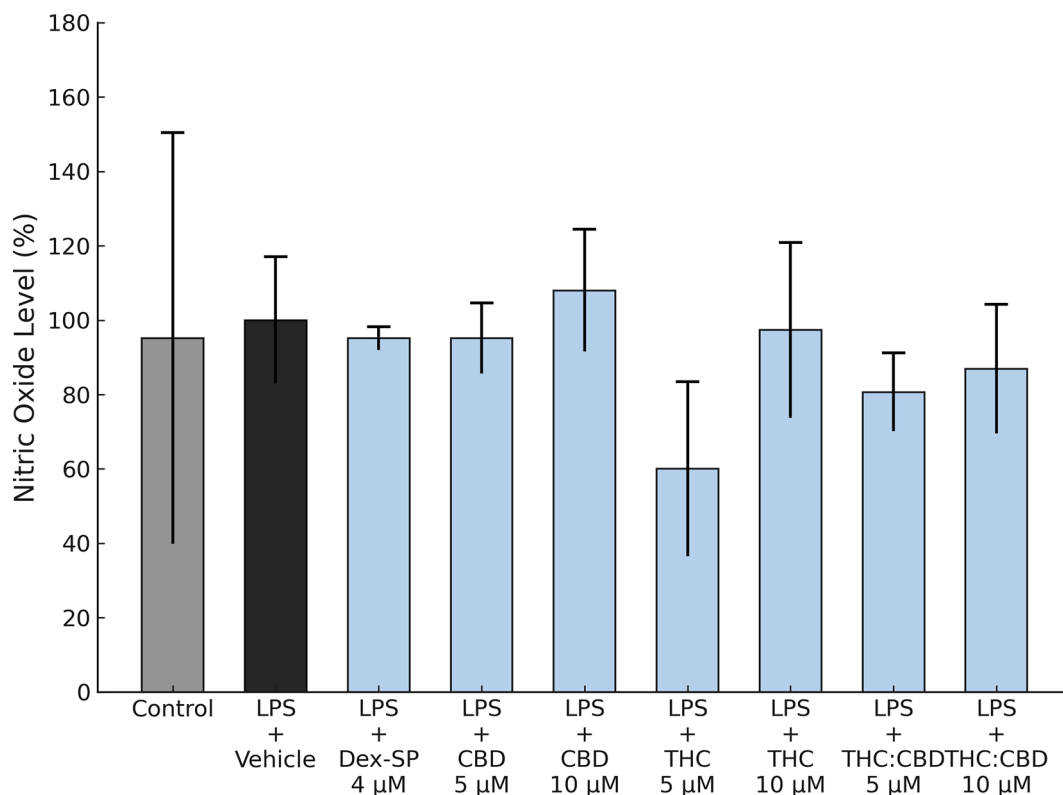


Figure 2. Effects of Dex-SP, CBD, and FSE-THC on NO Production in LPS-Stimulated BV-2 Microglial Cells. Interactions of Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (THC) (5 and 10 μ M), and FSE-THC:CBD (a compound containing 50% FSE-THC, standardized to 70%, and CBD) at 5 and 10 μ M concentrations in BV-2 microglial cells. Nitric oxide (NO) production was assessed after a 4 h treatment with LPS (100 ng/mL). None of the tested concentrations of Dex-SP or cannabinoids induced significant NO production. The results were based on three independent experiments, and each condition was tested in six technical replicates. Data are presented as mean $\pm$ SD, !!! $p < 0.05$ versus vehicle, ** $p < 0.05$ versus LPS + Vehicle, *** $p < 0.01$ versus LPS + Vehicle.

