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A novel insight into the neuroprotective effects of cannabidiol: maintained apelin/dopamine synthesis, NRF2 signaling, and AKT/CREB/BDNF gene expressions

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Neuroinflammation is a process associated with degeneration and loss of neurons in different parts of the brain. The most important damage mechanisms in its formation are oxidative stress and inflammation. This study aimed to investigate the protective effects of cannabidiol (CBD) against neuroinflammation through various mechanisms. Thirty-two female rats were randomly divided into 4 groups as control, lipopolysaccharide (LPS), LPS + CBD and CBD groups. After six hours following LPS administration, rats were sacrificed, brain and cerebellum tissues were obtained. Tissues were stained with hematoxylin-eosin for histopathological analysis. Apelin and tyrosine hydroxylase synthesis were determined immunohistochemically. Total oxidant status and total antioxidant status levels were measured, and an oxidative stress index was calculated. Protein kinase B (AKT), brain-derived neurotrophic factor (BDNF), cyclic-AMP response element-binding protein (CREB) and nuclear factor erythroid 2-related factor 2 (NRF2) mRNA expression levels were also determined. In the LPS group, hyperemia, degeneration, loss of neurons and gliosis were seen in all three tissues. Additionally, Purkinje cell loss in the cerebellum, as well as neuronal loss in the cerebral cortex and hippocampus, were found throughout the LPS group. The expressions of AKT, BDNF, CREB and NRF2, apelin and tyrosine hydroxylase synthesis all decreased significantly. CBD treatment reversed these changes and ameliorated oxidative stress parameters. CBD showed protective effects against neuroinflammation *via* regulating AKT, CREB, BDNF expressions, NRF2 signaling, apelin and tyrosine hydroxylase synthesis.

Key words: apelin, cannabidiol, dopamine, learning and memory, tyrosine hydroxylase

INTRODUCTION

Neuroinflammation is observed in various diseases, from acute cerebrovascular conditions to infections. It is responsible for diverse neurological symptoms, including damage to the cerebral cortex, cognitive decline due to hippocampal involvement, memory impairment, and cerebellar-related balance issues (Jurcau and Simion, 2022). At the cellular level, neuroinflammation involves the inflammatory response, oxidative stress, and apoptosis. Understanding these mechanisms is vital for developing effective treatments for preventing nervous system damage (Gu et al., 2021).

Apelin synthesized in the central nervous system (CNS), regulates neurotrophic factors, oxidative stress, neuroinflammation, and apoptosis through its receptor, APJ. Recently, studies conducted particularly in brain regions, such as the hippocampus, reveal

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protective properties of apelin. It has been found to enhance learning and memory *via* the protein kinase B (AKT)/cyclic-AMP response element binding protein (CREB)/brain-derived neurotrophic factor (BDNF) pathway, while also mitigating oxidative stress through the phospholipase C (PLC)/glycogen synthase kinase 3 beta (GSK3β)/nuclear factor erythroid 2-related factor 2 (NRF2) pathway (Duan et al., 2019; Jiang et al., 2019; Li et al., 2023).

AKT, an anti-apoptotic protein kinase, protects hippocampal tissue by activating the CREB/BDNF pathway through phosphorylation (Alfaris et al., 2021). CREB is a transcription factor that enhances synaptic potentiation and dendritic arborization while triggering BDNF expression, which then mediates anti-inflammatory, antioxidant, and anti-apoptotic mechanisms (Faria et al., 2014). BDNF is primarily found in the hippocampus, cortex, and basal ganglia and is crucial for synaptic plasticity, neuronal survival, and differentiation. Its role in protecting against hippocampal damage and memory deficits in neurodegenerative diseases has been studied extensively (Dechant and Neumann, 2003). BDNF binds to the Trk-B receptor, enhancing learning and memory through the phosphatidylinositol 3-kinase (PI3K)/AKT/CREB pathway while simultaneously acting as an anti-inflammatory agent by inhibiting nuclear factor kappa B (NF-κB) expression (Sun et al., 2021).

AKT activation not only enhances the CREB/BDNF pathway, but also increases the levels of antioxidant enzymes. This effect is mediated through NRF2, which is regulated by the downstream mechanism of PLC/GSK3β/NRF2 *via* GSK3β (Joshi et al., 2015). Stimulation of this pathway by the apelin/APJ system thus strengthens the antioxidant effect as nuclear transcription factor NRF2, translocates to the nucleus and binds to the antioxidant-response element, upregulating antioxidant activities (Loboda et al., 2016, Tapias et al., 2018).

Recent studies have also shown that BDNF promotes synaptic transmission by increasing release of dopamine from presynaptic neurons *via* Trk-B (Blöchl and Sirrenberg, 1996). In sympathetic nervous system neurons, however, studies have shown that neuronal damage may lead to a decrease in dopamine levels. This is due to a downregulation of tyrosine hydroxylase (TH) levels; the enzyme responsible for synthesis of levodopa (L-DOPA) and dopamine from tyrosine, which may affect learning and memory, especially in Parkinson's disease (Castillo Díaz et al., 2021).

Cannabidiol (CBD) is an active ingredient derived from the *Cannabis sativa* plant. CBD is a potent regulator of oxidative stress, through scavenging of reactive oxygen species (ROS) and reducing lipid peroxidation. CBD is therefore considered to be a putative therapeutic agent against neuronal damage and is used as an antiepileptic, however detailed molecular mechanisms regarding its tissue-protective effects on the CNS remain to be fully elucidated (Capasso et al., 2008; Kozela et al., 2017).

Our study aimed to investigate the neuroprotective effects of CBD against lipopolysaccharide (LPS)-induced neuroinflammation. We aimed to examine the involvement of apelin and dopamine synthesis, NRF2 signaling, and the AKT/CREB/BDNF pathways in an attempt to elucidate CBD-associated protective mechanisms.

METHODS

Experimental design

Thirty-two adult female albino Wistar rats weighing 250-350 g used in the experiments were housed at 21-22°C and 60% ± 5% humidity, subjected to 12-hour light: 12-hour dark cycle and fed with standard commercial feed *ad libitum* with water. All rats in this study were divided into 4 groups (each containing eight rats) after they were obtained from Suleyman Demirel University Experimental Animals Laboratory. The neuroinflammation model was implemented through LPS induction, as previously employed in other studies (Ozdamar Unal et al., 2022, Savran et al., 2019; 2020). CBD doses were also selected from previous studies (Fouad et al., 2013; Bing-Tian Xu et al., 2023). Rats were treated according to the following groups: Control group [0.5 ml saline (SF) and 0.1 ml solvent solution (0.9% NaCl + Tween 80) were administered intraperitoneally (i.p.) from the right and left inguinal regions respectively], LPS group [5 mg/kg dose LPS (Sigma Aldrich, ABD) dissolved in SF was applied i.p. from the right inguinal region, and 0.1 ml of solvent solution was applied i.p. from the left inguinal region simultaneously (Cao et al., 2017)], LPS+CBD group [5 mg/kg LPS was applied i.p. from the right inguinal region, and 5 mg/kg CBD in a 0.1 ml volume of solvent solution was applied i.p. from the left inguinal region], CBD group [0.5 ml SF was applied i.p. from the right inguinal region, and 5 mg/kg CBD in a 0.1 ml volume of solvent solution was applied i.p from the left inguinal region].

