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Cannabinoid Receptors and Neuroinflammation in Ischemic Stroke: A Systematic Review

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ABSTRACT

Purpose: Cannabinoids (CBs) have been shown to exert anti-inflammatory effects in Ischemic stroke (IS). This study aimed to systematically review preclinical studies exploring the anti-inflammatory capabilities of CBs in IS.
Methods: A comprehensive literature search was conducted across major databases (PubMed, EMBASE, Web of Science, Scopus, Google Scholar) for studies published up to February 2025. The search strategy employed combinations of terms for IS ("stroke," "cerebrovascular accident," "cerebral ischemia"), inflammation ("inflammation," "inflammatory response," "cytokine"), and cannabinoids ("cannabinoid," "cannabidiol," "CB1," "CB2") using Boolean operators. Primary outcomes were changes in inflammatory marker expression; secondary outcomes included infarct volume, neurological function, and histopathological outcomes. The inclusion criteria focused on preclinical studies reporting the effects of CB compounds on inflammation in rodent models of IS.
Results: The initial search identified 1326 studies. Following the removal of duplicates, 870 studies proceeded to the screening phase. Based on the title and abstract review, 826 studies were excluded. The full texts of 44 articles were then reviewed for eligibility, resulting in the exclusion of 16 studies for specific reasons. Ultimately, 28 studies met the inclusion criteria and were incorporated into the review. Available animal studies demonstrated anti-inflammatory action of CBs, often linked to the modulation of pro-inflammatory cytokines within the ischemic brain environment.
Conclusion: While preclinical data support the anti-inflammatory potential of CBs in IS, their translation to clinical practice remains limited. Key challenges include concerns over potential psychoactive side effects and significant uncertainty regarding optimal dosing strategies. Further research, specifically well-controlled clinical trials, is essential to establish the safety, efficacy, and therapeutic windows of CB-based interventions for IS.

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1. Introduction

Ischemic stroke (IS) is a leading cause of mortality and long-term disability worldwide, with limited therapeutic options beyond the critical time window for intervention.[1] The pathophysiology of stroke involves a multifaceted cascade of cellular and molecular events, including excitotoxicity, oxidative stress, and notably, neuroinflammation, which play a pivotal role in both acute injury progression and long-term recovery.[2] Post-stroke, a complex neuroinflammatory cascade emerges that has a dual effect on brain tissue recovery. This secondary inflammatory response simultaneously facilitates additional neural damage, causing neuronal demise, while also orchestrating essential recovery mechanisms through debris clearance and tissue repair initiation.[3,4] However, this same inflammation triggers the production of harmful pro-inflammatory mediators that can exacerbate neurological dysfunction.[5] Inflammatory responses following IS correlate with compromised BBB integrity, brain edema development, hemorrhagic conversion risk, and poorer clinical prognosis.[6,7]

Following brain ischemia, neuroinflammatory processes induce disruption mediated by glial cells, contributing to enhanced excitotoxicity and elevated ROS production.[8] Brain-resident immune cells, primarily microglia and astrocytes, demonstrate functional duality by establishing the inflammatory environment.[5] Under hypoxic conditions, necrotic neural cells release damage-associated molecular patterns, triggering microglial transformation toward the pro-inflammatory M1 phenotype with the increased expression of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), reactive oxygen species (ROS), chemokines (CCL2, CCL5, CXCL1), and matrix metalloproteinases (MMPs).[9] During later post-stroke phases, microglia transition to an M2 phenotype with anti-inflammatory properties supporting neural repair.[10] Astrocytes respond through proliferation and enlargement, regulating BBB function and promoting glial scar formation which initially reduces cerebral edema but eventually impedes axonal regeneration.[11,12]

Cannabinoids (CBs) have gained attention as potential therapeutic agents in IS. These compounds encompass plant-derived phyto-CBs, endogenous endo-CBs (eCBs), and synthetic CBs.[13,14] Within the central nervous system (CNS), eCBs participate in neural development, neurotransmitter secretion, and inflammatory processes.[15] CB receptor 1 (CB1R) and CB receptor 2 (CB2R) are primary receptors in stroke research, with CB1R modulation correlating with psychoactive effects and pain relief, while CB2R engagement produces neuroprotective and anti-inflammatory outcomes.[16,17] Preclinical studies demonstrated that modulation of eCB signaling and administration of exogenous CB compounds impact experimental stroke outcomes by affecting infarct dimensions, behavioral parameters, neuroinflammatory responses, cerebral edema, and BBB disruption.[18-20] This review assesses preclinical studies exploring the neuroprotective and anti-inflammatory capabilities of CBs in IS and discusses their therapeutic relevance.

1. Materials and Methods

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards. Ethical approval for the study was granted by the Research Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (Grant number: 74736). This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD420251104717.

2.1. Inclusion and Exclusion Criteria

All publications of experimental studies that were relevant to our topic and reported the effects of CB compounds on inflammation in rodent models of IS were included. Review articles, conference papers, letters to the editor, human studies, and studies involving non-rodent animals, *in vitro* or *ex vivo* investigations, studies that did not assess inflammatory markers, and non-English articles were excluded.

2.2. Search Strategy

The literature was searched across several independent databases, i.e., PubMed, EMBASE, Web of Science, Scopus, and Google Scholar, for studies published no later than February 2025 by an information specialist (S, S). The search strategy incorporated a combination of subject headings, synonyms, related terms, and variant spellings to ensure a comprehensive search. The core search structure combined three concept blocks using the Boolean operator "AND": (1) ischemic stroke terms (e.g., "stroke," "cerebrovascular accident," "cerebral ischemia," "MCAO"), (2) inflammation terms (e.g., "inflammation," "neuroinflammation," "cytokine," "interleukin," "TNF-α," "microglia," "astrocyte"), and (3) cannabinoid terms (e.g., "cannabinoid," "cannabidiol," "CBD," "CB1," "CB2," "JWH-133," "WIN55,212-2," "PEA"). Database-specific filters for animal studies (e.g., "animals" in PubMed, "nonhuman" in EMBASE) were applied where available. Duplicate records were identified and removed using reference management software (EndNote X9) followed by manual verification. Table S1 shows the complete search strategies for all databases. Moreover, the reference lists of the included articles were checked to identify additional eligible studies.

2.3. Selection Process and Data Extraction

The selection criteria included animal studies examining CB-induced anti-inflammatory responses in IS models. Each title and abstract was independently screened by two authors (I, E and A, M) and all disagreements between the authors were addressed and resolved at each stage through consultation with the third author (S, S).

2.4. Methodological Quality of Studies

Three independent reviewers (S, N; SP, H and H, S) assessed the quality and risk of bias of the selected studies using the collaborative approach to meta-analysis and review of experimental animal studies (CAMARADES) checklist. This checklist comprises ten criteria: Peer-reviewed publication, control of temperature, random allocation to treatment or control, blinded outcome assessment, use of an anesthetic without intrinsic neuroprotective activity, animal model, sample size calculation, compliance with animal welfare regulations, and statement of potential conflict of interests.[21]

2.5. Definition and Operationalization of Neuroinflammation and Outcome Specification

For the purposes of this systematic review, "neuroinflammation" was operationally defined as the CNS inflammatory response following IS, characterized by the activation of resident immune cells (microglia and astrocytes) and the production of inflammatory mediators. The following categories of inflammatory markers were most consistently evaluated across the included studies: (1) pro-inflammatory cytokines (TNF-α, IL-1β, IL-6); (2) anti-inflammatory cytokines (IL-10, TGF-β); (3) immune cell activation markers (Iba-1 for microglia, GFAP for astrocytes); (4) chemokines (MCP-1, MIP-1α, RANTES, CXCL1); (5) inflammatory enzymes (iNOS, COX-2); and (6) transcription factors (NF-κB). Reductions in pro-inflammatory markers and immune cell

activation, coupled with increases in anti-inflammatory mediators, were interpreted as anti-inflammatory neuroprotection, which was further correlated with structural outcomes (infarct volume, neuronal survival, BBB integrity) and functional outcomes (neurological scores, motor and cognitive performance).

Primary outcomes were specified a priori as changes in the expression of inflammatory markers (including cytokines, chemokines, inflammatory enzymes, and transcription factors) measured by any validated method (ELISA, Western blot, qRT-PCR, immunohistochemistry/immunofluorescence).

Secondary outcomes included: (1) infarct volume measured by TTC staining or MRI; (2) neurological function assessed by validated scales (e.g., Neurological Severity Score, rota-rod, adhesive removal test, Morris water maze); (3) histopathological outcomes (neuronal cell death, apoptosis, BBB integrity, white matter integrity); and (4) oxidative stress markers where reported.

2.6. PROSPERO Registration and Protocol Deviations

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD420251104717. The registered protocol specified: (1) a primary outcome of inflammatory marker modulation, (2) inclusion of peer-reviewed preclinical studies in rodent models, (3) a comprehensive search of five databases, and (4) quality assessment using the CAMARADES checklist. The following deviations from the registered protocol should be noted: Google Scholar was added as an additional database to capture gray literature and studies not indexed in major databases. All other procedural elements adhered to the registered protocol.

3. Results and Discussion

3.1. Literature Search

A total of 1326 studies were identified through a comprehensive search of bibliographic databases. Following the removal of duplicates, 870 studies proceeded to the screening phase. Based on the title and abstract review, 826 studies were excluded. The full texts of 44 articles were then reviewed for eligibility, resulting in the exclusion of 16 studies for specific reasons. Ultimately, 28 studies met the inclusion criteria and were incorporated into the review. Of the excluded studies, 5 studies did not involve drug administration,[22-26] 2 did not include CB agonists,[27-29] 7 did not assess inflammatory markers,[30-36] one study was *in vitro* investigations,[37] and one study was *ex vivo* investigations.[38] The selection process is shown in the PRISMA flow chart (Figure 1).