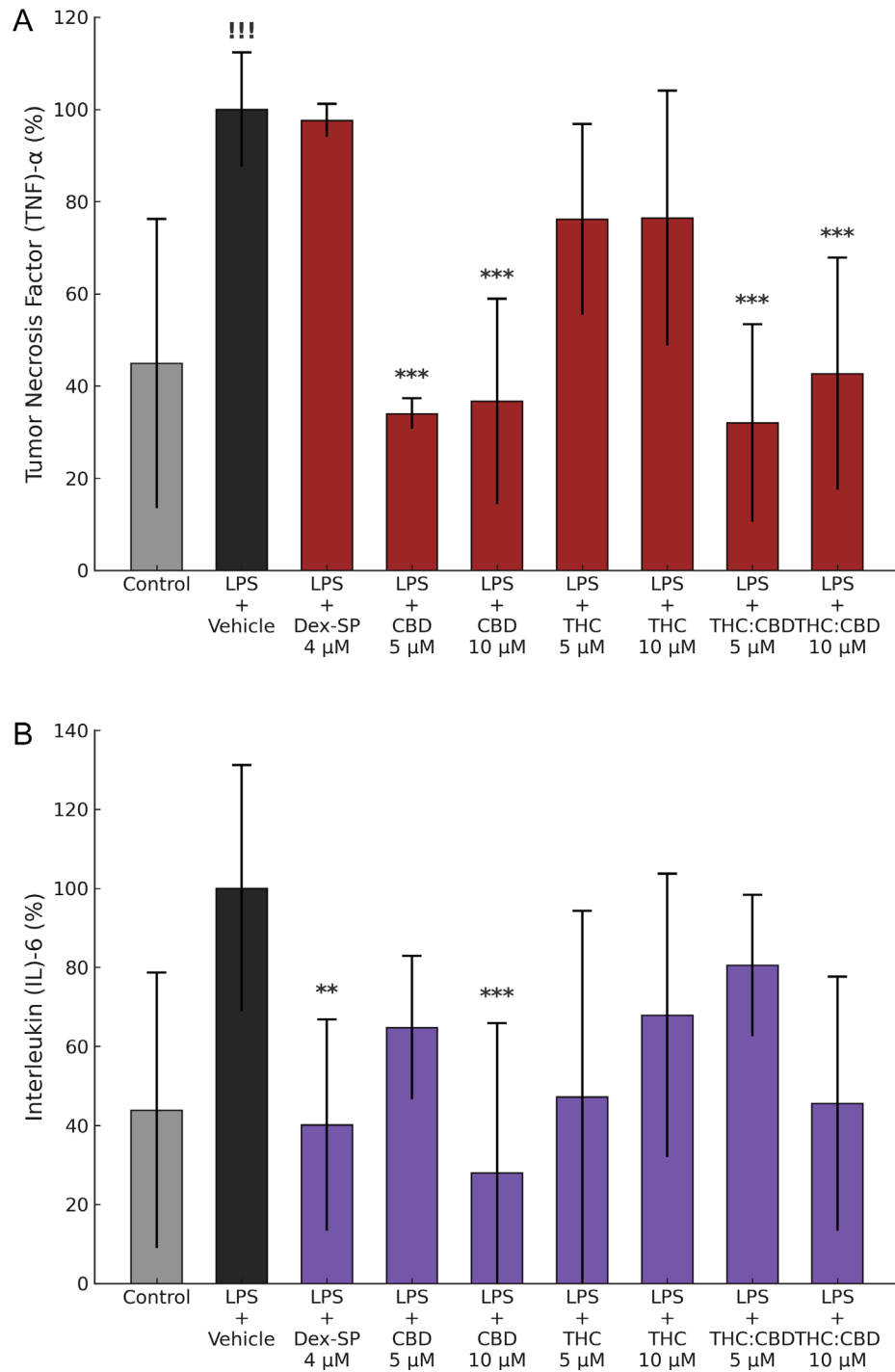


Figure 3. Dex-SP and Cannabinoids Modulate LPS-Induced TNF- α and IL-6 Production in BV-2 Microglial Cells. Effects of Dex-SP and cannabinoids on LPS-induced TNF- α and IL-6 production in BV-2 microglial cells. TNF- α and IL-6 levels were measured 4 h after LPS stimulation (100 ng/mL) and treatment with Dex-SP (4 μ M), CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M), and FSE-THC:CBD (5 and 10 μ M). (A) CBD (5 and 10 μ M) and FSE-THC:CBD (5 and 10 μ M) significantly reduced TNF- α production ($p < 0.01$). In contrast, Dex-SP (4 μ M) and FSE-THC 70% (5 and 10 μ M) did not inhibit TNF- α production. (B) IL-6 production was significantly reduced by Dex-SP (4 μ M) ($p < 0.05$) and CBD (10 μ M) ($p < 0.01$). The other cannabinoid treatments did not result in statistically significant reductions ($p > 0.05$). The results were based on three independent experiments, and each condition was tested in six technical replicates. Data are presented as mean $\pm$ SD, !!! $p < 0.05$ versus vehicle, ** $p < 0.05$ versus LPS + Vehicle, *** $p < 0.01$ versus LPS + Vehicle.