In all groups, drug administrations have been carried out only once. After six hours following the drug administration, experimental animals were sacrificed under anesthesia using 80-100 mg/kg ketamine (Alfamin, Alfasan IBV) and 8-10 mg/kg xylazine (Bioveta, Czech Republic). Following abdominal incision, euthanasia was performed by surgical exsanguination. Blood

was obtained from the v. cava inferior, and brain tissues were removed following decapitation. Half of the removed brain tissues were preserved in 10% buffered formalin under conditions suitable for histopathological and immunohistochemical analysis. Remaining brain tissues were stored at -20°C for biochemical and -80°C for genetic analysis.

All experiments conducted on animals in this study have been performed according to guidelines for Animal Research: Reporting in Vivo Experiments (ARRIVE) 2.0. The experimental protocol was approved by the local animal experiments ethics committee of Suleyman Demirel University with the number: 15.12.2022/100-08. Experiments were carried out in accordance with this protocol.

Reagents

CBD was obtained from Suleyman Demirel University, Natural Products Application and Research Center. The source of the CBD was the extract of *Cannabis sativa L.* (Cannabaceae). CBD content was >99.9, and the tetrahydrocannabinol content was <0.01. Limits of residual alcohol and heavy metals comply with USP and EU pharmacopeiae. LPS (L2630-100 mg) was obtained from Sigma Aldrich, Sweden and dissolved in SF.

Histology

Brains were delicately removed during the necropsy and fixed in 10% buffered formalin for histological examination. The tissues were then routinely processed with a fully automatic tissue processor, and a rotary microtome was used to cut 5 µm thick sections from the paraffin blocks (Leica RM2155, Leica Microsystems, Wetzlar, Germany). The sections were then deparaffinized, rehydrated with ethanol in decreasing concentrations, stained with hematoxylin-eosin (HE), cleaned in xylene, and cover slipped. The evaluation of histopathological alterations was done under a light microscope.

Histopathological lesions of different brain regions received semiquantitative scoring. Hyperemia, hemorrhage, gliosis, and neuronal damage were assessed for this purpose. According to the severity, the descriptions were assigned scores ranging from 0 to 3 (Aslankoc, 2022). The scoring system for the histology results is 0: No lesions, 1: mild damage lesions in <20% of the fields, 2: moderate damage lesions in 20-60% of the fields, 3: severe damage lesions in all fields. Histopathology was scored in a blinded manner. Score analyses were performed using ImageJ 1,48 version (National Institutes of Health, Bethesda, MD).

Immunohistochemistry

Sections taken onto polylysine coated slides were immunostained with apelin [Rabbit Anti-Apelin polyclonal antibody] (bs2425R), Bioss Antibodies, USA, 1/100 dilution], and TH [Rabbit Anti-Tyrosine Hydroxylase Polyclonal Antibody (bs-0016R) (Bioss Antibodies Inc., ABD)] using the streptavidin biotin technique. All primary antibodies were used at 1:100 dilutions. Sections were immunohistochemically stained with primary antibodies for 60 min and then incubated with biotinylated secondary antibodies and streptavidin-alkaline phosphatase conjugates. The secondary antibody and chromogen were obtained from a ready-to-use commercial kit [Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) from Abcam (Cambridge, UK)]. The antibody diluent solution was used for negative controls instead of primary antiserum. All tests were carried out using blinded samples. The following grading scale was applied for the semiquantitative analysis of the immunohistochemical scores: (0) negative staining, (1) focal and weak staining, (2) diffuse and weak staining, and (3) diffuse and marked staining. In each section, 10 separate areas were examined under 40X objective magnification. The Database Manual Cell Sens Life Science Imaging software system (Olympus Co., Tokyo, Japan) was used for microphotography and morphometric analysis.

Biochemical analyzes

In the determination of oxidative stress in brain tissue, samples were centrifuged at 10,000 rpm for 10 min after homogenization. Total antioxidant status (TAS) and total oxidant status (TOS) levels were measured using the colorimetric method developed by Erel from the supernatants obtained after homogenization with a Beckman Coulter AU 5800 automatic analyzer (Beckman Coulter, USA). An oxidative stress index (OSI) value was determined by calculating OSI = [(TOS, µmol H_2O_2 Eq/l) / (TAS, mmol Trolox Eq/l) × 100] (Erel, 2004; 2005).

RT qPCR analyzes

Using the manufacturer's protocol, RNA was isolated from homogenized tissues with the GeneAll Ri-

boEx (TM) RNA Isolation Kit (GeneAll Biotechnology, Seoul, Korea). The concentration and purity of the RNAs obtained were measured using the BioSpec-nano nanodrop (Shimadzu Ltd. Kyoto, Japan) device. 1 µg RNA was used for cDNA synthesis. cDNA synthesis, A.B.T.™ cDNA Synthesis Kit (Atlas Biotechnology, Turkey) was carried out in a thermal cycler according to the protocol. Primer designs were made by detecting specific mRNA sequences and testing possible primer sequences using the NCBI website. The primer sequences used are shown in Table 1. Expression levels of genes were measured in a Biorad CFX96 (California, USA) real-time PCR instrument using A.B.T.™ cDNA Synthesis Kit (Atlas Biotechnology, Turkey). The GAPDH gene was used as a housekeeping gene. The reaction mixture was prepared according to the manufacturer's protocol to a final volume of 20 µl. The resulting reaction mixture was placed in a real-time qPCR device with thermal cycling determined according to kit specifications in triplicates. PCR conditions, initial denaturation 95°C 300 sec 1 cycle, denaturation 95°C 15 sec and annealing/extension 60°C 30 sec were applied to 40 cycles. Relative mRNA levels were calculated by applying the $2^{-\Delta\Delta Ct}$ formula to the normalized results (Livak and Schmittgen, 2001). The entire study was performed in accordance with the MIQE protocol (Bustin et al., 2009).

Table 1. Primary sequences of genes and product size.

Genes	Primary sequence	Product size
GAPDH (HouseKeeping)	F: AGGTTGTCTCCTGTGACTTC R: CTGTTGCTGTAGCCATATTC	130 bp
BDNF	F: AGAATGAGGGCGTTTGCGTA R: CCTGGTGGAACATTGTGGCT	280 bp
CREB	F: TCAGCCGGGTACTACCATTC R: CCTCTCTCTTTCGTGCTGCT	282 bp
AKT	F: AGTCCCCACTCAACAACTTCT R: GAAGGTGCGCTCAATGACTG	119 bp
NRF2	F: GCCTTCCTCTGCTGCCATTAGTC R: TCATTGAACTCCACCGTGCCTTC	126 bp

(F) Forward; (R) Reverse; (GAPDH) Glyceraldehyde 3 phosphate dehydrogenase; (BDNF) brain-derived neurotrophic factor; (CREB) cAMP responsive element binding protein; (AKT) Protein Kinase B; (NRF2) Nuclear factor erythroid 2-related factor 2.

Statistical analyzes

Immunohistochemical and genetic scores, tissue TAS, TOS, and OSI levels were compared between the groups. For this purpose, the One-way ANOVA with *post-hoc* Bonferroni tests were implemented using SPSS-22.00 (SPSS Inc., Chicago, IL, USA) software. The level of significance was considered at $P<0.05$.

RESULTS

Histopathological and immunohistochemical results

The control group showed no findings during the histological investigations compared to the LPS group. However, LPS caused severe hyperemia, slight degeneration, and loss of neurons with minimal gliosis in the cortex. Additionally, Purkinje cell loss in the cerebellum was noticed in the LPS group compared to other groups. The lesions improved after receiving CBD treatment, but the results were insignificant (Fig. 1) (Table 2).