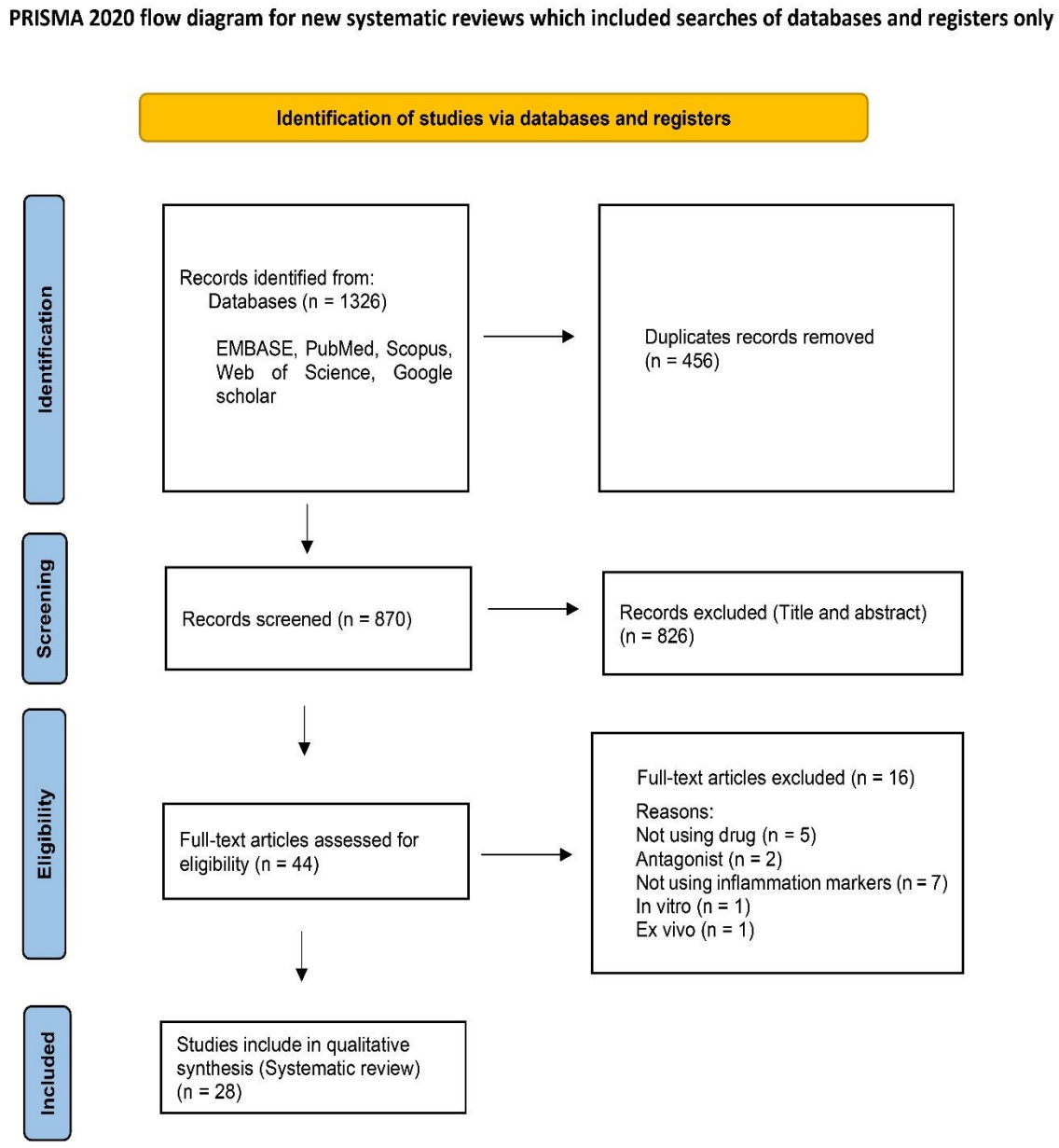

Figure 1. The PRISMA flow diagram detailing the quantity of studies screened, excluded, and ultimately included throughout each phase of the systematic review process

3.2. General characteristics of the studies and risk of bias

Among the 28 studies reviewed, treatment administration methods varied as follows: oral administration (PO) was employed in 3 studies,[39-41] subcutaneous (SC) injection in 2 studies,[42,43] intravenous (IV) injection in 2 studies,[44,45] and intracerebral (IC) injection in 1 study.[46] The remaining 20 studies utilized intraperitoneal (IP) administration.[47-66] Male C57BL/6J mice were utilized in 5 studies,[50,52,54,60,64] while male mice were used in one study.[44] Male Swiss mice were employed in two studies.[49,58] Male Sprague Dawley rats were included in 7 studies,[43,48,53,55,62,65,66] and male Wistar rats in 5 studies.[39-41,46,47] One study incorporated both male Sprague Dawley and Wistar rats.[66] Additionally, one study used postnatal Sprague Dawley rats,[57] and another used postnatal Wistar

rats.[61] Furthermore, one study involved male, female, and postnatal Sprague Dawley rats,[59] while another included male, female, and postnatal Wistar rats.[42] Across the studies reviewed, various experimental models of cerebral ischemia were employed. Specifically, deep hypothermic circulatory arrest (DHCA) was utilized in one study,[55] and photothrombotic stroke (PTS) in another.[52] The hypoxia-ischemia (HI) model was applied in 3 studies,[42,57,59] while 3 studies employed the permanent middle cerebral artery occlusion (pMCAO) model.[44,49,60] The bilateral common carotid artery occlusion (BCCAO) model was used in 7 studies.[39,41,51,56,58,64,65] The remaining 13 studies implemented the transient middle cerebral artery occlusion (tMCAO) model.[40,43,45-48,50,53,54,62,63,66] The studies varied in the type, dosage, and duration of treatment administration. Inflammatory markers were assessed using a range of methodologies, including quantitative real-time PCR (qRTPCR),[43,50,53] RTPCR,[45] immunohistochemistry,[39,41,47,48,52,53,57,58,60,61,64] immunofluorescence,[43,51,54,62,66] ELISA,[44,50,51,54-56,62] Cytometric bead array (CBA),[49] Tunnel staining,[54] and Western blotting.[39-41,43,46,47,49,51,59,62-64] These techniques were applied across the included studies as summarized in Table 1.

Table 1. Summary of the included studies on CB compounds and neuroinflammation in IS models

Study (APA citation)	Number of animals	Animal strain	Age / weight	Model and method of induction	Study group	Intervention	Time between injury induction and start of medication	Administration	Measures	Target receptor	Inflammation pathway markers	Main conclusion
Ahmad, A. et al. (2012)	80	Male Wistar rats	Age NR; Weight 270–290 g	tMCAO	1) Sham + Vehicle (n=20); 2) tMCAO + Vehicle (n=20); 3) Sham + PEA (n=20); 4) tMCAO + PEA (n=20)	PEA (10 mg/kg)	1 h after tMCAO and 6 h after reperfusion	IP	Sensory-motor function test (NSS); Cell death (H&E); Infarct volume (TTCS); MCs (TBCTS); Neurotrophic factors (GDNF, BDNF); Apoptosis markers (BAX and BCL2), Inflammatory markers (iNOS), Transcription factor (NF-κB p65; IκB-α1; p-JNK) (Western blot)	NR	iNOS, and GFAP	PEA can potentially reduce brain damage or enhance functional recovery in injuries induced by reperfusion.
Garg, P. et al. (2010)	27	Male Sprague Dawley rats	Age NR; Weight 300–325 g	tMCAO	1) Sham + Vehicle (n=3); 2) tMCAO + Vehicle (n=3); 3) tMCAO + NAE 16:0 (n=3)	NAE 16:0, AM404, AM251 and CPZ (10 mg/kg)	Ppretreatment 6 h and before, 15 min, concomitant, 2 h and 3 h after tMCAO	IP	Sensory-motor function test (NSS); Infarct volume (TTCS); Cell death, Apoptosis (H&E and Tunnel staining); Apoptosis marker (Cas-3), Inflammatory markers (iNOS, nNOS and BACE1), Transcription factor (NF-κB) (Immunohistochemistry)	TRPV1 and CB1	iNOS and nNOS	NAE 16:0 exhibits a neuroprotective effect following cerebral ischemia by reducing inflammation and apoptosis, independent of CB1 and VR1receptor activation.
Zarruk, JG. et al. (2012)	68	Male Swiss mice	Age 56–70 days; Weight NR	pMCAO	1) Sham + Vehicle (n=5); 2) Sham + JWH-133 (n=5); 3) pMCAO + Vehicle (n=5); 4) pMCAO + JWH-133 (n=5)	JWH-133 (1.5 mg/kg) and SR144528 (3–5 mg/kg)	10 m and 3 h after pMCAO	IP	Sensory-motor function test (NSS); Infarct volume (TTCS); Inflammatory markers (IL-1β, TNF-α, IL-6, MIP –1α, MCP-1, RANTES, IL-12/IL-23p40, IL-10) (Cytometric bead array); (iNOS and COX-2) (Western blot)	CB2	IL-1β, TNF-α, IL-6, MIP –1α, MCP-1,CCL-5, IL-12/IL-23p40, IL-10, iNOS and COX-2	A selective CB2R agonist (JWH-133) leads to neuroprotection after focal brain ischemia by inactivating microglia and macrophages and reducing inflammatory factors.

Khaksar, S. et al. (2017)	204	Male Wistar rats	Age 56–84 days; Weight 250–350 g	tMCAO	1) Control (n=5); 2) tMCAO (n=5); 3) Vehicle (n=5); 4) tMCAO + CBD (50 ng) (n=5); 3) tMCAO + CBD (100 ng) (n=5); 4) tMCAO + CBD (200 ng) (n=5)	CBD (50, 100, and 200 ng/rat)	5 consecutive days before tMCAO	IC	Sensory-motor function test (NSS and Rota-rod test); Infarct volume (TTCS); Brain edema BBBP (EBS); and Inflammatory markers (TNFR1, TNF-α), Transcription factor (NF-κB) (Western blot)	NR	TNF-α	CBD administration causes a reduction in the expression of TNF-α, TNFR1, and NF-κB as well as reduces infarction, brain oedema, and BBB permeability.
Lavayen, B. P. et al. (2023)	152	Male C57BL/6J mice	Age 84–112 days; Weight 28–32 g	tMCAO	1) tMCAO + Vehicle (n=9); 2) tMCAO + VCE-004.8 (n=9); 3) Sham + Vehicle (n=5); 4) Sham + VCE-004.8 (n=5)	VCE-004.8 (10 and 20 mg/kg)	4h and 6h after tMCAO	IP	Motor function test (Open field, Vertical grid and Grip test); Infarct volume (TTCS); BBBPM and Inflammatory markers (iNOS;IL-1β; PTGS2; CXCL1; ICAM1; CCL2; VEGF and Ly6G) (q-RTPCR), (IgG) (ELISA)	NR	IL-1β, CXCL1, CCL2, PTGS2, and iNOS,	Treatment with VCE-004.8 could significantly reduce the level of IgG and active MMP-9 in the brain of mice.
Rahmani, MR. et al. (2018)	78	Male mice	Age NR; Weight 25–35 g	pMCAO	1) Intact (n=13); 2) pMCAO (n=13); 3) pMCAO + Vehicle (n=13); 4) pMCAO + JZL-184 (n=13); 5) pMCAO + rTPA (n=13); 6) pMCAO + JZL-184 + rTPA (n=13)	JZL-184 (4 mg/kg)	Immediately and 5 h after pMCAO	IV	Sensory-motor function test (NSS and Adhesive removal test); Infarct volume (TTCS); Brain edema and Inflammatory markers (MMP9, IL-10 and TNF-α) (ELISA)	NR	IL-10 and TNF-α	JZL can be used to improve the effects of r-tPA after 3 h of stroke onset.
Su, Sh. H. et al. (2017)	50	Male Sprague-Dawley rats	Age NR; Weight 240±10 g	BCCAO	1) Sham + Vehicle (n=5); 2) BCCAo + Vehicle (n=5); 3) BCCAo + WIN55,212-2 (n=5); 4) BCCAo + URB597 (n=5); 5) BCCAo + WIN55,212-2 + URB597 (n=5)	WIN55,212-2 (0.3-3 mg/kg) and URB597(0.06-1.5 mg/kg)	24 h after BCCAO daily for 12 weeks	IP	Free radical and Inflammatory markers (TNF-α, IL-1β) (ELISA); (OX-42) (Immunofluorescence), (iNOS, COX-2); Transcription factor (NF-κB p65, IκB-a, p-IKKa/β) (Western blot) (NF-κB p65) (Immunofluorescence); Oxidative stress (ROS) (DHES)	NR	OX-42, TNF-α, IL-1β; iNOS and COX-2	Administration of WIN55,212-2 and URB597 inhibits the NF-κB activation pathway and mitigates the neurological impairment in CCH-induced rats.
Yokubaitis, C. G. et al. 2021	NR	Male C57B/6 mice	Age 42–56 days; Weight 19–24 g	PTS	NR	BCP (3–30 mg/kg) and CBD (3–30 mg/kg)	1 h before PTS	IP	Motor function test (Grip strength test); Infarct volume (TTCS); Inflammatory marker (Iba-1) (Immunohistochemistry)	NR	Iba-1	Single treatment with BCP or CBD promotes Iba-1 immunofluorescence, while combination treatment of BCP and CBD reduces infarct size and microglial activation.
Yu, S. J. et al. (2015)	30	Male Sprague-Dawley rats	Age NR; Weight NR	tMCAO	Pretreatment: 1) AM1241 + tMCAO (n=7); 2) pioglitazone + tMCAO (n=7) Post treatment: 1) tMCAO + vehicle (n=6); 2) tMCAO + AM1241 (n=5); 3) tMCAO + pioglitazone (n=5)	AM1241 (2.5 mg/kg) and pioglitazone (1 mg/kg)	5 min prior to tMCAO from days 2 to 5 after tMCAO	IP	Sensory-motor function test (Body asymmetry and NSS); Infarct volume (TTCS and H&E); Inflammatory markers (Iba-1, CD4, CD8) ((Immunocytochemistry and qRTPCR) (TLR4, CB1 and CB2) (qRTPCR);	CB2	Iba-1, CD4 and CD8	Delayed post-treatment with pioglitazone can significantly induce behavioral recovery and reduce Iba-1 expression in the stroke cortex of rats. In contrast, AM1241 pretreatment causes a significant reduction in the brain infarction and neurological deficits.