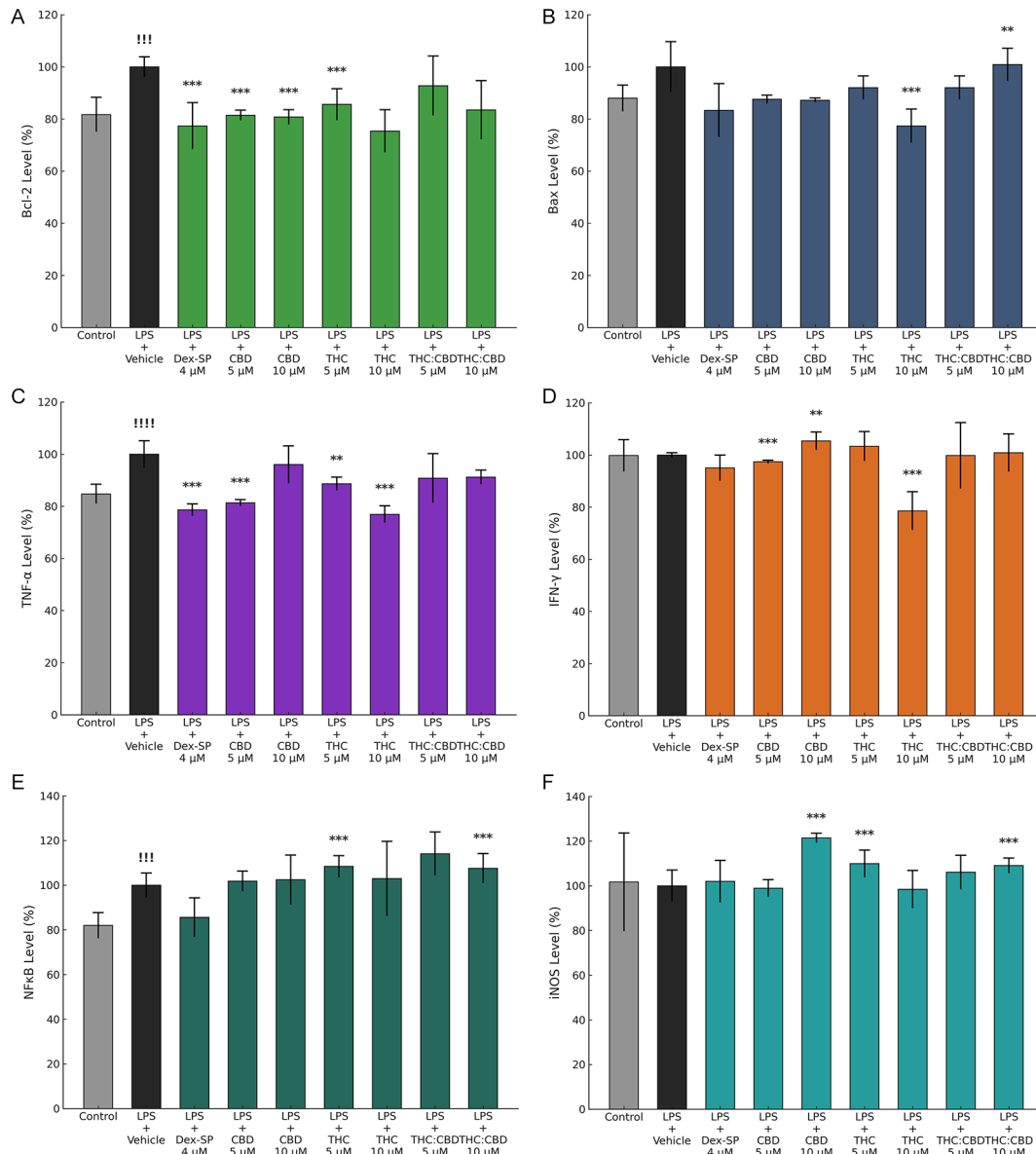


Figure 4. Immunocytochemistry Analysis of Inflammatory and Apoptotic Markers in BV-2 Microglial Cells. Immunocytochemistry (ICC) analysis revealed significant Bcl-2 reduction with Dex-SP (4 μM), CBD (5 and 10 μM), and FSE-THC 70% (10 μM) ($p < 0.01$) (Panel A). Bax levels decreased with FSE-THC 70% (10 μM) ($p < 0.01$) and FSE-THC: CBD (10 μM) ($p < 0.05$) (Panel B). TNF-α was significantly reduced, indicating anti-inflammatory effects, with Dex-SP (4 μM), CBD (5 μM), FSE-THC 70% (5 μM), and FSE-THC 70% (10 μM) ($p < 0.01$) (Panel C). IFN-γ levels varied; CBD (10 μM) increased ($p < 0.05$), CBD (5 μM) decreased ($p < 0.01$), and FSE-THC 70% (10 μM) reduced IFN-γ ($p < 0.01$) (Panel D). NF-κB was significantly elevated in FSE-THC 70% (5 μM) and FSE-THC: CBD (10 μM) ($p < 0.01$) (Panel E). iNOS expression was altered in the CBD (10 μM), FSE-THC 70% (5 μM), and FSE-THC: CBD (10 μM) groups ($p < 0.01$) (Panel F). The results were based on three independent experiments, and each condition was tested in six technical replicates. Data are presented as mean $\pm$ SD, !!! $p < 0.05$ versus vehicle, !!!! $p < 0.01$ versus vehicle, ** $p < 0.05$ versus LPS + Vehicle, *** $p < 0.01$ versus LPS + Vehicle.

anti-apoptotic Bcl-2 with Dex-SP (4 μM), CBD (5 and 10 μM), and FSE-THC 70% (10 μM) ($p < 0.01$) (Figure 4, Panel A). FSE-THC 70% (10 μM) ($p < 0.01$), and FSE-THC: CBD (10 μM) ($p < 0.05$) treatments decreased the levels of pro-apoptotic Bax (Figure 4, Panel B). Dex-SP (4 μM) ($p < 0.01$), CBD (5 μM) ($p < 0.01$), FSE-THC 70% (5 μM) ($p < 0.05$) and FSE-THC 70% (10 μM) ($p < 0.01$), treatments were found to significantly decrease TNF-α levels, indicating their anti-inflammatory properties (Figure 4, Panel C). The response of Interferon Gamma (IFN)-γ expression to treatments varied, with CBD (10 μM) ($p < 0.05$) minor increases or CBD (5 μM) ($p < 0.01$) minor decreases, and may decrease with FSE-THC (70%) (10 μM) ($p < 0.01$) (Figure 4, Panel D). There was a significant increase in Nuclear Factor

Kappa B Subunit 1 (NF- κ B) production in the FSE-THC (70%) (5 μ M) and FSE-THC:CBD (10 μ M) ($p < 0,01$) groups (Figure 4, Panel E). iNOS production was significantly altered in the CBD (10 μ M), FSE-THC (70%) (5 μ M), and FSE-THC:CBD (10 μ M) groups ($p < 0,01$) groups (Figure 4, Panel F).

The most efficacious treatments for modulating inflammation are CBD (10 μ M) because of their capacity to attenuate IFN- γ , and iNOS, Dex-SP (4 μ M) because of their capacity to attenuate TNF- α , and FSE-THC 70% (10 μ M), because of their capacity to attenuate Bax, TNF- α , and IFN- γ , which are critical mediators of the inflammatory response. All three treatments demonstrated a reduction in Bcl-2, which may represent a potential mechanism through which they attenuate prolonged microglial activation and facilitate the resolution of inflammation. However, if this reduction is accompanied by a concomitant decrease in Bax (as observed with FSE-THC 70% at 5 μ M), it may indicate a shift in the balance between apoptosis and cellular viability (Table 1).

4. Discussion

In our study, none of the tested treatments significantly affected cell viability. Treatment with Dex-SP (4 μ M) significantly reduced LPS-induced release of TNF- α (ICC), IL-6, and Bcl-2. In contrast, CBD significantly decreased LPS-induced release of TNF- α (5 and 10 μ M), IL-6 (10 μ M), Bcl-2 (5 and 10 μ M), IFN- γ (5 and 10 μ M), and iNOS (10 μ M). Additionally, FSE-THC 70% significantly reduced Bcl-2 (5 μ M), Bax (10 μ M), TNF- α (ICC) (5 and 10 μ M), IFN- γ (10 μ M), NF- κ B (5 μ M), and iNOS (5 μ M). Finally, FSE-THC:CBD significantly decreased TNF- α (5 and 10 μ M), Bax (10 μ M), NF- κ B (10 μ M), and iNOS (10 μ M).

We stimulated BV-2 microglial cells using LPS, leading to a notable release of Bcl-2, TNF- α and NF- κ B. These compounds are widely acknowledged as critical mediators in inflammatory processes.⁵⁴ CBD (5 and 10 μ M) and FSE-THC 70% (5 and 10 μ M) exhibited the greatest ability to modulate inflammatory and apoptotic mediators, significantly reducing TNF- α , IL-6, IFN- γ , NF- κ B, iNOS, and Bax, whereas FSE-THC:CBD (5 and 10 μ M) showed an intermediate response. In comparison, Dex-SP (4 μ M) effectively reduced TNF- α and IL-6 but had no significant impact on IFN- γ , NF- κ B, or iNOS, suggesting that phytocannabinoids, particularly CBD and FSE-THC 70%, may represent promising strategies for neuroinflammation modulation.

Previous studies have demonstrated that the endogenous cannabinoid system, which comprises compounds such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), plays a crucial role in protecting neurons from damage. This protection was achieved by reducing cellular apoptosis and enhancing cell viability.⁵⁵ Another study highlighted the antioxidant and neuroprotective properties of CBD and Cannabigerol (CBG) in rat astrocytes and cortices, demonstrating their effectiveness in reducing oxidative stress and preventing apoptosis. CBD outperformed CBG, with both impacting the neurokinin 3 receptor, indicating a potential multi-target mechanism.⁵⁶ To the best of our knowledge, this is the first study to compare the effects of Dex-SP, CBD, and FSE-THC 70%, both individually and in combination, on the viability of BV-2 microglial cells.