The cortex, hippocampus and cerebellum exhibited decreased apelin expression in the LPS group during the immunohistochemical examination when compared to all other groups. In the LPS+CBD group, apelin expressions were significantly lower compared to the control group in the cortex and hippocampus regions but not in the cerebellum (Fig. 2). Although noticeable TH expressions were observed in control and CBD treated groups, marked decreases in expression were seen in LPS administered groups. CBD treatment caused an increase in TH expressions in the LPS + CBD group (Fig. 3) (Table 2).

Biochemical results showed that in the LPS group, TOS and OSI levels increased while TAS levels decreased when compared to the control group. In the LPS+CBD group, TOS and OSI levels were lower respectively, while TAS levels were higher when compared to the LPS group ($P>0.05$). In the CBD group, TOS and OSI levels decreased, and TAS levels increased when compared to the LPS group. TOS was also found to be significantly lower when compared to the control group ($P=0.015$) (Fig. 4).

RT qPCR results showed that in the LPS group, BDNF, CREB, AKT, and NRF2 expressions decreased significantly when compared to the control group. All these mRNA expressions increased both in the LPS+CBD and CBD when compared to the LPS group (Fig. 5, Table 3).

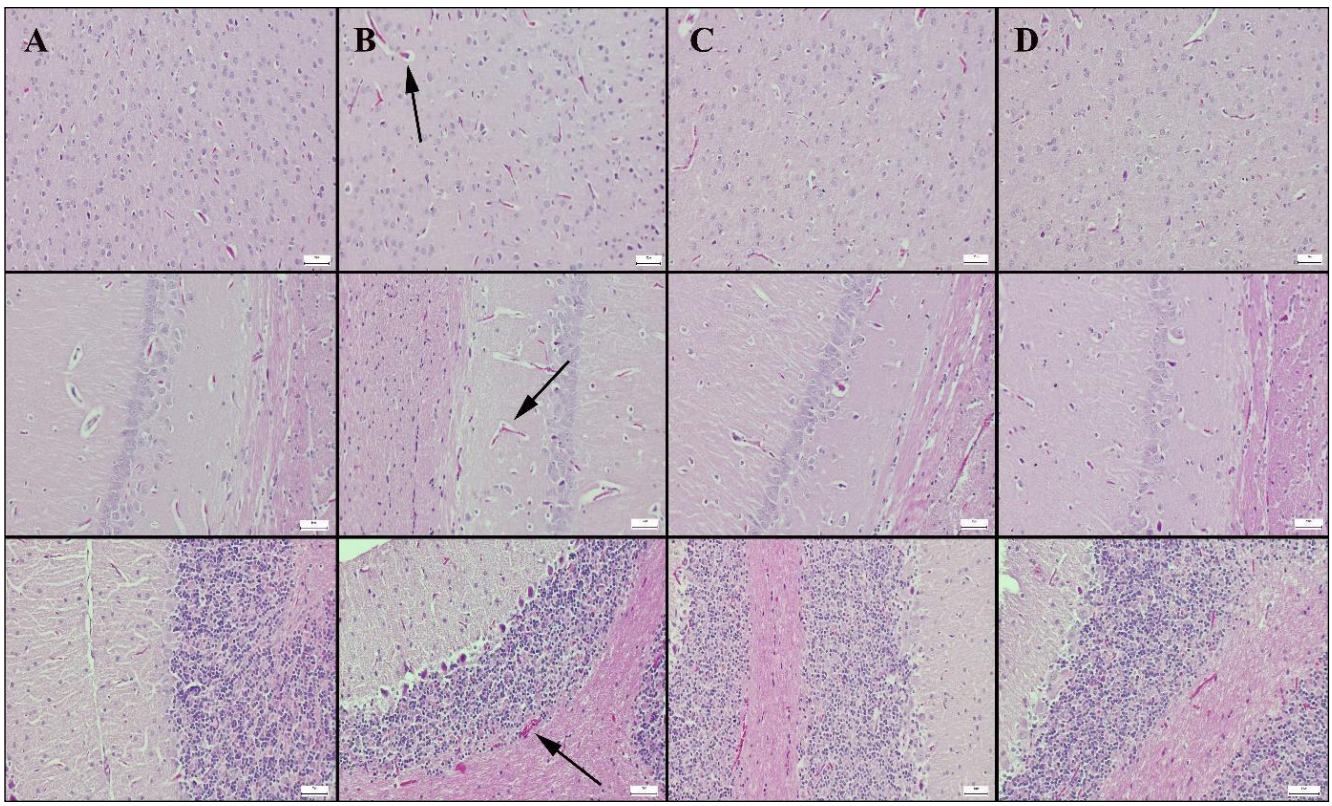

Fig. 1. Histopathological findings of cortex (upper row), hippocampus (medium row) and cerebellum (below row) tissues between the groups. (A) Normal tissue histoarchitecture in control group, (B) marked hyperemia (arrow) in LPS group, (C) decrease in pathological findings in LPS+CBD group, (D) normal brain, hippocampus and cerebellum histology in CBD group, HE, scale bars = 50 μm.