Fernandez-Lopez, D. et al. (2012)	24	Male Sprague-Dawley rats	Age 7 days; Weight NR	tMCAO	1) MCAO + Vehicle (n=12); 2) MCAO + WIN (n=12)	WIN55,212-2 (1 mg/kg)	Immediately after tMCAO, second injection 4 h later twice daily until sacrifice at 24 or 72 h after tMCAO	SC	Infarct volume (Immunofluorescence); Inflammatory markers (Iba-1) (Immunofluorescence); TNF-α, IL-1β, GATMCP-1, MIP-1a), Receptors (CB2R, CB1R, CCR2 and CX3CR1) (qRT-PCR); Iba-1 (Western blot)	CB1 and CB2	Iba-1, CD4, CD8, IL-1β and TNF-α	WIN decreased microglial activation at this point and attenuated infarct volume and microglial accumulation and proliferation in the injured cortex 72 hours after MCAO. cannabinoid agonist WIN protects against neonatal focal stroke in part due to inhibitory effects on microglia.
Serra, MP. et al. (2022)	44	Male Wistar rats	Age NR; Weight 210±20 g	BCCAO	1) Sham + Vehicle (n=11); 2) Sham + BCP (n=11); 3) BCCAO + BCP (n=11); 4) BCCAO + Vehicle (n=11)	BCP (40 mg/rat)	6 h befor BCCAO	PO	Inflammatory markers (TRPV1, Iba1 and GFAP) (Western blot and Immunohistochemistry); (BDNF and trkB) (Western blot and Immunohistochemistry)	TRPV1	GFAP and Iba1	BCP pretreatment can modulate the BCCAO/R-triggered cerebral inflammation through the regulation of TRPV1 and the BDNF-trkB system.
Yang, M. et al. (2017)	50	Male C57BL/6 mice	Age NR; Weight 20–25 g	tMCAO	1) Sham (n=10); 2) tMCAO (n=10); 3) tMCAO + BCP (8 mg/kg) (n=10); 4) tMCAO + BCP (24 mg/kg) (n=10); 5) tMCAO + BCP (72 mg/kg) (n=10)	BCP (8, 24, 72 mg/kg)	Once daily for 3 consecutive days before tMCAO and 2 h after tMCAO	IP	Infarct volume (TTCS); Inflammatory markers (TNF-α, IL-1β) (Immunofluorescence, Tunel Staining and ELISA); Necroptosis markers (RIPK1, RIPK3, p-MLKL) (qRT-PCR and Western blot); Cell death (H&E);	NR	IL-1β and TNF-α	BCP (24, 72 mg/kg) decreasedinfarct volumes, neuronal necrosis, expression of RIPK1, RIPK3, and MLKL phosphorylation after I/R injury. Also,BCP decreased levels of HMGB1, TLR4, IL-1β, and TNF-α.
Yu, MH. et al. (2023)	24	Male Sprague Dawley rats	Age 224–280 days; Weight 500–550 g	DHCA	1) Control (n = 8); 2) DHCA (n = 8); 3) WIN (n = 8)	WIN55,212-2 (1 mg/kg)	24 h before the CPB and DHCA	IP	Cell death (H&E staining), Inflammatory markers (IL-1β, IL-6, TNF-α) (ELISA); (CB1R), Apoptosis marker (Caspase-3) (Western blot) Oxidative stress (SOD) (Immunofluorescence, ELISA and Western blot)	CB1	IL-1β, IL-6 and TNF-α	WIN55, 212-2 reduced injury of the hippocampus in rats undergoing DHCA, related to lowered levels of IL-1β, IL-6, and TNF-α and an enhanced SOD level. Also, WIN55, 212-2 treatment increased the content of SOD in the hippocampus. The protein expression of caspase-3 was decreased and CB1R expression was upregulated in the hippocampus via WIN55, 212-2.
Cheng, L. et al. (2019)	100	Male Mongolian gerbils	Age 84 days; Weight 50–70 g	BCCAO	1) Sham (n=10); 2) Ischemia (n=10); 3) Ischemia + NITyr (1 mg/kg) (n=10); 4) Ischemia + NITyr (5 mg/kg) (n=10); 5) Ischemia + NITyr (10 mg/kg) (n=10); 6) Ischemia + Nimodipine (n=10); 7) ischemia+CB1 antagonist AM251 (1 mg/kg) (n=10);8) CB2 antagonist AM630 (1 mg/kg) (n=10); 9) NITyr +	NITyr (1, 5 and 10 mg/kg), AM251 (1 mg/kg), AM630 (1 mg/kg), Nimodipine (10 mg/kg)	12, 24, 48, 72, 96 and 120 h after BCCAO	IP	Motor function (OFT and RRT); Spatial memory (MWM); Cell death (H&E staining); Oxidative stress (CAT, SOD, GPx, MDA), Inflammatory markers (IL-1β,IL-6, TNF-α) (ELISA); Receptors (CB2 and CB1), Transcription factors (p-PI3K, p-Akt, PI3K and Akt) (Western blot)	CB1 and CB2	IL-1β, IL-6, TNF-α	NITyr failed to enhance spontaneous locomotor activity or reduce anxiety in the OFT but improved motor coordination in the Rotarod Test (RRT) and spatial memory in the MWM. It reduced ischemia-induced neural loss in the hippocampus, attenuated inflammation and oxidative stress, and upregulated PI3K and p-Akt expression in the hippocampus. The CB2 receptor antagonist AM630, but not the CB1 receptor

					AM251 and NITyr+ AM630 (n=10); 10) ischemia+Nimodipine (10 mg/kg) (n=10)							antagonist AM251, reversed these effects, except for oxidative stress, which was reversed by AM251.
Carloni, S. et al. (2020)	70	Postnatal Sprague-Dawley rats	Age 7 days; Weight NR	HI	1) Sham (n=8); 2) HI (n=10); 3) HI + URB447 1h-PRE (n=10); 4) HI + SR141716A (n=10); 5) HI + WIN-55,212-2 (n=10); 6) HI + URB447 + WIN-55,212-2 (n=10); 7) HI + URB447 30min-POST (n=10); 8) HI + URB447 3h-POST (n=10); 9) Sham (Histology; n=8); 10) HI (Histology; n=8); 11) HI + URB447 3h-POST (Histology; n=8)	(WIN-55,212-2, URB447 and SR141716A) 1 mg/kg	1 h and 30 min before HI, 3 h after HI, 30 min after HI	IP	Infarct volum (TBS); Inflammatory markers (Iba-1, and GFAP), White matter injury assessment (MBP) (Immunohistochemistry)	NR	Iba-1 and GFAP	URB447, when administered before the onset of brain injury, prevented brain tissue damage. SR141716A also had similar protective effects, while the WIN-55,212-2 reduced the protective effects of URB447. Even when URB447 was administered three hours after the onset of brain injury, it still prevented nerve cell damage and white matter damage.These findings suggest that URB447 may be used as an effective drug in treating brain damage caused by oxygen deprivation in newborns.
Schiavon, AP. et al. (2014)	81	Male Swiss mice	Age 49–63 days; Weight 30–40 g	BCCAO	1) Vehicle + sham (n=15); 2) Vehicle + ischemia (n=23); 3) CBD 3 mg/kg + ischemia (n=11); 4) CBD 10 mg/kg + ischemia (n=14); 5) CBD 30 mg/kg + ischemia (n=11)	CBD (3, 10, and 30 mg/kg)	30 min before and 3, 24, and 48 h after BCCAO	IP	Spatial memory (MWM); Cell death (Nissl Staining and FJCS); Inflammation markers (GFAP) (Immunohistochemistry);	NR	GFAP	CBD, at doses ranging from 3 to 30 mg/kg, improved spatial learning performance in BCCAO. CBD (10 and 30 mg/kg) reduced neurodegeneration in the hippocampus, and the 30 mg/kg dose also reduced GFAP. These results suggest that CBD can protect against neuronal death induced by stroke and likely acts by inhibiting reactive astrogliosis.
Gupta, B. et al. (2020)	144	Male and Female Postnatal Sprague-Dawley dams	Age 7 days; Weight NR	HI	1) Placebo (n=20); 2) JWH133 (n=20); 3) HIE + Placebo (n=20); 4) HIE + JWH133 (n=20); 5) HIE + Hypothermia + Placebo (n=20); 6) HIE + Hypothermia + JWH133 (n=20); TUNEL experiments (n=24)	JWH133 (1.5 mg/kg)	Following HI	IP	Apoptosis (TUNEL staining); Inflammatory markers (IL-6, TNFα, MIP1α, RANTES, TNF-α, IL-10), Receptor (CB2R) (Western blot)	CB2	TNF-α, MIP-1α, IL-6, CCL-5, TGF-β and IL-10	Treatment with JWH133, both alone and in combination with hypothermia, demonstrated neuroprotective effects through reducing inflammation, limiting DNA damage, and modulating CB2 receptor expression.