The inhibitory effects of CBD 5 and 10 μ M on the release of TNF- α were statistically superior to Dex-SP 4 μ M. On the other hand, IL-6 release was more effectively inhibited by both Dex-SP 4 μ M and CBD 10 μ M. THC and CBD have been reported to exert distinct pharmacological actions on known cannabinoid receptors, displaying differential effects on exposed cells. THC functions as a partial agonist for both CB1 and CB2 receptors, while CBD shows a markedly low affinity for these receptors.⁵⁷ CB2 receptors, found on a range of immune cells, including primary microglia and the BV-2 microglial cell line, are considered key mediators in cannabinoid-driven immunomodulation.⁵⁸ The immunosuppressive effects of THC have been linked to CB2 receptors, as demonstrated through knockout system studies.⁵⁹ Consequently, studies have demonstrated that CB2 agonists exhibit immunosuppressive effects in rat primary microglial cultures.⁶⁰ It has been demonstrated that rat primary microglial cells express CB1 receptors, and the activation of these receptors induces the production of NO.⁶¹ To assess whether various compositions of natural cannabinoids mediate immunosuppression in our system, we applied them prior to exposure to LPS and compared this response with that obtained using Dex-SP.

We observed that CBD (5 μ M) and FSE-TSH 70% (5 and 10 μ M) reduce TNF- α expression at the cytoplasmic level to a similar extent as Dex-SP. The precise mechanisms underlying these findings remain unclear. One potential explanation is that THC acts as a weak agonist for the CB2 receptor⁶² because that the CB1 is almost absent in BV-2 cells.⁶³ Additionally, several studies indicate that certain terpenoids may interact with components of the cannabinoid signaling system.⁶⁴

Immunocytochemistry (ICC) analysis revealed that CBD (5 and 10 μ M), FSE-THC 70% (5 and 10 μ M), and FSE-THC:CBD (5 and 10 μ M) exerted an anti-inflammatory effect comparable to Dex-SP (4 μ M). Similarly, CBD (5 and 10 μ M)

and FSE-THC 70% (5 μ M) demonstrated a similar ability to Dex-SP (4 μ M) in regulating cell survival by suppressing LPS-induced Bcl-2 expression. This reduction in Bcl-2 may reflect a shift in cell fate, promoting a less inflammatory environment and facilitating the clearance of hyperactivated or dysfunctional microglial cells, thereby contributing to inflammation resolution. Likewise, FSE-THC 70% (10 μ M) and FSE-THC:CBD (10 μ M) exhibited a statistically significant effect in reducing LPS-induced Bax expression.

In our model, ICC analysis of IFN- γ and iNOS showed no detectable changes, nor were any alterations observed in NO production. This may be due to the 4-hour LPS exposure, which might have been insufficient to induce a measurable response. Studies suggest that a 6-hour time point is more appropriate for capturing early microglial activation in neuroinflammatory models.⁶⁵ Similarly, early production of pro-inflammatory cytokines, such as IL-6 and TNF- α , has been reported shortly after intracerebral hemorrhage, highlighting the rapid onset of neuroinflammatory responses in the central nervous system.⁶⁶

Proinflammatory cytokines are key mediators of the immune response, often peaking within the first six hours of an inflammatory stimulus. This rapid release is a hallmark of acute inflammation, which occurs in response to infections, injuries, or other inflammatory triggers. For instance, IL-1 β and TNF- α are among the earliest cytokines released upon exposure to stimuli such as LPS or ionizing radiation, reflecting the swift activation of the inflammatory cascade.⁶⁷ Their early secretion is crucial for initiating immune responses by recruiting immune cells to the site of injury and amplifying cytokine production.⁶⁸ Accurately modeling acute neuroinflammatory responses and assessing pharmacological interventions before secondary changes occur are essential for developing effective therapeutic strategies. LPS-induced models combined with pharmacological agents targeting specific neuroinflammatory pathways provide a valuable framework for studying the acute phase of neuroinflammation.⁶⁹

Dex-SP, a widely used anti-inflammatory drug, has also been employed clinically to manage various neuroinflammatory processes.⁷⁰ Dex-SP inhibits inflammatory responses by regulating the activity of transcription factors such as nuclear factor kappa B (NF- κ B),⁷¹ which was observed in our study. In neuroinflammatory processes, NF- κ B plays a crucial role in neurodegeneration mediated by neuroinflammation.⁷² The upregulation of NF- κ B is associated with some neuroinflammatory disease, and targeting its inhibition could potentially serve as an effective therapeutic strategy for treating Alzheimer's disease,⁷³ Parkinson's disease,⁷⁴ and chronic pain.⁷⁵ Evidence suggests that proinflammatory cytokines, including TNF- α and IL-6 significantly contribute to the NF- κ B signaling pathway.⁷⁶ NF- κ B is known to activate these cytokines in cells, thereby triggering an inflammatory response.⁷⁶ Prior research has shown that in LPS-stimulated BV2 cells, the levels of proinflammatory cytokines TNF- α and IL-6 are elevated.⁷⁷ Elevated levels of pro-inflammatory cytokines in BV2 cells are believed to play a direct role in the development of neuroinflammation.⁷⁸ In the present study, Dex-SP (4 μ M) significantly decreased the expression of Bcl-2 and TNF- α in LPS-stimulated BV2 cells.

IL-1 β is an important pro-inflammatory cytokine in microglial activation; however, in this study, we prioritized the quantification of IL-6 and TNF- α , which are rapidly induced and play central roles in the inflammatory cascade. TNF- α is a key upstream regulator of the inflammatory response, capable of inducing both IL-1 β and IL-6 expression,^{52,53} while IL-6 serves as a crucial modulator of neuroinflammation and is strongly associated with microglial activation. Given their well-documented roles in neuroinflammatory processes, the measurement of IL-6 and TNF- α provided a comprehensive assessment of the inflammatory response in our model. Future studies could complement these findings by including IL-1 β measurements to further characterize the cytokine profile over time.

On the other hand, cannabinoid derivatives, particularly CBD, have been shown to modulate inflammation, yet there are still gaps in understanding their mechanisms of action or biological responses. It has been reported that the combination of THC and CBD can enhance these properties.⁷⁹ To date, there are no published studies comparing the anti-inflammatory properties of cannabinoids with Dex-SP in a neuroinflammation model using microglial cells. However, there are studies in other cell types showing that extracts are more potent than CBD alone,⁸⁰ which aligns with our findings.