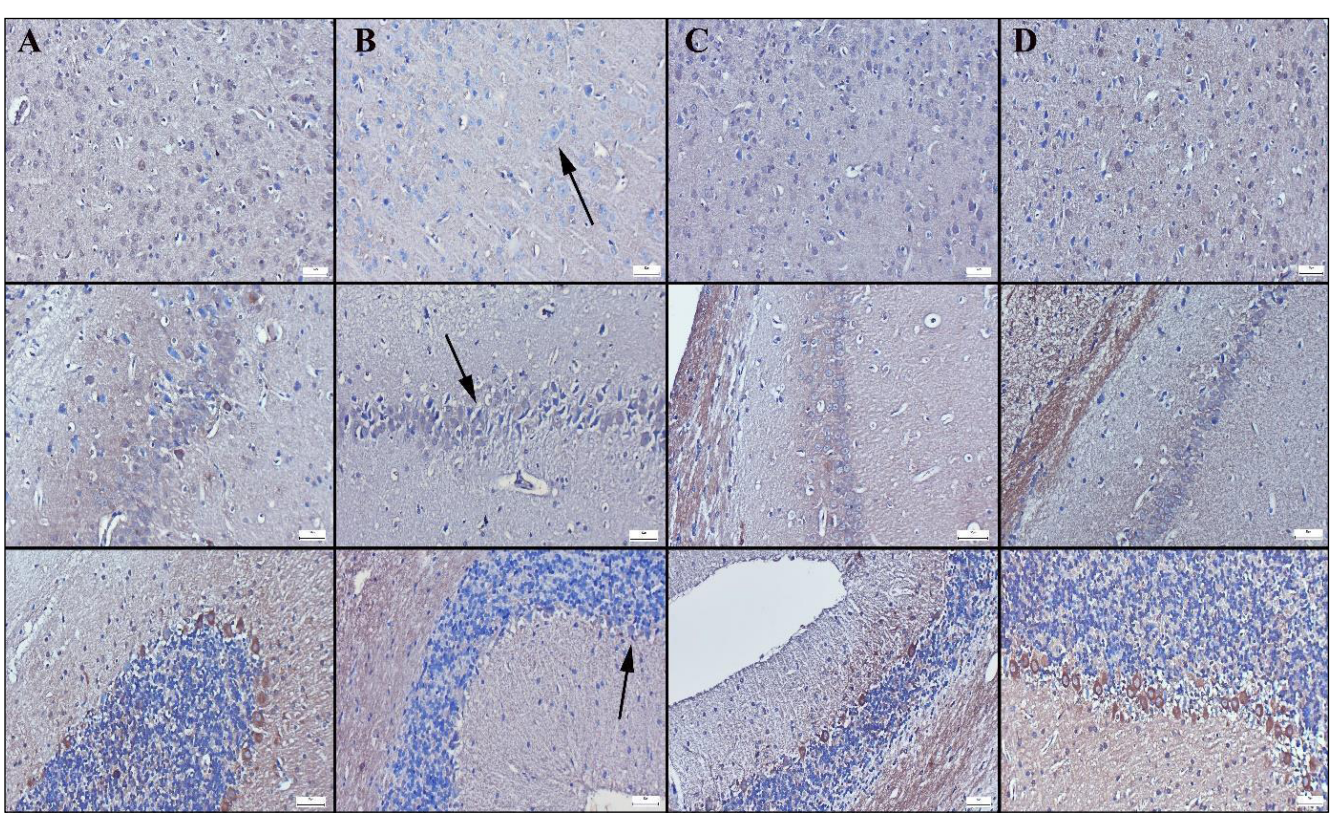

Fig. 2. Apelin expressions in cortex (upper row), hippocampus (medium row) and cerebellum (below row) tissues between the groups. (A) Marked expressions in control group, (B) marked decrease of expressions (arrows) in LPS group, (C) moderate increase of expressions in LPS+CBD group, (D) marked increase of expressions in CBD group, Streptavidin biotin peroxidase method, scale bars = 50 μm.

Table 2. Statistical analysis results of histopathological and immunohistochemical scores between the groups.

		Control	LPS	LPS+CBD	CBD
HP	Cortex *F*3,28=14.075, *P*<001	0.00±0.00	1.00±0.53	0.62±0.51	0.00±0.00
			p<0.001[a]	**p=0.014[a]**	p>0.05[a]
				p=0.321[b]	**p<0.001[b]**
					p=0.014[c]
	Hippocampus *F*3,28=8.136, *P*<001	0.00±0.00	0.87±0.64	0.50±0.18	0.00±0.00
			p<0.001[a]	p=0.141[a]	p>0.05[a]
				p=0.498[b]	**p<0.001[b]**
					p=0.141[c]
	Cerebellum *F*3,28=10.818, *P*<001	0.00±0.00	1.00±0.75	0.75±0.46	0.00±0.00
			p<0.001[a]	**p=0.013[a]**	p>0.05[a]
				p>0.05[b]	**p<0.001[b]**
					p=0.013[c]
Apelin	Cortex *F*3,28=17.203, *P*<001	2.75±0.46	1.00±0.53	2.00±0.53	2.37±0.51
			p<0.001[a]	**p=0.041[a]**	p=0.93[a]
				p=0.003[b]	**p<0.001[b]**
					p=0.93[c]
	Hippocampus *F*3,28=27.190, *P*<001	2.25±0.46	0.37±0.18	1.37±0.51	2.25±0.46
			p<0.001[a]	**p=0.005[a]**	p>0.05[a]
				p<0.001[b]	**p<0.001[b]**
					p=0.02[c]
	Cerebellum *F*3,28=21.086, *P*<001	2.75±0.46	1.12±0.64	2.25±0.46	2.87±0.35
			p<0.001[a]	p=0.307[a]	p>0.05[a]
				p<0.001[b]	**p<0.001[b]**
					p=0.1[c]
TH	Cortex *F*3,28=31.218, *P*<001	2.75±0.46	0.62±0.51	2.62±0.51	2.50±0.53
			p<0.001[a]	p>0.05[a]	p>0.05[a]
				p<0.001[b]	**p<0.001[b]**
					p>0.05[c]
	Hippocampus *F*3,28=32.709, *P*<001	2.25±0.46	0.50±0.18	2.37±0.51	2.75±0.46
			p<0.001[a]	p>0.05[a]	**p=0.032[a]**
				p<0.001[b]	**p<0.001[b]**
					p=0.848[c]
	Cerebellum *F*3,28=22.221, *P*<001	2.62±0.51	0.87±0.64	2.62±0.51	2.75±0.46
			p<0.001[a]	p>0.05[a]	p>0.05[a]
				p<0.001[b]	**p<0.001[b]**
					p>0.05[c]

Data expressed mean ± standard deviation (SD). One-way ANOVA *post-hoc* Bonferroni test. a: compared to the control group, b: compared to the LPS group, c: compared to the LPS+CBD group. Statistically significant p values have been outlined in bold. (LPS) Lipopolysaccharide; (CBD) Cannabidiol; (HP) Histopathology; (TH) Tyrosine hydroxylase.

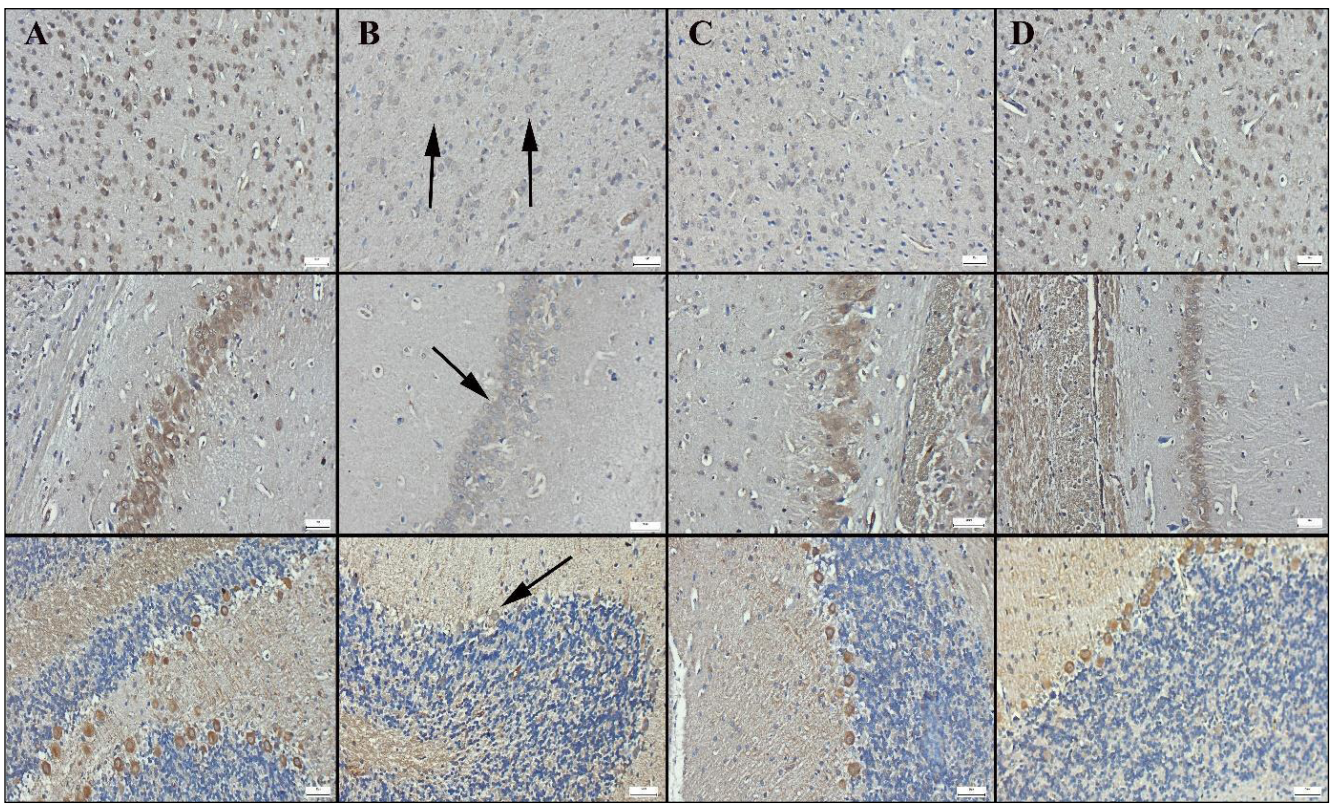

Fig. 3. TH expressions in cortex (upper row), hippocampus (medium row) and cerebellum (below row) tissues between the groups. (A) Marked expressions in control group, (B) marked decrease of expressions (arrows) in LPS group, (C) increased expressions in LPS+CBD group, (D) marked expression in CBD group, Streptavidin biotin peroxidase method, scale bars = 50 µm.