Caltana, L. et al. (2015)	60	Male C57/Bl 6 mice	Age NR; Weight 25–32 g	pMCAO	1) Control + Saline (n=10); 2) Control + ACEA (n=10); 3) Control + AM251 (n=10); 4) MCAo + Saline (n=10); 5) MCAo + ACEA (n=10); 6) MCAo + AM251 (n=10)	ACEA (1 mg/kg), AM251 (1 mg/kg)	3, 24 and 48 h after pMCAO	IP	Sensory-motor function test (NSS, Corner and cylinder tests); Morphological analysis; (TBS, FJBS, Immunohistochemistry, Lectin staining and Ultrastructural analysis); (GFAP) (Immunohistochemistry)	CB1	GFAP	Treatment with ACEA decreased astrocytic inflammatory response, neuronal cell death, and dendritic loss. Conversely, the use of AM251 resulted in an increase in these indicators. Motor function assessment tests revealed that ischemic animals progressively experienced motor impairments, a trend that only ACEA treatment was able to reverse.
Caltagirone, C. et al. (2015)	80	Male Wistar rats	Age NR; Weight 270–290 g	tMCAO	1) tMCAO + Vehicle: the rats (n =20); 2) tMCAO + co-ultraPEALut (n=20); 3) Sham + Vehicle (n=20); 4) Sham+ co-ultraPEALut (n=20)	Co-ultraPEALut (1 mg/kg) /	1 h after ischemia and 6 h after tMCAO	PO	Sensory-motor function test (NSS); Infarct volum (H&E, TTCS and Immunohistochemistry); Inflammatory marker (GFAP); Apoptosis markers (Bax and Bcl-2), Neurotrophic factors (BDNF and GDNF) (Western blot)	NR	GFAB	Co-ultraPEALut administration indicates neuroprotective and anti-inflammatory effects in a rat model of cerebral ischemia and ameliorate neurological outcomes in stroke patients undergoing rehabilitation. co-ultraPEALut decline infarct area, histological damage, and neurological deficits in rats undergoing MCAO. co-ultraPEALut administration was related to important enhancment in neurological status, pain, cognitive function, spasticity, and independence in daily activities.
Kong, D. et al.(2021)	60	Male and Female mice	Age NR; Weight 20–25 g	tMCAO	1) Sham (n=6); 2) Vehicle + 1h after tMCAO (n=6); 3) PEA (30 mg/kg) 1h after tMCAO (n=6); 4) PEA (30 mg/kg) + GW6471 (2 mg/kg) 1h after tMCAO (n=6); 5) Vehicle + 2h after tMCAO (n=6); 6) PEA (30 mg/kg) 2h after tMCAO (n=6); 7) PEA (30 mg/kg) + GW6471 (2 mg/kg) 2h after tMCAO (n=6); 8) Vehicle + 3h after tMCAO (n=6); 9) PEA (30 mg/kg) 3h after tMCAO (n=6); 10) PEA (30 mg/kg) + GW6471 (2 mg/kg) 3h after tMCAO (n=6);	PEA (30 mg/kg) and GW6471 (2 mg/kg)	0, 1 or 2 h after tFCI	IV	Infarct volume (TTCS); BBB disruption (TRITC-Dextran); Inflammatory markers (IL-1β, TNFa and IL-6) (RTPCR);	NR	IL-1β, TNFα, and IL-6	PEA did not affect inflammatory mediators but offered a strong protection against BBB breakdown in the early stages after tMCAO.

Poddighe, L. et al. (2018)	112	Male Wistar rats	Age NR; Weight 210±20 g	BCCAO	1) Vehicle (n=60); 2) BCP (n=52)	BCP (40 mg/rat)	6 h before BCCAO	PO	HPLC (AEA, 2-AG, PEA, OEA and lipoperoxides); Apoptosis marker (GAPDH), (CB1, CB2, PPAR-α) (Western blot); Inflammatory marker (COX-2) (Western blot and immunohistochemistry)	CB1, CB2 and PPAR-α	COX-2	Pretreatment with single dose of BCP, 6 h before the BCCAO/R model displayed neuroprotection. BCP acts via moderating the endocannabinoid system, declining oxidative stress, potentiation, and preserving DHA levels in the frontal cortex. This is mediated by receptors of CB2 and PPAR-α, inducing BCP as a potential complementary food for the control of oxidative stress and inflammation in cerebral ischemia.
Ceprián, M. et al. (2016)	88	Postnatal Wistar rats	7 to 9 days	tMCAO	1) Sham (n=28); 2) MCAO-V (n=34); 3) MCAO-C (n=26)	CBD (5 mg/kg)	30 min after tMCAO	IP	Sensory-motor function test (NGT, Wire hang test, Grip test, Beam test, Adhesive removal test, CRT); Non-spatial working memory (NORT); Infarct volum (MRI and H-MRS); Cell death (TUNEL, immunohistochemistry); Inflammatory markers (Iba1 and GFAP) (immunohistochemistry)	NR	Iba-1 and GFAP	Administration of CBD after injury led to long functional improvement, decline neuronal loss and astrogliosis, and moderating neuroinflammation, apoptosis, excitotoxicity, and metabolic disorder.
Chen, K. et al (2024)	12	Male Sprague-Dawley rats	Age NR; Weigth 270–300 g	tMCAO	1) sham + Vehicle (n=3); 2) sham + CBD (n=3); 3) tMCAO + Vehicle (n=3); 4)tMCAO + CBD (n=3)	CBD (5 mg/kg)	After 2 h of tMCAO	IP	Cell death (H&E staining); Inflammatory markers (iNOS) (Western blot) (Iba-1) (Immunofluorescence and Western blotting), (IL-1β, TNF-α) (ELISA); Transcription factor (NF-κB, TFAM and CKS1B) (Western blot)	NR	Iba-1, IL-1β, TNF-α and iNOS	CBD is able to decrease pro-inflammatory cytokine levels, alleviated oxidative stress, and decrease NF-κB phosphorylation.
Vill, M. et al (2024)	70	Male and Female Postnatal Wistar rats	Age NR; Weight 16.02-16.9 g	tMCAO	1) tMCAO + Vehicle (n=24); 2) tMCAO + VCE (n=26); 3) tMCAO + VCE 12h (n=10); 4) tMCAO + VCE 18h (n=10)	VCE-004.8 (5 mg/kg)	30 min, 12, and 18 h after tMCAO for three days	IP	Infarct volume (MRI); Apoptosis (TUNEL staining); Motor function (OFT, CRT, Geotaxis and Grasp test); Oxidative stress (Nitrosylation), Inflammatory marker (TNF-α), Receptor (TLR-4) (Western blot)	NR	TNF-α	VCE-004.8 exerts anti-inflammatory effects by activating PPARγ and CB2 receptors and modulating the HIF-1 pathway, which prevents BBB disruption.These advantages were achieved at a reduced dose and with a wider therapeutic range compared to all adult animal models.
Pottier, G. et al (2017)	40	Male Sprague Dawley rats and Male Wistar rats	Age NR; Weight 300 g	tMCAO	1) control (n=6); 2) MCAO + JWH133 (n=7); 3) MCAO + Vehicle (n=5); 4) ex vivo (n=12); 5) LPS (n=6);) 6) AMPA (n=4)	JWH-133 (1.5 mg/kg)	daily for a total of seven days from 1 h after tMCAO	IP	Brain lesion (MRI and PET), Receptors (CB2, CD11b and TSPO) (Immunofluorescence), Inflammatory markers (GFAP and Iba-1) (Immunofluorescence); Sensory-motor function test (NSS)	CB2	Iba-1 and GFAP	[11C]A-836339 PET imaging doese not indicate changes in CB2R expression after cerebral ischemia, LPS, or AMPA-induced neuroinflammation. Activation of CB2R with JWH133 decreases TSPO expression and neuroinflammatory response at day 7 post-MCAO.
Mori, MA. et al. (2016)	38	Male C57BL/6 mice	Age 60-90 days;	BCCAO	Sham + Vehicle (n=12); 2) BCCAO + Vehicle (n=13); 3)	CBD (10 mg/kg)	30 min before and 3, 24, and	IP	Locomotor activity (OFT), Anxiety (EZM), Spatial memory (YMT and OLT), Depression	NR	Iba-1 and GFAP	The CBD treatment causes prevention of cognitive and emotional impairments,

			Weight 25-30 g		BCCAO+CBD (n=13)		48 h after BCCAO		(FST); Cell death (Nissl, Kluver-Barrera staining), (DCX and MAP-2) (Western blot); Inflamatory markers (Iba-1) Immunohistochemical), (GFAP) (Western blot); neurotrophic factors (BDNF) (Western blot); Apoptosis marker (caspase-9) (Western blot);			reduces hippocampal neurodegeneration, and protects white matter in BCCAO mice. CBD decreases Iba-1 and GFAP in CA1 and CA2/CA3 areas. CBD promotes hippocampal neuroplasticity via enhancing DCX-IR neurons, MAP-2 expression, and BDNF levels. CBD reduces levels of caspase-9. As a result, CBD can mitigate long-term consequences of brain ischemia.
Pazos, MR. et al. (2012)	74	Male and Female Postnatal Wistar rats	7 to10 days	HI	1) Sham + vehicle (n=15); 2) Sham + CBD (n=16); 3) HI + vehicle (n=21); 4) HI + CBD (n=22)	CBD (1 mg/kg)	10 min after HI	SC	Motor function (Rotarod test, CRT); Spatial working memory (NOR) Infarct volume (MRI); Cell death (Nissl staining); Inflamatory marker (TNF-α) (Western blot)	NR	TNF-α	CBD results in significant and long-lasting neuroprotection. The CBD-treated group exhibits improvements in motor coordination, forelimb use, and memory function, accompanied by a reduction in brain lesion volume. There is a decrease in neuronal loss, oxidative stress, and excitotoxicity. Furthermore, CBD reduces TNF-α expression. Importantly, no side effects are observed in the CBD-treated sham group. Therefore, CBD holds neuroprotective potential for neonatal brain injury by targeting mechanisms of inflammation, oxidative stress, and excitotoxicity.
Wang, DP.et al. (2018)	60	Male Sprague-Dawley rats	Age 30 days; Weight 250±10 g	BCCAO	1) Sham (n=20); 2) 2VO (n=20); 3) 2VO + WIN (n=20)	WIN55,212-2 (1 mg/kg)	30 min after 2VO for 4 weeks	IP	Spatial memory (MWM); Recognition memory (NOR); Inflammatory markers (TNF-α, IL-1β) (RTPCR and Western blot); Apoptosis marker (cas-3) (Western blot); Autophagy markers (LC3 and Beclin-1) (Western blot)	NR	TNF-α, IL-1β	Treatment with WIN55,212-2 suppresses autophagy and inflammation, improves memory performance, reduces anxiety-like behavior, and ameliorates cognitive deficits. This effect is associated with the inhibition of TNF-α and IL-1β expression.

Abbreviations: NR: Not reported; PEA: Palmitoylethanolamide; IP: Intraperitoneal; IC: Intracerebral; CBD: Cannabidiol; CB1: Cannabinoid type 1 receptor; CB2: Cannabinoid type 2 receptor; VR1: Vanilloid receptor; TTCS: Triphenyl tetrazolium chloride staining; EBS: Evans blue staining; GDNF: Glial cell line-derived neurotrophic factor; BDNF: Brain-derived neurotrophic factor; TBS: Toluidine blue stained; TBCTS: Toluidine blue chymase and tryptase staining; MCs: mast cells; NF-κB p65: nuclear factor kappa B; IκB-α1: NF-κB p65 inhibitor of kappa B alpha 1; p-JNK: Phosphorylated c-Jun N-terminal kinase; iNOS: Inducible Nitric Oxide Synthase; tMCAO: Transient middle cerebral artery occlusion; pMCAO: Permanent middle cerebral artery occlusion; BCCAo: Bilateral common carotid artery occlusion; CCH: Chronic cerebral hypoperfusion; PTS: Photothrombotic stroke; IL-1β: interleukin-1β; IL-6: interleukin 6; IL12/IL-23p40: Interleukin-12/Interleukin-23 subunit p40; IL-10: interleukin 10; TNF-α: Tumor necrosis factor α; MIP–1α:macrophage inflammatory peptide –1α; MCP-1: Monocyte chemoattractant protein-1; RANTES: regulated on activation normal T-cell expressed and secreted; COX-2: Cyclooxygenase-2; NSS: Neurological severity score; JWH-133: CB2R agonist; SR144528: CB2R antagonist; VCE-004.8: Dual ligand agonist for PPARγ/CB2; IgG: Immunoglobulin G; BBBP: Blood-brain barrier permeability; PTGS2: Prostaglandin-endoperoxide synthase 2; Cxcl1: CXC motif chemokine ligand 1; Icam1: Intercellular adhesion molecule 1; Ccl2: CC motif chemokine ligand 2; VEGF: Vascular endothelial growth factor; Ly6g: Lymphocyte antigen 6G; MMP-9: Matrix metalloproteinase-9; PPAR-γ: Peroxisome proliferator-activated receptor γ; DHES: Dihydroethidium staining; ROS: Reactive oxygen species; OX-42: Cluster of differentiation molecule 11b; GFAP: Glial fibrillary acidic protein; IκB-a: NF-κB p65 inhibitor of kappa B alpha; IKK a/β: Phosphorylated IκB kinase a/β; COX-2: Cyclooxygenase-2; WIN55,212-2: Cannabinoid receptor agonist; URB597: Fatty acid amide hydrolase inhibitor; Iba-1: Ionized calcium-binding adapter molecule 1; AM1241: CB2 receptor agonist; pioglitazone: PPA-γ receptor agonist; TLR4: Toll-like receptor 4; CD4: Cluster of differentiation 4; CD8: Cluster of