The expression of Bax, IFN- γ , NF- κ B, and iNOS was significantly reduced in microglial cells exposed to cannabinoids compared to that in cells treated with Dex-SP (4 μ M). These differences may be attributed to the distinct modulatory mechanisms of cannabinoids, in contrast to the effects of corticosteroids, such as Dex-SP, on BV-2 microglial cell activity. Previous research has also shown that CBD can exert anti-inflammatory effects on LPS-stimulated microglial cells.⁸¹ The specific molecular mechanisms underlying CBD's actions have yet to be fully elucidated, and more in-depth studies directly comparing the effectiveness and therapeutic potential of CBD, other cannabinoids, and their mixtures are needed. Our study demonstrated a greater anti-inflammatory effect with the presence of FSE-THC, in line with findings reported in other studies.⁷⁹

The limitation of our study was that no RNA level tests were conducted; hence, the effect of the observed changes on genetic expression was not determined. The exposure duration to LPS was brief, so we were unable to detect changes in the response after the 6-hour period. Moreover, a broader range of inflammatory proteins, such as TLR4 and MyD88, and modulators, including IL-1 β , IL-4, IL-10, and Nerve Growth Factor (NGF), are required to further validate the roles of Dex-SP and cannabinoids in the regulation of neuroinflammation in LPS-stimulated BV2 cells.

5. Conclusion

Our findings indicated that Dex-SP has an anti-inflammatory effect on LPS-stimulated BV2 cells. Furthermore, cannabinoids, particularly at certain concentrations and combinations, demonstrate efficacy comparable to or even superior to that of Dex-SP in reducing certain inflammatory mediators. This includes a reduction in the expression of anti-apoptotic protein as Bcl-2, pro-apoptotic proteins such as Bax, as well as proinflammatory cytokines such as TNF- α , IFN- γ , NF- κ B and iNOS. This study will aid in elucidating the therapeutic effects of cannabinoids on processes associated with neuroinflammation. Further research is needed to understand the mechanisms and determine the combinations of cannabinoids that significantly affect inflammation modulation.

Ethics

Ethical approval and consent were not required.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

CrediT authorship contribution statement

Conceptualization J.-M.Q., R.-H. B., L.E.D., and M.X.L.; methodology J.-M.Q., R.-H. B. and L.E.D.; writing—original draft preparation, J.-M.Q., R.-H. B. and L.E.D.; writing—review and editing, J.-M.Q., R.-H. B. and L.E.D.; supervision, J.-M.Q., R.-H. B. and L.E.D.; project administration, R.-H.B.; funding acquisition, R.-H. B., L.E.D., and M.X.L. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Zenodo: Assessing neuroinflammation in vitro in a microglial model: advancing cannabinoid-based therapeutic insights. <https://doi.org/10.5281/zenodo.14901695>.⁸²

This project contains the following underlying data:

- [Data_experiments.xlsx](#)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

We would like to thank the Universidad de La Sabana.

References

1. Jin MY, Everett ES, Abd-Elsayed A: **Microbiological and Physiological Effects of Pain.** *Curr. Pain Headache Rep.* 2023 Jun; 27(6): 165–73. Epub 2023/04/22. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Ji RR, Nackley A, Huh Y, et al.: **Neuroinflammation and Central Sensitization in Chronic and Widespread Pain.** *Anesthesiology.* 2018 Aug; 129(2): 343–66. Epub 2018/02/21. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Ikeda H, Kiritoshi T, Murase K: **Contribution of microglia and astrocytes to the central sensitization, inflammatory and neuropathic pain in the juvenile rat.** *Mol. Pain.* 2012; 8: 43.
4. Tsuda M: **Microglia-Mediated Regulation of Neuropathic Pain: Molecular and Cellular Mechanisms.** *Biol. Pharm. Bull.* 2019; 42(12): 1959–68. Epub 2019/12/04. [PubMed Abstract](#)