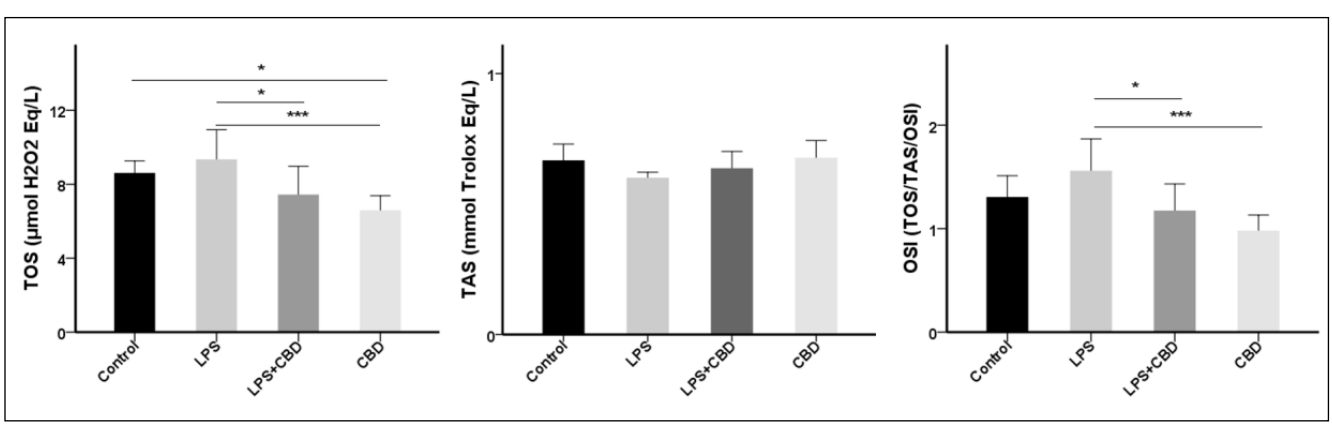

Fig. 4. Oxidative stress parameters of brain cortex tissues. In order to demonstrate the protective effects of CBD against neuroinflammation, levels of TOS, TAS, and OSI were determined. Significant differences between groups have been represented with lines. The stars above the lines indicate the degree of significance between the groups at the beginning and end of the lines. "*" represents p<0.05, "**" represents p<0.01, "***" represents p<0.001. Values are presented as means ± SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA, *post-hoc* Bonferroni test. LPS: Lipopolysaccharide, CBD: Cannabidiol, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index.

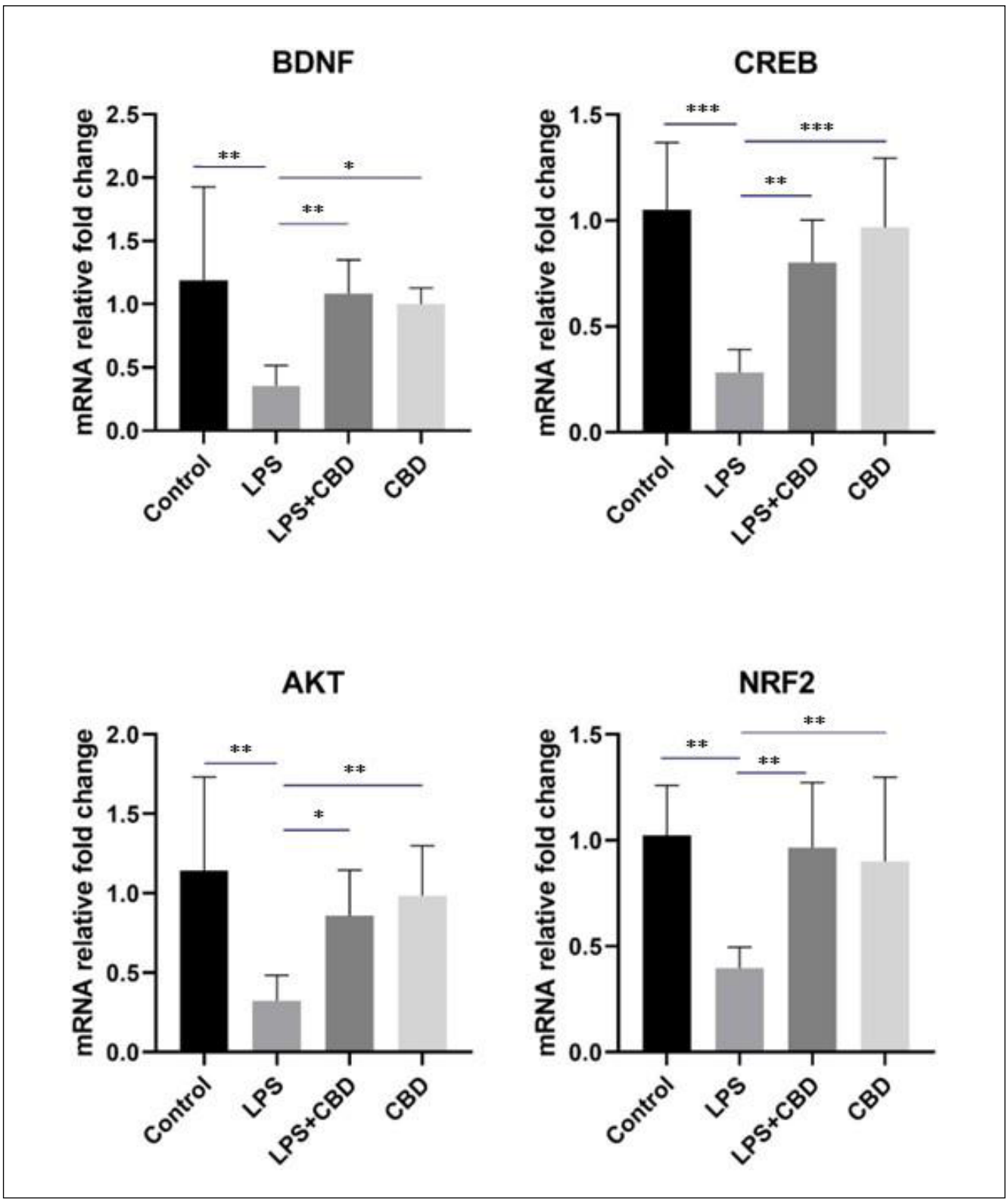

Fig. 5. mRNA relative fold change graph of genes. The expressions of the specified genes were examined using qRT-PCR in order to elucidate intracellular molecular mechanisms influenced by CBD. Significant differences between groups have been represented with lines. The stars above the lines indicate the degree of significance between the groups at the beginning and end of the lines. "*" represents $p<0.05$, "**" represents $p<0.01$, "***" represents $p<0.001$. Statistical analysis was performed with one-way ANOVA, *post-hoc* Bonferroni test. LPS: Lipopolysaccharide, CBD: Cannabidiol, BDNF: brain-derived neurotrophic factor, CREB: cAMP responsive element binding protein, AKT: Protein kinase B, NRF2: Nuclear factor erythroid 2-related factor 2.