differentiation 8; CD11b: Cluster of differentiation 11b; nNOS: Neuronal nitric oxide synthase; Cas-3: Cysteine-aspartic acid protease-3; BACE1: Beta secretase 1; BCCAO/R: Bilateral common carotid artery occlusion followed by reperfusion; BCP: Beta-caryophyllene; CCA: Common carotid arteries; RIPK3: Receptor-interaction protein kinase-3; ELISA: Enzyme-Linked Immunosorbent Assay; CPB: Cardiopulmonary bypass; DHCA : Deep hypothermic circulatory arrest; OFT: Open field test; RRT: RotaRod test; MWM: Morris water maze; NITyr :N-Linoleyltyrosine; HI: Hypoxia-ischemia; PND:postnatal day ; MMSE: Mini Mental State Examination; NRS: Numeric Rating Scale; OCR: Oxygen consumption rate; CRT: cylinder rearing test; H+-MRS: proton magnetic resonance spectroscopy; MCAO: middle cerebral artery occlusion; MRI: Magnetic resonance imaging; NOR: novel object recognition; PAIS: perinatal arterial ischemic stroke; S.C.: subcutaneously; HI: Hypoxia-Ischemia; NORT: novel object recognition test; BDNF: brain-derived neurotrophic factor; GDNF: glial cell-derived neurotrophic factor; OGD: Oxygen-Glucose Deprivation; PEA: Palmitoylethanolamide; MCAO-V: MCAO with vehicle treatment; MCAO-C: MCAO with cannabidiol treatment; BBB: Blood-brain barrier; SC: Subcutaneously; PO: Oral administration; FJCS: Fluorojade-C Staining; FJBS: Fluoro-Jade B; OS: Administration route; mOL: Mature oligodendrocyte; WM: White matter; NGT: Negative geotaxis test; PPAR-α: Peroxisome proliferator-activated receptor α; GPR18: G protein-coupled receptor; GPR119: G protein-coupled receptor 119; GPR55: G protein-coupled receptor 55; EZM: Elevated zero maze; YMT: Y-Maze test; OLT: Object location test; DCX: Doublecortin; MAP-2: Microtubule-associated protein 2; LC3: microtubule-associated protein 1 light chain 3.

3.3. Study Quality

In the present study, the CAMARADES quality checklist was employed to evaluate the internal and external validity of the included studies. Of the 28 studies reviewed, 24 reported the control of temperature, 20 disclosed potential conflicts of interest, and 21 described random allocation to treatment or control. 17 studies implemented blinded assessment of outcomes, while only one study mentioned allocation concealment. All the selected studies reported the use of anesthetics without neuroprotective properties, employed appropriate animal models, and demonstrated compliance with animal welfare. Notably, none of the studies provided information regarding the methodology for sample size calculation. The quality assessment results are presented in Table 2.

Table 2: Evaluation of the included studies based on CAMARADES checklist. The overall scores for each study are indicated in bold

Author, years	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	score
(Ahmad, A. et al. 2012)	X	X	X	–	X	X	X	–	X	–	**7**
(Garg, P. et al. 2010)	X	X	X	–	–	X	X	–	X	–	**6**
(Zarruk, JG. et al. 2012)	X	X	X	X	X	X	X	–	X	–	**8**
(Khaksar, S. et al. 2017)	X	X	X	–	–	X	X	–	X	X	**7**
(Lavayen, B. P. et al. 2023)	X	–	–	–	–	X	X	–	X	X	**5**
(Rahmani, MR. et al. 2018)	X	X	X	–	–	X	X	–	X	–	**6**
(Su, Sh. H. et al. 2017)	X	–	X	–	X	X	X	–	X	X	**7**
(Yokubaitis, C. G. et al. 2021)	X	X	–	–	X	X	X	–	X	X	**7**
(Yu, S. J. et al. 2015)	X	X	–	–	X	X	X	–	X	–	**6**
(Fernandez-Lopez, D. et al. 2012)	X	X	–	–	–	X	X	–	X	–	**5**
(Serra, MP. et al. 2022)	X	X	X	–	X	X	X	–	X	X	**8**
(Yang, M. et al. 2017)	X	X	X	–	X	X	X	–	X	X	**8**

(Yu, MH. et al. 2023)	X	X	X	–	–	X	X	–	X	X	**7**
(Cheng, L. et al. 2019)	X	X	–	–	–	X	X	–	X	X	**6**
(Carloni, S. et al. 2020)	X	–	X	–	X	X	X	–	X	X	**7**
(Schiavon, AP. et al. 2014)	X	X	X	–	X	X	X	–	X	X	**8**
(Gupta, B. et al. 2020)	X	X	X	–	–	X	X	–	X	X	**7**
(Caltana, L. et al. 2015)	X	X	X	–	–	X	X	–	X	X	**7**
(Caltagirone, C. et al. 2015)	X	X	X	–	X	X	X	–	X	X	**8**
(Kong, D. et al. 2021)	X	–	X	–	X	X	X	–	X	X	**7**
(Poddighe, L. et al. 2018)	X	X	X	–	X	X	X	–	X	X	**8**
(Ceprián, M. et al. 2016)	X	X	X	–	X	X	X	–	X	–	**7**
(Chen, K. et al 2024)	X	X	–	–	X	X	X	–	X	X	**7**
(Villa, M. et al 2024)	X	X	X	–	X	X	X	–	X	X	**8**
(Pottier, G. et al 2017)	X	X	–	–	–	X	X	–	X	X	**6**
(Mori, MA. et al. 2016)	X	X	X	–	X	X	X	–	X	X	**8**
(Pazos, MR. et al. 2012)	X	X	X	–	X	X	X	–	X	–	**7**
(Wang, DP.et al. 2018)	X	X	X	–	–	X	X	–	X	X	**7**

Studies fulfilling the criteria of: (1) Peer-reviewed publication; (2) Control of temperature; (3) Random allocation to treatment or control; (4) Allocation concealment; (5) Blinded assessment of outcome; (6) Use of anesthetic without significant intrinsic neuroprotective activity; (7) Appropriate animal model; (8) Sample size calculation; (9) Compliance with animal welfare regulations; and (10) Statement of potential conflict of interests.

4. Discussion

Understanding the pathways targeted by CBs in the post-stroke inflammatory response is key to developing treatments that reduce further brain injury and improve patient recovery. This systematic study classifies the studies into three primary groups according to their mechanisms of action: 1) agonists of CB1Rs, 2) agonists of CB2Rs, and 3) compounds with indirect effects. ACEA is about 2000 times more selective for CB1R than for CB2R, making it a valuable tool for studying CB1R-specific effects.[67] Preclinical investigation in C57Bl/6 mice subjected to MCAO showed that CB1 agonist ACEA administration decreased the number of stellate and ameboid microglia, but the CB1R antagonist AM251 did not alter the ischemia-induced inflammatory response.[60] JZL-184 exerts its effects mainly through the activation of CB1Rs.[68] Rahmani *et al*.[44] investigated that the administration of JZL, whether by itself or with r-tPA, resulted in reduced brain edema and infarct volume, and better performance on behavioral tests. JZL was able to reduce the levels of TNF-α while enhancing IL-10 expression. Hence, JZL may serve as a candidate for combination therapy with tPA to attenuate its secondary side effects in cases where administration occurs after the 3 h period. JWH-133 is an artificially created compound that serves as a strong and highly specific activator of the CB2R. The neuroprotective effects of JWH-133 were linked to the activation of the PI3K/Akt signaling pathway, suggesting that JWH-133 protects the neonatal brain by reducing cell death and inflammation.[59] Zarruk *et al*.[49] demonstrated that a single acute dose of JWH-133 markedly reduced

the protein levels of iNOS, IL-6, TNF-α, MIP-1α, and RANTES, while IL-12 and MCP-1 remained unaffected, 24 h post-ischemia. This neuroprotective effect was blocked by the CB2R-selective antagonist SR144528 and was not observed in CB2R knockout mice, indicating that JWH‑133-mediated protection is specifically dependent on CB2R activation. In another work by Gupta *et al.*[59], combining JWH-133 with hypothermia immediately after hypoxic-ischemic encephalopathy in rats reduced TNF-α, MIP1α, while IL-6, IL-10, and TGF-β remained unchanged. Hypothermia alone, as well as in combination with JWH-133, reduced CCL-5 at 24 h, reinforcing the idea that JWH-133 potentiates the neuroprotective effects of hypothermia. Pottier *et al.*[66] investigated that the activation of CB2Rs with JWH133 attenuated neuroinflammation, as reflected by a reduction in [^{18}F] DPA-714 uptake on PET at day 7 following MCAO. AM1241 is a selective CB2R agonist, exhibiting 82-fold selectivity over the CB1R.[69] According to Yu *et al.*[53], the delayed administration of pioglitazone (an anti-inflammatory PPAR-γ agonist) decreased Iba-1 expression in the stroke-affected cortex, whereas delayed treatment with AM1241 did not. In fact, neuroprotection by AM1241 occurred before the upregulation of Iba-1 and TLR4. Yang *et al.*[54] reported that B-caryophyllene (BCP) can induce neuroprotective effects by inhibiting both necroptosis and inflammation in cerebral ischemia-reperfusion injury models. Two additional important studies by Serra *et al.*[39] and Poddighe *et al.*[41] found that BCP can exert protective effects through the modulation of TRPV1, CB1, CB2, and PPAR-α receptors, resulting in reduced inflammation. Yokubaitis *et al.*[52] observed that co-administration of BCP with CBD markedly attenuated microglial Iba-1 expression 3 days post-stroke in mice, whereas BCP alone had no effect, suggesting a synergistic anti-inflammatory effect. VCE-004.8 has shown neuroprotective effects in tMCAO models. Villa *et al.*[63], found more pronounced at lower doses and a broader therapeutic window in neonatal models, likely through the activation of PPAR-γ and CBR2 and modulation of the HIF-1 pathway. Lavayen *et al.*[50], also reported that VCE-004.8 reduced neuroinflammation by decreasing the expression of IL-1β, CXCL1, CCL2, and PTGS2 after treatment. Cheng *et al.*[56] demonstrated that N-Linoleyltyrosine (NITyr) reduces inflammation and oxidative stress following stroke by downregulating pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 through CB2R activation. WIN55,212-2, a synthetic CBR agonist, strongly activates both CB1R and CB2R and modulates key signaling pathways including p42 and p44 MAP kinase, exhibiting greater CB1R affinity than THC. An *et al.*[70] reported that WIN55,212-2 activates CB1R and CB2R at low concentrations, but at higher doses, it directly blocks GIRK1/2 channels. Su *et al.*[51] found that treatment with WIN55, 212-2 and URB597 (a fatty acid amide hydrolase inhibitor) reduced inflammation and oxidative stress in chronic cerebral hypoperfusion, suggesting their neuroprotective action through the inhibition of NF-κB pathway. In another work by Wang *et al.*[65], chronic treatment with WIN55,212-2 for 4 weeks decreased neuroinflammation by lowering pro-inflammatory cytokines TNF-α and IL-1β and offered neuroprotection by modulating autophagy and inflammatory pathways. The study by Fernandez-Lopez *et al.*[43] showed that WIN55,212-2 treatment effectively reduced microglial activation 72 h after stroke, despite no changes in TNF-α and IL-1β levels at 24 h, suggesting its neuroprotective effects mainly stem from modulating microglial activity. One possible explanation for these conflicting data is that cytokine levels were analyzed exclusively at 24 h, even though microglial activation showed greatest decline at 72 h after injury. Yu *et al.*[55] reported that pretreatment with WIN55,212‑2 attenuated caspase‑3, restored SOD and lowered IL‑1β, IL‑6, and TNF‑α in the hippocampus of deep hypothermic circulatory arrest (DHCA) rat models through a CB1R-dependent mechanism.[71] CBD is recognized for its various health benefits without inducing psychoactive effects. It modulates CB1R and CB2R activity indirectly by raising the concentrations of natural eCBs like anandamide and 2-arachidonoylglycerol.[72] It can reduce inflammation by modulating immune system activity, including suppressing cytokine production, inhibiting T cell proliferation