5. Kanda H, Kobayashi K, Yamanaka H, *et al.*: **Microglial TNF α Induces COX2 and PG12 Synthase Expression in Spinal Endothelial Cells during Neuropathic Pain.** *eneuro*. 2017; 4(2): ENEURO.0064-17.2017.
[Publisher Full Text](#)
6. Kaur D, Sharma V, Deshmukh R: **Activation of microglia and astrocytes: a roadway to neuroinflammation and Alzheimer's disease.** *Inflammopharmacology*. 2019 Aug; 27(4): 663–77. Epub 2019/03/16.
[Publisher Full Text](#) | [PubMed Abstract](#)
7. Lee K-M, Jeon S-M, Cho H-J: **Interleukin-6 induces microglial CX3CR1 expression in the spinal cord after peripheral nerve injury through the activation of p38 MAPK.** *Eur. J. Pain*. 2010; 14(7): 682.e1–682.12.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Xu K, Wu Y, Tian Z, *et al.*: **Microglial Cannabinoid CB(2) Receptors in Pain Modulation.** *Int. J. Mol. Sci.* 2023 Jan 25; 24(3). Epub 2023/02/12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Cristino L, Bisogno T, Di Marzo V: **Cannabinoids and the expanded endocannabinoid system in neurological disorders.** *Nat. Rev. Neurol.* 2020 Jan; 16(1): 9–29. Epub 2019/12/14.
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Zou S, Kumar U: **Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System.** *Int. J. Mol. Sci.* 2018 Mar 13; 19(3). Epub 2018/03/14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Almogi-Hazan O, Or R: **Cannabis, the Endocannabinoid System and Immunity-the Journey from the Bedside to the Bench and Back.** *Int. J. Mol. Sci.* 2020 Jun 23; 21(12). Epub 2020/06/27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Atalay S, Jarocka-Karpowicz I, Skrzydlewska E: **Antioxidative and Anti-Inflammatory Properties of Cannabidiol.** *Antioxidants (Basel, Switzerland)*. 2019 Dec 25; 9(1). Epub 2019/12/29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Hinz B, Ramer R: **Anti-tumour actions of cannabinoids.** *Br. J. Pharmacol.* 2019 May; 176(10): 1384–94. Epub 2018/07/19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. Laws JS 3rd, Smid SD: **Evaluating Cannabis sativa L.'s neuroprotection potential: From bench to bedside.** *Phytomedicine: international journal of phytotherapy and phytopharmacology*. 2022 Dec; 107: 154485. Epub 2022/10/10.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Pellati F, Borgonetti V, Brighenti V, *et al.*: **Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer.** *Biomed. Res. Int.* 2018; 2018: 1691428. Epub 2019/01/11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Bennici A, Mannucci C, Calapai F, *et al.*: **Safety of Medical Cannabis in Neuropathic Chronic Pain Management.** *Molecules (Basel, Switzerland)*. 2021 Oct 16; 26(20). Epub 2021/10/24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Rock EM, Parker LA: **Constituents of Cannabis Sativa.** *Adv. Exp. Med. Biol.* 2021; 1264: 1–13. Epub 2020/12/18.
[Publisher Full Text](#) | [PubMed Abstract](#)
18. Christensen C, Alles M, Rose M, *et al.*: **Clinical Research Evidence Supporting Administration and Dosing Recommendations of Medicinal Cannabis as Analgesic in Cancer Patients.** *J. Clin. Med.* 2022 Dec 30; 12(1). Epub 2023/01/09.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Jones É, Vlachou S: **A Critical Review of the Role of the Cannabinoid Compounds Δ (9)-Tetrahydrocannabinol (Δ (9)-THC) and Cannabidiol (CBD) and their Combination in Multiple Sclerosis Treatment.** *Molecules (Basel, Switzerland)*. 2020 Oct 25; 25(21). Epub 2020/10/30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Marinelli S, Marrone MC, Di Domenico M, *et al.*: **Endocannabinoid signaling in microglia.** *Glia*. 2023 Jan; 71(1): 71–90. Epub 2022/10/13.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Kasatkina LA, Rittchen S, Sturm EM: **Neuroprotective and Immunomodulatory Action of the Endocannabinoid System under Neuroinflammation.** *Int. J. Mol. Sci.* 2021 May 21; 22(11). Epub 2021/06/03.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Iannotti FA, Vitale RM: **The Endocannabinoid System and PPARs: Focus on Their Signalling Crosstalk, Action and Transcriptional Regulation.** *Cells*. 2021 Mar 7; 10(3). Epub 2021/04/04.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Stasiłowicz A, Tomala A, Podolak I, *et al.*: **Cannabis sativa L. as a Natural Drug Meeting the Criteria of a Multitarget Approach to Treatment.** *Int. J. Mol. Sci.* 2021 Jan 14; 22(2). Epub 2021/01/21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Patil V, Bohara R, Krishna Kanala V, *et al.*: **Models and approaches to comprehend and address glial inflammation following spinal cord injury.** *Drug Discov. Today*. 2023 Jul 22; 28(10): 103722. Epub 2023/07/24.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Yang J, Hamade M, Wu Q, *et al.*: **Current and Future Biomarkers in Multiple Sclerosis.** *Int. J. Mol. Sci.* 2022 May 24; 23(11). Epub 2022/06/11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Spiteri AG, Wishart CL, Pamphlett R, *et al.*: **Microglia and monocytes in inflammatory CNS disease: integrating phenotype and function.** *Acta Neuropathol.* 2022 Feb; 143(2): 179–224. Epub 2021/12/03.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Walter L, Stella N: **Cannabinoids and neuroinflammation.** *Br. J. Pharmacol.* 2004 Mar; 141(5): 775–85. Epub 2004/02/06.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Lowe H, Toyang N, Steele B, *et al.*: **The Endocannabinoid System: A Potential Target for the Treatment of Various Diseases.** *Int. J. Mol. Sci.* 2021 Aug 31; 22(17). Epub 2021/09/11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Henshaw FR, Dewsbury LS, Lim CK, *et al.*: **The Effects of Cannabinoids on Pro- and Anti-Inflammatory Cytokines: A Systematic Review of in vivo Studies.** *Cannabis Cannabinoid Res.* 2021 Jun; 6(3): 177–95. Epub 2021/05/18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Cabral GA, Griffin-Thomas L: **Cannabinoids as therapeutic agents for ablating neuroinflammatory disease.** *Endocr Metab Immune Disord Drug Targets*. 2008 Sep; 8(3): 159–72. Epub 2008/09/11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Wang X, Lin C, Jin S, *et al.*: **Cannabidiol alleviates neuroinflammation and attenuates neuropathic pain via targeting FKBP5.** *Brain Behav. Immun.* 2023 Jul; 111: 365–75. Epub 2023/05/18.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Hagan N, Kane JL, Grover D, *et al.*: **CSF1R signaling is a regulator of pathogenesis in progressive MS.** *Cell Death Dis.* 2020 Oct 23; 11(10): 904. M.F., D.O. were employees of Sanofi at the time that their research was conducted. C.M. and O.B. collaborate with Sanofi and received research support funds from Sanofi. Epub 2020/10/25.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Lee SG, Kang H: **Saussurea Lappa Clarke Extract Exhibits Potent Antioxidant Effect and Attenuates Neuroinflammatory Responses in Lipopolysaccharide-Stimulated Microglial Cells.** *Trop. J. Pharm. Res.* 2020; 19(9): 1911–1917.
34. Asatryan L, Ostrovskaya O, Lieu D, *et al.*: **Ethanol differentially modulates P2X4 and P2X7 receptor activity and function in BV2 microglial cells.** *Neuropharmacology*. 2018 Jan; 128: 11–21. Epub 2017/09/26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
35. Henn A, Lund S, Hedtjärn M, *et al.*: **The suitability of BV2 cells as alternative model system for primary microglia cultures or for animal experiments examining brain inflammation.** *ALTEX - Alternatives to animal experimentation*. 2009 05/01; 26(2): 83–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Ribes S, Adam N, Schütze S, *et al.*: **The nucleotide-binding oligomerization domain-containing-2 ligand muramyl dipeptide enhances phagocytosis and intracellular killing of *Escherichia coli* K1 by Toll-like receptor agonists in microglial cells.** *J. Neuroimmunol.* 2012; 252(1): 16–23.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Kim SM, Ha JS, Han AR, *et al.*: **Effects of α -lipoic acid on LPS-induced neuroinflammation and NLRP3 inflammasome activation through the regulation of BV-2 microglial cells activation.** *BMB Rep.* 2019 Oct; 52(10): 613–8. Epub 2019/04/04.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Nam HY, Nam JH, Yoon G, *et al.*: **Ibrutinib suppresses LPS-induced neuroinflammatory responses in BV2 microglial cells and wild-type mice.** *J. Neuroinflammation*. 2018 2018/09/19; 15(1): 271.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Sargent J, Taylor C: **Appraisal of the MTT assay as a rapid test of chemosensitivity in acute myeloid leukaemia.** *Br. J. Cancer*. 1989; 60(2): 206–210.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Lee S, Park J-Y, Lee W-H, *et al.*: **Lipocalin-2 is an autocrine mediator of reactive astrocytosis.** 2009; 29(1): 234–249.
41. Juknat A, Gao F, Coppola G, *et al.*: **miRNA expression profiles and molecular networks in resting and LPS-activated BV-2 microglia-Effect of cannabinoids.** *PLoS one*. 2019; 14(2): e0212039. Epub 2019/02/12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Li Y, Zhou D, Ren Y, *et al.*: **Mir223 restrains autophagy and promotes CNS inflammation by targeting ATG16L1.** *Autophagy*.