Table 3. Mean±SD and statistical analysis of genes.

	Control Mean±SD	LPS Mean±SD	LPS+CBD Mean±SD	CBD Mean±SD	p value
BDNF *F*3,28=6.974, *P*=001	1.19±0.73	0.36±0.16	1.08±0.26	1.00±0.13	*P*=0.002[a]
					P=0.007[b]
					P=0.020[c]
CREB *F*3,28=14.653, *P*<001	1.05±0.32	0.28±0.11	0.80±0.20	0.97±0.33	*P*<0.001[a]
					P=0.002[b]
					P<0.001[c]
AKT *F*3,28=7.309, *P*=001	1.14±0.59	0.32±0.16	0.86±0.29	0.98±0.31	*P*=0.001[a]
					P=0.044[b]
					P=0.008[c]
NRF2 *F*3,28=8.296, *P*<001	1.02±0.24	0.39±0.01	0.96±0.31	0.90±0.40	*P*=0.001[a]
					P=0.002[b]
					P=0.008[c]

Statistical analysis of mRNA relative fold change Ct values of genes was performed with one-way ANOVA *post-hoc* Bonferroni test. [a] Control *vs.* LPS, [b] LPS *vs.* LPS+CBD, [c] LPS *vs.* CBD. (LPS) Lipopolysaccharide; (CBD) Cannabidiol; (BDNF) brain-derived neurotrophic factor; (CREB) cAMP responsive element binding protein; (AKT) Protein kinase B; (NRF2) Nuclear factor erythroid 2-related factor 2.

DISCUSSION

The results presented in this study have the potential to enhance the molecular understanding of CBD's neuroprotective effects, contributing to the existing literature. In particular, the Association of CBD's well-documented anti-inflammatory effects with its ability to enhance apelin and dopamine synthesis highlights the innovative aspect of this research. Furthermore, demonstrating the positive effects of CBD on oxidative stress linked to the APJ receptor, AKT, and NRF2 signaling pathways is also among the significant findings of our study.

It is well-established that pathological events in brain tissue are associated with oxidative stress, inflammation, and apoptosis, which can mutually exacerbate each other. This cascade of events has been demonstrated to result in cumulative damage to cells, causing widespread tissue damage, particularly in acute events (Gu et al., 2021).

One of the mechanisms through which oxidative damage occurs in brain tissue is the binding of prooxidant substances to their receptors. However, since antioxidant enzymes are mainly utilized during times of damage, their levels tend to be low, leading to a shift in the balance of antioxidant-oxidant ratios leaning towards a state of higher oxidant levels (Teleanu et al., 2022). In this study, we observed similar results, with decreases in TAS levels and increases in OSI levels in the injury group. This was alleviated by CBD treatment, indicating the antioxidant property of the drug, which has been corroborated by other groups. However, since there was no increase in TAS levels in the CBD group compared to the control group, it is suggested that the protective effect of CBD may have been due to the suppression of the inflammatory process. It is worth noting that our study utilized an acute injury model of short duration with only a single dose administered. It is possible that longer, repeated use of CBD may lead to an increase in antioxidant enzyme synthesis, as observed in a rat model of endometriosis by Okten et al. (2023) as they found that 7 days of CBD treatment significantly increased TAS levels.

In the acute inflammatory model, the development of neuroinflammation is apparent by pathological changes such as hyperemia, degeneration, neuronal loss, and gliosis in the cortex, hippocampus, and cerebellum regions of the injury group. These pathological processes may interrupt learning and memory mechanisms. Moreover, Purkinje cells in the cerebellar region play a crucial role in the formation of motor memory by sending information to the vestibular nucleus in the brainstem and then to the cortex during vestibulo-ocular reflex learning (Jang et al., 2020). CBD treatment results in the regression of all pathological

processes, indicating the drug's anti-inflammatory and neuroprotective effects on brain tissue. Consistent with this, Garcia-Baos et al. (2021) reported that CBD improves neuroinflammation and cognitive functions in their study. It should be noted that such drugs may not only have a protective effect on tissues but also increase the amount of anti-inflammatory proteins synthesized from tissues. Conversely, it should not be forgotten that their levels may decrease with neuronal loss (Kim et al., 2020).

Apelin is a protein associated with the prevention of neuronal damage, especially within the CNS. It binds to the APJ receptor on the cell surface and exerts neuronal anti-apoptotic and antiedema effects through the PI3K/AKT pathway (Liu et al., 2020). Furthermore, apelin can block the nod-like receptor pyrin domain-containing 3 inflammasome through AMPK signaling and prevent oxidative stress with its anti-inflammatory effect and phospholipase C-mediated NRF2 activation (Zhang et al., 2018; Duan et al., 2019). This study demonstrates that the reduction in apelin levels observed in the damaged groups, resulting from neuronal degeneration and loss, is consistent with the evidence of oxidative stress and neuroinflammation. This suggests that the neuroprotective effect of CBD is associated with the upregulation of apelin expression in remaining intact neurons. The protective role of apelin in neuroinflammation in previous studies also supports our findings (Luo et al., 2019; Xu et al., 2019; Hu et al., 2022).

Observation of the effects of apelin in all three parts of the brain demonstrates its potential to support various functions such as locomotor control *via* cortex protection, balance regulation *via* cerebellum protection, and cognitive processes, including learning and memory *via* hippocampal protection. Given the crucial role of the hippocampus in cognitive functions, any damage to this region can have negative impacts on daily activities and cognitive abilities (Jiang et al., 2019). Histopathological and immunohistochemical analyses have confirmed that CBD can protect hippocampal tissues and thereby maintain cognitive function.

The AKT/CREB/BDNF pathway plays a critical role in the mechanisms underlying learning and memory, and has been shown to exert a protective effect on brain tissue. In this study, significant inhibition of these gene expressions was observed in the damage group, which was reversed with CBD treatment, suggesting that the drug may contribute to reducing the damage caused by oxidative stress and neuroinflammation in affected cells. In accordance with these findings, Winstone et al. (2023) demonstrated that CBD has protective effects through BDNF mRNA expression. Additionally, CBD was shown to activate the AKT/GSK3β pathway as conducted by Corrê et al. (2022) which further supports our findings. If levels of gene expression were increased in the CBD-administered groups compared to the control group, it could be hypothesized that CBD also has a direct effect on gene synthesis. The findings of this study suggest that CBD is neuroprotective and may facilitate the continuity of learning and memory through its antioxidant and anti-inflammatory properties. CBD and its effective parts in neuroprotection mechanisms are summarized in Fig. 6. A significant limitation in our study was the absence of cognitive tests related to learning and memory function. Therefore, we aim to complement the molecular findings within this study with clinical tests in future studies. The results presented in this study have the potential to enhance the molecular understanding of CBD's neuroprotective effects, contributing to existing literature. In particular, the association of CBD's well-documented anti-inflammatory effects and its ability to enhance apelin and dopamine synthesis highlights the innovative aspect of this research. Furthermore, demonstrating the positive effects of CBD on oxidative stress linked to the APJ receptor, AKT, and NRF2 signaling pathways is also among the significant findings of our study.