and migration, and interacting with CB1R and CB2R.[73] In cell culture studies, CBD has been reported to decrease the secretion of pro-inflammatory markers such as TNF-α, IL-6, IL-1β, and MMP-9 from activated microglia, while at the same time promoting anti-inflammatory cytokines like IL-10.[7]. In mice subjected to bilateral common carotid artery occlusion (BCCAO), repeated CBD prevented hippocampal neurodegeneration, decreased GFAP immunoreactivity, improved spatial memory, and enhanced hippocampal plasticity by promoting neurogenesis, increasing MAP-2 and BDNF levels, and reducing neuroinflammation.[64] In another similar work, short-term CBD treatment mitigated hippocampal neuroinflammation in BCCAO mice, as evidenced by reduced Iba-1 and GFAP immunoreactivity.[64] Ceprián *et al*.[61] showed that treatment with 5 mg/kg CBD in a neonatal brain injury model led to long-term motor improvement, reduced neuronal death, and decreased astrogliosis and neuroinflammation, as evidenced by lower Iba-1 and GFAP levels. Comparable findings were reported by Chen *et al*.[62], who demonstrated that CBD can reduce iNOS, Iba-1, TNF-α, and IL-1β expression and inhibit NF-κB phosphorylation, contributing to reduced inflammation and oxidative stress. Similarly, a low dose of CBD (1 mg/kg) was able to decrease TNF-α expression without any observed side effects in a neonatal HI model.[42] CBD reduced infarct volume, edema, and BBB permeability while downregulating TNFR1 in core and penumbra, with the most consistent effects at 100 ng/rat (i.c.v.), and improved neurological scores. Along with these neuroprotective effects, CBD down-regulated the expression of TNF-α in ischemic brain regions.[46] The study conducted by schiavon *et al*.[58] reported that CBD (at 30 mg/kg dose) can reduce GFAP levels 1 week post-BCCAO by reducing reactive astrogliosis. These results imply that CBD might offer neuroprotection against neuronal death caused by ischemia, likely through the suppression of reactive astrogliosis. Another study found that when the same CBD treatment was used in a focal ischemia model, it reduces the levels of high-mobility group box 1.[74] CBD can also reduce the HI-induced elevation of TNFα levels, while causing no detectable side effects.[42] This finding supports the anti-inflammatory properties of CBD, consistent with previous results seen in newborn mice forebrain slices subjected to oxygen-glucose deprivation.[75] URB447 is a synthetic CB compound that targets both CB1R and CB2R, functioning as a CBR1 antagonist and CBR2 agonist simultaneously.[76] Calroni *et al*.[57] demonstrated a robust neuroprotective effect achieved with URB447 after HI in Neonatal Rats. CB1R antagonism (URB447, SR141716A) was protective and WIN55, 212‑2 co‑administration diminished URB447's benefit, indicating that CB1R activation during early development may exacerbate injury or counteract CB2‑mediated anti‑inflammatory signaling. Zhang *et al*.[77] also suggests that activating both CB1R and CB2R at the same time do not achieve maximum neuroprotection, unlike the enhanced effect seen when CB1 is inhibited and CB2 is activated, simultaneously. Palmitoylethanolamide (PEA) interacts with CB-related receptors (GPR55, GPR119) and naturally activates PPAR-α, helping to suppress inflammation almost as effectively as synthetic PPAR-α agonist GW7647.[78] It is continuously expressed in brain ependymal cells that proliferate and express GFAP upon MCAO-induced ischemia.[79] Studies have recently revealed that PEA can reduce peripheral inflammation and inhibit mast cell degranulation primarily through PPAR-α activation, leading to decreased nitric oxide level, neutrophil migration, and proinflammatory markers such as iNOS and COX-2.[41] Ahmad *et al*.[47] found that the administration of PEA inhibited NF-κB activation and IkB-a degradation after tMCAO in rats, and attenuated the damage-induced dysregulation of iNOS and GFAP. Oral Co-ultraPEALut (a coultramicronized composite containing PEA and the antioxidant flavonoid luteolin) reduced GFAP upregulation and induced region-specific increase in BDNF and GDNF levels around the injury. Here, PEA acts via PPAR activation, decreasing neutrophil infiltration and pro-inflammatory markers (iNOS, COX-2), while luteolin exerts antioxidant and anti-inflammatory effects by suppressing NF-κB activity and reducing pro-inflammatory markers. Together, they can synergistically enhance

post-injury outcomes.[40] *In vitro* data by Kong *et al*.[45] demonstrated that PEA could preserve BBB integrity after transient focal cerebral ischemia. Early protection (0–4 h) was PPARα‑independent, whereas late‑phase protection (>6 h) was partially PPARα‑dependent. In MCAO/R mice, immediate PEA administration further decreased the pro-inflammatory cytokines and improved BBB integrity through a PPARα-independent mechanism involving ROCK2 inhibition.[45] N-acyl ethanolamines (NAEs) classically target CB1R, CB2R, and VR1R, but increasing data show that their actions are not limited to CB or vanilloid receptors and involve additional receptor pathways.[47] In a study by Grag *et al*.[48] NAE 16:0 conferred strong neuroprotection in focal ischemia/reperfusion by reducing pyknotic nuclei and TUNEL‑positive cells and suppressing iNOS and NF‑κB. The current research showed that NAE 16:0, offers neuroprotective effects in stroke models by engaging an intracellular mechanism independent of CB1R and VR1R, primarily through anti-apoptotic signaling.

Overall, our findings indicate an association between the modulation of pro-inflammatory markers (TNF-α, IL-1β, IL-6) and anti-inflammatory mediators (IL-10, TGF-β), alongside indicators of immune cell activation (e.g., Iba1, GFAP) and chemokines (e.g., MCP-1) by CB compounds, and the observed neuroprotective effects. Specifically, the reduction in pro-inflammatory signaling and immune cell activation, coupled with an increase in anti-inflammatory mediators, is correlated with decreased neuronal damage, reduced infarct volume, and improved neurological function.

Strengths, Limitations, and Recommendations

Strengths

This systematic review has several notable strengths. First, it presents a detailed contemporary synthesis of preclinical evidence evaluating CB-based interventions targeting neuroinflammation in ischemic stroke. The inclusion of diverse CB classes, selective CBR1 and CBR2 agonists, eCB modulators, phyto-CBs, and synthetic analogues offers a broad perspective on the mechanistic range of CB signaling in stroke pathology. Additionally, the review incorporates multiple ischemic stroke models (tMCAO, pMCAO, BCCAO, HI, photothrombosis, DHCA), enhancing the biological relevance of the findings across developmental stages and injury severities. Rigorous study selection, dual reviewer screening, and assessment of methodological quality using the CAMARADES checklist further strengthen the internal validity of this review. Collectively, these features allow for a robust evaluation of how CB-based therapies modulate neuroinflammatory mediators, microglial activation, astrocytic responses, and downstream neuroprotective outcomes.

Limitations

A primary limitation of this systematic review is the inability to perform a quantitative meta-analysis. Significant heterogeneity among the included studies, including variations in the stroke models employed (e.g., tMCAO, pMCAO, BCCAO), the types of CB compounds investigated (e.g., CBR1/CBR2 agonists, the eCB system, phyto-CBs, and synthetic analogues), dosing regimens, administration timing, and diverse outcome measures (e.g., neurological, histopathological, inflammatory, and behavioral), rendered statistical data integration infeasible and potentially misleading.

Moreover, as previous studies indicated that the use of CB compounds can have positive effects in animal models of IS; however, the absence of sample size calculations and inadequate reporting of allocation concealment in a significant portion of the included studies raise the possibility of overestimating treatment effects, thereby increasing the risk of bias. Therefore, the results should be interpreted with caution, and preclinical studies with more rigorous methodological designs are essential. Of note, most evidence originates from rodent models, which may not completely reflect the complexity of human neuroinflammatory responses. Many studies assessed inflammatory markers at single or limited time points, making it difficult to capture the temporal dynamics of CB effects. Finally, the majority of compounds have not been tested clinically, and some (particularly potent CB1 agonists) carry psychoactive or hemodynamic risks that may restrict translation.

Publication bias represents a notable concern in this body of literature. Preclinical studies reporting positive or significant results are more likely to be published than those with null or negative findings (the "file drawer problem"), and this review cannot rule out the existence of unpublished studies showing no anti-inflammatory effect of CBs in stroke models. Furthermore, the inclusion of only English-language articles may have introduced language bias, potentially excluding relevant non-English publications from non-English speaking research communities with active stroke research (e.g., Chinese, Japanese, German, or French journals). The inclusion of Google Scholar, while enhancing search comprehensiveness, also introduces methodological challenges. Google Scholar lacks transparent, reproducible search syntax and does not support systematic duplicate removal or consistent sorting by relevance. Although it may capture gray literature and non-indexed studies, the absence of quality filtering and the potential inclusion of preprints, theses, or non-peer-reviewed reports necessitate cautious interpretation of studies identified exclusively through this source. In this review, no unique studies were included solely from Google Scholar that were not also identified in other databases; thus, the impact on the overall synthesis is likely minimal.

Recommendations for Future Research

Future investigations should prioritize standardized methodological practices, including transparent reporting of randomization, blinding, and sample size calculations, to enhance reproducibility and translational relevance. Comparative studies evaluating different CBR targets (CB1, CB2, TRPV1, PPAR-α, GPR55) within identical stroke models would help clarify mechanistic specificity. Dose–response analyses and optimization of therapeutic windows are critically needed, particularly for distinguishing acute versus delayed anti-inflammatory effects. Studies should also incorporate longitudinal assessment of neuroinflammation, integrating molecular, cellular, behavioral, and imaging outcomes. Given the promising anti-inflammatory and neuroprotective effects demonstrated in neonatal and adult models, carefully designed early-phase clinical trials—focusing initially on non-psychoactive agents such as CBD, PEA, and CB2-selective agonists—are warranted. Finally, exploring combinatorial strategies (e.g., CBs plus hypothermia or reperfusion therapies) may reveal synergistic benefits and more feasible translational pathways.