- 2019 Mar; **15**(3): 478–92. Epub 2018/09/14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Standoli S, Rapino C, Di Meo C, *et al.*: **Sphingosine Kinases at the Intersection of Pro-Inflammatory LPS and Anti-Inflammatory Endocannabinoid Signaling in BV2 Mouse Microglia Cells.** *Int. J. Mol. Sci.* 2023 May 9; **24**(10). Epub 2023/05/27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. Cankara FN, Çelik ZB, Günaydin C: **Cannabinoid receptors modulate LPS-induced increase of class-II transactivator expression levels in a microglial cell line.** *J. Recept. Signal Transduct. Res.* 2021 Jun; **41**(3): 209–16. Epub 2021/01/07.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Khan MA, Mujahid M: **Recent Advances in Electrochemical and Optical Biosensors Designed for Detection of Interleukin 6.** *Sensors (Basel).* 2020 Jan 23; **20**(3). Epub 2020/01/26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Brunsics B: **Modelling multivalent protein binding-kinetics.** 2022.
 47. Swanson SJ, Mytych D: **The use of surface plasmon resonance for the detection and characterization of antibodies. Detection and Quantification of Antibodies to Biopharmaceuticals: Practical and Applied Considerations.** 2011; 193–209.
[Publisher Full Text](#)
 48. O'Shannessy DJ, Brigham-Burke M, Peck K: **Immobilization chemistries suitable for use in the BIAcore surface plasmon resonance detector.** *Anal. Biochem.* 1992; **205**(1): 132–136.
[PubMed Abstract](#)
 49. Andersson K, Areskoug D, Hardenborg E: **Exploring buffer space for molecular interactions.** *J. Mol. Recognit.* 1999; **12**(5): 310–315.
[PubMed Abstract](#)
 50. Schasfoort RB: **Handbook of surface plasmon resonance: Royal Society of Chemistry.** 2017.
 51. Bustos RH, Zapata C, Esteban E, *et al.*: **Label-free quantification of anti-TNF- α in patients treated with adalimumab using an optical biosensor.** *Sensors.* 2018; **18**(3): 691.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Cenalmor A, Pascual E, Gil-Manso S, *et al.*: **Evaluation of Anti-Neuroinflammatory Activity of Isatin Derivatives in Activated Microglia.** *Molecules (Basel, Switzerland).* 2023 Jun 20; **28**(12). Epub 2023/06/28.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 53. Atta AA, Ibrahim WW, Mohamed AF, *et al.*: **Microglia polarization in nociceptive pain: mechanisms and perspectives.** *Inflammopharmacology.* 2023 Jun; **31**(3): 1053–67. Epub 2023/04/18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. Suzumura A, Takeuchi H, Zhang G, *et al.*: **Roles of glia-derived cytokines on neuronal degeneration and regeneration.** *Ann. N. Y. Acad. Sci.* 2006; **1088**(1): 219–229.
 55. Guo S, Liu Y, Ma R, *et al.*: **Neuroprotective effect of endogenous cannabinoids on ischemic brain injury induced by the excess microglia-mediated inflammation.** *Am. J. Transl. Res.* 2016; **8**(6): 2631–40. Epub 2016/07/12.
[PubMed Abstract](#) | [Free Full Text](#)
 56. di Giacomo V, Chiavari A, Recinella L, *et al.*: **Antioxidant and Neuroprotective Effects Induced by Cannabidiol and Cannabigerol in Rat CTX-TNA2 Astrocytes and Isolated Cortexes.** *Int. J. Mol. Sci.* 2020 May 18; **21**(10). Epub 2020/05/24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 57. Pertwee R: **The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ^9 -tetrahydrocannabinol, cannabidiol and Δ^9 -tetrahydrocannabivarin.** *Br. J. Pharmacol.* 2008; **153**(2): 199–215.
[PubMed Abstract](#)
 58. Rathod SS, Agrawal YO, Nakhate KT, *et al.*: **Neuroinflammation in the Central Nervous System: Exploring the Evolving Influence of Endocannabinoid System.** *Biomedicine.* 2023; **11**(10): 2642.
 59. Buckley NE, McCoy KL, Mezey É, *et al.*: **Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB2 receptor.** *Eur. J. Pharmacol.* 2000; **396**(2–3): 141–149.
[PubMed Abstract](#) | [Publisher Full Text](#)
 60. Romero-Sandoval EA, Horvath R, Landry RP, *et al.*: **Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation.** *Mol. Pain.* 2009; **5**.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 61. Coelho AA, Vila-Verde C, Sartim AG, *et al.*: **Inducible nitric oxide synthase inhibition in the medial prefrontal cortex attenuates the anxiogenic-like effect of acute restraint stress via CB1 receptors.** 2022; **13**: 923177.
 62. Cohen G, Gover O, Schwartz B: **Phytocannabinoids Reduce Inflammation of Primed Macrophages and Enteric Glial Cells: An in vitro Study.** *Int. J. Mol. Sci.* 2023; **24**(19): 14628.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 63. Pietr M, Kozela E, Levy R, *et al.*: **Differential changes in GPR55 during microglial cell activation.** 2009; **583**(12): 2071–2076.
 64. Dvorakova M, Wilson S, Corey W, *et al.*: **A critical evaluation of terpenoid signaling at cannabinoid CB1 receptors in a neuronal model.** 2022; **27**(17): 5655.
 65. Qiu T, Guo J, Wang L, *et al.*: **Dynamic microglial activation is associated with LPS-induced depressive-like behavior in mice: An [18F] DPA-714 PET imaging study.** *Bosn. J. Basic Med. Sci.* 2022 Jul 29; **22**(4): 649–59. Epub 2022/02/04.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 66. Wei CJ, Li YL, Zhu ZL, *et al.*: **Inhibition of activator protein 1 attenuates neuroinflammation and brain injury after experimental intracerebral hemorrhage.** *CNS Neurosci. Ther.* 2019 Oct; **25**(10): 1182–8. Epub 2019/08/09.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 67. Wang C, Wu Y, Liu C, *et al.*: **Nervonic acid alleviates radiation-induced early phase lung inflammation by targeting macrophages activation in mice.** *Front. Immunol.* 2024; **15**: 1405020. Epub 2024/12/26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 68. Reikerås O: **Immune depression in musculoskeletal trauma. Inflammation research: official journal of the European Histamine Research Society [et al].** 2010 Jun; **59**(6): 409–14. Epub 2010/02/12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 69. Vizuete AFK, Fróes F, Seady M, *et al.*: **Early effects of LPS-induced neuroinflammation on the rat hippocampal glycolytic pathway.** *J. Neuroinflammation.* 2022 Oct 11; **19**(1): 255. Epub 2022/10/12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 70. Chaudhuri A: **Adjunctive dexamethasone treatment in acute bacterial meningitis.** *Lancet Neurol.* 2004; **3**(1): 54–62.
[Publisher Full Text](#)
 71. Sehnert B, Burkhardt H, Dübel S, *et al.*: **Cell-type targeted NF- κ B inhibition for the treatment of inflammatory diseases.** *Cells.* 2020; **9**(7): 1627.
 72. Husain I, Akhtar M, Vohora D, *et al.*: **Rosuvastatin attenuates high-salt and cholesterol diet induced neuroinflammation and cognitive impairment via preventing nuclear factor KappaB pathway.** 2017; **42**: 2404–2416.
 73. Thawkar BS, Kaur G: **Inhibitors of NF- κ B and P2X7/NLRP3/Caspase 1 pathway in microglia: Novel therapeutic opportunities in neuroinflammation induced early-stage Alzheimer's disease.** *J. Neuroimmunol.* 2019; **326**: 62–74.
[PubMed Abstract](#) | [Publisher Full Text](#)
 74. Singh SS, Rai SN, Birla H, *et al.*: **NF- κ B-mediated neuroinflammation in Parkinson's disease and potential therapeutic effect of polyphenols.** *Neurotox. Res.* 2020; **37**: 491–507.
[PubMed Abstract](#) | [Publisher Full Text](#)
 75. Wang J, Yin C, Pan Y, *et al.*: **CXCL13 contributes to chronic pain of a mouse model of CRPS-I via CXCR5-mediated NF- κ B activation and pro-inflammatory cytokine production in spinal cord dorsal horn.** 2023; **20**(1): 1–20.
 76. Zhang T, Ma C, Zhang Z, *et al.*: **NF- κ B signaling in inflammation and cancer.** 2021; **2**(4): 618–653.
 77. Li C, Wu Y, Huang M-Y, *et al.*: **Characterization of Inflammatory Signals in BV-2 Microglia in Response to Wnt3a.** *Biomedicine.* 2023; **11**(4): 1121.
 78. Scipioni L, Ciarra Mellano F, Carnicelli V, *et al.*: **Microglial endocannabinoid signalling in AD.** 2022; **11**(7): 1237.
 79. Al-Ghezi ZZ, Busbee PB, Algheta H, *et al.*: **Combination of cannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), mitigates experimental autoimmune encephalomyelitis (EAE) by altering the gut microbiome.** *Brain Behavior, Immunity.* 2019; **82**: 25–35.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 80. Wang B, Li D, Fiselier A, *et al.*: **High-CBD cannabis extracts inhibit the expression of proinflammatory factors via miRNA-mediated silencing in human small intestinal epithelial cells.** *Heliyon.* 2023; **9**: e18817.
[Publisher Full Text](#)
 81. Yousaf M, Chang D, Liu Y, *et al.*: **Neuroprotection of cannabidiol, its synthetic derivatives and combination preparations against microglia-mediated neuroinflammation in neurological disorders.** *Molecules.* 2022; **27**(15): 4961.
[Publisher Full Text](#)
 82. Quintero J-M, Bustos R-H, Diaz L-E, *et al.*: **Assessing neuroinflammation in vitro in a microglial model: advancing cannabinoid-based therapeutic insights.** 2025.