CONCLUSIONS

In conclusion, CBD has shown neuroprotective effects against neuroinflammation in various regions of the brain tissue *via* the AKT/CREB/BDNF pathway. Because of this protective feature, learning and memory-related gene expressions were preserved in intact brain tissues. In future studies, the intracellular pathways used in this protective effect mechanism should be further elucidated.

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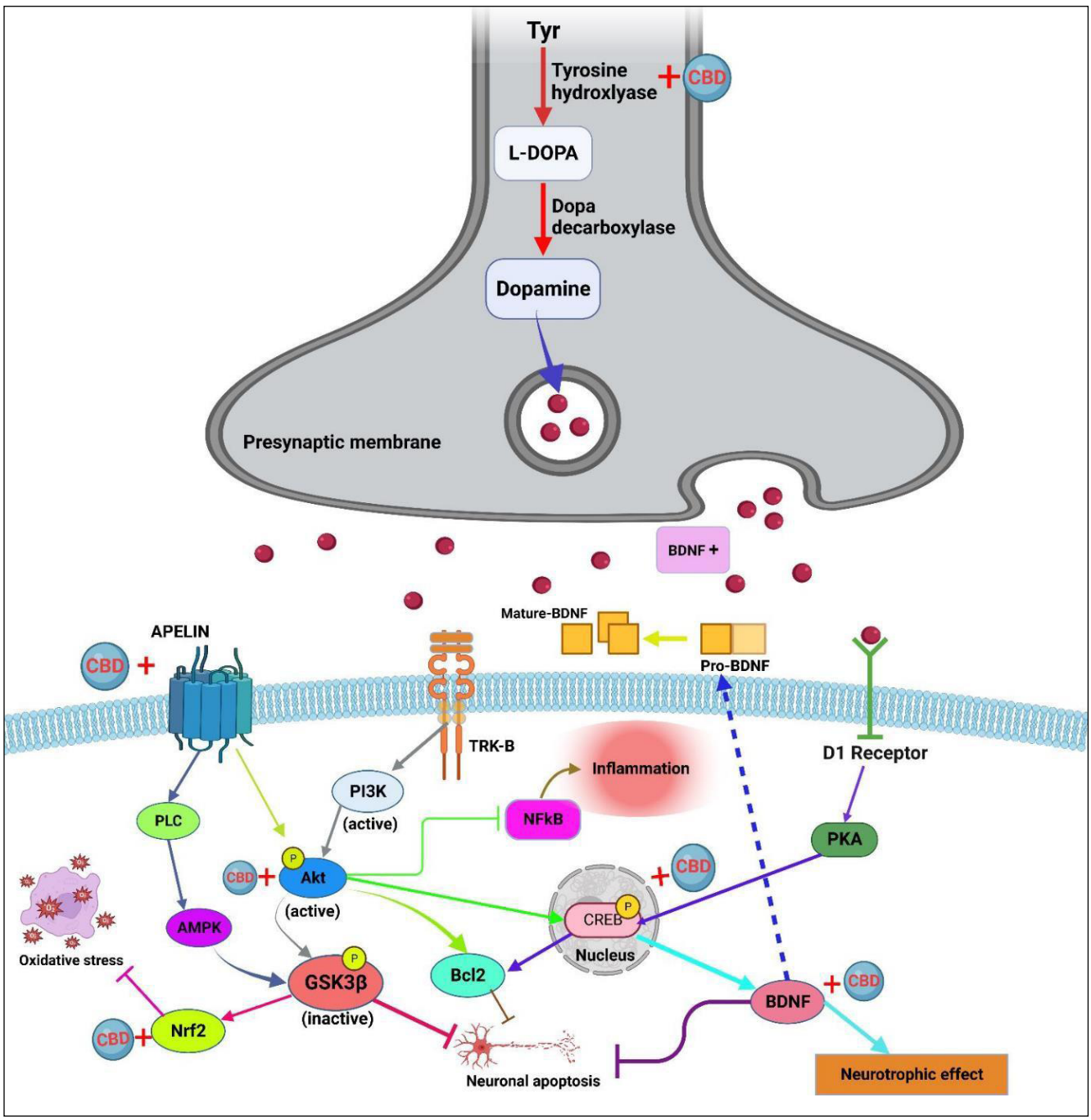

Fig. 6. The relationship between the action mechanism of CBD and the factors related with neuroprotection. CBD has the potential to positively impact presynaptic dopamine release by increasing tyrosine hydroxylase enzyme activity. TH activity leads to an increase in CREB followed by BDNF gene expression, activating the PI3K/Akt/GSK3β pathway through the Trk-B receptor, thus demonstrating a neuroprotective effect. Additionally, the increase it induces in Apelin synthesis positively affects NRF2 expression *via* the APJ receptor, again through GSK3β, explaining its antioxidant effect. CBD: Cannabidiol, PI3K: Phosphatidylinositol-3-kinase, PKA: Protein kinase A, AKT: Protein Kinase B, NRF2: Nuclear factor erythroid 2-related factor 2, AMPK: AMP-activated kinase, PLC: Phospholipase C, IP3: Inositol triphosphate, GSK3ß: Glycogen synthase kinase 3 beta, BDNF: Brain-derived neurotrophic factor, CREB: Cyclic-AMP response element binding protein, DA: Dopamine, TRK-B: Tropomyosin receptor kinase B, NF-κB: Nuclear factor kappa B, Bcl-2: B-cell lymphoma 2.

REFERENCES

Alfaris NA, Alshammari GM, Altamimi Dha JZ, Alagal RI, Aldera H, Alkhateeb May MA (2021) Ellagic acid prevents streptozotocin-induced hippocampal damage and memory loss in rats by stimulating Nrf2 and nuclear factor-kB, and activating insulin receptor substrate/PI3K/Akt axis. J Physiol Pharmacol 72: 503–515.

Aslankoc R, Ozmen O, Yalcin A (2022) Astaxanthin ameliorates damage to the cerebral cortex, hippocampus and cerebellar cortex caused by methotrexate. Biotech Histochem 97: 382–393.

Blöchl A, Sirrenberg C (1996) Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and p75(Lntr) receptors. J Biological Chem 271: 21100–21107.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: Minimum ınformation for publication of quantitative real-time PCR experiments. Clin Chem 55: 611–622.

Cao C, Gao T, Cheng M, Xi F, Zhao C, Yu W (2017) Mild hypothermia ameliorates muscle wasting in septic rats associated with hypothalamic AMPK-induced autophagy and neuropeptides. Biochem Biophys Res Commun 490: 882–888.

Capasso R, Borrelli F, Aviello G, Romano B, Scalisi C, Capasso F, Izzo AA (2008) Cannabidiol, extracted from Cannabis sativa, selectively inhibits inflammatory hypermotility in mice. Br J Pharmacol 154: 1001.

Castillo Díaz F, Caffino L, Fumagalli F (2021) Bidirectional role of dopamine in learning and memory-active forgetting. Neurosci Biobehav Rev 131: 953–963.

Corrê MDS, de Freitas BS, Machado GDB, Pires VN, Bromberg E, Hallak JEC, Zuardi AW, Crippa JAS, Schröder N (2022) Cannabidiol reverses memory impairments and activates components of the Akt/GSK3β pathway in an experimental model of estrogen depletion. Behav Brain Res. 417: 113555.