5. Conclusion

CBs have shown promising neuroprotective and anti-inflammatory effects in IS models. By modulating CBRs expressed on glial cells and neurons, CBs reduce neuroinflammation, oxidative stress, and excitotoxicity, which are key contributors to stroke-induced brain damage. Neuroinflammatory modulation by CBs is largely mediated via CBR1 and CBR2, which are present both in neuronal populations and on brain-resident immune cells like

microglia. Preclinical studies consistently report that CBs decrease infarct size, improve neurological outcomes, and mitigate secondary neuronal injury. Despite these encouraging findings, challenges remain in translating CB-based therapies to clinical practice due to side effects and dosage optimization. Nevertheless, targeting the eCB system represents a compelling therapeutic strategy to attenuate neuroinflammation and promote recovery after stroke. Further research is needed to develop safe and effective CB treatments for stroke patients and caution is essential when extrapolating these preclinical findings to human stroke patients, due to inherent biological differences and complexities.

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Conflicts of interest

The authors declare no conflicts of interest.

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References

.1.Chen R, Ovbiagele B, Feng W. Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcomes. *Am J Med Sci* 2016;351(4):380-6. 10.1016/j.amjms.2016.01.011

.2Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011;17(7):796-808. doi: 10.1038/nm

.3Mageed SSA, Ammar RM, Nassar NN, Moawad H, Kamel AS. Role of PI3K/Akt axis in mitigating hippocampal ischemia-reperfusion injury via CB1 receptor stimulation by paracetamol and FAAH inhibitor in rat .*Neuropharmacology* 2022;207:108935. doi: 10.1016/j.neuropharm

.4Zhang W, Tian T, Gong S-X, Huang W-Q, Zhou Q-Y, Wang A-P, Tian Y. Microglia-associated neuroinflammation is a potential therapeutic target for ischemic stroke. *Neural Regen Res* 2021;16(1):6 .11-doi: 10.4103/1673-5374.286954

.5Jayaraj RL, Azimullah S, Beiram R, Jalal FY, Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation* 2019;16:1-24. doi: 10.1186/s12974-019-1516-2

.6Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron* 2010;67(2):181-98. doi: 10.1016/j.neuron.2010.07.002

.7Okada T, Suzuki H, Travis ZD, Zhang JH. The stroke-induced blood-brain barrier disruption: current progress of inspection technique, mechanism, and therapeutic target. *Curr Neuropharmacol* 2020;18(12):1187-212. doi: 10.2174/1570159X18666200528143301

.8Doll DN, Barr TL, Simpkins JW. Cytokines: their role in stroke and potential use as biomarkers and therapeutic targets. *Aging Dis* 2014;5 .294:(5)doi: 10.14336/AD.2014.0500294

.9Ye Y, Zeng Z, Jin T, Zhang H, Xiong X, Gu L. The role of high mobility group box 1 in ischemic stroke. *Front Cell Neurosci* 2019;13:127. doi: 10.3389/fncel.2019.00127

.10Xia C-Y, Zhang S, Gao Y, Wang Z-Z, Chen N-H. Selective modulation of microglia polarization to M2 phenotype for stroke treatment. *Int Immunopharmacol* 2015;25(2):377-82. doi: 10.1016/j.intimp.2015.02.019

.11Przykaza Ł. Understanding the connection between common stroke comorbidities, their associated inflammation, and the course of the cerebral ischemia/reperfusion cascade. *Front Immunol* 2021;12:782569. doi: 10.3389/fimmu.2021.782569

.12Li L, Zhou J, Han L, Wu X, Shi Y, Cui W, et al. The specific role of reactive astrocytes in stroke. *Front Cell Neurosci* 2022;16:850866. doi: 10.3389/fncel.2022.850866

.13Pratt M, Stevens A, Thuku M, Butler C, Skidmore B, Wieland LS, et al. Benefits and harms of medical cannabis: a scoping review of systematic reviews. *Syst Rev* 2019;8:1-35. doi: 10.1186/s13643-019-12-43x

.14Zou S, Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. *Int J Mol Sci* 2018;19(3):833. doi: 10.3390/ijms19030833

.15Cristino L, Bisogno T, Di Marzo V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nat Rev Neurol* 2020;16(1):9-29. doi: 10.1038/s41582-019-0284-z

.16Morales P, Hurst DP, Reggio PH. Molecular targets of the phytocannabinoids: a complex picture. *Prog Chem Org Nat Prod* 2017:103-31. doi: 10.1007/978-3-319-45541-9_4

.17Coelho MP, Duarte P, Calado M, Almeida AJ, Reis CP, Gaspar MM. The current role of cannabis and cannabinoids in health: A comprehensive review of their therapeutic potential. *Life Sci* 2023 .329:121838; doi: 10.1016/j.lfs.2023.121838

.18Pellegrini-Giampietro DE, Mannaioni G, Bagetta G. Post-ischemic brain damage: the endocannabinoid system in the mechanisms of neuronal death. *FEBS J* 2009;276(1):2-12. doi: 10.1111/j.1742-4658.2008.06765.x

.19Hayakawa K, Mishima K, Fujiwara M. Therapeutic potential of non-psychotropic cannabidiol in ischemic stroke. *Pharmaceuticals* 2010;3(7):2197-212. doi: 10.3390/ph3072197

.20England TJ, Hind WH, Rasid NA, O'sullivan SE. Cannabinoids in experimental stroke :a systematic review and meta-analysis. *J Cereb Blood Flow Metab* 2015;35(3):348-58. doi: 10.1038/jcbfm.2014.218

.21Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias .*Stroke* 2004;35(5):1203-8. doi: 10.1161/01.STR.0000125719.25853.20

.22Kossatz E, Maldonado R, Robledo P. CB2 cannabinoid receptors modulate HIF-1α and TIM-3 expression in a hypoxia-ischemia mouse model. *Eur Neuropsychopharmacol* 2016;26(12):1972-88. doi : /10.1016j.euroneuro.2016.10.003

.23Hosoya T, Fukumoto D, Kakiuchi T, Nishiyama S, Yamamoto S, Ohba H, et al. In vivo TSPO and cannabinoid receptor type 2 availability early in post-stroke neuroinflammation in rats: a positron emission tomography study. *J Neuroinflammation* 2017;14(1):69. doi: 10.1186/s12974-017-0851-4

.24Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *J Neurosci* 200 .75-7767:(21)23;3doi: 10.1523/JNEUROSCI.23-21-07767.2003

.25Vandeputte C, Casteels C, Struys T, Koole M, van Veghel D, Evens N, et al. Small-animal PET imaging of the type 1 and type 2 cannabinoid receptors in a photothrombotic stroke model. *Eur J Nucl Med Mol Imaging* 2012;39(11):1796-806. doi: 10.1007/s00259-012-2209-6

.26Ashton JC, Rahman RM, Nair SM, Sutherland BA, Glass M, Appleton I. Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. *Neurosci Lett* 2007;412(2):114-7. doi: 10.1016/j.neulet.2006.10.053

.27Anthony Jalin AM, Rajasekaran M, Prather PL, Kwon JS, Gajulapati V, Choi Y, et al. Non-selective cannabinoid receptor antagonists, hinokiresinols reduce infiltration of microglia/macrophages into ischemic brain lesions in rat via modulating 2-arachidonolyglycerol-induced migration and mitochondrial activity. *PLoS One* 2015;10(10):e0141600. doi: 10.1371/journal.pone.0141600

.28Ju C, Hwang S, Cho G-S, Kondaji G, Song S, Prather PL, et al. Differential anti-ischemic efficacy and therapeutic time window of trans-and cis-hinokiresinols: Stereo-specific antioxidant and anti-inflammatory activities. *Neuropharmacology* 2013;67:465-75. doi: 10.1016/j.neuropharm.2012.12.006

.29Reichenbach ZW, Li H, Ward SJ, Tuma RF. The CB1 antagonist, SR141716A, is protective in permanent photothrombotic cerebral ischemia. *Neurosci Lett* 2016;630:9-15. doi: 10.1016/j.neulet.2016.07.041

.30Ceprián M, Vargas C, García-Toscano L, Penna F, Jiménez-Sánchez L, Achicallende S, et al. Cannabidiol administration prevents hypoxia-ischemia-induced hypomyelination in newborn rats. *Front Pharmacol* 2019;10:1131. doi: 10.3389/fphar.2019.01131

.31Wolff V, Schlagowski A-I, Rouyer O, Charles A-L, Singh F, Auger C, et al. Tetrahydrocannabinol induces brain mitochondrial respiratory chain dysfunction and increases oxidative stress: A potential mechanism involved in cannabis-related stroke. *Biomed Res Int* 2015;2015(1):323706. doi: 10.1155/2015/323706

.32Suzuki N, Suzuki M, Murakami K, Hamajo K, Tsukamoto T, Shimojo M. Cerebroprotective effects of TAK-937, a cannabinoid receptor agonist, on ischemic brain damage in middle cerebral artery occluded rats and non-human primates. *Brain Res* 2012;1430:93-100. doi: 10.1016/j.brainres.2011.10.044

.33Shirazi RS, Vyssotski M, Lagutin K, Thompson D, MacDonald C, Luscombe V, et al. Neuroprotective activity of new Δ3-N-acylethanolamines in a focal ischemia stroke model. *Lipids* 2022;57(1):17-31. doi: 10.1002/lipd.12326

.34Wang S, Yu L, Guo H, Zuo W, Guo Y, Liu H, et al. Gastrodin ameliorates post-stroke depressive-like behaviors through cannabinoid-1 receptor-dependent PKA/rhoA signaling pathway. *Mol Neurobiol* 2025;62(1):366-85. doi: 10.1007/s12035-024-04267-5

.35Wang S, Sun H, Liu S, Wang T, Guan J, Jia J. Role of hypothalamic cannabinoid receptors in post-stroke depression in rats. *Brain Res Bull* 2016;121:91-7. doi: 10.1016/j.brainresbull.2016.01.006

.36Zhang M, Mahadevan A, Amere M, Li H, Ganea D, Tuma RF. Unique effects of compounds active at both cannabinoid and serotonin receptors during stroke. *Transl Stroke Res* 2012;3(3):348-56. doi: 10.1007/s12975-012-0197-2

.37Tian X, Peng J, Zhong J, Yang M, Pang J, Lou J, et al. β-Caryophyllene protects in vitro neurovascular unit against oxygen-glucose deprivation and re-oxygenation-induced injury. *J Neurochem* 2016;139(5):757-68. doi: 10.1111/jnc.13833

.38Contartese A, Valoti M, Corelli F, Pasquini S, Mugnaini C, Pessina F, et al. A novel CB2 agonist, COR167, potently protects rat brain cortical slices against OGD and reperfusion injury. *Pharmacol Res* 2012;66(6):555-63. doi: 10.1016/j.phrs.2012.08.003

.39Serra MP, Boi M, Carta A, Murru E, Carta G, Banni S, Quartu M. Anti-Inflammatory Effect of Beta-Caryophyllene Mediated by the Involvement of TRPV1, BDNF and trkB in the Rat Cerebral Cortex after Hypoperfusion/Reperfusion. *Int J Mol Sci* 2022;23(7). doi: 10.3390/ijms23073633

.40Caltagirone C, Cisari C, Schievano C, Di Paola R, Cordaro M, Bruschetta G, et al. Co-ultramicronized Palmitoylethanolamide/Luteolin in the Treatment of Cerebral Ischemia: from Rodent to Man. *Transl Stroke Res* 2016;7(1):54-69. doi: 10.1007/s12975-015-0440-8