Open Peer Review

Current Peer Review Status: ?

Version 1

Reviewer Report 30 June 2025

<https://doi.org/10.5256/f1000research.178547.r387464>

© 2025 dos Santos Pereira M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Maurício dos Santos Pereira

Broad Institute Stanley Center for Psychiatric Research, Cambridge, Massachusetts, USA

The manuscript titled 'Assessing neuroinflammation in vitro in a microglial model: advancing cannabinoid-based therapeutic insights' presents a well-designed and timely investigation into the anti-inflammatory potential of cannabinoid compounds using the BV-2 microglial cell line. The authors employed robust techniques including MTT assays, nitric oxide quantification, immunocytochemistry (ICC), and surface plasmon resonance imaging (SPRi) to assess the impact of CBD, FSE-THC, and their combination on LPS-induced neuroinflammatory responses.

Key strengths of the manuscript include:

- Integration of complementary readouts (cell viability, cytokine production, apoptotic and inflammatory markers).
- Use of real-time SPRi technology, offering enhanced temporal resolution.
- Clear experimental design with appropriate controls and statistical analysis.
- High relevance to cannabinoid-based therapeutic research for neuroinflammatory conditions.

Major Concerns

1. Lack of Mechanistic Insight: The study would benefit from further mechanistic exploration using receptor antagonists or gene expression analyses.
2. Limited Time-Point Analysis: The 6-hour endpoint limits interpretation. 4h is necessary to start cytokine production but the peak varies between cytokines. Also, the lack of absolute values doesn't help checking if 4h of LPS was enough to induce a high cytokine response.
3. Lack of detailed explanations of cell manipulation for SPRi technology. Because it is not a standard tool to analyze cytokine and show compelling reasoning for its use, the paper could be a reference for its use in BV2 microglial cells. So, details of the protocol should be addressed.
4. Use of BV-2 Cell Line Only: While common, this model has limitations compared to primary microglia. These should be more explicitly discussed.
5. No Inclusion of IL-1 β Data: Given its relevance, the exclusion of IL-1 β is a gap. Even preliminary or supplementary data would help.

Minor Concerns

1. Abbreviations: Ensure all abbreviations are defined upon first use, in both abstract and

main text.

2. SPR Method Description: Consider summarizing highly technical details and moving them to supplementary methods (while improving the description on how your biological samples were treated in terms of volume, dilution if any, etc.)
3. Supplementary Figures: ICC images should be clearer, with consistent labeling and higher resolution.
4. Statistical Methods: Include effect sizes or confidence intervals. A supplementary table of statistical results would help.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neuroimmunology, neuropharmacology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research