Dechant G, Neumann H (2003) Neurotrophins. Adv Exp Med Biol 513: 303–334.

Duan J, Cui J, Yang Z, Guo C, Cao J, Xi M, Weng Y, Yin Y, Wang Y, Wei G, Qiao B, Wen A (2019) Neuroprotective effect of Apelin 13 on ischemic stroke by activating AMPK/GSK-3β/Nrf2 signaling. J Neuroinflammation 16: 24.

Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37: 277–285.

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38: 1103–1111.

Faria MC, Gonçalves GS, Rocha NP, Moraes EN, Bicalho MA, Gualberto Cintra MT, Jardim de Paula J, José Ravic de Miranda LF, Clayton de Souza Ferreira A, Teixeira AL, Gomes KB, Carvalho M das G, et al. (2014) Increased plasma levels of BDNF and inflammatory markers in Alzheimer's disease. J Psychiatr Res 53: 166–172.

Fouad AA, Albuali WH, Al-Mulhim AS, Jreisat I (2013) Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. Environ Toxicol Pharmacol 36: 347–357.

García-Baos A, Puig-Reyne X, García-Algar Ó, Valverde O (2021) Cannabidiol attenuates cognitive deficits and neuroinflammation induced by early alcohol exposure in a mice model. Biomed Pharmacother 141: 111813.

Gu M, Mei X, Zhao Y (2021) Sepsis and cerebral dysfunction: BBB damage, neuroinflammation, oxidative stress, apoptosis and autophagy as key mediators and the potential therapeutic approaches. Neurotoc Res 39: 489–503.

Hu S, Shen P, Chen B, Tian SW, You Y (2022) Apelin-13 reduces lipopolysaccharide-induced neuroinflammation and cognitive impairment via promoting glucocorticoid receptor expression and nuclear translocation. Neurosci Lett 788: 136850.

Jang DC, Shim HG, Kim SJ (2020) Intrinsic plasticity of cerebellar Purkinje cells contributes to motor memory consolidation. J Neurosci 40: 4145.

Jiang C, Hanif K, Han L, Luo H, Xiang Y, Qu X, Liu H, Liu C, Li G, Qin X (2019) Apelin-13 suppresses neuroinflammation against cognitive deficit in a streptozotocin-ınduced rat model of Alzheimer's disease through activation of BDNF-TrkB signaling pathway. Front Pharmacol 10: 395.

Joshi G, Gan KA, Johnson DA, Johnson JA (2015) Increased AD-like pathology in the APP/ PS1ΔE9 mouse model lacking Nrf2 through modulation of autophagy. Neurobiol Aging 36: 664.

Jurcau A, Simion A (2022) Neuroinflammation in cerebral ıschemia and ıschemia/reperfusion injuries: from pathophysiology to therapeutic strategies. Int Mol J Sci 23: 14.

Kim Y, Lee S, Zhang H, Lee S, Kim H, Kim Y, Won MH, Kim YM, Kwon YG (2020) CLEC14A deficiency exacerbates neuronal loss by increasing blood-brain barrier permeability and inflammation. J Neuroinflammation 17.

Kozela E, Juknat A, Vogel Z (2017) Modulation of astrocyte activity by cannabidiol, a nonpsychoactive cannabinoid. Int J Mol Sci 18.

Li A, Zhao Q, Chen L, Li Z (2023) Apelin/APJ system: an emerging therapeutic target for neurological diseases. Mol Biol Rep 50: 1639–1653.

Liu W, Niu F, Sha H, Liu L, Lv Z, Gong W, Yan M (2020) Apelin-13 / APJ system delays intervertebral disc degeneration by activating the PI3K / AKT signaling pathway. Eur Rev Med Pharmacol Sci 24: 2820–2828.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. methods 25: 402–408.

Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J (2016) Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci 73: 3221.

Luo H, Xiang Y, Qu X, Liu H, Liu C, Li G, Han L, Qin X (2019) Apelin-13 suppresses neuroinflammation against cognitive deficit in a streptozotocin-induced rat model of Alzheimer's disease through activation of BDNF-TrkB signaling pathway. Front Pharmacol 10: 395.

Okten SB, Cetin C, Tok OE, Guler EM, Taha SH, Ozcan P, Ficicioglu C (2023) Cannabidiol as a potential novel treatment for endometriosis by its anti-inflammatory, antioxidative and anti-angiogenic effects in an experimental rat model. Reprod Biomed 46: 865–875.

Ozdamar Unal G, Asci H, Erzurumlu Y, Ilhan I, Hasseyid N, Ozmen O (2022) Dexpanthenol may protect the brain against lipopolysaccharide induced neuroinflammation via antioxidant action and regulating CREB/BDNF signaling. Immunopharmacol Immunotoxicol 44: 186–193.

Savran M, Aslankoc R, Ozmen O, Erzurumlu Y, Savas HB, Temel EN, Kosar PA, Boztepe S (2020) Agomelatine could prevent brain and cerebellum injury against LPS-induced neuroinflammation in rats. Cytokine 127: 154957.

Savran M, Ozmen O, Erzurumlu Y, Savas HB, Asci S, Kaynak M (2019) The ımpact of prophylactic lacosamide on LPS-ınduced neuroinflammation in aged rats. İnflammation. 42: 1913–1924.

Sun Y, Zhang H, Wu Z, Yu X, Yin Y, Qian S, Wang Z, Huang J, Wang W, Liu T, Xue W, Chen G (2021) Quercitrin rapidly alleviated depression-like behaviors in lipopolysaccharide-treated mice: the ınvolvement of PI3K/AKT/NF-κB signaling suppression and CREB/BDNF signaling restoration in the hippocampus. ACS Chem Neurosci 12: 3387–3396.

Tapias VE, Zainuddin S, Ahuja M, Stack C, Elipenahli C, Vignisse J, Gerges M, Starkova N, Xu H, Starkov AA, Bettendorff L, Hushpulian DM, et al. (2018) Benfotiamine treatment activates the Nrf2/ARE pathway and is neuroprotective in a transgenic mouse model of tauopathy. Hum Mol Genet 27: 2874.

Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladacenco O, Roza E, Costăchescu B, Grumezescu AM, Teleanu RI (2022) An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. İnt J Mol Sci 23.

Winstone J, Shafique H, Clemmer ME, Mackie K, Wager-Miller J (2023) Effects of tetrahydrocannabinol and cannabidiol on brain-derived neurotrophic factor and tropomyosin receptor kinase B expression in the adolescent hippocampus. Cannabis Cannabinoid Res 8: 612–622.

Xu BT, Li MF, Chen KC, Li X, Cai NB, Xu JP, Wang HT (2023) Mitofusin-2 mediates cannabidiol-induced neuroprotection against cerebral ischemia in rats. Acta Pharmacol Sin 44: 499–512.

Xu W, Li T, Gao L, Zheng J, Yan J, Zhang J, Shao A (2019) Apelin-13/APJ system attenuates early brain injury via suppression of endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation and oxidative stress in an AMPK-dependent manner after subarachnoid hemorrhage in rats. J Neuroinflammation 16: 247.

Zhang H, Chen S, Zeng M, Lin D, Wang Y, Wen X, Xu C, Yang L, Fan X, Gong Y, Zhang H, Kong X (2018) Apelin-13 administration protects against LPS-ınduced acute lung ınjury by ınhibiting NF-κB pathway and NLRP3 ınflammasome activation. Cell Physiol Biochem 49: 1918–1932.