.41Poddighe L, Carta G, Serra MP, Melis T, Boi M, Lisai S, et al. Acute administration of beta-caryophyllene prevents endocannabinoid system activation during transient common carotid artery occlusion and reperfusion. *Lipids Health Dis* 2018;17(1):23. doi: 10.1186/s12944-018-0661-4

.42Pazos MR, Cinquina V, Gómez A, Layunta R, Santos M, Fernández-Ruiz J, Martínez-Orgado J. Cannabidiol administration after hypoxia–ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. *Neuropharmacology* 2012;63(5):776-83. doi: 10.1016/j.neuropharm

.43Fernández-López D, Faustino J, Derugin N, Wendland M, Lizasoain I, Moro MA, Vexler ZS. Reduced infarct size and accumulation of microglia in rats treated with WIN 55,212-2 after neonatal stroke. *Neuroscience* 2012;207:307-15. doi: 10.1016/j.neuroscience.2012.01.008

.44Rahmani MR, Shamsizadeh A, Hakimizadeh E, Allahtavakoli M. Monoacylglycerol Lipase Inhibitor is Safe when Combined with Delayed r-tPA Administration in Treatment of Stroke. *Inflammation* 2018;41(6):2052-9. doi: 10.1007/s10753-018-0848-x

.45Kong D, Xie B, Li Y, Xu Y. PEA prevented early BBB disruption after cerebral ischaemic/reperfusion (I/R) injury through regulation of ROCK/MLC signaling. *Biochem Biophys Res Commun* 2021;566:164-9. doi: 10.1016/j.bbrc.2021.06.019

.46Khaksar S, Bigdeli MR. Intra-cerebral cannabidiol infusion-induced neuroprotection is partly associated with the TNF-α/TNFR1/NF-κB pathway in transient focal cerebral ischaemia. *Brain Inj* 2017;31(13-14):1932-43. doi: 10.1080/02699052.2017.1358397

.47Ahmad A, Genovese T, Impellizzeri D, Crupi R, Velardi E, Marino A, et al. Reduction of ischemic brain injury by administration of palmitoylethanolamide after transient middle cerebral artery occlusion in rats. *Brain Research* 2012;1477:45-58. doi: https://doi.org/10.1016/j.brainres.2012.08.006

.48Garg P, Duncan RS ,Kaja S, Koulen P. Intracellular mechanisms of N-acylethanolamine-mediated neuroprotection in a rat model of stroke. *Neuroscience* 2010;166(1):252-62. doi: 10.1016/j.neuroscience.2009.11.069

.49Zarruk JG, Fernández-López D, García-Yébenes I, García-Gutiérrez MS, Vivancos J, Nombela F, et al. Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. *Stroke* 2012;43(1):211-9 .

.50Lavayen BP, Yang C, Larochelle J, Liu L, Tishko RJ, de Oliveira ACP, et al. Neuroprotection by the cannabidiol aminoquinone VCE-004.8 in experimental ischemic stroke in mice. *Neurochem Int* 2023;165:105508. doi: 10.1016/j.neuint.2023.105508

.51Su SH, Wu YF, Lin Q, Hai J. Cannabinoid receptor agonist WIN55,212-2 and fatty acid amide hydrolase inhibitor URB597 ameliorate neuroinflammatory responses in chronic cerebral hypoperfusion model by blocking NF-κB pathways. *Naunyn Schmiedebergs Arch Pharmacol* 2017;390(12):1189-200. doi: 10/1007.s00210-017-1417-9

.52Yokubaitis CG, Jessani HN, Li H, Amodea AK, Ward SJ. Effects of Cannabidiol and Beta-Caryophyllene Alone or in Combination in a Mouse Model of Permanent Ischemia. *Int J Mol Sci* 2021;22(6). doi: 10.3390/ijms22062866

.53Yu SJ, Reiner D, Shen H, Wu KJ, Liu QR, Wang Y. Time-Dependent Protection of CB2 Receptor Agonist in Stroke. *PLoS One* 2015;10(7):e0132487. doi: 10.1371/journal.pone.0132487

.54Yang M, Lv Y, Tian X, Lou J, An R, Zhang Q, et al. Neuroprotective Effect of β-Caryophyllene on Cerebral Ischemia-Reperfusion Injury via Regulation of Necroptotic Neuronal Death and Inflammation: In Vivo and in Vitro. *Front Neurosci* 2017;11:583. doi: 10.3389/fnins.2017.00583

.55Yu MH, Yang Q, Zhang YP, Wang JH, Zhang RJ, Liu ZG, Liu XC. Cannabinoid Receptor Agonist WIN55, 212-2 Attenuates Injury in the Hippocampus of Rats after Deep Hypothermic Circulatory Arrest. *Brain Sci* 2023;13(3). doi: 10.3390/brainsci13030525

.56Cheng L, Li J, Zhou Y, Zheng Q, Ming X, Liu S. N-Linoleyltyrosine Protects against Transient Cerebral Ischemia in Gerbil via CB2 Receptor Involvement in PI3K/Akt Signaling Pathway. *Biol Pharm Bull* 2019;42(11):1867-76. doi: 10.1248/bpb.b19-00394

.57Carloni S, Crinelli R, Palma L, Álvarez FJ, Piomelli D, Duranti A, et al. The synthetic cannabinoid URB447 reduces brain injury and the associated white matter demyelination after hypoxia-ischemia in neonatal rats. *ACS Chem Neurosci* 2020;11(9):1291-9. doi: 10.1021/acschemneuro.0c00047

.58Schiavon AP, Soares LM, Bonato JM, Milani H, Guimarães FS, Weffort de Oliveira RM. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. *Neurotox Res* 2014;26(4):307-16. doi: 10.1007/s12640-014-94570-

.59Gupta B, Hornick MG, Briyal S, Donovan R, Prazad P, Gulati A. Anti-apoptotic and Immunomodulatory Effect of CB2 Agonist, JWH133, in a Neonatal Rat Model of Hypoxic-Ischemic Encephalopathy. *Front Pediatr* 2020;8:65. doi: 10.3389/fped.2020.00065

.60Caltana L, Saez TM, Aronne MP, Brusco A. Cannabinoid receptor type 1 agonist ACEA improves motor recovery and protects neurons in ischemic stroke in mice. *J Neurochem* 2015;135(3):616-29. doi: 10.1111/jnc.13288

.61Ceprián M, Jiménez-Sánchez L, Vargas C, Barata L, Hind W, Martínez-Orgado J. Cannabidiol reduces brain damage and improves functional recovery in a neonatal rat model of arterial ischemic stroke. *Neuropharmacology* 2017;116:151-9. doi: 10.1016/j.neuropharm.2016.12.017

.62Chen K, Xu B, Xiao X, Long L, Zhao Q, Fang Z, et al. Involvement of CKS1B in the anti-inflammatory effects of cannabidiol in experimental stroke models. *Exp Neurol* 2024;373:114654. doi: 10.1016/j.expneurol.2023.114654

.63Villa M, Martínez-Vega M, Silva L, Romero A, de Hoz-Rivera M, Prados ME, et al. Neuroprotective effects of VCE-004.8 in a rat model of neonatal stroke. *Eur J Pharmacol* 2024;972:176554. doi: 10.1016/j.ejphar.2024.176554

.64Mori MA, Meyer E, Soares LM, Milani H, Guimarães FS, de Oliveira RMW. Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. *Prog Neuropsychopharmacol Biol Psychiatry* 2017;75:94-105. doi: 10.1016/j.pnpbp.2016.11.005

.65Wang DP, Yin H, Kang K, Lin Q, Su SH, Hai J. The potential protective effects of cannabinoid receptor agonist WIN55,212-2 on cognitive dysfunction is associated with the suppression of autophagy and inflammation in an experimental model of vascular dementia. *Psychiatry Res* 2018;267:281-8. doi: 10.1016/j.psychres.2018.06.012

.66Pottier G, Gómez-Vallejo V, Padro D, Boisgard R, Dollé F, Llop J, et al. PET imaging of cannabinoid type 2 receptors with [(11)C]A-836339 did not evidence changes following neuroinflammation in rats. *J Cereb Blood Flow Metab* 2017;37(3):1163-78. doi: 10.1177/0271678x16685105

.67Takheaw N, Jindaphun K, Pata S, Laopajon W, Kasinrerk W. Cannabinoid Receptor 1 Agonist ACEA and Cannabinoid Receptor 2 Agonist GW833972A Attenuates Cell-Mediated Immunity by Different Biological Mechanisms. *Cells* 2023;12(6). doi: 10.3390/cells12060848

.68Zhong P, Wang W, Pan B, Liu X, Zhang Z, Long JZ, et al. Monoacylglycerol Lipase Inhibition Blocks Chronic Stress-Induced Depressive-Like Behaviors via Activation of mTOR Signaling. *Neuropsychopharmacology* 2014;39(7):1763-76. doi: 10.1038/npp.2014.24

.69Li S ,Yang P, Wu Z, Huang W, Zhu X, Zhong L. The effects and mechanisms of AM1241 in alleviating cerebral ischemia-reperfusion injury. *Brain Res Bull* 2024;215:111025. doi: 10.1016/j.brainresbull.2024.111025

.70An D, Peigneur S, Tytgat J. WIN55,212-2, a Dual Modulator of Cannabinoid Receptors and G Protein-Coupled Inward Rectifier Potassium Channels. *Biomedicines* 2021;9(5). doi: 10.3390/biomedicines9050484

.71Sun J, Fang YQ, Ren H, Chen T, Guo JJ, Yan J, et al. WIN55,212-2 protects oligodendrocyte precursor cells in stroke penumbra following permanent focal cerebral ischemia in rats. *Acta Pharmacol Sin* 2013;34(1):119-28. doi: 10.1038/aps.2012.141

.72Di Marzo V, Piscitelli F. The Endocannabinoid System and its Modulation by Phytocannabinoids. *Neurotherapeutics* 2015;12(4):692-8. doi: 10.1007/s13311-015-0374-6

.73Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. *Antioxidants (Basel)* 2019;9(1). doi: 10.3390/antiox9010021

.74Hayakawa K, Irie K, Sano K ,Watanabe T, Higuchi S, Enoki M, et al. Therapeutic time window of cannabidiol treatment on delayed ischemic damage via high-mobility group box1-inhibiting mechanism. *Biol Pharm Bull* 2009;32(9):1538-44. doi: 10.1248/bpb.32.1538

.75Castillo A, Tolón MR ,Fernández-Ruiz J, Romero J, Martinez-Orgado J. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors. *Neurobiol Dis* 2010;37(2):434-40. doi: 10.1016/j.nbd.2009.10.023

.76Béjot Y, Mossiat C, Giroud M, Marie C. Circulating and Brain BDNF Levels in Stroke Rats. Relevance to Clinical Studies. *PLoS One* 2011;6:e29405. doi: 10.1371/journal.pone.0029405

.77Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D, Tuma RF. Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. *Neuroscience* 2008;152(3):753-60. doi: 10.1016/j.neuroscience.2008.01.022

.78Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, Piomelli D. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol Pharmacol* 2005;67(1):15-9. doi: 10.1124/mol.104.006353

.79Maisel M, Herr A, Milosevic J ,Hermann A, Habisch HJ, Schwarz S, et al. Transcription profiling of adult and fetal human neuroprogenitors identifies divergent paths to maintain the neuroprogenitor cell state. *Stem Cells* 2007;25(5):1231-40. doi: 10.1634/stemcells.2006-0617